Global Scientific JOURNALS

GSJ: Volume 11, Issue 3, March 2023, Online: ISSN 2320-9186
www.globalscientificjournal.com

MOLECULAR STUDY OF PKD1 GENE IN IRAQI PEOPLE SUFFERED WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

Yamama A. Zedan[1] and Owayes M. Hamed[1]
Department of Biology, College of Science,
University of Mosul,
Mosul-Iraq
*Corresponding author*s: yamamaayamamaa@gmail.com&*Owsbio31@uomosul.edu.iq*

Abstract:
The objectives of this study are to determine the genetic pattern, estimate gene frequency, estimate the prevalence of PKD, and provide a suitable plan for genetic counseling tailored to these participants in the study. A serious, life-threatening monogenic illness with a high rate of morbidity and mortality is autosomal dominant polycystic kidney disease (ADPKD). This condition affects between 1 in 400 and 1 in 1000 people worldwide. The polycystic kidney disease (PKD)1 and PKD2 genes have been substantially implicated in the development of ADPKD. However, it is unclear how harmful the various PDK1 polymorphisms are for the emergence of ADPKD. In this research, a total of 71 patients who were living in Iraq were enrolled. This study demonstrated that the PKD1 gene abnormalities cause the disease to be transmitted via an autosomal dominant gene. According to the results, the proportion of patients with (ADPKD), who carried the wild genotype GG was largest (42%) and the proportion who observed the mutant genotype CC was the lowest (27%), while the proportion of patients carrying the heterogeneous GC was (31%) as compared to the healthy group. The results, shows an increase in the levels of biochemical parameters in ADPKD patients, Glucose (70 mg\dl), Urea (27 mg\dl), creatinine (0.98 mg\dl), albumin (4.39 g\dl), GOT (21 IU\L), GPT (22 U\L) total bilirubin (0.59 mg\dl) and Calcium (10.31 mg\dl).
Key words: *PKD1* gene, T-ARMS-PCR, Polymorphism, mutation and ADPKD

Introduction:

One of the most prevalent and potentially fatal hereditary diseases in people, particularly in, is renal disease that is autosomal dominant and polycystic (ADPKD),which has a prevalence of 16–49% worldwide([1]..Mutations that are homozygous or heterozygous are two ways that human ADPKD might manifest. Humans who are heterozygous for the mutation continue to beclinically healthy until renal function fails after a period of ages, however homozygous fetuses will die before birth (often after the age of seven)[(2)].The polycystin-1 (PC1) protein develops an earlystop codon at position 3284 of the amino acid as a resultOn the exon 29 of the PKD1 gene's sequence position c.10063 of the point mutation (C to A transversion).The PC1 protein is produced by

the PKD1 gene. incorporates this stop codon early in the related mRNA transcript This protein's C-terminus will be reduced by around 25%[3].PC1, a transmembrane protein, is essential for the formation of tubules when the kidney is developing and is probably alsotubular involvement repair in adults. It interacts with other proteins to regulate a variety of cell behaviors to govern tube lumen size[4].

Several cysts filled with fluid will occasionallydevelop in the liver, pancreas, and uterus before birth due to the PC1 protein's impairment of function, which increases the multiplicity and death Contains renal tubular epithelial cells [5].Chronic increasing cysts of this size and number harm the renal parenchyma irreparably, which can result in renal failure and persistent kidney disease (CKD) [6].

With a frequency of roughly 1 in 800, polycystic renal disease that is autosomal dominant (ADPKD)is a prevalent congenital inherited illness [7].ADPKD frequently affects adults; 10% of people with advanced-renal stage disease and 50% of ADPKD patients who are 60 years of age or older proceed to ESRD have this condition. In addition to the kidneys, ADPKD also has an impact on the liver, brain, connective tissue, and digestive tract.Changes to the exon sequences or base changes in the polycystic kidney disease (PKD1) gene, which is located at 16p13.3, are significant contributors to the etiology of ADPKD[8] ·. There is still no viable treatment for ADPKD, a hereditary condition with a high incidence and serious consequences.Therefore, it is essential to identify carriers of and correctly diagnose patients. A recent diagnostic method for The process of genetic screening involves multiplex ligation-dependent probe amplification (MLPA).which is steadily taking over as the primary technique for detecting exon deletion and duplication[9].The PKD1 and PKD2 genes have been shown to include a variety of mutations. In the current investigation, we used MLPA to check individuals with ADPKD for potential mutations caused by exon deletion and exon duplication.to recognize and prevent potential data with erroneous positives, we added additional ways of verification to the MLPA results[10].The polycystin-1 protein is encoded by the 16th chromosome's short arm (16p13.3). has been identified as the location of PKD1. The PKD1 gene is very big, with 52 kb of genomic DNA and 46 exons. [11].14.1 kb of the gene's mRNA transcript, which has an open reading frame (ORF) of 12909 bp, can become a protein after translation with The 4302 amino acids [12].

