









Whole-Exome Sequencing (WES) results of 50 patients with chronic kidney diseases: a perspective of Alport syndrome

Cüneyd Yavaş^{1,2,3*} , Cemal Ün² , Evrim Çelebi³ , Alper Gezdirici³ , Mustafa Doğan³ , Ezgi Gökpınar İli³ , Tunay Doğan³ , Nehir Özdemir Özgentürk⁴ 

SUMMARY

OBJECTIVE: Chronic kidney disease (CKD) remains one of the major common health problems, and the number of people affected by the disease is progressively increasing in Turkey and worldwide. This study aimed to investigate molecular defects in Alport syndrome (AS) and other genes in patients with clinically suspected CKD using whole-exome sequencing (WES).

METHODS: Patients with clinical suspicion of CKD were included in the study. Molecular genetic analyses were performed on genomic DNA by using WES.

RESULTS: A total of 15 with 5 different pathogenic or likely pathogenic variants were identified in CKD patients, with a diagnostic rate of 30%. Eight variants of uncertain significance were also detected. In this study, 10 variants were described for the first time. As a result, we detected variants associated with CKD in our study population and found AS as the most common CKD after other related kidney diseases.

CONCLUSIONS: Our results suggest that in heterogeneous diseases such as CKD, WES analysis enables accurate identification of underlying molecular defects promptly. Although CKD accounts for 10–14% of all renal dysfunction, molecular genetic diagnosis is necessary for optimal long-term treatment, prognosis, and effective genetic counseling.

KEYWORDS: Chronic kidney disease. Alport syndrome. Variant. Whole-exome sequencing.

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem, with increasing incidence and prevalence¹ and affecting more than 10% of people worldwide². CKD is associated with disease enclosing many disorders such as metabolic and end-stage renal failure and is a leading cause of death³, showing one of the highest increases in mortality for 10 years⁴. However, it is generally not recognized by people and specialists⁵. Moreover, clinical trials in nephrology are still insufficient for diagnosis, which has limited therapeutic options to alter disease progression and high costs for the health system⁶.

The description of hereditary (genetic) risk factors for CKD can enable early detection and increase our understanding of the pathogenesis of the disease. Genetic factors include both Mendelian (monogenic) and polygenic risk. Recent clinical sequencing studies have detected diagnostic variants in 10–25% of patients of various CKD populations,

suggesting a high burden of Mendelian disorders among patients with nephropathy⁷.

Alport syndrome (AS) is the most common CKD after other related kidney diseases⁸. Recent and comprehensive studies have reported that most patients with CKD have variants in AS-related genes⁹. AS is a well-defined genetic disorder characterized by kidney failure, hematuria, hearing impairment, and vision abnormalities¹⁰ and it is one of the hereditary causes of nephropathies and can genetically be transmitted from one generation to the next as a recessive dominant or X-linked⁸ because of variants in the *COL4A3*, *COL4A4*, and *COL4A5* genes, which encode, respectively, $\alpha3$, $\alpha4$, and $\alpha5$ chains of collagen type IV that consist of glomerular basement membrane in the kidney¹⁰. With the development of technologies such as massively parallel sequencing, it is possible to treat complex diseases that are difficult to diagnose more cheaply and precisely⁹. Our study aimed to detect variant in AS genes in CKD and contribute new variants to the literature.

¹Yildiz Technical University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics – Istanbul, Turkey.

²Ege University, Faculty of Science, Department of Biology Molecular Biology Section – Bornova (İzmir), Turkey.

³Başakşehir Çam and Sakura City Hospital, Department of Medical Genetics – Istanbul, Turkey.

⁴Yildiz Technical University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics – Istanbul, Turkey.

*Corresponding author: cuneydyavas@gmail.com

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METHODS

A total of 50 unrelated patients with a clinical CKD were included in the study after obtaining informed consent from the patients and their parents for medical examination and genomic analysis. The procedures adhered to the Declaration of Helsinki, and the Ethics Committee approved the relevant studies of the Basaksehir Cam and Sakura City Hospital (E-96317027-000-8273 2021.03.02/KAEEK). All the patients met the generally accepted clinical diagnostic criteria for abnormalities of kidney structure or function present more than 3 months.

