# Whole-Exome Sequencing Application for Genetic Diagnosis of Kidney Diseases: A Study from Southwest of Iran

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## Introduction

The kidneys have important and vital functions and provide maintenance of overall body health. Many kidney diseases are caused by single gene defects. Kidney disease is categorized in a heterogeneous group of disorders affecting the kidney, both in structure and function. The end stage of kidney disease is known as kidney failure. Kidney disease is divided into two forms: AKI and CKD (1). Among kidney diseases, polycystic kidney disease (PKD; also known as polycystic kidney syndrome) is revealed to have a genetic background (2). PKD is characterized by the presence of cysts in kidneys. The development and growth of cysts causes abnormalities in the renal tubules. PKD is a clinically and genetically heterogeneous disease (2,3). Clinical characteristics of autosomal dominant and recessive forms of PKD are variable in their penetrance. A range from neonatal death to incidence in old age was reported for PKD (2,4). Disease causing variants in three genes including *PKD1*, *PKD2,* and *PKDH1* can cause PKD. The pathogenic variations in the *PKD1* and *PKD2* genes can cause an autosomal dominant (ADPKD) pattern of inheritance. The pathogenic variations in *PKDH1* can cause an autosomal recessive (ARPKD) pattern of inheritance (2). There are more than 500 monogenic causes of CKD (5) and numerous genes are listed in next-generation sequencing (NGS) panels for PKD, including *ALG9*, *ANKS6*, *ATP6V0A4*, *BICC1*, *GANAB*, *GLIS3*, *HNF1B*, *INVS*, *LRP5*, *MUC1*, *NOTCH2*, *NPHP3*, *OFD1*, *PKD1*, *PKD2*, *PKHD1*, *SEC61A1*, *TMEM231*, *TSC1*, *TSC2*, *UMOD*, and *ZNF423*, *etc.* (2). For classification, pre-prognosis, monitoring and treatment, and identification of the etiology of the disease is necessary. It helps us understand the scenario of the disorder's causes and select the best approach for drug treatment (6). NGS is a powerful technique that enables rapid and cost-effective parallel sequencing of large panels of genes or whole-exome sequences. However, the targeted panel sequencing approach related to the Whole-Exome Sequencing (WES) is confirmed to provide deep coverage of specific sequences, but WES can provide an opportunity for novel variation detection (7–9). Regarding the polygenic entity of kidney diseases, which means many genes are involved in the pathogenesis of disease, for example, for FSGS, pathogenic variants in more than 20 podocyte-specific genes, such as *NPHS1*, *NPHS2*, *WT-1*, *LAMB2*, *CD2AP*, *TRPC6*, *ACTN4,* and *INF2,* were announced (10). In fact, conventional Sanger sequencing may be very time consuming and expensive, so using NGS would be helpful. In our study, we identified probable pathogenic variations associated with CKD using WES.

## Materials and Methods

### Patient Reports

In total, 22 enrolled patients and their parents were recruited from Southwest Iran, with demographic information shown in Table 1. All patients were referred to the Narges Medical Genetics and Prenatal Diagnosis Laboratory, Ahvaz, Iran between 2017 and 2020. Genetic counseling for all patients was conducted by genetic specialists from the laboratory. This research study was approved by the Ahvaz Jundishapur University of Medical Sciences. For the studies, informed consent was obtained from all of the families. All of the experiments were conducted in accordance with the relevant guidelines and regulations.

### DNA Extraction

First, 10 ml of peripheral blood was withdrawn from each of the enrolled patients and their parents, and collected in EDTA tubes. Genomic DNA was extracted using the standard salting out protocol. The quality and quantity of the extracted DNA samples were checked by gel electrophoresis and NanoDrop.

### Exome Sequencing

WES was applied for the patients by Macrogen Co., Korea. Samples were subject to Exome enrichment with the SureSelect v6, followed by sequencing using the Illumina HiSeq 2000 genome analyzer platform.

