

Investigation of Distribution of PKD1, PKD2 and GANAB Genes in Autosomal Dominant Polycystic Kidney Patients in Turkish Population

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Abstract

Objective: Mutational screening of the PKD1 and PKD2 genes, which are known to be associated with the severity of the disease, and the GANAB gene, which is a strong candidate gene, which is thought to be involved in the genetic basis of ADPKD, were performed in 16 cases admitted with the indication of autosomal dominant polycystic kidney disease (ADPKD).

Materials and Methods: A total of 16 cases who were diagnosed with ADPKD and met the criteria were evaluated. After DNA isolation from peripheral blood samples of the case group, PCR was performed with primers designed specifically for PKD1, PKD2 and GANAB genes. In this study, targeted resequencing method was applied by using MiSeq (Illumina, San Diego, CA) system for mutation screening of the aforementioned genes. In the analysis of detected variants, in silico analyzes, database searches and literature reviews were performed. For this, Varsome, Polyphen2, HGMD-Public, PubMed, Google search, Clinvar, EXAC and 1,000 Genomes studies were used.

Results: As a result of the sequence analysis of PKD1, PKD2 and GANAB genes from 16 cases diagnosed with ADPKD, at least one of the 3 genes analyzed for all patients and at least one disease-related variant was detected. The changes found are all heterozygous changes.

Six PKD1 polymorphism were found in 4 patients. In 11 of 16 patients, 14 different variants for the PKD1 gene (2 frameshifts, 5 missense, 3 nonsense, 1 splice region and 3 synonyms) were detected. In 6 of 16 patients, 6 different variants (3 nonsense, 1 missense, 2 synonyms) were detected for the PKD2 gene with a pathogenic effect. As a result of the GANAB gene sequence analysis, 6 different variants (1 missense, 3 synonyms and 2 noncoding) were determined for 5 patients. Of the 27 non-polymorphic variants detected for these 3 genes, 5 are novel mutations that have not been reported before. Of these 5 mutations, 3 were found in the PKD1 gene, and 1 in each of the PKD2 and GANAB genes. While 6 of these mutations in 16 patients are de novo (fresh mutation), 10 of them have autosomal dominant inheritance.

Conclusion: Our study results shed light on the genotype-phenotype correlation, the effect of genotype on clinical progression, and mutations not previously reported in the literature. In addition, our study results support that the GANAB gene is a strong candidate gene that plays a role in the genetic basis of ADPKD. It is recommended that similar studies be conducted with patient groups with a higher number of patients.

Keywords: Autosomal Dominant Polycystic Kidney Disease; PKD1 Gene; PKD2 Gene; GANAB Gene

Objective

Autosomal dominant/adult type polycystic kidney disease (ADPKD) is the most common form of hereditary renal cystic diseases. The kidneys end up looking like bubble-wrap kidneys in this disease. Its incidence is reported to be between 1/400-1000. ADPKD was found in the etiology of renal failure in 10-15% of hemodialysis patients [1].

The clinical features of ADPKD are variable and different in each patient. Flank pain is occurred due to large cysts caused compression of structures around the kidney or stretching of the kidney capsule. Hematuria may also be the presenting symptom of ADPKD. It is usually severe and recurrent. Urinary tract infection (UTI), kidney stones, heavy exercise, or mild trauma may caused hematuria. Nocturia and polyuria due to concentration defect are common symptoms. Polyuria may initially be mild and unnoticed, but in future that may worsen with increasing age and disease progression [2]. Hypertension is a common symptom seen in more than 50 percent of patients. Complications of hypertension, such as left ventricular hypertrophy (LVH) and LV diastolic dysfunction, may occur earlier, possibly due to increased renin secretion due to renal ischemia caused of cysts enlargement [3]. In past, had kidney stones is an important symptom of ADPKD, which occurs in approximately 20% of patients. The most appropriate method for the diagnosis of stones in ADPKD is Computed Tomography (CT) scanning [4]. The main complication of ADPKD is renal failure. About 45% of patients develop End-Stage Renal Disease (ESRD) at the age about 60 years. The age of onset of Chronic Renal Failure (CRF) varies between 2 and 80 years. These variations are caused from mutations in different genes or different mutations in the same gene. There are also important differences in the clinical course of the disease between affected members in the same family. This situation cannot be explained by locus or mutational heterogeneity. For example, family variations in the age of onset of ESRD average 15 years, but in some cases more than 30 years [5].

Cysts develop not only in the kidneys, but also in the liver, pancreas, ovaries, seminal vesicle, spleen and central nervous system. Polycystic Liver Disease is the most common extrarenal manifestation of ADPKD. Cysts are usually simple and solitary. Hepatomegaly is a common early finding in patients with Polycystic Liver Disease. Rare complications related to liver cyst enlargement such as; pain, early satiety, gastroesophageal reflux, dyspnea, orthopnea, ascites, obstructive jaundice and portal hypertension [6]. Pancreatic cysts are asymptomatic and seen generally rarely, ADPKD with incidence of 9% among ADPKD patients over 30 years of age. Pancreatic cysts are more common in patients with PKD2 mutations [7]. Intracranial aneurysm is seen in 9-12% of patients with ADPKD. The recommended method for the diagnosis and follow-up of intracerebral aneurysm is Magnetic Resonance (MR) imaging [8]. The major non-cystic extrarenal manifestations of ADPKD are heart valve disease and diverticular disease of the gastrointestinal tract. Mitral valve prolapse is the most common valve abnormality with incidence of 26% in cases with valve defect, followed by aortic regurgitation and myxomatous valve degeneration [9].