The study's objective,is to detect the abnormality change in sequence in ADPKD patients who have PKD1 inIrqi population.

Case Study:

This study included (71) patient of an age group ranging from (20-55) years of reviews to the private pathological analysis laboratories in the city of Mosul for a period ranging from June to September of 2022, and it was relied on these clinical cases of the disease to choose Samples . The samples two groups were formed. parts according tobiochemical results: The first group: this group included 20 person among persons who did not suffer from any health problems and were considered as a control group, The second group: This group included 71 patienthave kidney disease, based on biochemical results..

Collection of Blood sample

5 ml of venous Blood was taken outfrom these patient and separated into two groups, the first component was put into tubes with EDTA anticoagulantto extract DNA, and a second group was put into tubes free of any anticoagulant. The tubes were left for one hour until the blood clotted, after which a centrifugation was carried out for a period of (10) ten minutes at a speed of (3000) cycles/minute to obtain the blood serum on which the biochemical tests were conducted.

DNA extraction:

DNA was extracted from the blood of (71) patient with control groups who were subjected to this study, using the modified method presented by (Iranpur and Esmailizadeh., 2010).

Genotyping:

Tetra–ARMS-PCR Reactions:

Adjustments are made to the DNA concentration in all research samplesafter being measured by biodrop by diluting them with TE buffer solution to achieve the necessary concentration for performing PCR reactions and was (25) ng/microliter for each sample.Four Primer addition for each primer reaction (F-outer and R-outer) for the whole gene, forward outer-reverse inner for the normal allele, forward outer-reverse inner) for the mutant allele.

The PCR reaction mixture is prepared by mixing the nucleic acid of each sample and the primer designated for the mutations under study using the master-components mix'sin a 0.2-ml PCR-tube produced by the English by Biolaps Company. Mix for five to three seconds in the Microfuge to make sure that the components of the reaction are mixed, Then, the PCR tubes were put insidethe thermocycler within the special program for each mutation, then the reaction product is injected into the pits of agarose gel that has been made with a concentration of 2%, by Including the Ladder DNA created by Biolaps Company, inside one of the first holes, after which the samples are migrated After operating the electrophoresis apparatus for 45 minutes, which followed the bands are imaged using a gel-documentation device.

Determination of the genetic variation of the PKD1 gene byTetra–ARMS-PCR technology

The presence of the G C mutation for PKD1 gene was discovered by adding 4 µl (100 nanogram) DNA templates and 1 µl (10 picomol) each of mutation specialized primer for PKD1 gene mutation supplied the ingredients of the master mix by the Korean business Macrogen

The presence of the AG mutation of for PKD1 gene by adding 4 µl (100 nanogram) of template DNA and 1 l (10 picompl) of each primer designed for a particular mutation., which was designed by the researcher using Pimer 3 software and used For the first time on this gene, it was produced by the Macrogen Company of Korea, and it was included into the master mix's composition. The final reaction volume was 20 µl (Ya-Jane Lee et al., 2010)[13].

Table 1: Shows the primers used to determine genetic variation at the locus (rs17467825) using PCR technology.

Primer	Sequence	Band size	Annealing
F-outer	5-TGTGTGGCAGGAGAGGAGAGAG-3	400 bp	59
R-outer	5-AGAGGCAGACGAGGAGCACT-3		
F-inner	5-GCCTCGTGGAGAAGGAGGT-3	276 bp	
R-inner	5-CCAGCGGGCCACCTGGTGC-3	170 bp	

The multiplication reaction was then carried out in the thermocycler using the reaction's custom protocol, as indicated in Table 2.:

Table 2: Shows the program adopted in the ARMS-PCR technique to identify the mutation (rs17467825)**.**

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation.	95.0.	5.0. min.	1

2.	Denaturation.	95.0.	45.0. sec.	35
3.	Annealing.	59.0.	1.0. min.	
4.	Extension.	72.0.	1.0. min.	
5.	Final extension.	72.0.	7.0. min.	1
6.	Stop reaction.	4.0.	3.0. min	1

The optimal temperature for the primer bonding in this reaction was determined using the Gradient program on the thermocycler device; the gradient was (5) and the mean temperature was (59) C. The temperature of 59°C was used because it provided the greatest results. Afterwards, the PCR reaction was separated by 2% agarose gel**.**

nucleotide determination sequencing of DNA-sequencing-based amplified fragments technology:

The gene's nitrogenous base sequence was determined for the PKD1 gene samples that were included in the study for the purpose of verifying the validity of the designed primer for the purpose of detecting the presence of any additional discrepancies in the gene. These genes were obtained using a Genetic Analyzer 3130 device provided by the Japanese firm Hitachi. The National Center for Biotechnology Information's NCBI gene sequence database was searched against these gene sequences, and the results were compared. The BLAST tool was used to examine the findings.(Morteza Bagheri*et al.*,2019)[14].

