Sahin *et al.*
Egyptian Journal of Medical Human Genetics (2023) 24:59
https://doi.org/10.1186/s43042-023-00441-x

Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

Expanding the genotype–phenotype correlations in Alport syndrome: novel mutations, digenic inheritance, and genetic modifiers

Ibrahim Sahin[1,2*], Nefise Kandemir[1] and Hanife Saat[1]

Abstract

Background Alport syndrome (AS) is the second most prevalent genetic cause of kidney failure, behind autosomal-dominant polycystic kidney disease, affecting at least one in 5000 individuals worldwide. AS is caused by COL4A3, COL4A4, and COL4A5 mutations. It is characterized as three distinct disorders of type IV collagen 3/4/5 based on a genetic evaluation: X-linked, autosomal, and digenic. About two-thirds of AS cases are X-linked (XLAS), 15% are autosomal recessive (ARAS), and 20% are autosomal dominant (ADAS). The spectrum of phenotypes associated with AS ranges from increasing renal disease with extrarenal abnormalities to isolated hematuria. Coinherited genetic mutations contribute significantly to clinical severity and variability.

Methods In this study, an AS panel (COL4A3/COL4A4/COL4A5) and clinical exome sequencing (CES) were performed on 18 patients.

Results Nineteen specific AS mutations, including 15 novel mutations, were found in these 18 cases, which included 17 Turkish families and 1 Syrian family. Digenic inheritance was observed in one patient, and eight coinherited genetic mutations were discovered.

Conclusions This research reveals many novel AS mutations and shows robust genotype–phenotype heterogeneity in the disease. The results expand the clinical and molecular scope of AS and clarify the ADAS and digenic AS phenotypes, further enhancing our understanding of the complex nature of AS and its association with genetic modifiers. The data broaden the spectrum of AS-related gene mutations and provide new insights on genotype–phenotype correlations in AS.

Keywords Alport syndrome, COL4A3, COL4A4, COL4A5, Digenic, Genetic modifier

Background

Alport syndrome (AS) is the second most prevalent genetic cause of kidney failure, behind autosomal-dominant polycystic kidney disease, affecting at least one in 5000 people [1]. AS is characterized by a spectrum of phenotypes, including progressive renal failure with extrarenal abnormalities and isolated hematuria, as well as ocular and auricular alterations. Atypical clinical symptoms include leiomyomatosis and vascular abnormalities [2].

*Correspondence:
Ibrahim Sahin
ibrahimsahinmd@gmail.com
[1] Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey
[2] Department of Molecular Medicine, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain

Mutations in the genes encoding alpha-3 (COL4A3), alpha-4 (COL4A4), and alpha-5 (COL4A5) of type 4 collagen are responsible for AS [3]. About two-thirds of AS cases are X-linked (XLAS), 15% are autosomal recessive (ARAS), and 20% are autosomal dominant (ADAS) [4]. Compared to patients with XLAS, ADAS patients develop end-stage renal disease (ESRD) at a slower rate and are less prone to extrarenal symptoms [5].

Type IV collagen (COL4) is the structural component of basement membranes (BMs) throughout the human body. Collagen chains are organized in the triple helix configurations α1α1α2, α3α4α5, and α5α5α6. These heterotrimers are generated by the self-association of non-collagenous carboxy-terminal domains in each chain, followed by the folding of collagenous domains containing Gly-X–Y triplet repeats into triple helical structures [2]. The synthesis of the α3α4α5 triple helix is compromised by the absence of one type IV collagen chain. Pathogenic variants of the genes encoding the alpha-3, alpha-4, and alpha-5 chains disrupt the normal collagen network in the BMs of the cochlea, eye, Bowman's capsule, glomerulus, and distal and collecting tubules of the kidney [6]. In AS, the GBM is more vulnerable to proteolytic degradation, resulting in the activation of podocytes and endothelin receptors' adhesion kinase, glomerular inflammation, tubulointerstitial fibrosis, and ESRD [7].

Clinical outcomes are classified based on the organ or structure affected, such as the kidney, inner ear, eyes, smooth muscles, and cardiovascular system. Alport syndrome (AS) is distinguished by hematuria, predominantly microscopic hematuria. Extensive hematuria is the most prominent sign, followed by foam in the urine. Proteinuria is preceded by microalbuminuria and often presents in the later disease stages, indicating renal impairment. Thus, proteinuria is indicative of end-stage renal disease (ESRD). Bilateral sensorineural hearing loss (SNHL) is often one of the first signs of AS. A significant association exists between the severity of SNHL and renal function impairment. In up to forty percent of individuals, ocular symptoms may precede proteinuria. Vision impairment is more prevalent in adults. Leiomyomatosis is a rare clinical sign of AS, characterized by benign smooth muscle cell nodular tumors [2, 8, 9].