The polycystin 1 gene (Polycystin 1, PKD1) encodes transcript which is approximately 14-kb and contains 46 exons. The genomic region encoding PKD1 consists of complex segmental duplications (six duplicate copies, three-quarters of the 5' direction of the gene) and these regions exist as pseudogenes at on chromosome 16 [10]. "ADPKD Mutation Database" lists total of 1,650 "likely pathogenic" and "pathogenic" PKD1 gene mutations from approximately 2,450 families with PKD1-related ADPKD [11]. The polycystin-1 protein consists of 4,303 amino acids with a non-glycosylated molecular mass of 460 kd. The protein has a large extracellular region and 11 transmembrane domains with a short cytoplasmic tail. Polycystin-1 is mostly expressed in tubule epithelium in the kidney and epithelial cells in most organs. Its expression is also present in the endothelium and smooth, skeletal and cardiac muscles these are suggested polycystin-1' downregulation has a direct role in many

of the extrarenal manifestations of the disease [12]. Pathogenic PKD1 variants caused inactivation of one allele and develop cysts due to non-functional protein. The threshold hypothesis argues that cysts can develop when the presence of polycystin-1 protein below a certain level. This threshold can be induced with loss of the normal allele with somatic mutation or other non-genetic changes and this lead to cyst development [13].

The polycystin 2 gene (Polycystin 2, PKD2) consists of 15 exons and this is a gene with a transcript product of approximately 5.5 kb. According to the "ADPKD Mutation Database", 250 different PKD2 pathogenic variants have been identified from approximately 550 families [14]. The polycystin-2 protein consists of 968 amino acids and six cytoplasmic N- and C-terminated transmembrane domains. It shares a homology site with polycystin-1 in the transmembrane domain. Although polycystin-2 expression is widespread, it continues at a consistent level in adulthood [15]. As happen in PKD1, possible cause of disease in mutation of PKD2 is associated with the absence or complete loss of functional protein below a certain threshold.

Neutral Alpha-Glucosidase AB (Glucosidase, Alpha, Neutral AB, GANAB) gene consists of a 21.9 kb genomic region. The longest transcript variant has 25 exons and encodes the longest protein isoform [16]. 11 pathogenic variants of GANAB have been described in 12 families with ADPKD [17]. GANAB encodes Glucosidase II α , a protein of calreticulin that is involved in transcoding glycans of glycosylated proteins. This protein has an important role in the quality control of the membrane and secreted proteins [18].

The diagnosis of ADPKD is provided by imaging methods such as ultrasonography, computed tomography and magnetic resonance imaging [19]. However, in some cases molecular diagnosis may be necessary. These are; 1) For the definitive diagnosis of young individuals in families with suspected ADPKD by imaging methods, in cases such as possible donors for relatives 2) In patients with a negative family history in terms of ADPKD, due to overlap with other cystic diseases of the kidney 3) Because it may be associated with hypomorphic allele (loss of gene function) and/or oligogenic inheritance (trait affected by more than one gene) in families affected by early-onset polycystic kidney disease 4) In patients applying for genetic counseling for preimplantation genetic diagnosis [20].

In this study, it is aimed to evaluate the effects of mutations that can be detected by mutation analysis of the said genes on the course of ADPKD and its clinical presentation.

Materials and Methods

A total of 19 patients, 14 male and 5 female, who applied to Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Nephrology were included in the study. The inclusion criterias;

- Those who have this disease in their family
- Those with clinical findings that should be suspected of this disease
- Those who have multiple cysts during imaging examination such as ultrasonography or computed tomography performed for another purpose.

The detailed anamnesis and family history information of the patients were reached. The appropriate inheritance pattern was determined after the pedigrees of the patients were drawn. At the same time, the patients' past laboratory information and radiological information were investigated. Their consent was obtained by signing the Informed Voluntary Consent Form. Afterwards, 2 ml peripheral blood samples were taken from the patients in 2 EDTA tubes and stored at +4 0C. DNA isolation was performed with the Roche High Pure PCR Template Preparation Kit. For each sample obtained after isolation, DNA quantity and purity were measured using a spectrophotometer (Nanodrop ND-1000). After DNA isolation from peripheral blood samples of the patients, PCR was performed with primers designed specifically for PKD1, PKD2 and GANAB genes. In this study, targeted resequencing method was applied by using MiSeq (Illumina, San Diego, CA) system for mutation screening of the aforementioned genes. The MiSeq workflow consists of software and manufacturer-recommended cluster, sequencing, and analysis and steps. Sequences obtained with MiSeq Reporter analysis software were compared with reference sequences registered in databases. The program marks points that differ from the reference sequence, and these sequence differences are considered as disease-causing and non-disease changes at the time of reporting. Detected variants were evaluated by database searches, in silico analyzes, and literature reviews. For this, Varsome, Polyphen2, HGMD-Public, PubMed, Google search, Clinvar, EXAC and 1,000 Genomes studies were used.