Table (3) : shows the primers belonging to the for PKD1 gene and on which the DNA sequencing test was conducted.

	Primer	Sequence
PKD1 gene	PKD-Exon25-F	5′GGTGGTTGAGCTTCCCGG 3′
	PKD-Exon25-R	5′ ATGTAGGTCACCAGGCACAC3′

Biochemical test:

This study also include some biochemical parameters used as a kidney frailer markers like (glucose -urea -creatinine -albumin -got -gpt -total bilirubin -calcium -cholesterol -triglycerides -s.iron -uibc -phosphorus).

Result and discussion.

The results showed, as in Figure (1), the existence of a relationship between Iraqi people Polycystic kidney disease that is autosomal dominant (ADPKD) and the mutation of PKD1 Gene, As shown in Table (4).

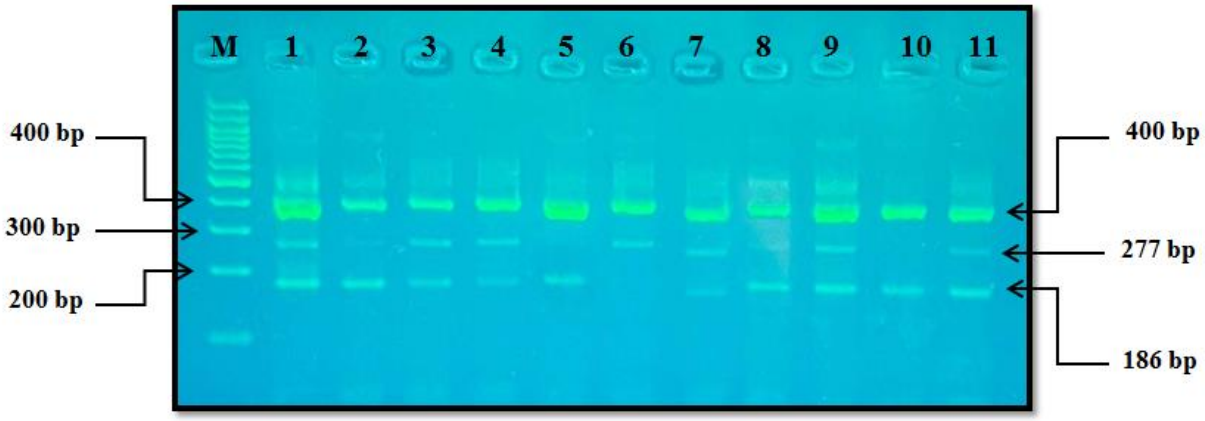


Figure (1): the relationship between Iraqi people Polycystic kidney disease that is autosomal dominant (ADPKD)and the mutation of PKD1 Gene, with three band in PCR product. 400 bp fpr universal band, 277 bp for wild type allele and 186 for mutant type allele, samples (1) wild genotype GG, samples (1,3,4,7,9,11) have hetero-genotype GC and samples (2,5,8,10) have mutant genotype CC.

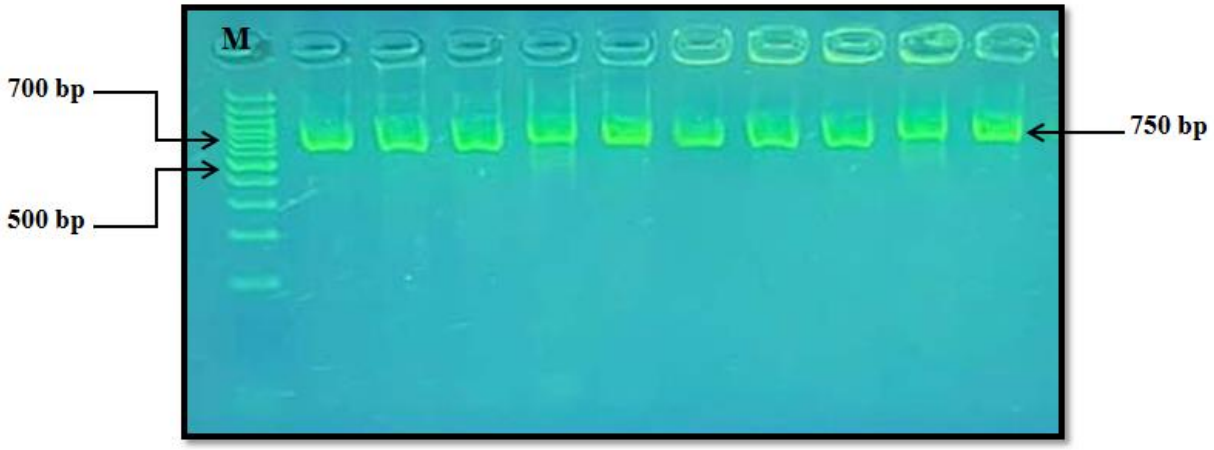
Table (4): Distribution of the percentage of allelic observations and genotypes of the PKD1 gene mutation among a group of healthy groups and Iraqi people with (ADPKD), knowing that the G allele is the normal allele and the C allele is the mutant allele:

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
GG	30	42	18	85	P = 0.0227	11.4000	1.4043 to 92.5469
GC	22	31	2	10			
CC	19	27	1	5			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
G	82	57	38	90	P = 0.0005	7.1667	2.3767 to 21.6104
C	82	43	4	4			

According to the study's results, the proportion of patients with (ADPKD), who observed the wild genotype GG was largest (42%) and the proportion who observed the mutant genotype CC was lowest (27%), and the proportion of observing for the heterogeneous GC was (31%) comparison to healthy group, who observed the wild genotype GG was largest (42%) and the proportion who observed the mutant genotype CC was lowest (27%), and the proportion of observing for the heterogeneous GC was (31%), The allelic observation rate in patients with (ADPKD)was 57 percent for the mutant G allele and 43percent for the normal c allele.

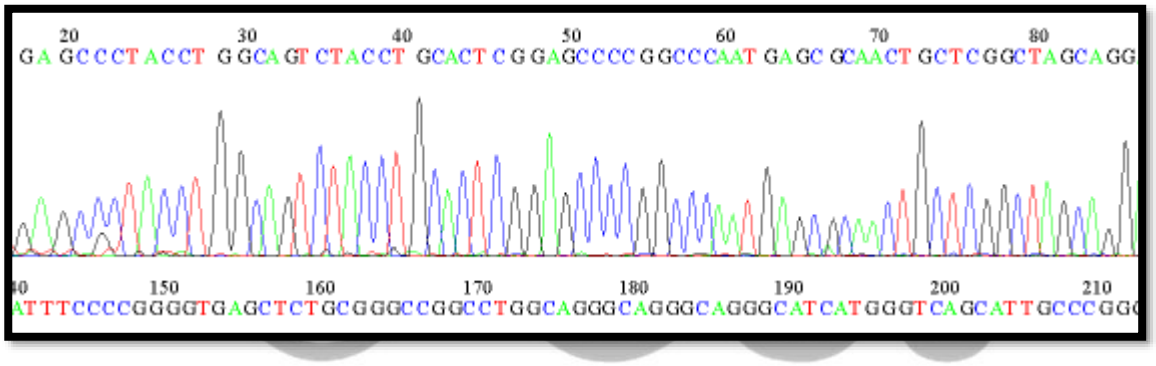
sing DNA sequencing technology to determine the nucleotide sequence of amplified portions:

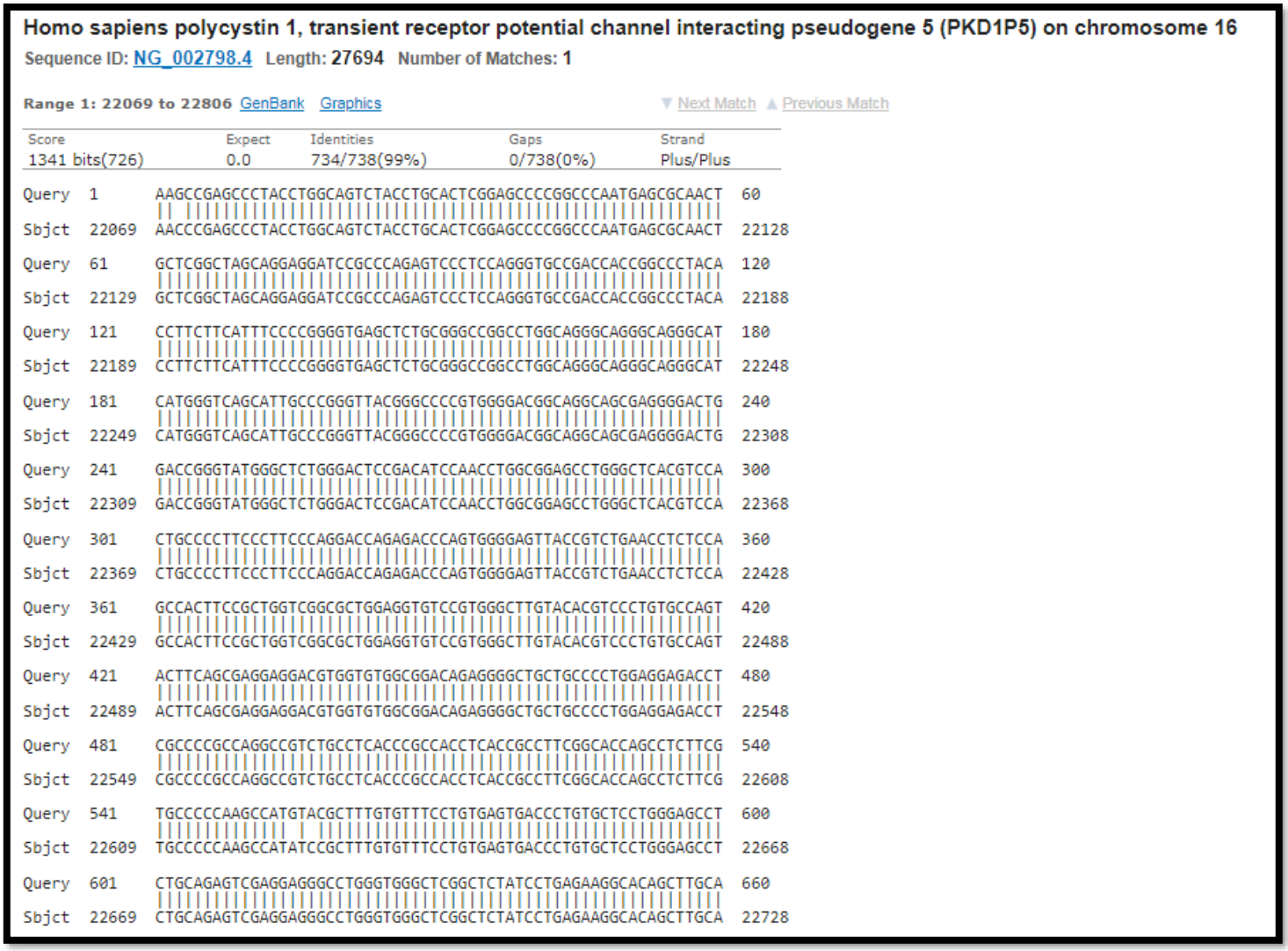
Figure (2)show the PCR reaction 750 bp, for PKD1 gene, on which the nucleotide sequence identification test was performed.



