# Exon Sequencing of *PKD1* Gene in an Iranian Patient with Autosomal-Dominant Polycystic Kidney Disease

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#### ABSTRACT

**Introduction:** Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic kidney disorders with the incidence of 1 in 1,000 births. ADPKD is genetically heterogeneous with two genes identified: *PKD1* (16p13.3, 46 exons) and *PKD2* (4q21, 15 exons). Eighty five percent of the patients with ADPKD have at least one mutation in the *PKD1* gene. Genetic studies have demonstrated an important allelic variability among patients, but very few data are known about the genetic variation among Iranian populations. **Methods:** In this study, exon direct sequencing of *PKD1* was performed in a seven-year old boy with ADPKD and in his parents. The patient's father was ADPKD who was affected without any kidney dysfunction, and the patient's mother was congenitally missing one kidney. **Results:** Molecular genetic testing found a mutation in all three members of this family. It was a missense mutation GTG>ATG at position 3057 in exon 25 of *PKD1*. On the other hand, two novel missense mutations were reported just in the 7-year-old boy: ACA>GCA found in exon 15 at codon 2241 and CAC>AAC found in exon 38 at codon 3710. For checking the pathogenicity of these mutations, exons 15, 25, and 38 of 50 unrelated normal cases were sequenced. **Conclusion:** our findings suggested that GTG>ATG is a polymorphism with high frequency (60%) as well as ACA>GCA and CAC>AAC are polymorphisms with frequencies of 14% and 22%, respectively in the population of Southwest Iran. *Iran. Biomed. J. 18 (3): 143-150, 2014* 

Keywords: Autosomal dominant polycystic kidney disease (ADPKD), Polycystic kidney diseases (PKD), PKD1 gene, Iran

# INTRODUCTION

mong all renal diseases, autosomal dominant polycystic kidney disease (ADPKD, OMIM ID: 173900) is the most common inherited kidney disorder [1] that accounts for more than 10% of all cases of end-stage renal diseases (ESRD) [2]. ADPKD is characterized by numerous enlarged fluidfilled epithelial cysts typically in both kidneys and in some cases in other organs. The major extra-renal complications of ADPKD include hepatic cysts, pancreatic cysts, ovarian cysts, prostatic cysts, and cardiac valve disease. In ADPKD, the sensing mechanisms for tubule size seem to be lost; therefore, the cysts are developed and enlarged progressively [3]. PKD causes the progressive cyst formation and ultimately results in renal failure. In ESRD of PKD, many patients depend on either hemodialysis to suitable treatment has been developed yet [4]. At present, two causal genes, PKD1 (MIM 601313) and PKD2 (MIM 173910) have been identified for ADPKD that are located respectively on chromosome 16 (16p13.3) and chromosome 4 (4q21) [5-7]. PKD1 gene has 46 exons and encodes a transcript with approximately 14.2 kb in length (NM 000296.3). This gene is extended to 50 kb of the genomic DNA [8] and codes polycystin-1 protein (4302 aa) [9]. The PKD1 gene is mutated in 85% of all ADPKD cases [8]. On the other hand. PKD2 encodes a 3-kb open reading frame and has 15 exons which extends to 70 kb genomic areas and produces polycystin-2 protein (968 aa). The PKD2 gene is mutated in about 15% of ADPKD cases [3]. The exact function of polycystin remains unknown, and the mechanisms of mutations in polycystin led to the pathogenesis of the ADPKD also

attenuate renal failure or transplantation; however, no

remain unclear [10]. Polycystin-1 is a receptor protein for cell-cell/matrix interactions that plays crucial roles in the regulation of cell proliferation and apoptosis [11]. Polycystin-2 functions as a transient receptor potential ion channel and regulates the intracellular Ca<sup>2+</sup> concentration. Polycystin-1 and polycystin-2 interact together to form a functional complex. Their complex acts as a flow-dependent mechanosensor that regulates the differentiated state of tubular epithelial cells [12-14]. Polycystin-1 and polycystin-2 are colocalized in primary cilia and mediate Ca<sup>2+</sup> signaling as a mechanosensor. Multiple mechanisms have been shown to contribute to PKD, including increased proliferation, apoptosis as well as loss of differentiation and polarity. Approximately 50% of individuals with ADPKD have ESRD by age 60 years [15], but the actual age of onset of ESRD depends on the contributed gene. If ADPKD occurs because of PKD1 mutations, the ESRD may appear as early as the age of 53, but if the PKD1 is contributed, the age of onset of the ESRD is about 69 [16].

Based on the Human Gene Mutation Database, so far, more than 970 pathogenic mutations have been known for *PKD1* (Table 1). Until now, about 1,920 mutations have been identified in the *PKD1* gene (available through the ADPKD Mutation Database website [http://pkdb.mayo.edu/]). Most of these mutations are point mutations or deletion/insertion

mutations that introduce frame shifts and stop codons leading to premature termination. The most likely effect of these types of mutations is loss of polycystin-1 function completely.

Early diagnosis of ADPKD at first is established by ultrasound imaging with age-related cyst number criteria [17], but unfortunately, for younger at-risk individuals and patients with PKD2 mutations, ultrasonography may be insufficient for providing a definite diagnosis [18]. In these cases, mutation screening, a direct and an effective method, has been established as the applicable method to all cases suspected of ADPKD. This method plays a vital role in the evaluation of related potential kidney donors with doubtful imaging data in individuals with a negative family history, and even in cases of early age of onset of ADPKD. In the present study, we report cases of mutations discovered from the direct mutation screening of all coding exons in the PKD1 gene in one Iranian family with ADPKD. To verify the pathogenic effect of detected change, 50 unrelated healthy individuals were selected regarding gender, old, and ethnicity for sequence analysis of exon 15, 25, and 38 of the PKD1 gene. However, very little information is known about how missense mutations might alter the structure of polycystin-1 and mechanical properties we predicted the effects of these mutations on protein polycystin-1 structure.

<b>Table I</b> the number and type of no	athogenic mutations according t	o Human ( sene Mutation	Listabace site for PKIII dene
		o muman dene mutation	Database site for I ADI gen
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Primer	Sequence	Length	TM	GC%	Product (bp)
Pkd1-15-F	5'-CTGTCCCGGTTCACTCACT-3'	19	58.95	57.89	210
Pkd1-15-R	5'-CTCAGAGCCTGAAAGGCAGT-3'	20	59.68	55.00	219
Pkd1-25-F	5'-GAGACTGCGACATCCAACCT-3'	20	59.75	55.00	200
Pkd1-25-R	5'-TTCTCAGGATAGAGCCGAGC-3'	20	58.68	55.00	390
Pkd1-38-F	5'- AGGGTGTGTGCTGCCATTAC -3'	20	60.61	55.00	272
Pkd1-38-R	5'- GGGTCTGGCTGGACTAAAGG -3'	20	59.75	60.00	3/3

TM, melting temperature

Mutation type	Total No. of mutation	
<ul> <li>Nucleotide substitution</li> </ul>		
Missense/nonsense	450	
Splicing	77	
Regulatory	0	
Small deletions	235	
<ul> <li>Small insertions</li> </ul>	119	
Small indels	12	
<ul> <li>Gross deletions</li> </ul>	62	
<ul> <li>Gross insertions</li> </ul>	9	
• Complex	8	
• Repeats	0	
Total	972	



Fig. 1. Pedigree of the ADPKD-affected family. The proband is a seven-year-old boy affected by ADPKD (arrow in the pedigree).