Genomic DNA was extracted from peripheral blood, and whole-exome sequencing (WES) was performed by capturing the coding regions and splice sites of target genes (101 genes) via the Twist Human Comprehensive Exome kit panel. After library enrichment and quality control, the samples were sequenced on the BGI platform (Shenzhen, China) with 100-bp paired-end reads at an average sequencing depth of $\times 100$. Annotation of detected variants was performed using www.genomize.com.tr (version 6.14.4), interval, Franklin, VarSome, ClinVar, OMIM, and Pubmed. Variants with a frequency higher than 0.1% were filtered out. dbNSFP (contains MetaLR, MetaSVM, MetaRNN, REVEL, SIFT, PolyPhen-2, LRT, Mutation Taster 4.2 versions) was used to predict variants' pathogenicity (deleteriousness). Detected variants were classified as "pathogenic," "likely pathogenic (LP)," or "variants of uncertain significance (VUS)" according to the international guidelines of the ACMG. These variants are listed in Tables 1 and 2.

RESULTS

When 23 of 50 patients who underwent WES due to CKD were evaluated together, it was seen that 15 (65%) patients were older than 17 years and 8 (35%) were younger than 17 years (Tables 1 and 2). Of the 23 detected variants, 10 are novel types. Variants in the genes *COL4A3* (c.4153+1del, c.4123C>T, c.4222A>G, c.1061C>T), *PKD1* (c.6260_6263dup, c.7401del, c.4168C>T), *PKD2* (c.872T>C), *LMX1B* (c.237G>T), and *PLCE1* (c.5410_5411del) were described for the first time in this report (Tables 1 and 2). The genes with variants in 23 patients were *COL4A3*, *COL4A4*, *COL4A5*, *COQ8B*, *FN1*, *LMX1B*, *MEFV*, *PKD1*, *PKD2*, *PLCE1*, and *SLC34A1*. Variants were found in *PKD1* in 7 of 23 patients, in *COL4A3* in 5, in *COL4A4* in 1, and in *COL4A5* in 2 patients.

Within the scope of the study, the WES results of 50 patients diagnosed with CKD were evaluated. Pathogenic/LP variants were detected in 15 (30%) of 50 patients following ACMG criteria (Figure 1). Of these 15 patients, 4 (27%) had pathogenic/LP variants in genes other than AS genes (*COL4A3*, *COL4A4*, or *COL4A5*). Variants in genes associated with AS were detected in 8 (35%) of 23 patients (Table 2).

Variants in the AS-related genes *COL4A3*, *COL4A4*, and *COL4A5* were found in 8 (35%) of 23 CKD patients who underwent WES (Table 2). Variants were detected in the *COL4A3* gene in five of these eight patients, in the *COL4A4* gene in one, and in the *COL4A5* gene in two patients. One of

Table 1. Characteristics of detected variations.