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**Table 1. Demographic data**

| Characteristics | % |
|---|---|
| **Sex** | |
| Female | 35 |
| Male | 65 |
| **Age (yrs)** | |
| <10 | 88 |
| 11–20 | 8 |
| 21–30 | 4 |
| **Ethnicity** | |
| Lur | 38 |
| Fars | 12 |
| Arab | 44 |
| Kurd | 6 |
| **Consanguinity** | |
| Consanguineous | 87 |
| Nonconsanguineous | 13 |

### Sanger Validation

Targeted regions of the genes were amplified by PCR using primers designed by the software Oligo 7. The PCR reactions were conducted using Master Mix (Ampliqon, Denmark). An initial five denaturations at 95°C for 5 minutes were followed by 35 cycles at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

PCR products were directly sequenced and analyzed by the ABI Prism 3700 automated genetic analyzer (Applied Biosystems). The results were analyzed with Chromas LITE 2.1.1, then compared with the reported gene sequence using the BLASTN program. The presence of the detected mutation was confirmed by parent analysis and bidirectional sequencing.

### *In-Silico* Analysis

There are many tools for predicting the pathogenicity of variations in genes coding and noncoding regions. In this study we used some of these tools for evaluating the disease causing potentiality of the variations.

## Results

We found disease-causing variants in *PKHD1*, *PKD1*, *PKD2L2*, *NPHS1*, *NPHP3*, *CD2AP*, *COL4A4*, and *DCDC2* genes. All variants were analyzed in terms of pathogenicity using different tools, including MutationTaster, PredictSNP1, PredictSNP2, Proven, Gnomad, GME, PolyPhen, VarSome, and I-Mutant (Table 2).

As Figure 1 shows, among 15 patients reported in Table 2; 53% of patients (eight) have pathogenic variations in the *PKHD1* gene, 13% (two) in *PKD1*, 7% (one) in *NPHS1*, 7% (one) in *NPHP3*, 7% (one) in *CD2AP*, 7% (one) in *COL4A4*, and 7% (one) in *DCDC2*.

### Discussion

In this study we demonstrated that WES works efficiently for the genetic profiling of kidney diseases. We evaluated WES in 22 Iranian patients with a history of clinically different inherited kidney diseases. Samples from family members were taken to perform segregation analysis where possible. We found two disease-causing variants (c.11626_11635delGCCGGCCGCG and c.1687C>T) in the *PKD1* gene. According to the *in-silico* analysis using MutationTaster, American College of Medical Genetics (ACMG) classification *etc*., the variants are disease causing and pathogenic. In addition, the variants were not present in our homemade exome database and the public SNP databases, including dbSNP, ExAC, and GnomAD. Due to the proximity of the pseudogenes to the *PKD1* gene, it has always been difficult to identify the pathogenic variants in *PKD1* (11,12). Several assays indicated that WES is a proper technique that can be used in a relatively short time and at a low cost to identify single-gene disease and genetic heterogeneity complaints in patients with ADPKD, in comparison with standard diagnostics on the basis of Sanger sequencing and multiplex ligation-dependent probe amplification assays (13,14). Multiplex ligation-dependent probe amplification is clinically suggested only for patients whose disease-causing variants have not been determined through NGS investigation (15). Ranjzad *et al.* (16) used targeted NGS for detecting novel pathogenic variants in Iranian families with ADPKD and demonstrated NGS can significantly reduce the cost and time for the simultaneous sequence analysis of *PKD1* and *PKD2*. Mallawaarachchi *et al.* (11) analyzed 28 unique pedigrees with ADPKD and reported that WGS is able to overcome technical challenges created by pseudogenes proximal to *PKD1*. However, *PKD1* is inherently difficult to sequence using WES (13). The clinical genetic diagnosis of ADPKD significantly affects the quality of patients' lives and renal transplantation, in which the presence of a familial pathogenic variant in a transplant phenotypically normal donor is screened. Also, in patients with an identified family pathogenic variant, we can prevent transmission to children *via* current prenatal diagnostic techniques (17,18). At the result of *in-silico* analysis, we found eight disease-causing variants, including four reported pathogenic single nucleotide variants (rs137852949, rs398124503, rs745770404, and CM100562) and three novel probable disease-causing variants in *PKHD1* gene. These variants were not present in our exome database and had no homozygotes with low allele frequency, or were not found in the public SNP databases, including ExAC and GenomAD. Efforts to prevent severe ARPKD complications from the embryonic development period have led to several approaches, including second-trimester sonography and molecular genetic analysis for prenatal diagnosis (19). Because of the large size of *PKHD1*, genetic heterogeneity, and broad phenotypic of cystic and PKDs, WES is an efficient approach for pre- and postnatal diagnosis of ARPKD (20,21). Obeidova *et al.* (22) used NGS for clinical analysis of ARPKD in 24 families, and reported that NGS of the *PKHD1* gene is a very convenient procedure, with high precision for molecular diagnosis in patients with very similar clinical symptoms to ARPKD. We found one disease-causing deletion (rs751527253) in the *NPHP3* gene. The *in-silico* analysis using MutationTaster and ACMG classification showed this variant is disease causing and pathogenic. Early diagnosis of nephronophthisis 3 is dependent on clinical and imaging findings, because of the similarity of symptoms in patients with renal problems, the exact diagnosis of these patients through NGS is important (23,24). In this study we detected some potentially pathogenic variants *via in-silico* analysis using MutationTaster, ACMG classification, *etc*. in *CD2AP*, *NPHS1*, and *COL4A4* genes in patients with different clinical symptoms of kidney disease. Bekheirnia *et al.* analyzed WES in 62 patients with