#### MATERIALS AND METHODS

# Finding the PKD1 mutations in patient's family.

*Case report.* The patient was a seven-year-old boy, the only child of consanguineous parents (31 and 35 years old first cousin couple). After obtaining an informed consent, all participants were questioned about their personal medical history, and a family tree was drawn (Fig. 1). On sonography of the abdomen of the proband, the liver was normal in size and normal in echotexture, but contained multiple simple cysts; the largest was  $27 \times 27 \times 25$  mm located in the right lobe. On sonography of the kidneys and bladder, both kidneys were normal in position but enlarged in size. Bilateral increased medullary echogenicities due to multiple small parenchymal cysts were seen. There were no stone or hydronephrosis. The lengths of the right and the left kidneys were 112 and 111 mm, respectively, and the parenchymal thickness of both right and left was 11 mm. Serum creatinine level was 0.7 mg/dl. Blood urea nitrogen and urinalysis were normal, and the diagnosis of ADPKD was evoked; therefore, a genetic conformation was asked. In regard to the patient's parents, the father was affected by ADPKD and had a few cysts in his kidneys with no clinical symptoms, while the mother was healthy with no kidney cysts, but congenitally she had only one kidney.

Sample collection, DNA isolation and polymerase chain reaction. Peripheral blood (10 ml) was collected from the patient and his parents in the EDTA tubes. DNA was extracted by the standard salting out protocol, and the quality of the extracted DNA was verified by gel electrophoresis (Fig. 2). Mutations were analyzed based on the PCR-direct sequencing method. *PKD1* gene-specific PCR primers were synthesized from the region between exons 1 and 46, and a part of the flanking intron sequences were amplified with designed primer pairs (Table 2). The PCR was

conducted under the following conditions: 100 ng genomic DNA, 200 µM dNTP, 1.5 mm MgCl, 2.5 units SuperTaq polymerase (Genfanavaran, Iran), and 25 pmol of each primer. For amplification of exons and flanking introns, primers were designed by the Primer3 website (http://primer3.ut.ee/). Amplification was carried out in 25 µl volumes and 35 cycles: 93°C for 1 min., 60°C for 30 s and 72°C for 45 s. To amplify the full length of each exon, primer positions were chosen on the flanking intron close to the splice sites. The concentration of the primers and Taq polymerase for optimal reaction efficiency was determined empirically. The annealing temperature ranged from 60 to 65°C for each primer set. The PCR products were checked by electrophoresis on 1.2% agarose gels and then purified and directly sequenced.

Direct sequencing of PKD1 exons of patient's family. Direct sequencing of the exons and the flanking intron sequences were the same as performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on an ABI Prism 3700 automated genetic analyzer (Applied Biosystems). The same PCR primers were used for sequencing reactions, which were performed with forward and reverse primers. Finally, the sequences were compared to the reported gene sequence using the BLASTN program.

Sample collection, DNA isolation and Polymerase chain reaction of controls. Peripheral blood (5 ml) was collected from 50 ethnicities matched unrelated male controls without any kidney disease in EDTA tubes. DNA was extracted by standard salting out protocol. The PCR was conducted for exons 15, 25, and 38 in the following conditions: 100 ng genomic DNA, 200  $\mu$ M dNTP, 1.5 mM MgCl, 2.5 units SuperTaq polymerase (Genfanavaran, Iran), and 25  $\mu$ l pmol each primer. Amplification was carried out in 25



**Fig. 2.** Gel electrophoresis of genomic DNA. Lanes 1 and 2, genomes extracted from normal samples; lanes 3-5, genomes extracted from ADPKD family samples, and M is 1 kb marker.