The proposed classification of thin basement membrane nephropathy and some cases of focal segmental glomerulosclerosis as AS would increase the number of affected persons. Specifically, the reclassification approach identifies AS as three distinct disorders of type IV collagen 3/4/5 based on a genetic evaluation: X-linked, autosomal, and digenic [6].

Individuals with ARAS and male patients with XLAS generally exhibit severe disease manifestations. Due to the phenomenon of X inactivation, women with XLAS have a variable phenotype. Patients with heterozygous disease-causing mutations in COL4A3 or COL4A4 exhibit a wide range of clinical symptoms, ranging from asymptomatic to hematuria alone or proteinuria and renal failure in addition to hematuria and sensorineural hearing loss [5].

The differential diagnosis of AS includes immunoglobulin A nephropathy, thin GBM disease, medullary cystic disease, multi-cystic renal dysplasia, and polycystic kidney disease. Thin basement membrane (TBM) disease, a collagen 4-associated nephropathy strongly linked to AS, is a critical diagnostic indicator for AS patients. The same genes seem to be involved in many individuals with the illness. Unlike AS, there are fewer extrarenal symptoms, the symptoms are milder, and renal damage is infrequent [10].

In this study, 18 individuals underwent next-generation sequencing to determine the significance of the ADAS phenotype, digenic inheritance, genetic modifiers, and clinical variability in AS. Our findings expand the known spectrum of gene mutations and provide new insights into genotype–phenotype correlations in AS (Table 1, Fig. 2).

Material and methods

Patients

Consent for the publishing of the research and any extra associated material was obtained from the patients who participated in the study or their parents. Data were gathered for the 18 patients referred to our clinic (Table 1). Between January 2017 and December 2020, the patients underwent the Alport Syndrome Panel (COL4A3/COL4A4/COL4A5) or clinical exome sequencing (CES) at the Ankara Central Genetic Laboratory (Turkey).

Next-generation sequencing (NGS), interpretations, descriptive statistics, and graphics

The patients' genomic DNA was extracted using the QIAamp DNA Blood Midi Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions from blood samples collected in EDTA tubes. A NanoDrop 1000 spectrophotometer and Qubit 4 fluorometer (Thermo Fisher Scientific Inc., MA, USA) were used to quantify the DNA samples.

The AS panel was performed with Multiplicom ALPORT MASTR (Multiplicom N.V., Niel, Belgium) on the Illumina MiSeq (Illumina Inc., San Diego, CA, USA), and CES was performed with a Sophia Clinical Exome Kit (Sophia Genetics, Saint-Sulp) on the Illumina NextSeq 500 (Illumina Inc., San Diego, CA, USA). The data were analyzed using SOPHiA-DDM-v4 software (Sophia Genetics, Saint-Sulp). The NGS approach has been configured and optimized on Sophia-DDM-v4 to