**Table 2. *In-silico* pathogenicity evaluation of the variant**

| Gene | Phenotype (OMIM) | Variation | Mutation Taster | PredictSNP1 | PredictSNP2 | Proven (cutoff = -2.5) | ACMG classification | GnomAD | GME | Stability (I-Mutantv2.0) | PolyPhen | Mutation assessor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *PKD1* | Polycystic kidney disease 1 | p.A3876Pfs*66 c.11626_11635del GCCGGCCGCG | Disease causing | — | — | — | Pathogenic | Not found | Not found | — | — | — |
| *PKD1* | Polycystic kidney disease 1 | p.Q563× c.1687C>T CM020486 | Disease causing | — | 60% benign | — | Pathogenic | Not found | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.T904N c.2711C>A | Polymorphism | 65% benign | 89% benign | Neutral | Uncertain significance | Not found | Not found | Decrease (RI=0) | Benign | Medium |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.R496× C.1486C>T CM032309 rs137852949 | Disease causing hom=0 | — | 68% benign | — | Pathogenic | 0 hom | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.R496× C.1486C>T CM032309 rs137852949 | Disease causing Hom=0 | — | 68% benign | — | Pathogenic | 0 hom | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.W365× c.1095G>A CM100562 | Disease causing | — | 58% benign | — | Pathogenic | Not found | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.H459Q c.1377T>G | Polymorphism | 83% benign | 89% benign | Deleterious | Uncertain significance | Not found | Not found | Decrease (RI=4) | Benign | Neutral |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | p.R328× c.982C>T rs398124503 CM032306 CM100565 | Disease causing hom=0 | — | — | — | Pathogenic | 0 hom | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | c.2279G>A p.R760H rs745770404 CM020957 | Disease causing hom=0 | 74% benign | 82% deleterious | Neutral | Pathogenic | 0 hom | 0 hom | Deacrease (RI=4) | Probably damaging | Low |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | c.1233+3A>T | Disease causing | — | — | — | Uncertain significance | Not found | Not found | — | — | — |
| *PKHD1* | Polycystic kidney disease 4, with or without hepatic disease | c.6491–1G>A rs1554132790 | Disease causing | — | — | — | Pathogenic | Not found | Not found | — | — | — |
| *NPHP3* | Meckel_syndrome_type_7\| Renal-hepatic-pancreatic_dysplasia\| Nephronophthisis\| Polycystic_kidney_dysplasia) | c.2694–2_2694–1del rs751527253 CD082161 | Disease causing | — | — | — | Pathogenic | 0 hom | Not found | — | — | — |
| *NPHS1* | Nephrotic, type 1 syndrome | c.585_586insA p.$^{19}$F6Ifs*16 | Disease causing | — | — | — | Pathogenic | Not found | Not found | — | — | — |
| *COL4A4* | Alport syndrome | c.3679G>C p.G1227R | Disease causing | 63% benign | 87% deleterious | Deleterious | Likely pathogenic | Not found | Not found | Increase (RI=1) | Probably damaging | — |
| *DCDC2* | Nephronophthisis | c.663T>G p.S221R | Disease causing | 71% benign | 63% benign | Neutral | Uncertain significance | Not found | Not found | Increase (RI=5) | Benign | Low |

OMIM, Online Mendelian Inheritance in Man; ACMG, American College of Medical Genetics; SNP, single nucleotide polymorphism; Hom, number of homozygotes; RI, the value of reliability index.