P	G	Age	Gene	Variant	Variant type	Zygosity	ACMG classification	Patient frequency	Novelty
P1	F	37	<i>PKD2</i>	c.872T>C (p.Leu291Pro)	Missense mutation	Het	LP	N/A	Novel
P2	M	6	<i>LMX1B</i>	c.237G>T (p.Glu79Asp)	Missense mutation	Het	P	N/A	Novel
P4	F	9	<i>MEFV</i>	c.2080A>G (p.Met694Val) c.442G>C (p.Glu148Gln)	Missense mutation Missense mutation	Het Het	P B	$f = 0.000282$ $f = 0.0711$	rs61752717 rs3743930
P5	M	2	<i>FN1</i>	c.4151T>C (p.Ile1384Thr)	Missense mutation	Het	V	$f = 0.0000159$	rs768047478
P8	M	0	<i>PLCE1</i>	c.5410_5411del (p.Phe1804LeufsTer15)	Small deletion (frame shift)	Homo	P	N/A	Novel
P9	M	11	<i>COQ8B</i>	c.1027C>T (p.Arg343Trp)	Missense mutation	Homo	LP	$f = 0.000004$	rs398122981
P10	F	51	<i>PKD1</i>	c.7666C>T (p.Gln2556Ter)	Nonsense mutation	Het	P	N/A	rs1567184366
P12	M	38	<i>PKD1</i>	c.8302G>A (p.Val2768Met)	Missense mutation	Het	P	$f = 0.0000081$	rs1456510041
P13	F	6	<i>PKD1</i>	c.2308C>T (p.Arg770Trp)	Missense mutation	Het	V	$f = 0.0000147$	rs771288493
P15	M	29	<i>PKD1</i>	c.6260_6263dup (p.Arg2089ProfsTer20)	Small insertion (frame shift)	Het	P	N/A	Novel
P17	F	58	<i>SLC34A1</i>	c.1325C>T (p.Pro442Leu)	Missense mutation	Het	V	$f = 0.0000159$	rs760010857
P18	F	21	<i>PKD1</i>	c.7401del (p.Asn2468ThrfsTer152)	Small deletion (frame shift)	Het	P	N/A	Novel
P19	F	32	<i>PKD1</i>	c.4168C>T (p.Gln1390Ter)	Nonsense mutation	Het	P	N/A	Novel
P23	M	9	<i>CD2AP</i>	c.902A>T (p.Lys301Met)	Missense mutation	Het	P	$f = 0.000191$	rs141778404
P24	M	57	<i>PKD1</i>	c.3877G>A (p.Val1293Ile)	Missense mutation	Het	V	$f = 0.0000168$	rs774353552

P: patient; G: gender; F: female; M: male; N/A: not available. The genes, variant types, zygosity, pathogenicity, frequency, and database numbers of the detected variations were determined according to the ACMG guideline. Six variations were detected, which were novels.

Table 2. Clinical characteristics of the patients and detected Alport syndrome genes.

P	G	Age	Clinical or pathological diagnosis before WES-based analysis	Spot urine protein/creatinine ratio (0–150 mg/g)	Serum albumin (35–52 g/L)	Serum creatinine (0.5–0.9 mg/dL)	Serum globulin (26–46 g/L)	Alport gene	Variant	Variant type	Zygoty	ACMG classification	Patient frequency	Novelty
P3	F	7	SRNS	190↑	25↓	0.51	17↓	COL4A3	c.3829G>A (p.Gly1277Ser)	Missense mutation	Het	P	f = 0.000377	rs190598500
P7	F	17	Pancytopenia, bleeding diathesis, NS	178↑	34↓	0.43↓	23↓	COL4A3	c.4421T>C (p.Leu1474Pro)	Missense mutation	Het	P	f = 0.0027	rs200302125
P11	F	9	SRNS, FSGS	4137↑	34↓	0.68↑	22↓	COL4A3	c.4153+1del	Splicing deletion	Homo	LP	N/A	Novel
P14	F	0	NS	130	26↓	1.48↑	15↓	COL4A3	c.4123C>T (p.Pro1375Ser)	Missense mutation	Het	LP	N/A	Novel
P22	F	10	NS	151↑	46	0.38↓	21↓	COL4A3	c.3182G>A (p.Gly1061Asp)	Missense mutation	Het	VUS	f = 0.000197	rs202078295
P20	M	2	FSGS, NS	38157↑	23↓	0.19↓	20↓	COL4A4	c.665C>T (p.Pro222Leu)	Missense mutation	Het	VUS	f = 0.000122	rs773533313
P6	F	5	SRNS	8812↑	38	0.18↓	21↓	COL4A5	c.4222A>G (p.Thr1408Ala)	Missense mutation	Het	VUS	N/A	Novel
P21	F	6	NS	7664↑	25↓	0.38↓	19↓	COL4A5	c.1061C>T (p.Thr354Ile)	Missense mutation	Het	VUS	N/A	Novel