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Table 2. Primers are listed above for ampli	ification of PKD1 gene that has be	een designed according reference	e sequence NT-037887
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pkd1-F1-1-F Gatgeogregicetant pkd1-F20-1-F gategegegegegege pkd1-F21-F Glegenggangecegag pkd1-F20-2-R GACCAGGCCTAACCAG pkd1-F21-F Glegenggangecegag pkd1-F20-3-R CACAGCGGCTAACTGCAGC pkd1-F31-F gategegiteggentagegege pkd1-F20-3-R CACAGCGGTAACTGCAGCC pkd1-F31-F gategegiteggentagegege pkd1-F20-3-R CACAGCGGTAACTGCAGCC pkd1-F31-F gategegiteggentage pkd1-F20-3-R CACAGCGGTAACTGCAGCC pkd1-F31-F gategegiteggentage pkd1-F20-3-R CACAGCGGTAACTGCAGCC pkd1-F51-F cntagencethocaccaga pkd1 pkd1-F21-F gategegegegegetage pkd1-F21-F gategegegegegegegegegege pkd1-F51-F cntagencethocaccaga pkd1 pkd1-F51-F cntagencethocaccaga pkd2 pkd1-F51-F cntagencethocacc	Primer	Sequence	Primer	Sequence
pkd1-F21-F. Gigeanggaegueceng pkd1-F22-F. Gigeanggaegueceng pkd1-F22-F. Gigeanggaegueceng pkd1-F23-F. Gigeanggaegueceng pkd1-F23-F. Gigeanggaegueceng pkd1-F23-F. CACGCCTAGGGCAGGCAGG pkd1-F23-F. CACGCCTAGGGCAGG pkd1-F23-F. Categocctengent pkd1-F21-F. gaeguegaeguesengae pkd1-F21-F. gaeguegaeguesengae pkd1-F21-F. gaeguegaeguegeseng pkd1-F21-F. gaeguegaeguegeseng pkd1-F21-F. gaeguegaeguegeseng pkd1-F21-F. Gaeguegeeguegeseng pkd1-F21-F. Categocctencenet pkd1-F21-F. Gaeguegeeguegeseng pkd1-F21-F. Categocctenceacene pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Categocctenceacene pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Categocctenceacene pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Categocctenceacene pkd1-F21-F. Categocctenceacene pkd1-F21-F. Categocctence pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. Categocctence pkd1-F21-F. Gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. gaeguegeeguegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseng pkd1-F21-F. GaeGuegeegeeseeseeseeseeseeseeseeseeseeseesee	pkd1-E1-1-F	Gatgccagtccctcatcg	pkd1-E20-1-F	tgacagggcagagggttg
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pkd1=23-1-R gatestgggargggerangeg pkd1=20-3-F GCTCACGCTAGTGTGCTC gatestggarggerangegg pkd1=20-4-F GCGCAGCACGTGTCACCAG pkd1=23-1-R acagetagerangeggargg pkd1=20-4-F ggscgaracggetagggarg pkd1=24-1-R ggggeracagetagg pkd1=24-1-R ggggarggarggerange pkd1=25-1-R gggarggarggerangeg pkd1=25-1-R gggarggarggerange pkd1=25-1-R ctaggarggarggarggerange pkd1=25-1-R ctaggarggarggarggerange pkd1=25-1-R ctaggarggarggarggerange pkd1=22-2-F ctagGACGTGTCGGAG pkd1=22-2-F CTGGGACGCCGGAGGG pkd1=22-2-F ACTTCGTGTGGAATGCCATC pkd1=6-1-R CAGCACATAGCGAGG pkd1=22-2-F ACTTCGTTGGAATGCCATC pkd1=6-2-R agggtgtranggtrange pkd1=22-3-F TGCTTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGCTTTGGTTGGAATGCCAGG pkd1=22-3-F TGGTTTGGAATGCCAGG pkd1=22-3-F TGGTTTGGAATGCCAGG pkd1=22-3-F TGGTTTGGAATGCCAGG pkd1=22-3-F TGGTTTGGAATGCCAGG pkd1=22-3-F TGGCTTGGAAGGCCT pkd1=23-1-F gggarggarggarggggggggggggggggggggggggg	pkd1-E2-1-F	Gtgcagaggaagcccgag	pkd1-E20-2-R	GACCAGGGCCAACGAGTA
pkd1-E3-1-F gagetgegengengeg pkd1-E20-3-R CACAGCAACTCACGC pkd1-E4-1-F gggettecategetti pkd1-E20-4-F CTAGCACCTAACTGCACGCC pkd1-E4-1-F gggettecategetti pkd1-E20-4-F CTAGCACCTAACTGCACCCC gdd1-E4-1-F gggettecategetti pkd1-E21-1-F ggftgagetgeggetget gdd1-E5-1-F catagacettecatecate pkd1-E5-1-F catagacettecatecate pkd1-E5-1-F catagacettecatecate pkd1-E2-1-F ggettecategetgetge pkd1-E22-1-R CCTGTGTCTGGAATGCCATC pkd1-E6-1-R CAGCACATAGCGAG pkd1-E22-2-R CATAGGAGCCTCTGCACCAG pkd1-E6-1-R CAGCACATAGCGACGG pkd1-E22-2-R CATAGGAGCCTCTGCACCAG pkd1-E6-2-R agggtgetaeggetagetget pkd1-E22-3-R ggatecategetagetgetgetgetgetgetgetgetgetgetgetgetgetg	pkd1-E2-1-R	gactcaggtgtgggcttcag	pkd1-E20-3-F	GCTCACCGCTAGTGTGCTC
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	pkd1-E3-1-R	acagctgagcagcaagagg	pkd1-E20-4-F	CTAGCACGTAACTGCACCCC
	pkd1-E4-1-F	ggggcttccatcagcttt	pkd1-E20-4-R	gacagaacggctgaggctac
kd1-E5-1-F catagaaccatcccaga pk1-E2-1-R aggaggaggaaggaa pk1-E2-1-F ctcccttatoctccctgb ctcctatoctccctgb pk1-E5-1-F ggaccaggggagcaggat pk1-E2-1-F ctccttatoctccctgb pk1-E5-1-F ggaccagggaggacggat pk1-E2-1-F ctccttatoctccctgb pk1-E5-1-F ctcccttatoctccdgb pk1-E2-2-F CTGGACTCTCGACGCG pk1-E2-2-F CTGGACGCTTCGACGCG pk1-E2-2-F CTGGACGCTTCGACGCG pk1-E2-2-F GTCTTCCTGCACCAG pk1-E2-2-F ggacaggggaggaa pk1-E2-1-F ctccctctatoctccdgb pk1-E2-2-F ggacaggggggggggggggggggggggggggggggggg	pkd1-E4-1-R	gagggcagaagggatattgg	pkd1-E21-1-F	gtgtagagaggagggcgtgt
hcl1-E5-1-R cccccitraccccitige pkd1-E2-1-F cccccitracccitige pkd1-E5-1-R CAGCACTATAGGATGCCATC pkd1-E5-1-R CAGCACTATAGGATGCGATG pkd1-E2-2-R AATCCCTTTCGGTATGCCATC pkd1-E5-1-R CAGCACATAGGATGCGAG pkd1-E22-3-F TGCTTTCGGTTGGCATGCACAG pkd1-E22-3-F TGCTTTCGGTTGGCATGCACG pkd1-E22-3-F TGCTTTCGGTTGGCATGCACGA pkd1-E22-3-F TGCTTTCGGTTGGCATGCACGA pkd1-E22-3-F TGCTTTCGGTTGGCATGCACGA pkd1-E22-3-F TGCTTTCGGTTGGCATGCACGA pkd1-E22-3-F TGCTTTCGGTTGGCATGCACGACGACGACGCCT pkd1-E5-1-F gtgggaggagggagggg pkd1-E22-1-F gtggacqcaggttgtae pkd1-E22-1-F gtggacqcaggttgtae pkd1-E22-1-F gtggccacgaggttgtae pkd1-E22-1-F gtggccacgaggtgtae pkd1-E22-7-F TGGCAGGCACACAACAAA pkd1-E11-1-F cagtggggagtcggggg pkd1-E25-2-F TGGCGGGCCTTCGCCTC pkd1-E12-1-R gtgggagtacgggggagggagg pkd1-E25-2-F GGGGGCCTTCCCCTCT gtgGGGTCTTCCTTGGCTC pkd1-E22-1-F GCGGGGCTTTCCCTCTGGCTC pkd1-E22-1-F GCGGGGCTTTCCTTGGCTC pkd1-E22-1-F GCAGGACCCCCGCCTGAAGG pkd1-E22-1-F GGGGGCCTTTCCCTCT gtgGGGTCTTGCTTGGTGGCCCCTG pkd1-E22-1-F GCAGGACGCCCTGGGTG pkd1-E22-1-F GCAGGGGCTTGGCTTGGTGGC pkd1-E22-1-F GtgGGGGCTTGGCTTGGTGGC pkd1-E22-1-F GtgGGGGCTTGGCTGG pkd1-E22-1-F GtgGGGGTCTGGCCGG pkd1-E22-1-F GtgGGGGCTGGCC pkd1-E22-1-F GtgGGGGCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E5-1-F	catagaccetteccaccaga	pkd1-E21-1-R	aggaggaggcagaggaa
kd1-E5c-1F gazcageageageage	pkd1-E5-1-R	ctgggaaggacagagctgg	pkd1-E22-1-F	cetecetetacetecetgte
