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IDENTIFICATION OF C/T GENETIC MARKER IN AUTOSOMAL POLYCYSTIC KIDENY DISEASE AMONG SOUTH INDIAN POPULATION (MADURAI)

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Abstract: Autosomal dominant polycystic kidney disease (ADPKD) is a common disease among different population in worldwide. Mutations in the PKD1 gene on 16p13.3 are responsible for ~85% of cases of polycytic kidney disease. Until now, more than 1000 single nucleotide polymorphism (SNP) have been reported as genetic marker in different population. Hence, the study aimed to C/T polymorphism in PKD1 gene. Three hundred South Indian patients with clinically proven ADPKD were selected for C/T (PKD1 gene) gene polymorphism is identified with PCR, RFLP. Allelic frequency calculated using *Hardy-Weinberg equilibrium (p+q=1)* and the significant difference is found by *chi-square* test. C/T polymorphism at position 4058 in exon 45 of the PKD1 gene with ADPKD revealed that "TT", "CT" genotype and frequency mutant allele "T" is found to be significantly (at $p<0.001$) higher in patients compare to control subjects. The study concluded that, C/T transition mutation in exon 45 among South Indian patients is described. It is a single nucleotide polymorphism and autosomal dominant mutation, which is found to be equal distribution in both male and female. It is also demonstrated that "two –hit model of cytogenesis due to not only mutation in PKD1 also includes mutation in PKD2.

Keywords: Polycystin, Chronic kidney disease, Genotype, Mutation, Recessive allele

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease in human affecting all ethnic groups worldwide with an incidence of 1 in 500 to 1in 1,000 (Igarashi and Somlo, 2002). It occurs in both children and adults. The clinical manifestations include abdominal mass, chromic flank or back pain, gross hematuria, urinary tract infection and urolithiasis. In addition to causing progressive renal failure, renal cysts can be complicated by hemorrhage, rupture, infection, nephrolithiasis and intractable pain. Systemic hypertension is also very common occurring in more than 75% of patients. Increased blood pressure has been attributed to activation of the renin-angiotensin system. End stage renal disease usually occurs within 5 to 10 yrs after development of renal insufficiency. The ADPKD is caused by mutation in either PKD1 or PKD2 genes, with PKD1 mutation underlying over 85% of cases (Tazon-Vega *et al.,* 2007). Hence, the study focused on PKD1 gene C/T polymorphism. PKD1 gene was mapped to the short arm of 16th (16p13.3) chromosome, consisting of 46 exons distributed over 52kb of genomic DNA which encodes a 14.1kb mRNA transcript to be translated intron, a protein composed of 4302 amino acids transcript with an open reading frame (ORF) of 12, 909bp (Bogdonova *et al.*, 2002). The PKD1 and PKD2 gene codes proteins called polycytin1 and polycystin2, which are plays a vital role in cell –cell and cell-matrix interaction. Thus a defect in polycystin 1 and 2 were leads to alteration in the differentiation of epithelial cells and abnormal phenotypic expression of ADPKD1 and ADPKD2 (Hughes *et al.,* 1995). Thus a defect in polycystin 1 and 2 were leads to alteration in the differentiation of epithelial cells and abnormal phenotypic expression of ADPKD1 and ADPKD2 (Hughes *et al.,* 1995); it is genetically heterogeneous with two genes identified, PKD1 (16P13.3) and PKD2 (4q21) (*R*advind *et al.,* 1992, Igarishi and Somlo, 2002). Linkage analysis studies have revealed that the mutation of PKD1 is responsible for 85%, whereas mutation in PKD2 15% of the familial ADPKD (Gogusev *et al.,* 2003). Several research reports were stating that, gene polymorphism or mutations like frameshift, transition, transversion, insertion, addition, deletion, duplication, and silence, either in PKD1 or PKD2 leads to ADPKD **(Table:1).** Hence the study was focused on PKD1 (C/T) and PKD2 (G/C) single nucleotide polymorphism in patients with autosomal polycystic kidney disease among South Indian (Madurai) population.