igure (3): shows the PCR reaction product of PKD1 gene in patients with ADPKD disease and the reaction product 750 bp, M is the Ladder with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel.

he results of a sequencing test for the amplified of PKD1gene had differences in a number of nucleotides, however exon 5 had no differences. These findings are depicted in the following figures:





Homo sapiens polycystin 1, transient receptor potential channel interacting pseudogene 5 (PKD1P5) on chromosome 16

Sequence ID: NG_002798.4 Length: 27694 Number of Matches: 1

Range 1: 22069 to 22806 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1341 bits(726)	0.0	734/738(99%)	0/738(0%)	Plus/Plus

```
Query  1      AAGCCGAGCCCTACCTGGCAGTCTACCTGCACTCGGAGCCCCGGCCCAATGAGCGCAACT  60
              ||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22069  AACCCGAGCCCTACCTGGCAGTCTACCTGCACTCGGAGCCCCGGCCCAATGAGCGCAACT  22128

Query  61     GCTCGGCTAGCAGGAGGATCCGCCCAGAGTCCCTCCAGGGTGCCGACCACCGGCCCTACA  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22129  GCTCGGCTAGCAGGAGGATCCGCCCAGAGTCCCTCCAGGGTGCCGACCACCGGCCCTACA  22188

Query  121    CCTTCTTCATTTCCCCGGGGTGAGCTCTGCGGGCCGGCCTGGCAGGGCAGGGCAGGGCAT  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22189  CCTTCTTCATTTCCCCGGGGTGAGCTCTGCGGGCCGGCCTGGCAGGGCAGGGCAGGGCAT  22248

Query  181    CATGGGTCAGCATTGCCCGGGTTACGGGCCCCGTGGGGACGGCAGGCAGCGAGGGGACTG  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22249  CATGGGTCAGCATTGCCCGGGTTACGGGCCCCGTGGGGACGGCAGGCAGCGAGGGGACTG  22308

Query  241    GACCGGGTATGGGCTCTGGGACTCCGACATCCAACCTGGCGGAGCCTGGGCTCACGTCCA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22309  GACCGGGTATGGGCTCTGGGACTCCGACATCCAACCTGGCGGAGCCTGGGCTCACGTCCA  22368

Query  301    CTGCCCCTTCCCTTCCCAGGACCAGAGACCCAGTGGGGAGTTACCGTCTGAACCTCTCCA  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22369  CTGCCCCTTCCCTTCCCAGGACCAGAGACCCAGTGGGGAGTTACCGTCTGAACCTCTCCA  22428

Query  361    GCCACTTCCGCTGGTCGGCGCTGGAGGTGTCCGTGGGCTTGTACACGTCCCTGTGCCAGT  420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22429  GCCACTTCCGCTGGTCGGCGCTGGAGGTGTCCGTGGGCTTGTACACGTCCCTGTGCCAGT  22488

Query  421    ACTTCAGCGAGGAGGACGTGGTGTGGCGGACAGAGGGGCTGCTGCCCCTGGAGGAGACCT  480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22489  ACTTCAGCGAGGAGGACGTGGTGTGGCGGACAGAGGGGCTGCTGCCCCTGGAGGAGACCT  22548

Query  481    CGCCCCGCCAGGCCGTCTGCCTCACCCGCCACCTCACCGCCTTCGGCACCAGCCTCTTCG  540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22549  CGCCCCGCCAGGCCGTCTGCCTCACCCGCCACCTCACCGCCTTCGGCACCAGCCTCTTCG  22608

Query  541    TGCCCCCAAGCCATGTACGCTTTGTGTTTCCTGTGAGTGACCCTGTGCTCCTGGGAGCCT  600
              |||||||||||||| | ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22609  TGCCCCCAAGCCATATCCGCTTTGTGTTTCCTGTGAGTGACCCTGTGCTCCTGGGAGCCT  22668

Query  601    CTGCAGAGTCGAGGAGGGCCTGGGTGGGCTCGGCTCTATCCTGAGAAGGCACAGCTTGCA  660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22669  CTGCAGAGTCGAGGAGGGCCTGGGTGGGCTCGGCTCTATCCTGAGAAGGCACAGCTTGCA  22728
```