Results

According to determined study criteria, 14 (74%) of the 19 patients included in the project were male and 5 (26%) were female. The mean age of disease onset of the patients was 34.5, the mean age at diagnosis was 38, and the mean age at diagnosis by genetic test analysis was 48.3.

According to the anamnesis data obtained, the most common complaint of the patients at the first admission to the hospital due to ADPKD was the presence of flank pain (10/19) (52.6%). The first admission complaint was hypertension in 3 of 19 patients, headache in 1 patient, and pollakiuria in 1 patient. In addition, 3 of 19 patients were diagnosed with ADPKD incidentally (15.8%). In this disease, the age range of onset of pathogenesis in the patient and the age of onset of symptoms is quite wide (21). The presence of the affected organ/system was questioned due to the long process of disease. Eleven of the patients had hypertension, 2 had retinopathy, 1 had anemia, and 1 had both diabetes mellitus and coronary artery disease. No additional involvement was detected in 6 patients. When taking anamnesis, the previous surgery was questioned in order to take knowledge about the medical history of the patients. While 2 of the patients (10.5%) had a history of renal transplantation, 3

of them had inguinal hernia (15.7%), 1 each of anal fissure, tonsillectomy, septoplasty, nephrectomy, cholecystectomy, bypass and lumbar hernia.

One of the most important steps in the evaluation of genetic diseases is to determine the inheritance pattern. At this stage, the family history information of the patients was questioned. There was consanguinity between the parents in 5 of the patients, and the degree of consanguinity in all of them was determined as 1st degree cousin marriage (26,3%). Parents of 12 patients were from the same village (63.1%). One of the most important clues of autosomal/X-linked dominant inheritance, "presence of similar cases in the family" was questioned. There was a positive family history in 17 of the patients (89.4%). In one of them, 9 of the family members had a similar story, in one of them 4 family members, in 2 of them 3 family members, in 10 of them 2 family members, and in 3 of them 1 family member had a similar disease. In addition, 11 patients had the disease in their first degree relatives, 2 had 2nd degree relatives, and 4 had both 1st degree and 2nd degree relatives with similar disease. In this group of disease, ESRD has been reported to start at an average age of 58 years [22]. In 5 of the patients, there was a family member in need of dialysis before the age of 58 (all 1st degree relatives).

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8
Age (years)	50	65	60	26	41	57	52	58
Presenting complaint	Incidental	Flank pain	Control for urinary calculus	Flank pain	Stomachache	Hypertension	Incidental	Flank pain
Presenting age (years)	35	35	25	15	38	41	-	38
Diagnosed age (years)	35	35	26	15	38	41	45	38
Affected Organ/System	Hypertension, unilateral retinopathy	Anemia	Hypertension, benign prostatic hypertrophy	Hypertension, retinopathy	-	Hypertension	Hypertension	-
Chronic Disease	Hypertension, CRF, arrhythmia	Anemia	Hypertension, gout	Hypertension	-	Hypertension, hyperlipidemia, CRF	-	CRF, heart failure
Past Surgery	umbilical hernia, cholecystectomy	-	-	Tonsillectomy, septoplasty	-	Unilateral radical nephrectomy	Bilateral inguinal hernia	renal transplantation (2 times)
Parental Consanguinity	Degree	-	-	-	-	-	-	-
	Same village	-	+	+	+	+	+	+
Similar Individual in the Family	Sister and brother	Sister and niece	2 daughter	Brother and father	Sister and father	Brother and mother	son	Maternal uncle

Table 1A: Summary of Patients' Anamnesis and Family History Information.

Patient ID	P9	P10	P11	P12	P13	P14	P15	P16
Age (years)	41	56	30	77	59	51	42	33
Presenting complaint	Polyuria, Urge	Flank pain	Flank pain, headache	İncidental	Flank pain	Flank pain	Family history of pkd	Hypertension, tachycardia
Presenting age (years)	37	46	20	63	55	46	26	32
Diagnosed age (years)	37	46	22	77	55	46	26	32
Affected Organ/System	Hypertension	-	Hypertension	Hypertension, coronary artery disease, diabetes mellitus	-	-	Hypertension, cyst in the liver	Hypertension
Chronic Disease	Hypertension, CRF, ESRD	CRF	Varicose veins in the legs, reactive hypoglycemia, hypertension	Hypertension, coronary artery disease, diabetes mellitus	Benign prostatic hypertrophy	Diabetes mellitus, dyspnea	Diabetes mellitus	Hypertension
Past Surgery	Bilateral inguinal hernia	-	Anal fissure	By-pass	İnguinal Hernia, Lumbar Hernia	Renal Transplantation	-	-
Parental Consanguinity	Degree		1 st degree cousin	1 st degree cousin			1 st degree cousin	
	Same village	+	+		+	+		+
Similar Individual in the Family	Sister, father and paternal aunt	4 sister, 2 brother, 3 daughter	Sister and father	-	Brother and maternal uncle	-	Brother, Maternal Uncle And Maternal Aunt	Aunt's daughter, aunt's son

Table 1b: Summary of Patients' Anamnesis and Family History Information.