$      kd1-E5-1-R \\      CTAGGACTTGGGAGCGG \\      kd1-E22-2-R \\      cTAGGACTTGGACTGGGAGCGG \\      kd1-E22-3-F \\      CTAGGACTTGGCACCAG \\      kd1-E22-3-F \\      TGCTTTCCTTGCCACCAG \\      kd1-E22-3-R \\      gracetgeoragetgac \\      gractgeoragetgac \\      gracetgeoragetgac \\      gracetgeoragetgrac \\      gracetgeoragetgac \\      gracetgeorage$	pkd1-E6-1-F	gagccaggaggagcagaac	pkd1-E22-1-R	CCTGTGTCTGGAATGCCATC
$      kd1-E52-F \\ kd1-E52-F \\ cTGGGACTTCGGAGACGG \\      kd1-E22-3F \\ acatigacegigacegigtage \\      gagatigacegigace \\      gd1-E7-1-R \\ acatigacegigace \\      gd1-E7-1-R \\ acatigacegigace \\      gd1-E7-1-R \\ acatigacegigace \\      gd1-E2-1-R \\ acatigacegigace \\      gd1-E1-1-R \\ acatigacegigace \\      gd1-E1-1-R \\ acatigacegigace \\      gd1-E1-1-R \\ acatigacegigane \\      gd1-E1-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E2-1-R \\ acatigacegigane \\      gd1-E1-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E1-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E1-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E2-1-R \\ acatigacegigane \\      gd1-E1-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E1-2-1-R \\ acatigacegigane \\      gd1-E1-2-1-R \\ acatigacegigane \\      gd2-E2-1-R \\ acatigacegigane \\      gd1-E1-2-1-R \\ acatigacegigane \\      gd1-E1-2-1-R \\ acatigacegigacegigate \\      gd1-E2-1-R \\ acatigacegigaceging \\      gd1-E1-2-1-R \\ acatigacegigaceging \\      gd1-E1-2-1-R \\ acatigacegigacegiga \\      gd1-E1-2-1-R \\ acatigacegigacegig \\      gd1-E1-2-1-R \\ acatigacegigacegig \\      gd1-E2-1-R \\ acatigacecaceteqig \\      gd2-E-2-1-R \\ acatigacegigacegig \\      gd1-E2-1$	pkd1-E6-1-R	CAGCACATAGCGATGCGAG	pkd1-E22-2-F	AATCCCTTTCCCTTTGGCTA
kd1-E5-2-R aggregataggreggtig pkd1-E22-3-R tore the transformation of transforma	pkd1-E6-2-F	CTGGGACTTCGGAGACGG	pkd1-E22-2-R	CATAGGAGCCTCTGCACCAG
kd1-E7-1-F according cgtrige according	pkd1-E6-2-R	agggtgtcaacggtcagtgt	pkd1-E22-3-F	TGCTTTCTGTTTCATGGGCT
kd1=E27-1-R t ccttcciccipagactccc kd1=E27-1-F tcttcciccipagactccaact ttctaagatggaggagg kd1=E27-1-R ttctaagatggaggagg kd1=E27-1-R ttctaagatggaggagg kd1=E27-1-R ttctaagatggaggaggtg kd1=E27-1-R ttctaagatggaggaggtgag kd1=E27-1-R cagcagggcaggtgagg kd1=E27-1-R cagcagggcaggtgaggaggtgg kd1=E27-1-R cagcagggcaggtgaggagggaggggggggggggaggggggagggggaggggg	pkd1-E7-1-F	acactgaccgttgacaccc	pkd1-E22-3-R	gtaacccaggcaatgctgac
$        kd1-E8-1-F \\        greggagatgagagggg   kd1-E24-1-R \\      greggatgatagagecegge   kd1-E25-1-F \\      cagecaactiftigeatciggtge   kd1-E25-1-F \\      cagecaactiftigeatciggtge   kd1-E25-1-F \\      cagecaactiftigeatciggtgeaggetage   kd1-E25-2+F \\      cagecaactiftigeatciggtgeaggetaleg   kd1-E25-2+F \\      cagecaactiftigeatciggtgeaggetaleg   kd1-E25-2+F \\      cagecaactiftigeatciggtgeaggetaleg   kd1-E25-3+F \\      cagecaactiftiggtactiggtgeag   kd1-E25-3+F \\      cagecaactiggtgaggaggtgatg   kd1-E25-3+F \\      cagecaactiggtgaggatgetaleg   kd1-E25-3+F \\      cagecaactiggtgaggatgetaleg   kd1-E25-3+F \\      cagecaactiggtgaggatgetaleg   kd1-E25-3+F \\      cagecaactiftiggtaggatgetaleg   kd1-E25-3+F \\      cagecaactiftiggtaggatgetaleg   kd1-E25-3+F \\      radiacctgraggatgetaleg   kd1-E25-3+F \\      radiacctagtgaaggactigatg   kd1-E25-3+F \\      radiacctagtgaaggetaleg   kd1-E25-3+F \\      radiactigtgaaggetaleg   kd1-E25$	pkd1-E7-1-R	teetteeteetgagaeteee	pkd1-E23-1-F	gagactgcgacatccaacct
	pkd1-E8-1-F	gtgggaggatggaggagtg	pkd1-E23-1-R	ttetcaggatagageegage
	pkd1-E8-1-R	ctaaccacagccagcgtctc	pkd1-E24-1-F	geteggetetateetgagaa
pkd1-E29-1-R	pkd1-E9-1-F	gtctgttcgtcctggtgtcc	pkd1-E24-1-R	agtgeteacgaggteattee
	pkd1-E9-1-R	gcaggagggcaggttgtag	pkd1-E25-1-F	cagccatgtttgcatgtcac
$      pkd1-E10-1-R \\      cagtagacgtgaaagctcag \\      pkd1-E11-1-F \\      cagtagacgtgaagtcaggaacaga \\      pkd1-E11-1-R \\      gagagagtagagtgaagtacaga \\      pkd1-E12-1-F \\      atgaccgtgaggacgtgatg \\      pkd1-E12-2-F \\      ACCTCTCTCTCCAGCGTTTGA \\      pkd1-E12-2-F \\      ACCTCTCTCTCCAGCGTTTGA \\      pkd1-E12-2-F \\      acGGGAGTCTCTAGTGgtga \\      pkd1-E12-3-R \\      ggtcacgccattictgatg \\      pkd1-E12-3-R \\      ggtcacgccattictgatg \\      pkd1-E12-1-F \\      atgacgacgagcgagag \\      pkd1-E12-1-F \\      atgacgacgatgccagag \\      pkd1-E12-1-F \\      atgacgacgatgccagag \\      pkd1-E12-1-F \\      tigtgcacactccctgtgta \\      pkd1-E12-1-F \\      tigtgcacactccctgtgta \\      pkd1-E12-1-F \\      tigtgcacactccctgtgta \\      pkd1-E12-1-F \\      tigtgcacactccctgtgta \\      pkd1-E12-1-F \\      tigtgcacactcccttgta \\      pkd1-E12-1-F \\      tigtgcacactcccttgta \\      pkd1-E12-1-F \\      tigtgcacactcccttgta \\      pkd1-E12-1-F \\      tigtgcacactcccttgta \\      pkd1-E12-1-F \\      tigtgcacactccttgta \\      pkd1-E12-1-R \\      cccactrocccc \\      pkd1-E12-1-R \\      cccactroccccc \\      pkd1-E12-1-R \\      cccactrocccccc \\      pkd1-E12-1-R \\      cccactrocccccc \\      pkd1-E12-1-R \\      cccactrocccccccccccccccccccccccccccccccc$	pkd1-E10-1-F	ctctccttccctcctctt	pkd1-E25-1-R	CAGGAGGGAGGTCAGGCT
pkd1-E11-1-F cagatgggaactctgacg pkd1-E25-2-R AGATGAGAGAACGCAGCAG pkd1-E11-1-R gaggagatgcagggaacaga pkd1-E25-3-R TGACAAGCACATCTGGCTC pkd1-E12-1-R attaccgtgaggacgtgatg pkd1-E25-3-R GTGGACGCCTTTCCCTCT pkd1-E12-2-R CTGTGTGAGCACCCTGTGA pkd1-E25-3-R AGCGACGCCTGCC pkd1-E12-3-F GCAGGGCACCCTGTGTG pkd1-E25-5-F GCCGCCTGTAATCCCAACACTT pkd1-E12-3-F GCAGGGAGTCCTAGTGgtg pkd1-E25-5-R aattatategtgaaggctgag pkd1-E12-3-F ggtcacgccattctgatg pkd1-E26-1-R taggccagagccacc pkd1-E13-1-R taggccagagcgag pkd1-E27-1-R tctgtcctgtctggtgga tcttctgcttggtgt pkd1-E13-1-R taggccagagcgag pkd1-E27-1-R tctgtcctgtctggtgga tcttctgctctgtt pkd1-E15-1-R taggccagagcctgaa pkd1-E27-1-R tctgtcctgtctggtgga pkd1-E15-1-R taggccagagcctgaa pkd1-E27-1-R tctgtcctgtctggtgga pkd1-E15-1-R tctggcctgtagtgt pkd1-E27-1-R tgtgccacacctggts pkd1-E15-1-R taggccagagcctgaa pkd1-E27-1-R tgtgccacacccc pkd1-E15-1-R tggccagagcctgaa pkd1-E27-1-R tgtgccacacctcccc pkd1-E15-1-F cttgcctgttgtt pkd1-E28-2-R ggaacaagagcctgag pkd1-E16-1-F ggtctgtctgctct pkd1-E28-2-R ggaacaagagcctgaa pkd1-E28-2-R ggaacaagagcctgag pkd1-E16-1-F ggtctgtctgctCC pkd1-E28-2-R ggaacaagagcgggg pkd1-E16-1-R GATGTTGTCGCCCGTGTG pkd1-E29-1-F cctaggtctctgcacct pkd1-E16-1-R GATGTTGTGCCCCGTGTG pkd1-E29-1-F cctaggtcctgcactgt pkd1-E16-1-R GATGTTGTGCCCCGTGTG pkd1-E30-1-R ggtgctctcactgt pkd1-E16-1-R GATGTTGTGCCCCGTGT pkd1-E30-1-R ggtgggggggggggggggggggggggggggggggggg	pkd1-E10-1-R	cagcagacgtgaaagctcag	pkd1-E25-2-F	AGTGTGGCACGACAACAAAG