D sequence	Nucliotide	Location	Mutation type	Idendity	Gaps
(NG_002798.4)	C → G	(22071)	(Transversion)	(99%)	(0)
(NG_002798.4)	A → G	(22023)	(Transition)	(99%)	(0)
(NG_002798.4)	C → A	(22025)	(Transversion)	(99%)	(0)
(NG_002798.4)	→ A	(22122)	(Deletion)	(99%)	(1)
(NG_002798.4)	A → G	(22623)	(Transition)	(99%)	(0)

(NG_002798.4)	CA →	(22625)	(Transversion)	(99%)	(0)
(NG_002795.4)	-- → A	(15705)	(Addition)	(99%)	(1)
(NG_002795.4)	TC →	(15802)	(Transition)	(99%)	(0)
(NG_002795.4)	A → G	(16191)	(Transition)	(99%)	(0)
(NG_002798.4)	→ C	(22085)	(Addition)	(99%)	(1)
(NG_002798.4)	A → G	(22770)	(Transition)	(99%)	(0)
(NG_002800.3)	C → G	(12447)	(Transversion)	(99%)	(0)
(NG_002800.3)	T → C	(12881)	(Transition)	(99%)	(0)
(NG_002800.3)	G → A	(12972)	(Transition)	(99%)	(0)
(NG_002800.3)	A G	(12984)	(Transition)	(99%)	(0)

Table(5) : The result of matching with the nucleotide sequence of PKD1 gene was compared with the sequences of the original gene at the NCBI site

When the table (5) is observed, It exhibits the different the many genetic variants and their locations on the PKD1 gene after conducting a sequencing test and matching them up with the gene sequences on the NCBI website.

The results of this study also shows an increase in the levels of biochemical parameters in ADPKD patients, Glucose (70 mg\dl), Urea (27 mg\dl), creatinine (0.98 mg\dl), albumin (4.39 g\dl), GOT (21 IU\L), GPT (22 U\L) total bilirubin (0.59 mg\dl)and Calcium (10.31 mg\dl), compare with healthy group: Glucose (74 mg\dl), Urea (17 mg\dl), creatinine (0.7 mg\dl), albumin (3.3 g\dl), GOT (12.4 IU\L), GPT (10.5 U\L) total bilirubin (0.69 mg\dl) and Calcium (8.7 mg\dl).

Discussion:

Iraqi ADPKD patients' PKD1 mutation analysis has not before been reported. even if there are only a small number of ADPKD families (less than 100 in all prior investigations) as of late, Jin et al[15] .among 71 patients with ADPKD, PKD1 analysis has not been published. However, the absence in their pedigree analysis investigation restricted the ability to assess a condition's pathogenicity discovered mutations, particularly for those variants that were presumably pathogenic.The current study's overall PKD mutation detection..While no PKD mutations were found discovered among these patients, other mechanisms most likely caused a decrease in PC1 protein levels. According to earlier research, the PKD gene's 3′ UTR might bind to microRNA-17 and microRNA-93, which would then post-transcriptionally down-regulate the production of the gene[16].Furthermore, Zheng et al. discovered that PKD2's 3′ UTR might bind to protein 1 that binds to upstream elements, which would prevent translation of PKD2[17]. With a detection rate of 2.4% in our investigation, 2 significant deletion mutations in PKD1 were discovered, which was consistent with previously published results. With little pedigree information, it is difficult to assess the pathogenicity of missense mutations. However, not all pedigree data required for determining pathogenicity is readily accessible to researchers. Pathogenicity of missense mutations is assessed has been aided by the development of many predictionstechniques (SIFT, Mutation Taster, and PolyPhen-2). Pkd1 has two mutations (c.4984G>A and c.7544G>C), which were shown to be by the prediction tools, benign mutations, were identified through pedigree analysis as genetic defects. This is despite the fact that the infectivity