Patient ID		P17	P18	P19
Age (years)		51	47	20
Presenting complaint		Flank pain	Flank pain, hypertension	Flank pain, nephrolithiasis
Presenting age (years)		39	46	20
Diagnosed age (years)		43	48	21
Affected Organ/System		Hypertension	Hypertension	-
Chronic Disease		Hypertension	Hypertension	-
Past Surgery		-	-	-
Parental Consanguinity	Degree		1 st degree cousin	
	Same village	+		+
Similar Individual in the Family		Son and daughter	Mother, maternal aunt, grandmother, aunt's daughter	Father

Table 1c: Summary of Patients' Anamnesis and Family History Information.

The clinical findings of ADPKD are quite variable and systematic. The patients included in the study were examined and evaluated in detail. The most common finding (11/19) (57.8%) in the patients was hypertension. 4 patients (21%) had CRF, 1 had hepatomegaly developing on the basis of Hepatitis B and Hepatitis C.

Radiological examination, one of the most valuable diagnostic tools in the diagnosis of ADPKD, was evaluated for all patients participating in the study. Multiple cysts were seen in the kidney

in all patients. At the same time, cysts were detected in the liver in 10 patients (52,6%) and in the prostate in 1 patient. 6 of the patients included in the study had a brain MR image and none of them had aneurysms. The presence of diverticulum, which is one of the extrarenal findings of the disease, and colonoscopies performed in the history of the patient file record system were examined. Colonoscopy was performed for 1 patient who have complaints that have lead to colonoscopy indication, and sigmoid colon diverticulum was detected.

Patient ID	P1	P2	P3	P4
Clinical Findings	Hypertension, CRF, arrhythmia, retinopathy	Anemia	Hypertension	Hypertension
Radiological Findings				
Abdominal CT	Cyst in the liver, multiple cysts in both kidneys (largest right: 24cm, left: 31 cm), hypointensity in the peripheral zone of the prostate	-	Multiple cysts in the liver, multiple cysts in both kidneys (largest right: 20 cm, left: 20 cm), cortex medulla cannot be differentiated	Multiple cysts in the liver, increased size and multiple cysts in both kidneys, thinning of the kidney parenchyma
Urinary USG	-	Increased echogenicity of parenchyma, a few cysts in the right lobe of the liver, cysts in both kidneys (the largest right: 25 cm, left: 24 cm)	-	-

Voiding	-	-	-	-
Brain MRI	-	Hyperintense millimetric foci in peri-supraventricular white matter	-	Normal
Neck USG	Cortical nodule	-	-	-
Endoscopy	-	-	-	-
Colonoscopy	-	-	-	-
Others	Echocardiography > Light MY	Brain angiography > Normal	-	-

Table 2a: Summary of the Clinical and Radiological Findings of the Patients.

Patient ID	P5	P6	P7	P8
Clinical Findings	-	Hypertension, CRF, hyperlipidemia	Hypertension	Hepatomegaly, HBV + HCV
Radiological Findings				
Abdominal CT	Cyst in the liver	Intra-cystic hemorrhage in the right kidney, prostate calcification, cyst in the liver, multiple cysts in the right kidney (largest 6 cm)	Cysts in the liver	Cortex/medulla distinction could not be made in both kidneys and multiple cysts (largest 4 cm) were found in both kidneys.
Urinary USG	Multiple cysts in both kidneys (largest right: 4.5cm left: 3.5cm)	-	Multiple cysts in both kidneys (largest right: 19cm left: 21cm), cortex medulla distinction could not	Cysts in the prostate
Voiding	-	-	-	Right kidney grade 4 hydronephrosis, Left kidney grade 3 hydronephrosis
Brain MRI	Normal	-	-	Hyperintense lesions in peri-supraventricular white matter
Neck USG	-	-	-	Anechoic cyst in left thyroid lobe
Endoscopy	-	-	-	Hyperplastic polyp in the stomach
Colonoscopy	-	-	-	Diverticulum in the sigmoid colon
Others	-	Lung CT > Pleural effusion	-	-

Table 2b: Summary of the Clinical and Radiological Findings of the Patients.

Patient ID	P9	P10	P11	P12
Clinical Findings	Hypertension, CRF, ESRD	CRF	Headache, hypertension	Hypertension, CRF
Radiological Findings				
Abdominal CT	5-6 cysts in both kidneys	Multiple cysts in the liver; dilatation in the intrahepatic bile ducts, multiple cysts in both kidneys (largest right: 6 cm left: 4 cm)	Cysts in both kidneys (left largest 1.6 cm, right largest 1.5 cm)	Calcification in the liver
Urinary USG	-	-		Multiple cysts in both kidneys (largest right: 42mm left: 46mm)
Voiding	-	-		-
Brain MRI	-	Normal	Millimetric gliotic focus in the left frontal subcortical region	-
Neck USG	-	-	-	-
Endoscopy	-	Antral erythematous gastropathy	-	-
Colonoscopy	-	-	-	-
Others	Echocardiography > Mild MR + Mild TR + Left ventricular hypertrophy	-	-	Echocardiography > Mild MR and TR- Left ventricular hypertrophy

Table 2c: Summary of the Clinical and Radiological Findings of the Patients.