pkd1-E11-1-Rgaggagtucaggaacagapkd1-E25-3-FTTGACAAGCACATCTGGCTCpkd1-E12-1-Fattaccegtagaacgtaatgpkd1-E25-3-RGTGGACGCCTTCCCTCTpkd1-E12-2-FAACCTCTCCTGCAGCGTTGApkd1-E25-4-FAGTACTGGGAATGGACCCTGpkd1-E12-2-FCACGGGAGTCCTAGTGGTGpkd1-E25-5-FCGCCTGTAATGCACACCTTpkd1-E12-3-FGCAGGGAGTCCTAGTGgtapkd1-E25-5-Raaattcatacgtaaggcctagapkd1-E12-3-Fggtcacgccatttctgatgpkd1-E25-7-Fctgaccacgtgacctgacapkd1-E12-1-Ftaaggcgaagtcctcacapkd1-E25-7-Fctgaccacgtgacctggtapkd1-E13-1-Raagcagaaggcagagpkd1-E27-1-Fgtgtacacatccccctggtapkd1-E14-1-Fctggccattactpkd1-E28-1-Fctgctcctgtgtapkd1-E15-1-Fctgtccctgttactactpkd1-E28-1-Faggtacaagagcctggtapkd1-E15-1-Fctgtccctgttacactpkd1-E28-1-RctcacCTTCAGTGGCTCCpkd1-E15-1-Fctgtccctgttacattactpkd1-E28-1-RctcacCTTCAGTGGCTCCpkd1-E15-1-Fctgtcctacactgtgtctpkd1-E28-2-Rggacaagagacggtggpkd1-E16-1-RGATGTGTGCGCCGTCTGpkd1-E29-1-Fcaaagaccgcagagtggpkd1-E16-1-RGATGTGTGCACACACAACTTCpkd1-E30-1-Fcctacgttcctgggtcatcpkd1-E16-1-RGCACGAGTGCAACACAACTTCpkd1-E30-1-Fcctagcgaaaacactctgtpkd1-E16-3-FCGCAGGTGACACACAACTTCpkd1-E31-1-Fcctgcgagaacactctgtpkd1-E16-3-FGGAGTGAACACACAAGTTCpkd1-E31-1-Fcctgcgcagagacctggpkd1-E16-3-FGGACGTGAAGCAGCACAGCAGpkd1-E31-1-Fccagcagagacctggpkd1-E16-3-FGGACG	pkd1-E11-1-F	cagttgggcatctctgacg	pkd1-E25-2-R	AGATGAGGAGAACGCAGCAG
pkd1-E12-1-Fatgacogtagagagtgatpkd1-E25-3-RGTGGACGCCTTTCCCTCTpkd1-E12-1-RGTTGGTGGGACAGTAGAGGpkd1-E25-4-RAGTACTGGGAATGGAGCCTGpkd1-E12-2-FAACCTCTCCTGCACGTTTGApkd1-E25-4-RAGGGGTCTTGCTATGTGCpkd1-E12-3-Rgtcacccattctgatgpkd1-E25-5-FCGCCTGTAATCCCAACACTTpkd1-E12-3-Rgtcacccattctgatgpkd1-E25-7-RcGCCTGTAATCCCAACACTTpkd1-E13-1-Ftaaggcagagtoctccaapkd1-E25-7-Rcagacacagtgacctgcacpkd1-E13-1-Ftaaggcagagtoctccaapkd1-E27-1-Rcagacacagtgacctcccggtapkd1-E14-1-Fcttgcctgttcgtctgtcgcagcagpkd1-E27-1-Rctgtgcactaccctggtapkd1-E15-1-Fttgggacaagagcaggpkd1-E28-1-FctaccTCAGTGGCTCCCpkd1-E15-1-Fctgtcgctaccattpkd1-E28-1-Fagttaacatggcttgctpkd1-E15-1-Fctgtcgctgcacctgtgctpkd1-E28-1-Fagttaacatggcttgctpkd1-E16-1-Fgtgtcgtcaccattggctpkd1-E28-1-Fagttaacatggctggtggtpkd1-E16-1-Fgtgtcgtcaccattggctpkd1-E29-1-RcctaccTCAGTGCTCCCCCpkd1-E16-1-Fgtgtcgtcaccatggcgtpkd1-E29-1-Rcctacctgttcaccagaggggggggpkd1-E16-2-RCCCAGCAGCTGGGAACACAGCpkd1-E30-1-Fcctacctgttcaccagaggccagpkd1-E16-3-RCCCCAGATGCTGGGAAGAAGAGpkd1-E31-1-Rccagagagacaccctgtpkd1-E16-3-RCCCCAGATGCTGCGAGAGCTpkd1-E31-1-Rccagagagaccacctctgpkd1-E16-3-RCCCCAGATGCTGCAGGAGCTpkd1-E31-1-Ragtgaggagtcctctpkd1-E16-3-RCCCCAGATGGTGGTGGTATpkd1-E33-1-Ragtgaggagtggtggctcctpk	pkd1-E11-1-R	gaggagatgcagggaacaga	pkd1-E25-3-F	TTGACAAGCACATCTGGCTC
pkd1-E12-1-RGTTGTGGGGACGTAGAGGpkd1-E25-4-FAGTACTGGGATGGAGCCTGpkd1-E12-2-RCTGTGTGAGCACCTGTCTGpkd1-E25-5-FAGGGGTCTTGCTAGTATGTGCpkd1-E12-3-RGCAGGGAGTCCTAGTGgtgapkd1-E25-5-FcGCCTGTAATCCCAACACTTpkd1-E12-3-Rggtacagcattictgalgpkd1-E25-5-Raaattcatacgtgaaggctgagapkd1-E11-Ftagggcaagtcctccacapkd1-E26-1-Rcgacacatggacctggacpkd1-E13-1-Ftagggcaagtcctccacapkd1-E27-1-Fgtgtacacccctggtapkd1-E14-1-Fctcgccttcigctcgctgpkd1-E28-1-Faggtaacatgggctggcpkd1-E15-1-Fctgtcccggtacaccetpkd1-E28-1-Faggtaacatgggctggtpkd1-E15-1-Fctgtcccggtaaaggcagpkd1-E28-1-Faggtaacaaggagcaggggggpkd1-E16-1-Fgtgtgcctacacctgtgtapkd1-E28-1-FccacCTTCAGTGGCTCCCpkd1-E16-1-Fgtgtgctacacctgtgctcpkd1-E29-1-Fccaagacctggggtggpkd1-E16-1-Fgtgtgctacacctgtgctcpkd1-E29-1-Fccaagacctggaggtggpkd1-E16-2-FACTCAGCGTGGAAGTAApkd1-E30-1-Fcctagttcctgggtctctpkd1-E16-3-RCGCCAGATGCTGGTAAAGACpkd1-E30-1-Rcctagttcctgggacacccggagapkd1-E16-3-RCCCCAGATCCACAGGTAApkd1-E31-1-Fccagcaggaacacagaggpkd1-E16-3-RCCCCAGATCAAAGGTCAAAGCGpkd1-E31-1-Fccagcaggaaccccdgtgpkd1-E16-3-RGGACGTCAAAGGCCAAGACCpkd1-E31-1-Fccagcggaacacctcctgtpkd1-E16-3-RGGACGTCAAAGGCCAAGACCpkd1-E31-2-Rgagtgaggtgggctctpkd1-E16-4-RGGACGTCAAAGGCCAGACAAGACpkd1-E31-2-Rgagtgaggtgggtgctctpkd1-E	pkd1-E12-1-F	atgaccgtgaggacgtgatg	pkd1-E25-3-R	GTGGACGCCTTTCCCTCT
pkd1-E12-2-FAACCTCTCCTGCAGCTTTGApkd1-E25-5-FATGGGGTCTTGCTATGTTGCpkd1-E12-3-FGCAGGAGTCCTAGTGgtapkd1-E25-5-FCGCCTGTATCCCAACACTTpkd1-E12-3-FGCAGGGAGTCCTAGTGgtapkd1-E25-5-Faaattctategtaaggetagpkd1-E12-3-Fggtcacgccatttctgatgpkd1-E26-1-Ftctgtcctgctgagapkd1-E13-1-Ftaaggcaagagagaggeagapkd1-E27-1-Fgtgtcaccatcccctggtapkd1-E14-1-Fcttgccttctgctctgctgpkd1-E27-1-Fgtgtcaccatcccctggtapkd1-E15-1-Ftgtcccggttcactcatcpkd1-E28-1-RcccacTCCAGTGGTCCCCpkd1-E15-1-Ftgtcccggttcactcatcpkd1-E29-1-Fcagaccaggagaggagggpkd1-E16-1-Fgtgtcgctcacctgtgtcpkd1-E29-1-Fccaggccgaggtggpkd1-E16-1-Fgtgtcgctcacctgtgtcpkd1-E29-1-Fccaggccggaggtggpkd1-E16-1-Fgtgtcgctcacctgtgtcpkd1-E29-1-Fccaggccggaggtggpkd1-E16-1-Fgtgtcgctcacctgtccpkd1-E29-1-Fccaggccggaggtggpkd1-E16-1-FgtgtcgctcacctgtGCCCGCTGTpkd1-E29-1-Fccaggcggaggtggpkd1-E16-3-RCCCCAGGTGGACATGAGCpkd1-E30-1-Rgctctctcacacagaggapkd1-E16-3-FCGCAGGTCCAACACACTTCpkd1-E31-1-Fccaggtggagaccctgtpkd1-E16-3-RCCCCAGGTCAAAGGCGAApkd1-E31-1-Fccaggtggagacctggapkd1-E16-5-FCAATATCATCGTCACGGCTGpkd1-E31-2-FTCCTGTGTCCACGCGTGTGpkd1-E16-5-FCAATATCATCGTCACGGCTGpkd1-E31-2-FTCCTGTGCCAGCTGpkd1-E16-5-FCAATATCATCGTCACGGCTGpkd1-E31-2-FTCCTGTGTCCACGCGTGTGpkd1-E16-5-FCAATATCATCGT	pkd1-E12-1-R	GTTGGTGGGCACGTAGAGG	pkd1-E25-4-F	AGTACTGGGAATGGAGCCTG