of the majority each of the missense mutations analyzed by the prediction methods in this investigation was in agreement with the findings. Sorting analysis is advised as the gold standard to assess missense mutations' ability to cause disease as opposed to prediction techniques because ADPKD is a single-gene hereditary disease. however it was shown our study's pedigree analysis to be a non-pathogenic mutation. This may imply that more data from various the pedigrees needed to assess missense mutations' potential for disease. The substantial PKD1 allelic heterogeneity and the incidence private mutations in individuals with ADPKD indicate that mutations that are new occur often in this illness. In our study, 120 families (7.5%) had 9 de novo mutations in total, which is higher than the variety of 0.9% to 3.1% described In prior research [(18)].This disparity could be partially explained by a comprehensive gathering pedigree information from all of the families included in our research. We discovered that PKD1 mutant carriers generally experienced ESRD at a younger age than PKD2 mutation carriers. Additionally, individuals with truncating mutations had worse clinical outcomes than patients without these mutations, confirming the prior research' findings that there is a significant association between the type of mutation and the median age at ESRD startPKD1[(20)]. High allelic variability exists in PKD1, and no hotspot mutation location has yet been identified. This gene's mutations are typically highly varied and private. Therefore, patients with ADPKD require a thorough PKD1 mutation investigation[(21)]. among the mutation types identified in this study. According to earlier investigations, the truncating mutations the most prevalent type of sequence change in our patients. Inactivating these mutations and result in ADPKD by causing the polycystin-1 protein to be lost or produced at a lower dosage. Through its impact on splicing, the harmful missense variation (PKD1) is also projected to be truncating. This mutation eliminates the intron 38 donor splice site, resulting in the integration of this exon with the remaining 117 bytes of exon 39 and the omission of intron 38. An early stop codon in the mRNA causes lower-sized polycystin-1 protein that lacks the final C terminal amino acids, 585 of them, making it more prone to nonsense-mediated degradation. Studies have revealed that polycystin-1-expressing missing in transfected cells their Polycystin-2 and carboxyl terminus do not interact.. However, prior research using ADPKD animal models has demonstrated that a reduction in PKD1 expression is adequate to cause Vascular and cytogenesis abnormalities [(22)]. The C terminal region of PKD1 has a higher frequency of mutations than the N terminal part, according to this study. A systemic illness with a preference for the kidneys, ADPKD. Even within the same family, the severity of ADPKD and other diseases of the kidneys consequences vary among those affected[(23)]. Patients with ADPKD may also experience complications from other organs, including infertility and cardiovascular issues. Infertility in patient 7 who has the mutation p.Gln3395Ter, ESRD in the mother of patient 5 with p.Arg3719Gln mutations, and vascular problems in patient 9 who has the mutation p.Gln4042Ter are consistent with other study [(21)].although research has shown that Polycystic is produced in the flagella and cilia and aberrant proteins might cause infertility in men, individuals with ADPKD are typically fertile despite this[(25)]. Polycystin-1 is thought to be involved in cell-cell communication, Polycystin-2, however, is a calcium (Ca2+) permeable ion channel that may be involved in controlling internal calcium ion in cells levels. Additionally, polycystins 1 and 2 are expressed in endothelial cells and vascular smooth muscle, indicating that they directly contribute to the disease's vascular symptoms[(24)]. discover more mutations and broadening the range of PKD1 mutations can improve the diagnostic utility of molecular analysis in ADPKD patient screening and aid in the therapeutic reduction of mortality and morbidity related to renalproblems or another illness -related consequences.

Conclusion: According to this study, the observation of different genotypes and allelic frequency of PKD1 gene and detection some mutation by Sequence technique mutation in patients with ADPKD in Iraqi population.

References :

1- Guerra JM, Cardoso NC, Daniel AG, et al. Prevalence of autosomal dominant polycystic kidney disease in Persian and Persian-related cats in Brazil. Braz J Biol 2020; 81: 392–397.to further examine their pathogenicity using minigene splicing.

2 Young AE, Biller DS, Herrgesell EJ, et al. Feline polycystic kidney disease is linked to the PKD1 region. Mamm Genome 2005; 16: 59–65.

3 Lyons LA, Biller DS, Erdman CA, et al. Feline polycystic kidney disease mutation identified in PKD1. J Am Soc Nephrol 2004; 15: 2548–2555.

4 Zhou J. Polycystins and primary cilia: primers for cell cycle progression. Annu Rev Physiol 2009; 71: 83–113.

5 DomanjkoPetrič A, Černec D and Cotman M. Polycystic kidney disease: a review and occurrence in Slovenia with comparison between ultrasound and genetic testing. J Feline Med Surg 2008; 10: 115–119.

6 Lyons LA. Feline genetics: clinical applications and genetic testing. Top Companion Anim Med 2010; 25: 203–212.

7 TorraBalcells TR, Ars Criach AE. Molecular diagnosis of autosomal dominant polycystic kidney disease. Nefrologia. 2011; 31(1):35–43.