Patient ID	P13	P14	P15	P16
Clinical Findings	-	-	-	Hypertension
Radiological Findings				
Abdominal CT	-	-	-	-
Urinary USG	3-4 cysts in the left kidney (largest 47 mm)	Multiple cysts in both kidneys (largest right: 22 cm, left: 21 cm)	Multiple cysts in both kidneys (largest right: 6.5 cm, left: 6 cm)	Multiple cysts in both kidneys (right: 55 mm)
Voiding	-	-	-	-
Brain MRI	-	-	-	-
Neck USG	-	-	-	-
Endoscopy	-	-	-	-
Colonoscopy	-	-	-	-
Others	-	-	-	-

Table 2d: Summary of the Clinical and Radiological Findings of the Patients.

Patient ID	P17	P18	P19
Clinical Findings	Hypertension, CRF	Hypertension	-
Radiological Findings			
Abdominal CT	Multiple cysts in the liver	Multiple cysts in the liver	-
Urinary USG	Multiple cysts in both kidneys (largest right: 38 mm, left 35 mm), hyperechoic appearance in liver (hemangioma?)	Multiple cysts of 40 mm in diameter in both kidneys	Multiple cysts in both kidneys (right 67 mm, left 56 mm)
Voiding	-	-	-
Brain MRI	Chronic ischemic changes	-	-
Neck USG	-	-	-
Endoscopy	Antral gastrit	-	-
Colonoscopy	-	-	-
Others	Echocardiography > Mild MR + Mild TR	-	-

Table 2e: Summary of the Clinical and Radiological Findings of the Patients.

Although there is no specific criterion for the number of cysts occurring in ADPKD, it has been reported that there are a lot of cysts. (20)Only 3 of the patients included in this study had less than 10 cysts on the renal ultrasound image. It was reported as “multiple cysts” in all 16 other patients. Hypertension is one of the most common complications of the disease. During the treatment and follow-up, systolic and diastolic blood pressure are measured.

In this study, the mean systolic blood pressure of the patients was measured as 126.47 mm Hg, and the diastolic blood pressure was measured as 81.7 mm Hg on average. The mean height of the patients was 170.75 cm and their mean weight was 82.41 kg. According to this information, the body mass indexes of the patients were evaluated and the average was found to be 28.31 kg/m2.

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8
Gender	M	F	M	F	F	M	M	M
Age (years)	50	65	60	26	41	57	51	58
Cyst Count	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple
Blood Pressure Systole (mmHg)	80	82	85	76	78	70	92	75
Blood Pressure Diastole (mmHg)	120	137	151	106	116	100	147	125
Family History of Dialysis/Renal Transplantation Before Age 58	Brother and sister	-	-	-	-	Brother	-	-
Length (cm)	180	150	175	173	167	175	170	179
Weight (kg)	95	66	95	60	101	72	78	70

Table 3a: Summary of Patients’ Clinical Information.

Patient ID	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
Gender	M	F	M	M	M	M	F	M	M	M	M
Age (years)	41	56	30	77	58	51	42	34	51	48	20
Cyst Count	6_6	Multiple	2	Multiple	3_4	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple
Blood Pressure Systole (mmHg)	83	92	75	-	78	88	-	93	133	130	121
Blood Pressure Diastole (mmHg)	127	138	119	-	116	127	-	137	89	80	73
Family History of Dialysis/Renal Transplantation Before Age 58	Sister and father	-	-	-	-	-	-	-	-	Mother	Father
Length (cm)	170	160	180	-	-	170	-	-	-	-	-
Weight (kg)	76	75	100	-	-	101	-	-	-	-	-

Table 3b: Summary of Patients' Clinical Information.

Laboratory data are very helpful in detecting renal and extrarenal findings that may occur during the course of ADPKD disease. The mean BUN value of the patients included in the study was 51.9, the mean creatinine value was 2.57, the mean eGFR value was 68.7, the mean sodium value was 139.1, the mean potassium value was 4.68, the mean hemoglobin value was 13.38, and the mean hematocrit value was 42.91. Chronic Renal Failure developed in 11 of the patients, these patients had CRF for an average of 8.1 years. Seven of them (63.6%) had history of dialysis. Flank pain and proteinuria which are clinical findings of ADPKD, was present, respectively, in 3 of the patients (15.7%) and 10 of the patients (52.6%). Hematuria, renal cell carcinoma and nephrolithiasis were not detected in any of the patients. In the laboratory values obtained for the follow-up of ADPKD extrarenal findings, the mean Triglyceride value of the patients was 176.1, the mean total cholesterol value was 171.9, the mean LDL value was 114.33, and the mean HDL value was 40.93. Liver cyst development, which is one of the most common extrarenal complications, was seen in 10 of the patients (52.63%). Although intracranial aneurysm was not detected in any of the patients, sigmoid colon diverticulum was seen in only 1 patient.

As a result of the sequence analysis of PKD1, PKD2 and GANAB genes from 19 patients diagnosed with ADPKD by the Nephrology department of our hospital, at least one of the 3 genes analyzed for all patients and at least 1 variant responsible for the disease were detected. The mutation / polymorphism classification of the variants determined for these genes was made based on the minor allele frequency of the laboratory from which the service was recruited.