P: patient; G: gender; WES: Whole-Exome Sequencing; F: female; M: male; N/A: not available; SRNS: steroid-resistant nephrotic syndrome; NS: nephrotic syndrome; FSGS: focal segmental glomerulosclerosis. The genotype-phenotype correlation of the patients carrying the AS genes in which the variations were detected the most was calculated, and the damaging effects were examined according to the laboratory findings.

the novel variants detected in the *COL4A3* gene of two patients was homozygous and the other heterozygous, and both were not previously reported in the literature. These two novel variants were classified as LP according to the ACMG. Both heterozygous novel variants in the *COL4A5* genes of two patients were not previously reported in the literature. These variants were classified as VUS, according to the ACMG. Heterozygous variant in the *COL4A4* gene was detected in only one patient. The protein/creatinine ratio in the spot urine of patients with variants in the *COL4A4* and *COL4A5* genes was significantly higher than in patients with variants in the *COL4A3* gene.

Variants were found in genes other than the AS-related genes in 15 (65%) of 23 patients who underwent WES due to CKD and were found to have the variants (Table 1).

DISCUSSION

Frequently, it is clinically challenging to distinguish CKD type. In general, CKD diagnosis can be made based on morphological, pathological, and genetic examination after complaints such as persistent alterations in kidney structure or function, filtration disorders, proteinuria, and hematuria¹¹. The prevalence of all stages of CKD in studies varies between 7 and 12% in different regions of the world. Therefore, multiple patients with this monogenic form of CKD genetic testing emerge as an important tool in determining the cause of CKD, especially in children and young adults¹². To date, 101 genes thought to be responsible for the CKD phenotype have been identified

in the HPO database. Some gene groups responsible for the CKD phenotype stand out in the studies. Studies have shown that patients diagnosed with CKD have pathogenic variants, mostly in AS and polycystic disease (PD) genes¹³. The prominent gene groups in our study are genes related to AS and PD. Because of this, we focused on patients with detected variants in AS genes. In this study, the rate of patients diagnosed with AS gene was 35%. Furthermore, the frequency of disease-causing variants in CKD is likely to differ between different ethnic groups depending on age, gender, and the molecular analysis methods used for diagnosis¹⁴. Many CKD-associated genes have yet to be identified, and it is believed that unidentified CKD genes may play a role in populations with low detection rates¹⁵.

According to the U.S. Renal Data System (USRDS, <https://www.usrds.org/>), approximately 0.2% of adults and 3% of children with end-stage renal disease (ESRD) in the United States are diagnosed with AS. In this study, AS genes were found to be higher in patients with CKD and variant, with a rate of 35%. Also extensive studies, significant findings were observed at the rate of 24% as a diagnostic value⁹, but this rate was higher than the literature in our study (30% rate). In our study, dominant inheritance was more common than recessive and X-linked inheritance.

WES studies make it possible to identify new genes and detect significant variants from defined genes by rapidly and simultaneously examining genes related to monogenic CKD; the number of patients diagnosed with monogenic CKD can be expected to increase rapidly. In this study, we identified a possible pathogenic/pathogenic (i.e., high-risk) CKD-associated variant in 30% of our patients and provided a comprehensive description of these patients' clinical and variational characteristics. A total of 15 variants in 23 patients were of the high-risk type, 5 (33.3%) patients had *PKDI* variant, 4 (26.6%) had AS genes variant, and other genes (40.1%) had variant. For physicians, the most current challenge in the molecular genetic diagnosis of CKD is the evaluation of VUS, which perhaps represents a barrier to clinical interpretation. As a result, disaggregation analysis studies on family members will be particularly useful and, in some cases, even vital. In addition, extensive in silico analyses of the structural and functional impact on the protein product may be useful for some. We believe that our cases will contribute to developing the scientific literature on CKD. Clinically, there is no difference between patients with renal impairment (patients with VUS or no variant) and patients with confirmed CKD (i.e., patients with a probable pathogenic/pathogenic variant). However, among carriers of the AS gene variant, individuals with recessive inheritance and the *COL4A5* variant had a higher protein/creatinine ratio (Table 2).