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pkd1-E12-3-FGCAGGGAGTCCTAGTGgta ggtcacgccattictgatgpkd1-E25-5-R pkd1-E12-1-Faaattctatcgtgaaggctag aggccagagcctgaa ggtgacacccattictgatgpkd1-E13-1-Faagcagagcagaaggcagag pkd1-E14-1-Fpkd1-E27-1-Fctgtcacccaccpkd1-E14-1-Fctgccttctgctctgettpkd1-E27-1-Fgtgtaacatgcctgcaccpkd1-E15-1-Fctgtcccggtcactcactpkd1-E28-1-RctcacCTTCAGTGGCTCCpkd1-E15-1-Fctgtcccggtcactcactpkd1-E28-1-RctcacCTTCAGTGGCTCCpkd1-E16-1-Fgtgtcgctaccctggtcpkd1-E28-2-FCTGTGGCTGTCTCAGGGTGpkd1-E16-1-Fgtgtcgtcacctgigctpkd1-E29-1-Rccacagagcacgaggtggpkd1-E16-2-RCCAGGTGCACATGAGCpkd1-E29-1-Rccacagagcacgaggtggpkd1-E16-3-FCGACAGTGCTGGTGAAGTAApkd1-E30-1-Fccatgttccctgtpkd1-E16-3-FCGACAGTGCCACACACACTCpkd1-E30-1-Fccatgtcctggtctctpkd1-E16-3-RCCCAGATCCCACAGGTAGpkd1-E31-1-Rccgcaggaagacactcctgtpkd1-E16-3-FCGACGGTGCAAATGGCTCpkd1-E31-1-Rccgcaggaagacactcctgtpkd1-E16-5-FAATATCATCGTCACGGCTGpkd1-E31-1-Rccgcaggagagcacctctgtpkd1-E16-5-FGGAACTAAGGCCAAATGGCCpkd1-E31-1-Fccacgacggacacctcctgtpkd1-E16-5-FGGAACATAGCGCACACCTCApkd1-E31-1-Fccacgcagcacctcctctpkd1-E16-5-FGGAACATGGCAGGGTGTATpkd1-E32-1-Fagcccacctcactcctpkd1-E16-7-RCCAGCGCGACATGCCACACCCCpkd1-E33-2-RACGCCAACACACACACCpkd1-E16-7-RCCAGCGCGCGACATAGACCpkd1-E33-2-RACGCCGCGCAACACCpkd1-E16-7-FCTAACTCGC	pkd1-E12-2-R	CTGTGTGAGCACCCTGTCTG	pkd1-E25-5-F	CGCCTGTAATCCCAACACTT
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pkd1-E16-5-RGGTAÅATGGCTCGGAGGTCTpkd1-E32-1-Fagcccaccetcactectcpkd1-E16-6-FGGTGGCAGTGGTGTCGTATpkd1-E32-1-RACAGCAGCAGGCAGAGCACACetpkd1-E16-6-RGGATCTGAAAATGGACCAGCpkd1-E33-1-FCTCTGCTCACCTCGgtacgpkd1-E16-7-FCTCAGCCACGTACAACCTCApkd1-E33-1-RATGTCTTGCCAAAGACGGACpkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-2-RATCTCGTAGTCCTGGGGGCTCpkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E18-1-Ftgetttaaaactggatgggpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGTGTTGGGGGAGpkd1-E18-2-RGGAGCGTGAGGGTGAAACpkd1-E33-5-RCAGCAGGTGTTGGGGGAGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-FTTACTTCCGCGCACAACCGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-FTTACTTCCGCGTGTCAApkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-RCAGCAGGAGAGCGCAAACCGpkd1-E18-2-RGGAGCGTGAGGGGAGAACpkd1-E33-5-FTTACACCACAACAACCACCACCAACCACCACCACCACCAC	pkd1-E16-5-F	CAATATCATCGTCACGGCTG	pkd1-E31-2-R	gagtgagggtgggctcct
pkd1-E16-6-FGGTGGCAGTGGTGTCGTATpkd1-E32-1-RACAGCAGCAGGCAGCACACctpkd1-E16-6-RGGATCTGAAAATGGACCAGCpkd1-E33-1-FCTCTGCTCACCTCGgtacgpkd1-E16-7-FCTCAGCCACGTACAACCTCApkd1-E33-1-RATGTCTTGCCAAAGACGGACpkd1-E16-7-RCACCTGCAGCCCACTCACpkd1-E33-2-FGGCTGCAAGCAGACAGACAGATTTpkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttcttgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGTGTGGGGGGGpkd1-E18-2-RGGAGCGTGAGGGTGAAACpkd1-E33-5-RCAGCAGGTGTGGGGGGGpkd1-E18-2-RGGAGCGTGAGGGTGAAACCpkd1-E33-5-FTTACTTCCGCGGGGAGpkd1-E18-2-RGGAGCGTGAGGGTGAAACCpkd1-E33-5-RCAGCAGCACAACCACGACAGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-RCAGCAGCACAACCACGACAGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-RCAGCAGCACAACCACCACCACCACGACAGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-RCAGCACCACCAACCACCACCACCACGCACAACCACCACCA	pkd1-E16-5-R	GGTAAATGGCTCGGAGGTCT	pkd1-E32-1-F	ageceaeceteaeteete
pkd1-E16-6-RGGATCTGAAAATGGACCAGCpkd1-E33-1-FCTCTGCTCACCTCGgtacgpkd1-E16-7-FCTCAGCCACGTACAACCTCApkd1-E33-1-RATGTCTTGCCAAAGACGGACpkd1-E16-7-RCACCTGCAGCCCACTCACpkd1-E33-2-FGGCTGCAAGCAGACAGATTTpkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-2-RATCTCGTAGTCCTGGGGGCTCpkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E18-1-Ftgctttaaaactggatgggpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGTGTGGGGGAGpkd1-E18-2-RGGAGCGTGAGGGTGAAACpkd1-E33-5-FCTCACTGTGTGTCCGTGTCAGpkd1-E18-2-RGGAGCGTGAGGGTGAGAACpkd1-E33-5-FCTCACTGCTGCGCGCCAAACACGpkd1-E18-2-RGGAGCGTGAGGGGAGAACpkd1-E33-5-FCTCACCGTGTCAGGGGAGAGACpkd1-E18-2-RGGAGCGTGAGGGGAGAACpkd1-E33-5-FCTCACCGTGTCACGGCCACAGGCGAGAAC	pkd1-E16-6-F	GGTGGCAGTGGTGTCGTAT	pkd1-E32-1-R	ACAGCAGCAGGCACACct
pkd1-E16-7-FCTCAGCCACGTACAACCTCApkd1-E33-1-RATGTCTTGCCAAAGACGGACpkd1-E16-7-RCACCTGCAGCCCACTCACpkd1-E33-2-FGGCTGCAAGCAGACAGATTTpkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-2-RATCTCGTAGTCCTGGGGGCTCpkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E16-6-R	GGATCTGAAAATGGACCAGC	pkd1-E33-1-F	CTCTGCTCACCTCGgtacg
pkd1-E16-7-RCACCTGCAGCCCACTCACpkd1-E33-2-FGGCTGCAAGCAGACAGATTTpkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-2-RATCTCGTAGTCCTGGGGGCTCpkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E16-7-F	CTCAGCCACGTACAACCTCA	pkd1-E33-1-R	ATGTCTTGCCAAAGACGGAC
pkd1-E17-1-FGTGGCCTACCACTGGGACTpkd1-E33-2-RATCTCGTAGTCCTGGGGGCTCpkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E16-7-R	CACCTGCAGCCCACTCAC	pkd1-E33-2-F	GGCTGCAAGCAGACAGATTT
pkd1-E17-1-RCCCAAATGACACGACAAACApkd1-E33-3-FGCTGGGGGGCTGTTATTCTCpkd1-E17-2-FGAGTACCGCTGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E17-1-F	GTGGCCTACCACTGGGACT	pkd1-E33-2-R	ATCTCGTAGTCCTGGGGGCTC
pkd1-E1/-2-FGAGTACCGCTGGGGAGGTGTApkd1-E33-3-RAGTCGGTCAAACTGGGTGAGpkd1-E17-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatgggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E17-1-R	CCCAAATGACACGACAAACA	pkd1-E33-3-F	GCTGGGGGGCTGTTATTCTC
pkd1-E1/-2-Rgttctctgggctcatgggtpkd1-E33-4-FGGACAAGGTGTGAGCCTGAGpkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E17-2-F	GAGTACCGCTGGGAGGTGTA	pkd1-E33-3-R	AGTCGGTCAAACTGGGTGAG
pkd1-E18-1-Ftgctttaaaactggatggggpkd1-E33-4-RTGAAGCCCACAGACAGACAGpkd1-E18-1-RAGGGGCTCTTCCTCACTGTTpkd1-E33-5-FTTACTTTCTGCCGCTGTCAApkd1-E18-2-FCTTCCAAACCTGCCACAGTTpkd1-E33-5-RCAGCAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	pkd1-E17-2-R	gttetetgggeteatgggt	pKd1-E33-4-F	GGACAAGGIGIGAGCCIGAG
pkd1-E18-1-K     AGGGGCTCTTCCTCACTGTT     pkd1-E33-5-F     TTACTTTCTGCCGCTGTCAA       pkd1-E18-2-F     CTTCCAAACCTGCCACAGTT     pkd1-E33-5-R     CAGCAGGTGTTGGGGGGAG       pkd1-E18-2-R     GGAGCGTGAGGGGGAGAAC     pkd1-E33-6-F     CTCACTGTGTGTCTCGTGTCAG       pkd1 F19 1 F     TTCCAGCAGGCCAAATAGAC     pkd1 F23 6 P     TACACACAACCACCACACCACACCACACCACACCACAC	pkd1-E18-1-F	tgctttaaaactggatgggg	pKd1-E33-4-R	IGAAGUUUACAGACAGACAG
pkd1-E18-2-F     CTTCCAAACCTGCCACAGTT     pkd1-E33-5-R     CAGCAGGTGTGGGGGGAG       pkd1-E18-2-R     GGAGCGTGAGGGGGAGAAC     pkd1-E33-6-F     CTCACTGTGTGTCTCGTGTCAG       pkd1 E19 L F     TTCCAGCAGGCCAAATAGAC     pkd1-E33-6-F     TTCCAGCAGAGCAGAGCAGAGAGAGAGAGAGAGAGAGAGA	pKd1-E18-1-K	AUGUGUIUIICUICACIGIT	ркат-Езз-5-Е	
pka1-E10-2-K UUAUUUUUUUUUUUUUUAUAAU $pka1-E35-0-F$ UUAUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU	ркат-Ето-2-F		ркат-Езз-5-К	
	pku1-E18-2-K		pku1-E33-0-F	