The variants found were all heterozygous changes occurring in a single allele of the gene of interest. 32 different variants were detected from 19 patients. 20 different variants in the PKD1 gene were detected in 14 of 19 patients. Although 6 of the variants were pathogenic according to the ACMG (The American College of Medical Genetics and Genomics) classification, 4 variants of uncertain significance, 5 likely benign and 5 benign variant were detected. While 2 of these variants caused frameshift mutations, 11 were missense, 3 were nonsense, and 1 were splice region mutations. Also, 3 of them were synonyms. Six different variants

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
BUN	114	123	28,7	17	24	203	96,6	28,7	138	21	9	19,5	12,4	34,9	10	15,3	47	28	17,5
Creatinine	3,54	4,31	1,76	0,6	0,7	10,3	3,17	1,1	8,7	2,14	0,83	1,95	0,68	1,27	0,63	0,87	4,27	1,28	0,88
eGFR	19	10	41	126	107		21	73,6		25	118	32	105	65	109	113	15	66	123
Na	136	138	140	140	141	138	142	140	140	132	139	136	141	145	135	138	142	141	140
K	4	6	5	4	5	7	5	5	6	3	4	4	4	4	4	4	4,1	4,8	4,9
Hb/HCT	13,7/ 41,2	10,7/ 32,7	11,5/ 46	11,1/ 36,7	13,1/ 40,9	12,3/ 40,3	17/ 51,8	14,1/ 48,5	12/ 37,7	11,8/ 30,4	12,9/ 40,2	10,8/ 32,7	17,3/ 50,6	18,8/ 56,9	12,4/ 36,9	14,7/ 45,7	15,4/ 46,3	16,1/ 49,0	17,2/ 50,8
CRF (Year)	10	9	12	-	-	15	7	6	10	10	-	2	-	1	-	-	8	-	-
ESRD (Year)	-	+	-	-	-	3	-	+	3	-	-	-	-	-	-	-	1	-	-
Dialysis (Year)	-	+	-	-	-	2	-	20	2	2	-	?	-	-	-	-	+	-	-
Flank pain	-	-	-	-	+	-	-	-	-	-	+	?	-	-	-	-	-	-	+
Proteinuria	+	+	+	-	-	+	+	-	+	-	-	-	-	+	-	+	+	+	-
Hematuria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RCC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nephrolithiasis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Renal Findings.

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
Triglyceride	135	229	172	230	128	318	200	139	51	91	51	169	360	321	203	81	302	86	81
Total Cholesterol	164	191	189	139	197	194	138	172	149	175	174	122	223,4	181	189	147	191	182	150
LDL	113	142	156	114	153	126	89	103	90	126	101	70	140	119		102	116		84
HDL	45	40	38	41	41	37	32	57	49	32	63	20	25	50	45	35	29	53	46
Polycystic Liver	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	+	-
Intracranial Aneurysm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colon Diverticulum	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

Table 5a: Extrarenal Findings.

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
Triglyceride	135	229	172	230	128	318	200	139	51	91	51	169	360	321	203	81	302	86	81
Total Cholesterol	164	191	189	139	197	194	138	172	149	175	174	122	223,4	181	189	147	191	182	150
LDL	113	142	156	114	153	126	89	103	90	126	101	70	140	119		102	116		84
HDL	45	40	38	41	41	37	32	57	49	32	63	20	25	50	45	35	29	53	46
Polycystic Liver	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	+	-
Intracranial Aneurysm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colon Diverticulum	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

Table 5b: Extrarenal Findings.

with pathogenic effects for the PKD2 gene were detected in 6 of 16 patients. Of these variants, 3 were nonsense, 1 were missense, and 2 were synonymous. Although 3 of the variants were classified as pathogenic according to the ACMG classification, 1 of them was determined as likely benign and 1 as benign variant. As a result of the sequence analysis of the GANAB gene, 6 different variants were detected for 5 patients. 1 of these variants caused a missense variants, 3 of them led to synonymous and 2 of them led to non-coding variant.

Although 2 of the variants were classified as variants of uncertain significance according to the ACMG classification, 3 of them were identified as likely benign and 1 benign variant. 5 variants are novel mutations that have not been reported before in literature. Of these 5 mutations, 3 were determined in the PKD1 gene, and 1 in each of the PKD2 and GANAB genes. Pedigrees were drawn for all 19 patients and the inheritance pattern of the said variant was tried to be revealed. Accordingly, while 6 of these mutations were de novo, 13 of them had autosomal dominant inheritance.

Case id	Date of birth (year)	Age at testing (year)	Variant(s)						Zygosity	Is there another affected individual?	Mode of transmission
			GENE	Alteration	Exon/ intron number	Type	Acmg classification	Clinvar clinical significance			
P1	1969	50	PKD1	c.6730_6731delAG	exon 15	Frameshift	Pathogenic		Heterozygous	Mother, 2 siblings	Autosomal dominant
P2	1954	65	PKD1	c.11870G>A	exon 43	Missense	Likely Benign		Heterozygous	1 sibling, 1 nephew	De novo
P3	1959	60	PKD1	c.12133A>G	exon 44	Missense	Benign	Bening	Heterozygous	2 daughter	De novo
			PKD1	c.8898G>C	exon 24	Missense	Benign	Bening	Heterozygous		
			PKD1	c.9195_9196delG-TinsCC	exon 25	Missense	Likely Benign	Bening	Heterozygous		
			PKD2	c.1010T>A	exon 4	Nonsense	Pathogenic		Heterozygous		
P4	1993	26	PKD1	c.7302_7306delG-GGCC	exon 18	Frameshift	Pathogenic		Heterozygous	Father, 1 sibling	Autosomal dominant
P5	1978	41	PKD2	c.1010T>A	exon 4	Nonsense	Pathogenic		Heterozygous	Father, 1 sibling	Autosomal dominant
			GANAB	c.1026G>T	exon 10	Synonymos	Likely Beningn		Heterozygous		
P6	1962	57	PKD1	c.8017-2_8017-1del	exon 22	Splicing variant	Pathogenic	Pathogenic	Heterozygous	Mother, 1 sibling	Autosomal dominant
P7	1967	52	PKD2	c.784G>A	exon 3	Missense	Benign	Conflicting Interpretations Of Pathogenicity	Heterozygous	1 son	De novo
			GANAB	c.2322+18A>G	intron 19	Non coding	Uncertain Significance		Heterozygous		

Table 6a: Summary of Variants.