MATERIALS AND METHODS:

Three hundred patients with autosomal dominant polycystic kidney disease (ADPKD) were selected from Madurai Rajaji Government hospital and Kidney transplantation and research centre, Madurai (TN), India. The prevalence of ADPKD was found to be equally affected in males (151) and females (149) in the age group of 10-80 yrs. The age and sex matched healthy individuals as control subjects were selected from healthy individuals. The blood samples were collected in EDTA coated tubes and stored at -20^{o}C. Ethical clearance was also obtained from

Institutional ethical clearance committee, Lady Doak College and Ethical clearance committee certificate from Government Rajaji hospital, Madurai.

C/T single nucleotide polymorphism (SNP) analysis in polycystic kidney disease 1 gene:

Genomic DNA was prepared from peripheral blood cells using standardized protocol (Sambrooke *et al*., 1993); then the gDNA was subjected to polymerase chain reaction (PCR) using thermal cycler (Eppendorf, India). The PKD1 gene segment was amplified using specific primers forward (5' – AGC TGT ACG CCC TCA CTG G – 3') and reverse (5' – GGA ACA ACT CCA CCA TCT CG – 3') (Fermentas life sciences). PCR was performed using gDNA (0.3µg), taq DNA polymerase (1U), 10 pmol of each primer and dNTPs (200µM). PCR condition was used as follows: initial denaturation for 4 minutes at 94oC, anealing for 40 seconds, extension for 45 seconds at 72oC (Constantinides *et .al.,* 1997, Koptides *et. al*., 1999, Veeramuthumari *et al.,* 2011). PKD1 exon 45 at position 14058 C/T (Ala/Val) polymerphism was typed using PCR-restricted fragment length polymorphism method. Amplified PKD1 gene segment (298bp) was digested with *AvaII*, which acts on the "T" variation, but not on the "C" variation. If a "T" allele was at position 14058 (225/73bp) fragments were obtained. PCR and RFLP products were confirmed by using 1% and 1.2% agarose gel electrophoresis.

Statistical Analysis: Allelic frequency was calculated using hardy- weinberg equilibrium (p+q=1). The significance level of genotype and allele frequency was tested by chi-square test (χ^2).

Results and Discussion:

The survey reports also showed that the APDKD patients were also affected by associated complications like hematuria (12%), renal calculi (10%), urinary tract infection (13%), diabetic nephropathy (17%), cardiovascular problems (21%), renal osteodystrophy (13%) and anemia (14%). 72% of the patients have high blood pressure (hypertension). The study also analyzed the autosomal dominant polycystic kidney disease (ADPKD) associated complications like dyslipidemia, diabetes mellitus, hypertension, anemia and cardiovascular disease were commonly noted in ADPKD patients. The cause might be due to low sodium, iron, high calcium, potassium and increase in the level of Triglycerides, total cholesterol, low density lipoprotein and also decrease in the level of high density lipoprotein. Verghese *et al.,* (2008) also reported that ADPKD is a multisystem disorder; some patients could develop associated intracranial aneurysm, which might cause stroke and intracranial hemorrhage. Much of the morbidity of ADPKD is due to chronic hypertension. The present study also found that 72% of the patients having systemic high blood pressure (110/80mmHg) (Hateboer *et al.,* 2000, Bhardwaj *et al*., 2013, Ramana *et al.,* 2012) also reported that the common complications include hypertension, macroscopic hematuria, urinary tract infection, renal calculi, cardiac valve abnormalities, herniae of the anterior abdominal wall and cerebral berry aneurysms in ADPKD.

The study also found that both male (48%) and females (52%) were equally affected by autosomal dominant polycystic kidney disease (ADPKD); this might be due to the gene inheritance in both the sexes equally and some of the normal individuals also affected by ADPKD. It might follow simple Mendelian coinheritance (Constantinides *et al.,* 1997). The occurrence of ADPKD among South Indain (Madurai) population also showed that, the most of the patients were in the age group of 30 – 40 years. Autosomal dominant polycystic kidney disease most often initially presents in adults aged 20-40 years (Verghese *et al.,* 2008). Clinically, it also characterized by progressive formation and enlargement of cysts, typically leading to end-stage renal disease (ESRD) in late middle age. PKD1 and PKD1 patients were similar to previously described, with the mean age of ESRD being 75.5 years (Torra *et al.*, 1999). Hence the study also selected the patients within age group of 10-80 years. The results also found that 2 patients are in the age group of 80.