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volumes and 35 cycles: 93°C for 1 min., 60°C for 30 s, and 72°C for 45 s. The PCR products were qualified by electrophoresis on 1% agarose gel, and then the PCR products were purified and directly sequenced.

**Direct sequencing of PKD1 exons of controls.** Direct sequencing of the exons 15, 25, and 38 and the flanking intron sequences were performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) on an ABI Prism 3700 automated genetic analyzer (Applied Biosystems). The same PCR primers were used for sequence reactions. Sequencing reactions were performed with the forward primers. missense mutation GTG  $\rightarrow$  ATG substitution in the proband and his parent at exon 25, which causes an amino acid exchange of valine to methionine at codon 3057. Moreover, two heterozygous missense mutations were observed just in the patient: ACA $\rightarrow$  GCA in exon 15 at codon 2241 that changed threonine to alanine and CAC $\rightarrow$  AAC in exon 38 at codon 3710 that changed histidine to asparagine. The results of direct sequencing of *PKD1* exons 15, 25, and 38 in 50 normal individuals demonstrated ACA $\rightarrow$  GCA in 9 persons, GTG  $\rightarrow$ ATG in 30 persons, and CAC $\rightarrow$  AAC in 11 persons of the normal population. The results of sequencing exons 15, 25, and 38 are given in Figure 3.