P8	1961	58	GANAB	c.917A>G	exon 9	Missense	Benign	Bening	Heterozygous	-	De novo
P9	1948	41	PKD1	c.12133A>G	exon 44	Missense	Benign	Bening	Heterozygous	Father, 1 sibling, 1 aunt	Autosomal dominant
			PKD1	c.6949C>T	exon 16	Missense	Uncertain Significance		Heterozygous		
P10	1973	56	PKD1	c.12436G>A	exon 45	Missense	Likely Benign	Bening	Heterozygous	6 siblings, 3 daughters	Autosomal dominant
			PKD1	c.11097C>A	exon 38	Nonsense	Pathogenic		Heterozygous		
P11	1989	30	PKD1	c.7302_7306delGGGCG	exon 18	Frameshift	Pathogenic		Heterozygous	Father, 1 sibling	Autosomal dominant
P12	1944	77	PKD1	c.10529C>T	exon 35	Missense	Benign	Bening	Heterozygous	-	De novo
			GANAB	c.282T>C	exon 4	Synonymos	Likely Benign		Heterozygous		
			GANAB	c.2799C>T	exon 24	Synonymos	Likely Benign		Heterozygous		
P13	1962	59	PKD1	c.2081C>T	exon 10	Missense	Uncertain Significance		Heterozygous	2 borother, 1 uncle	Autosomal dominant
			PKD1	c.9795C>T	exon 29	Synonymos	Benign		Heterozygous		
			PKD2	c.960A>T	exon 4	Synonymos	Likely Benign		Heterozygous		
P14	1970	51	PKD1	c.6593C>T	exon 15	Missense	Likely Benign		Heterozygous	-	De novo
			PKD1	c.12024C>T	exon 44	Synonymos	Uncertain Significance		Heterozygous		
			PKD1	c.12273G>A	exon 45	Nonsense	Pathogenic		Heterozygous		
			GANAB	c.1937-7C>T	intron 17	Non coding	Uncertain Significance (novel mutation)		Heterozygous		

Table 6b: Summary of Variants.

P15	1979	42	PKD1	c.10143C>T	exon 31	Synonymos	Likely Benign		Heterozygous	1 sibling, 1 aunt, 1 uncle	Autosomal dominant
			PKD1	c.10351G>A	exon 33	Missense	Uncertain Significance		Heterozygous		
			PKD2	c.2326C>T	exon 12	Nonsense	Pathogenic		Heterozygous		
P16	1988	33	PKD1	c.10002T>A	exon 30	Nonsense	Pathogenic		Heterozygous	1 aunt, 2 cousin	Autosomal dominant
			PKD2	c.1830G>A	exon 8	Synonymos	Benign		Heterozygous		
P17	1969	51	PKD1	c.7302_7306delGGGCG	exon 18	Frameshift	Pathogenic		Heterozygous	1 daughter, 1 son	De novo
P18	1973	48	PKD1	c.10002T>A	exon 30	Nonsense	Pathogenic		Heterozygous	Mother, 2 aunt	Autosomal dominant
P19	2001	21	PKD1	c.12273G>A	exon 45	Nonsense	Pathogenic		Heterozygous	Father	Autosomal dominant

Table 6c: Summary of Variants.

Discussion

According to national studies in the United States, it has been reported that ADPKD is much more common in women than in men. It has been suggested that this is due to the detection of kidney cysts during routine ultrasound imaging of most women during pregnancy [23]. In our study, on the other hand, an increase in male gender was found at total rate of 3/4. The mutations detected in the PKD1 and PKD2 genes in our study were found at almost the same rate as the sex ratio (PKD1 gene mutation rate 10/13 and PKD2 gene mutation rate 2/3 for male gender, respectively). However, in terms of the GANAB gene, the gene mutation rate for the male gender was found to be 4/5. In this situation, it was thought that there might be an increased expressivity for male sex-selective in the GANAB gene.

The age at diagnosis of the disease has been reported as 30-40 years [1,23,24]. In our study, the mean age at diagnosis was found to be 38, same with the literature. 5 of the patients in the study group were diagnosed between the ages of 0-29, 6 of them were between the ages of 30-40, and 7 of them were diagnosed between the ages of 41-55. Only 1 of the patients (P12) was diagnosed at the age of 77. There was no individual with similar phenotype in the pedigree of this patient, and there was a de novo mutation. It was thought that this situation might have delayed the age of diagnosis. At the same time, one variant in the PKD1 gene and two variants in the GANAB gene were detected in the patient in question. The variant in the PKD1 gene (NM_001009944):c.10529C>T was classified as benign, but the variants in the GANAB gene [(NM_198334):c.282T>C and (NM_198334):c.2799C>T] were likely benign. In this case, it suggests the possibility that GANAB gene mutations may qualify as "late-onset" in ADPKD.