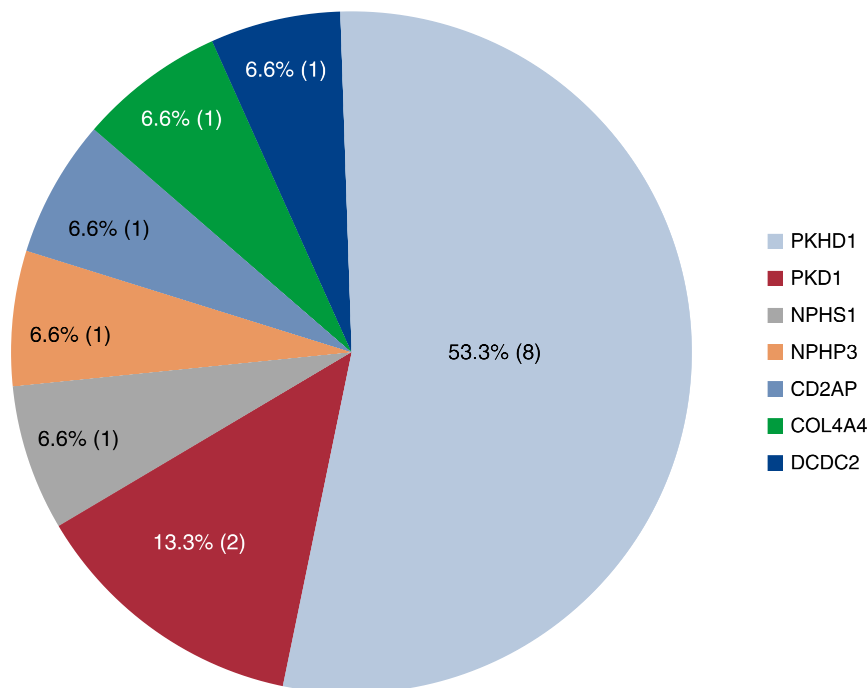


**Figure 1.** | **The contribution of the genes harboring the presented kidney disease–causing variants**. Most of the enrolled patients had pathogenic variants in the *PKHD1* gene.

congenital anomalies of the kidney and urinary tract. Nearly 5% of individuals with congenital anomalies of the kidney and urinary tract have pathogenic single-nucleotide variants in known key genes that can be uncovered by WES. In addition, 7% of these patients have pathogenic copy number variations that were extracted from WES data (25). Some reports also showed the potential of WES to find novel kidney disease–causing variants (26,27). We applied WES for patients with kidney disease from Southwest Iran populations and were able to detect pathogenic variations in 68% of the enrolled patients, although the disease-causing variants were not determined in 32% of patients. We confirmed that WES is a very advantageous procedure for identifying genes and mutations in kidney disease, and can accurately detect novel genes and variants; consequently, WES is emerging as a preferred diagnostic tool for hereditary disorders, including kidney disease.

### Disclosures

All authors have nothing to disclose.

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### Author Contributions

H. Galehdari, G. Shariati, and M. Zamani conceptualized the study; S. Negahdari, S. Sedighzadeh, T. Seifi, M. Zamani, and J. Zeighami were responsible for formal analysis; S. Sedighzadeh, T. Seifi, T. Yadegari, M. Zamani were responsible for the investigation; M. Zamani and J. Zeighami were responsible for the methodology; H. Galehdari, M. Hamid, A. Saberi, A. Sedaghat, and G. Shariati provided supervision; and S. Negahdari, S. Sedighzadeh, T. Seifi, and M. Zamani wrote the original draft.

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