Polycystic kidney disease (PKD) affects men and women equally, regardless of age, race or ethnic origin. It typically expresses itself in adults when they were in prime of life, between 30 and 40 years of age. It is passed from generation to generation by either the male or female parent. Each child of a PKD parent has a 50% chance of inheriting the disease (PKD News>Archive, 2000).

C/T marker analysis:

Genomic DNA was isolated from the patients and control subjects; after confirming the presence of genomic DNA with 0.7% agarose gel electrophoresis. It was subjected to polymerase chain reaction (PCR) and 298bp fragment was obtained, the amplified PCR product was digested with restriction enzyme called *AvaII*. The enzyme acts on the "T" variation but not on the "C" variation. If a "T" allele was present at position 4058, 225bp and 73bp were obtained. If it was homozygous mutant (CC) 225bp, 73bp; heterozygous mutant (CT) 298bp, 225bp, 73bp) and homozygous normal (TT) 298bp fragments were identified. The PCR and RLFP products were detected by 1.2% agarose gel electrophoresis.

Polycystic kidney disease 1 (PKD1) gene, which has the size of approximately 52kb containing 46 exons and encoding a 14 kb transcript. It defects account for about 85% of autosomal dominat polycystic kidney disease (ADPKD) (Thongnoppakhun *et al*., 1999; Chauvet *et al.,* 2002)). 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. The disease is characterized by the progressive formation and enlargement of fluid-filled cysts in both kidneys that leads to renal failure (Brown *et al.,* 2005). Cysts 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 (Igarashi and Somlo, 2002).

C/T polymorphism at position 4058 in exon 45 of the PKD1 gene among South Indian (Madurai) population with ADPKD revealed that the "TT" "CT" genotype and the frequency of "T" allele was found be significantly (p<0.05) **(Table: 2)** higher in the patients compared to control subjects. The study was demonstrated that ADPKD patients had higher frequencies of "T" allele and lower frequency of "C" allele than control subjects. The variation was at position 12173 of the PKD1, which was occupied by either C or T at the second position of codon 4058 (Hughes *et al*., 1995; Constantinides *et al.,* 1997).Hence; the amino acid residue encoded was either alanine or valine (Ala/Val4058).

The work was also done by Constantinides *et al.,* (1997) among Caucasian and Japanese population, he found that C/T4058 polymorphism with the association of ADPKD patients. The PKD1 gene is responsible for cause autosomal dominant polycystic kidney disease. It has been recently cloned and sequenced. ADPKD is a very frequent disorder among Caucasian population with an estimated incidence of approximately 1:100. It is characterized by genetic heterogeneity and three genes have been implicated in its pathogenesis called PKD1, PKD2 and PKD3 (Gabow 1993; Kimberling *et al.,* 1993; Peral *et al.,* 1996). PKD1 and PKD2 are on chromosomes 16 and 4 respectively. Recently several mutations were identified in both PKD1 and PKD 2 genes (Mochizuki *et al.,* 1996; Neophyton *et al*., 1996; Peral *et al.,* 1996; Rossetti *et al.,* 1996).

The PKD2 gene provides instructions for making a protein called polycystin-2. This protein is found in the kidneys before birth and in many adult tissues. The polycystin could be regulated by a larger and somewhat similar protein called polycystin-1, which is encoded by PKD1 gene. Polycystin-2 would be function as a channel spanning the cell membrane of kidney cells. In conjugation with polycystin-1, the channel transcripts positively charged atoms (ions) particularly calcium ions into the cells (http://ghr.nlm.nih.gov/gene=pkd2).

The influx of calcium ions triggers a cascade of chemical reactions inside the cell that might instruct the cell to undergo certain changes. Polycystin-1 and polycystin-2 work together to help regulate cell growth and division (proliferation), cell movement (migration) and interactions with other cells. The interaction of polycystin-1 and polycystin-2 in renal tubules promotes the normal development and function of the kidneys (Charron *et al.,* 2000). More than 250 mutations in PKD1 gene and more than 75 mutations in PKD2 gene have been identified in people with polycystic kidney disease. These mutations in PKD1 were responsible for about 85% and in PKD2 estimated to be responsible for 85% of ADPKD cases. The PKD3 gene is apparently responsible causing ADPKD (Almeida *et al.,* 1995; Bogdanova *et al.,* 1995; Daoust

et al., 1995; Constantinides *et al.,* 1997). Hence the current study focused on PKD1 gene C/T polymorphism in ADPKD among South Indian (Madurai) population.