Although various clinical symptoms of ADPKD have been reported, the most common ones are flank pain, hypertension, recurrent urinary tract infections, nephrolithiasis, and hematuria attacks. In literature, flank pain was reported as the most frequently reported symptom with a rate of 60-23% [25,26]. In our study, the most common reason for apply of patients was flank pain, which is compatible with the literature with a rate of 52.6%. The second most common reason for admission was hypertension, which was consistent with the literature [27].

Although ADPKD was diagnosed incidentally during ultrasonography in 3 of the patients (P1, P7 and P12), no symptoms

developed in these patients. The ages at diagnosis of these patients are 35, 45 and 77, respectively. Considering the average age at diagnosis of the disease, it can be suggested that the possible cause of this delayed diagnosis is the gene in which the mutation occurred. Among these patients, there is a change in the PKD1 gene in P1 (NM_001009944): c.6730_6731delAG, but this change causes a frameshift mutation and this is classified as pathogenic by ACMG. Although there are 2 changes in P7, one of them is the missense mutation in the PKD2 gene (NM_000297): c.784G>A and the noncoding mutation in the GANAB gene (NM_198334): c.2322+18A>G. Among these, the variant occurring in the PKD2 gene was classified as "benign" by ACMG and the mutation in the GANAB gene was classified as "clinical significance of uncertain". Both [(NM_198334):c.282T>C and (NM_198334):c.2799C>T] are synonymous mutations occurring in the GANAB gene. These changes have been classified as "likely benign" by ACMG. It was thought that there was a correlation between the age of onset the disease and GANAB gene mutations in our study group. However, this data should be supported by larger studies.

Because of the progression of the disease, renal transplantation indication occurs due to end-stage renal failure. The renal transplantation rate in the ADPKD group has been reported as 9.8% [28]. In our study, 2 of 16 patients (P8 and P14) had a history of renal transplantation, and this rate was found to be 10.5% for these patients. Among these patients, P8 had a missense mutation in the GANAB gene. For P14, 3 different variants were detected in the PKD1 gene, one of which was missense, one was synonymous and one was nonsense mutation. At the same time, a non-coding mutation in the GANAB gene was detected for this patient. While the GANAB gene mutation was common in both patients, it was thought that mutations in this gene might be responsible for the progressive course.

According to the pedigrees, autosomal dominant inheritance was determined in 12 of 14 patients with PKD1 gene mutation, and de novo mutation in 2 patients. This suggested that penetrance for the PKD1 gene was high. When the same situation was evaluated for PKD2 gene, autosomal dominant inheritance was determined in 4 of 6 patients and de novo mutation in 2 of them. Penetrance for the PKD2 gene is also over 50%, but for our patient group, there is a lower penetrance than for the PKD1 gene. For the GANAB gene, although autosomal dominant inheritance was determined

according to the pedigree in 1 of 5 patients, de novo mutation was detected in 4 patients. The penetrance of the GANAB gene was quite low. In our study, it is recommended to plan a larger study with a larger patient scale and molecular segregation analysis for the penetrance rate of the genes for which sequence analysis was performed.

With the new generation sequencing method used in this study, the rate of identification of novel mutations that were not previously reported in the literature has increased [29]. In our study, 7 new variants that were not previously reported in the literature were identified in a total of 8 patients. According to the ACMG criteria, 5 were classified as pathogenic and 1 as of uncertain clinical significance. Of these variants, 4 were identified in the PKD1 gene, 2 in the PKD2 gene and 1 in the GANAB gene. One of the 4 variants detected in the PKD1 gene has a frameshift mutation (NM_001009944): c.7302_7306delGGGCG other 3 variants (NM_001009944): c.11097C>A / (NM_001009944):c.12273G>A / (NM_001009944): c.10002T>A are nonsense mutations.

Of the 2 other variants in the PKD2 gene, (NM_000297): c.960A>T is a synonymous change, the other one (NM_000297): c.2326C>T is caused for the nonsense mutation.

The last variant detected in the study, which has not been reported before in the literature, is a change that occurs in the non-coding region (splice – intron), although it is defined in the GANAB gene (GANAB c.1937-7C>T), and its clinical significance is classified as uncertain. It is expected that the number of newly identified mutations will increase with new generation-based panel analyzes increase.

Conclusion

In conclusion, our study sheds light on the genotype-phenotype correlation, the effect of genotype on clinical progression and mutations that have not been reported before in the literature. It is recommended that, in future, similar studies with a higher number of patients. In addition, since there is limited information in the literature about the effect of the GANAB gene in ADPKD, future studies on this gene are promising.

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Conflict of Interest

The authors declare that *there is no conflict of interest* regarding the publication of this article.

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Ethics Declarations

All participants gave their informed consent and were studied under a protocol approved by the Health Sciences University Medical Ethic Committee. The study was approved by Afyonkarahisar Health Sciences University Ethics Committee at the meeting numbered 2021/8 dated 02.07.2021 with the code of 2011-KAEK-2 ethics committee.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created in this study.

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