8. Ong AC, Harris PC. Molecular pathogenesis of ADPKD: The polycystin complex gets complex. Kidney Int. 2005; 67(4):1234–1247.

9. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30(12):e57.

10. Ravine D, Gibson RN, Walker RG, Sheffield LJ, Kincaid-Smith P, Danks DM. Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. Lancet. 1994;

11. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San MJ, Gamble V, Harris PC. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell rec- ognition domains. Nature Genetics. 1995;10:151-160

12. Bogdanova N, Markoff A, Gerke V, McCluskey M, Horst J, Dworniczak B. Homologues to the first gene for autosomal dominant polycystic kidney disease are pseudogenes. Genomics. 2002;74:333-341

13.L.Ya-Jane ,Y.Hisn ,W.Min-Liang ,H.Wei-Li , Molecular detection of autosomal-dominant feline polycystic kidney disease by multiplex amplification refractory mutation system polymerase chain reaction ,Molecular Biology , J Vet Diagn Invest 22:424-428 (2010) .

14. B,Morteza , M,Khadijeh , A.Ali , N.Ahmed , R.Isa , Examining the Role of Polymorphisms in Exon 25 of the PKD1 Gene in the Pathogenesis of Autosomal Dominant Polycystic Kidney Disease in Iranian Patients ,Biochemistry & Molecular Biology ,Vol.8 ,No .2 , July 2019 (102 -109) .

15. Jin M, Xie Y, Chen Z, Liao Y, Li Z, Hu P, Qi Y, Yin Z, Li Q, Fu P, Chen X: System analysis of gene mutations and clinical phenotype in Chinese patients with autosomal-dominant polycystic kidney disease. Sci Rep 2016;6:35945.

16. Cloonan N, Brown MK, Steptoe AL, Wani S, Chan WL, Forrest AR, Kolle G, Gabrielli B, Grimmond SM: The miR-17-5p microRNA is a key regulator of the G1/S phase cell cycle transition. Genome Biol 2008;9:R127.

17. Zheng W, Shen F, Hu R, Roy B, Yang J, Wang Q, Zhang F, King JC, Sergi C, Liu SM, Cordat E, Tang J, Cao Y, Ali D, Chen XZ: Far Upstream Element-Binding Protein 1 Binds the 3' Untranslated Region of PKD2 and Suppresses Its Translation. J Am Soc Nephrol 2016;27:2645-2657

18. Koptides M, Mean R, Demetriou K, Constantinides R, Pierides A, Harris PC, Deltas CC: Screening of the PKD1 duplicated region reveals multiple single nucleotide polymorphisms and a de novo mutation in Hellenic polycystic kidney disease families. Hum Mutat2000;16:176.

19.Hateboer N, v Dijk MA, Bogdanova N, Coto E, Saggar-Malik AK, San Millan JL, Torra R, Breuning M, Ravine D: Comparison of phenotypes of polycystic kidney disease types 1 and 2. European PKD1-PKD2 Study Group. Lancet 1999;353:103-107.

20.Cornec-Le Gall E, Audrézet MP, Chen JM, Hourmant M, Morin MP, Perrichot R, Charasse C, Whebe B, Renaudineau E, Jousset P, Guillodo MP, Grall-Jezequel A, Saliou P, Férec C, Le Meur Y: Type of PKD1 mutation influences renal outcome in ADPKD. J Am Soc Nephrol 2013;24:1006-1013.

21. .FatehiR, Khosravi S, Abedi M, SalehiR, GheisariY. Heterozygosity analysis of polycystic kidney disease 1 gene microsatellite markers for linkage analysis of autosomal dominant polycystic kidney disease type 1 in the Iranian population. J Res Med Sci 2017;22:102

22. Gonzalez-Paredes FJ, Ramos-Trujillo E, Claverie-Martin F. Defective pre-mRNA splicing in PKD1 due to presumed missense and synonymous mutations causing autosomal dominant polycystic disease. Gene 2014;546:243-9.

23.Harris P, Torres VE. Polycystic Kidney Disease, Autosomal Dominant Synonym: ADPKD. GeneReviews®. NCBI Bookshel; 2002. Available from: https://www.ncbi.nlm.nih.gov/books/ NBK1246/. [Last update on 2018 Jul 19].

24. Rossetti S, Chauveau D, Kubly V, Slezak JM, Saggar-Malik AK, Pei Y, et al. Association of mutation position in polycystic kidney disease 1 (PKD1) gene and development of a vascular phenotype. Lancet 2003;361:2196-201

25. Grenhaa V, Pereirab BJ, Retroza E, Coelhoa H, Godinhoa R, Temidoa P, et al. Mechanisms of male infertility in autosomal dominant polycystic kidney disease. Acta Urol Port 2014;31:41-4.