Mutations in PKD1 and PKD2 genes include changes in single DNA building blocks (base pairs) and deletions or insertions of small number of base pairs in the gene. PKD2 gene mutations are predicted to result in the production of an abnormally small, non-functional version of the polycystin-2 protein. It leads to the cyst formation which likely disrupts the protein's interaction with polcystin-1 and alters signaling within the cell and in primary cilia. As a result, cells lining the renal tubules might grow and divide abnormally which is leading to the growth of numerous cysts characteristic of polycystic kidney disease (Gratham and Calvet, 2001).

Constantinides *et al.,* (1997) have been studied that Ala/Val4058 polymorphism in exon 45 of polycystic kidney disease 1 gene among Caucasians of Greek origin and on oriental of Japanese origin. They have been reported that "C" allele frequency observed to be hick (0.88) compared to "T" allele (0.12). However, The present study group showed the mutant "T" allele frequency (0.642) to be significantly higher in ADPKD patients than in the control subjects (0.4) and the normal "C" allelic frequency significantly decreased in ADPKD patients (0.7) than the control subjects (0.358) **(Table:2**) among South Indian (Madurai) population. This will be the first gene polymorphism reports on PKD1 gene among South Indian (Madurai) population. Therefore, in accordance with previously published reports; the present study also demonstrated an association between the PKD1 gene polymorphism and autosomal dominant polycystic kidney disease among South Indian (Madurai) population.

Hence, the present study concluded that (i) Abnormalities in basic lipid profile were also more frequent in ADPKD and it may constitutes a major atherogenic risk factor for the development of diabetes mellitus and cardiovascular disease which might be significantly decrease the life span of the patients with ADPKD. (ii) The elements like calcium, sodium, potassium and iron levels were also influenced in causing complications like hyponatremia, hypertension, hyperparathyroidism and renal osteodystrophy and anaemia in ADPKD patients among South Indian (Madurai) population. (iii) Association of Ala/Val4058 polymorphism showed that the severity of the ADPKD condition in ADPKD patients among South Indian (Madurai) population. This finding has important implications for our understanding of the pathogenesis of ADPKD (Santanu *et al.,* 2012).

Currently there is no effective therapy for ADPKD or ARPKD. Kidney transplantation and dialysis are the only methods that can be performed to prolong life. Murine models have been successfully employed to increase the understanding of the molecular mechanisms of polycystic kidney disease. These tests have been used to explore possible therapeutic techniques for polycystic kidney disease, mostly ADPKD, the more common form. Current treatments of

polycystic kidney disease involve caspase inhibitors, CDK inhibitors, Triptolide, and mTOR inhibitors (Torres *et al.,* 2007, Yadav, 2013, Paleti *et al.,* 2013, Margaret *et al.,* 2013).

The future study will be focused on PKD2 gene polymorphism and sequencing in the same population and further research on DNA based drug design by using bioinformatics data bases which might help the physicians in providing the better treatment for polycystic kidney disease patients.

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Table:1 Details of mutation identified:

Gene	Loci	Exon	Gene frequency (%)	Mutation	References
PKD1	16p13.3	46 (14.1kb)	85	Nonsense Frameshift Inframe Splicing Missense Insertion Deletion Nucleotide substitution	Rosetti *et al*.,(2001, 2002, 2003). Magistroni *et al.,* (2003) Boucher and Sandford, (2004) Ding *et al.,* 2002
PKD2	4q21-q23	15 (5kb)	15	Nonsense Frameshift Splicing Missense	Torra *et al.,* 1999, Rosetti *et al*.,(2001, 2002, 2003). Magistroni *et al.,* (2003) Boucher and Sandford, (2004)
PKD1	16p13.3	Exon 45	85	Single nucleotide polymorphism (SNP)	Presents study
PKD2	4q21-q23	Exon 1	15	Single nucleotide polymorphism (SNP) Insertion	Presents study

Table:2

Comparison of mutant and normal genotype and allelic frequency of PKD1 gene in control subjects and ADPKD subjects among South Indian (Madurai) population.

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

T/T – Homozygous mutant C/T – Heterozygous mutant C/C – Homozygous normal

*** denotes highly significant p<0.05 ** denotes significant p<0.05

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