## DISCUSSION

#### RESULTS

Direct sequencing analysis of the proband and his parents after comparison with the NCBI Reference sequence of *PKD1* gene demonstrated a heterozygous

ADPKD is the most common genetic and inherited kidney disease with an incidence of 1 in 1,000, characterized by an important allelic variety with many variants described [5]. A large number of methods



**Fig. 3.** The result of genetic sequencing showing the heterozygous ACA>GCA missense mutation in exon 15 (a), the heterozygous GTG>ATG missense mutation in exon 25 (b), and the heterozygous CAC>AAC missense mutation in exon 38 (c) of Patient's PKD1 gene (sequencing with forward primer).

have been used to screen mutations in ADPKD for research and clinical aims, but direct sequencing is still considered as the method of choice [19, 16]. The most frequently reported types of mutations with pathogenic significance are deletions, insertions, splices, frame shift, substitutions, and nonsense mutations [15]. PKD1 gene mutations are considered as an important factor causing ADPKD. However, some observations suggest that genetic susceptibility might contribute to the varied disease phenotype [20]. In about 85% of the patients, mutations occur in PKD1 gene and in 15% of the cases, *PKD2* has mutations [5]. However, genetic evaluation was performed to have an idea of causal mutation. Method of genotype analysis is variable according to the clinical background and family history; therefore, genetic analysis is not always required in all patients with ADPKD. The preliminary diagnosis is often made by ultrasound, CT scan, and MRI. Young individuals with positive family history are recommended to undertake genetic tests [5]. However, we performed whole gene sequencing of the *PKD1* gene for family members, including affected son, and his mildly affected father, who showed no clinical manifestation of kidney disease. In addition, his mother with known congenital agenesis of right kidney was screened for putative mutations in the PKD1 gene. As a result, we identified a genetic variant of PKD1 in ADPKD affected boy and his parents. It was a missense mutation G>A (V3057M) located in exon 25 of the PKD1 gene. In addition, we found two novel mutations just in affected boy, one of them was a heterozygous A>G missense mutation (ACA>GCA)/N in exon 15 at codon 2241 of PKD1 gene that changed threonine to alanine in polycystin-1 protein and the other was a heterozygous C>A missense mutation

(CAC>AAC)/N in exon 38 at codon 3710 of the PKD1 gene that changed histidine to asparagine. After that, for verifying the pathogenicity of these three mutations, we performed PCR and direct sequencing of exons 15, 25, and 38 and their flanking regions 50 unrelated controls. All of our controls were healthy males (sex matched with our patient) without any sign of kidney disease, and all of them were in the same ethnicity as the patient's family from Southwest Iran. Interestingly, the GTG>ATG mutation was observed in 30 unaffected individuals. These results support the idea that this mutation is a polymorphism with an incidence of 60% in the Southwest Iran. As mentioned mutation in the PKD1 database (http://pkdb. mayo.edu/), the occurrence of the mutation V3057M is rare, but our findings demonstrate that this mutation is very common in the southwest population of Iran. Moreover, our novel mutations (ACA>GCA and CAC>AAC) are polymorphisms with frequencies of 14% and 22% in control samples, respectively. Figure 4 schematically shows the locations of mutations found in the polycystin-1 domains.

The first mutation ACA>GCA changes threonine to alanine at codon 2241 in REJ (receptor for egg jelly) domain of the polycystin-1 protein. Although the REJ domain is found in the polycystin-1 and in the sperm REJ, the function of this domain is unknown. The domain is 600 amino acids long; therefore, it is probably composed of multiple structural domains. There are six completely conserved cysteine residues that may form disulfide bridges. This region contains tandem PKD-like domains [21, 22]. T2241A makes changes in this domain structure in comparison with the normal domain structure as shown in Figure 5a.



Fig. 4. Diagrams of polycystin-1 (upper) and PKD1 gene (lower) show domain structures and coding regions for the protein domains, respectively. Three PKD1 mutations (above polycystin-1) were identified by our groups.



**Fig. 5.** Three dimensional models of PKD1 gene produc. **Right:** predicting the 3D structure of normal REJ domain (a), GPS domain (b), and transmembrane and loop regions (c) of the polycystin-1 protein. **Left:** T2241A mutant REJ domain (a), V3057M mutant GPS domain (b), and H3710N mutant transmembrane and loop region (c) structures. The differences between two domain structures have shown with black circles. Pictures prepared by Phyre2 website.

The second mutation GTG>ATG causes the conversion of amino acids valine to methionine at codon 3057 in G-protein-coupled receptor proteolytic site domain (GPS domain) of polycystin-1. This domain is present in latrophilin/CL-1, sea urchin REJ, and polycystin and termed the GPS domain (for GPCR proteolytic site), because it contains a cleavage site in latrophilin [23]. Many surface receptors present thid domain [24]. Currently, there has been no evidence that this domain provides a cleavage site in any of the other receptors. However, the peptide bond that is cleaved in latrophilin is between amino acids leucine and threonine residues that are conserved in some of the other receptors [24]. GPS domains are about 50 residues long and contain either 2 or 4 cysteine residues that are likely to form disulfide bridges. Similar to REJ domain, the exact function of GPS domain is still unknown. V3057M makes some changes in this domain structure in comparison with

the normal domain structure as shown in Figure 5b.

The third mutation CAC>AAC changes histidine to asparagine at codon 3710 in transmembrane and loop regions of the polycystin1 protein. Hydropathy plot and computer-assisted analyses identified 9 to 11 transmembrane regions from amino acids 3075 to 4014 with intervening intracellular and extracellular loops [8]. We also tested the effect of H3710N mutation on domain structure (the structure of the polycystin-1 protein domain, Fig. 5c) that differs significantly from wild-type. However, functional prediction of transmembrane domains is limited by computer algorithms. Therefore, direct biochemical protein purification and crystallization studies are needed to elucidate definitively the structure [25-27].

We conclude that the pathogenicity of some allelic variants is difficult to prove. Nevertheless, it is a first step to future multicenter studies for better description of genetic variants in Iranian ADPKD patients.

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