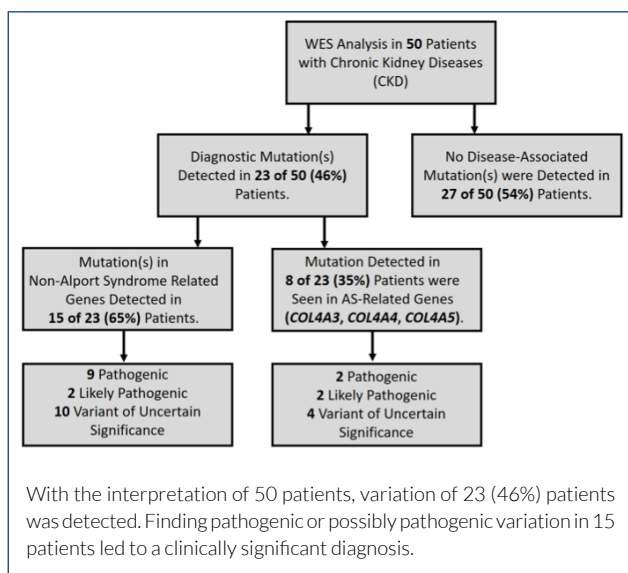


Figure 1. Flowchart of the Whole-Exome Sequencing analysis.

This study showed that for most patients, genetic diagnoses could be made clinically for patients. In this study, we manifest that our results highlight the potential of genetic findings to change the diagnostic value of consultation. For example, eight patients with the most detected variants of *COL4A3*, *COL4A4*, or *COL4A5* did not have clinical diagnoses of classically associated with AS. Although these patients do not have eye and ear nose and throat findings, due to the detection of variants, patients with clinical nephropathy variant are diagnosed and treated according to the variant type¹⁶. In addition, it was determined how effective the variants detected in AS-related genes were in the clinic shown in Table 2. Consistent with recent studies, we identified autosomal and X-linked forms of AS among patients with a clinical diagnosis of kidney disease, supporting the variable phenotypic expression of variants in type IV collagen genes^{9,17}.

To make a clinical diagnosis in nephrology, the need to “revise the disease ontology based on molecular classifiers” has been emphasized in some publications¹⁸. Our findings support the diagnostic utility of exome sequencing among the different clinical categories of kidney disease, the most detecting variant in AS-related genes. Therefore, looking at AS genes first in nephropathy patients in low-budget laboratories is recommended. It also highlights the potential of genetic testing to guide treatments for diseases accurately.

Large databases of controls, such as ExAC and gnomAD¹⁹, are of great value for interpreting detected variants and deciding clinical relevance based on their frequency in the population. New variant annotation algorithms that take into account genomic context to assess variant intolerance can facilitate variant classification in patients independent of previous clinical reports²⁰ and help standardize disease association interpretation. Therefore, many initiatives are currently examining the clinical relevance of genes and variants for diagnosing diseases (e.g.,

ClinGen (www.clinicalgenome.org) and will provide further evidence of the usefulness of genetic testing in various clinical settings²¹. When the novel variants detected in our study are analyzed according to population frequencies, and in silico analysis, it predicts that protein function may be impaired.

CONCLUSIONS

Large-scale genetic studies are needed to understand the genetic aspects of CKD in Turkey and other populations. Observational studies of CKD often reveal seemingly conflicting relationships between traditional risk factors and outcomes, making it difficult to predict outcomes in studies of CKD patients with normal kidney function. However, many completed CKD studies are limited by tentative results of uncertain clinical relevance or narrow eligibility criteria that limit external validity, and the implementation of proven treatments remains a challenge. Therefore, the nephrology community should capitalize on the recent interest in new approaches to experimental design, such as practical clinical trials²². Therefore, genetic approaches are important for treatment and diagnosis. Our study has shown that the most common CKD subtype is AS.

AUTHORS' CONTRIBUTIONS

CY: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **CÜ:** Data curation, Formal analysis. **EÇ:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **AG:** Data curation, Formal analysis. **MD:** Data curation, Formal analysis. **EGİ:** Data curation, Formal analysis. **TD:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **NÖÖ:** Data curation, Formal analysis.

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