Table 1 Demographic features and mutations of the patients

ID	Gender	Age	Indication	AS genes	AS mutations	AS inheritance	Mutation type	ACMG classification of AS mutations	Novelty
1	F	23	Alport syndrome	COL4A4	COL4A4, c.4684delT, p.Tyr1562Metfs*41	Heterozygous	Frameshift	Pathogenic	Yes
2	M	12	Alport syndrome	COL4A5	COL4A5, c.780 + 2 T > A	Hemyzygous	Splicing	Likely pathogenic	Yes
3	F	36	Alport syndrome	COL4A4	COL4A4, c.4485C > G, p.Tyr1495Ter	Heterozygous	Nonsense	Likely pathogenic	Yes
4	F	3	Alport syndrome	COL4A3	COL4A3, c.388-1G > T	Heterozygous	Splicing	pathogenic	No
5	F	22	Alport syndrome	COL4A5	COL4A5, c.81 + 1G > T	Heterozygous	Splicing	Likely pathogenic	Yes
6	F	48	Alport syndrome	COL4A4	COL4A4, c.3467del, p.Gln1156Argfs*12	Heterozygous	Frameshift	Likely pathogenic	Yes
7	M	29	Alport syndrome	COL4A4	COL4A4, c.4625G > A, p.Trp1542Ter	Homozygous	Nonsense	Likely pathogenic	Yes
8	M	10	Alport syndrome	COL4A3	COL4A3, c.3664G > C, p.Gly1222Arg	Heterozygous	Missense	Likely pathogenic	Yes
9	F	19	Alport syndrome	COL4A4	COL4A4, c.4294A > T, p.Lys1432Ter	Heterozygous	Nonsense	Likely pathogenic	Yes
10	F	44	Alport syndrome	COL4A3/COL4A5	COL4A3, c.3536del, p.Pro1179Glnfs*42/ COL4A5, c.3190_3192dup, p.Asp1064dup	Heterozygous/ heterozygous	Frameshift	Likely pathogenic/ likely pathogenic	Yes/yes
11	F	6	Alport syndrome	COL4A5	COL4A5, c.2692A > G, p.Met898Val	Heterozygous	Missense	VUS	No
12	F	35	Alport syndrome/ focal segmental glomerulosclerosis	COL4A4	COL4A4, c.763G > A, p.Gly255Arg	Heterozygous	Missense	VUS	Yes
13	M	36	Alport syndrome/ renal failure	COL4A4	COL4A4, c.4805_4809del, p.Leu1602fs	Heterozygous	Frameshift	Likely pathogenic	Yes
14	F	28	Alport syndrome/ short stature/Fanconi anemia	COL4A4	COL4A4, c.193-2del	Heterozygous	Splicing	Likely pathogenic	Yes
15	F	14	Alport syndrome	COL4A3	COL4A3, c.4347_4353delCCGACAC, p.Arg1450Valfs*77	Homozygous	Frameshift	Pathogenic	No
16	F	14	Alport syndrome	COL4A4	COL4A4, c.871G > A, p.Gly291Arg	Heterozygous	Missense	Likely pathogenic	Yes
17	M	34	Alport syndrome	COL4A3	COL4A3, c.3454G > C, p.Gly1152Arg	Heterozygous	Missense	Pathogenic	No
18	M	17	Alport syndrome	COL4A4	COL4A4, c.3512C > G, p.Ser1171Cys	Heterozygous	Missense	VUS	Yes

AS Alport syndrome, *M* male, *F* female, *ACMG* American College of Medical Genetics and Genomics, *VUS* variant of uncertain significance, *N/A* not applicable

ensure sufficient read coverage to detect deletions and duplications.

The variants were filtered using the Genome Aggregation Database and our in-house database's minor allele frequency threshold of 0.0005. Several pathogenic prediction approaches were applied to variants using the VarSome and Franklin tools (Genoox). Splicing variations were analyzed with the Human Splicing Finder.

The ClinVar and Leiden Open Variation Databases were searched for reports on all potential disease-causing variations. Using Sanger sequencing, the potential disease-causing mutations were validated, and their segregation was analyzed. In line with the American College of Medical Genetics and Genomics, variants were classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign [11]. Pathogenic, likely pathogenic, and strong VUS (supports clinical phenotype and no other mutations found to be responsible) variants were included in the research. The presence of two disease-causing mutations indicated a digenic inheritance pattern [12, 13]. Variants were classified into the following categories: missense, frameshift, nonsense, and splicing. The data were visualized with IGV 2.7.2 software (Broad Institute). In addition, descriptive statistical analyses were calculated, and graphics were created with Python 3.10.6 (IPython 7.29.0).

Results

The mean age of the participants was 23.89 years, with a minimum age of 3 and a maximum of 48. There were significantly more female participants ($n=12$, 66.67%) than male participants ($n=6$, 33%) (Fig. 1).

Eighteen specific Alport mutations were found in the 18 cases, representing 17 Turkish families and one Syrian family. Two of them were homozygous (11.11%), 14 were heterozygous (77.77%), one was hemizygous (5.55%), and one was digenic inheritance (5.55%) (Table 1 and Fig. 2).

The most commonly mutated gene was COL4A4. Ten mutations were detected in COL4A4 (55.56%), four in COL4A3 (22.22%), three in COL4A5 (16.67%), and one in COL4A3/COL4A5 (5.56%). Recurrent or founder mutations or mosaicisms were not detected in the study. Detected pathogenic and likely pathogenic mutations were validated through Sanger sequencing in the patient and all other available family members. Fifteen mutations were novel and have never been reported before. They belong to evolutionary highly conserved regions, and in silico tools showed that they are disease-causing. Nonsense and frameshift mutations may be predicted to cause nonsense-mediated mRNA decay.

The coinherited genetic mutations were detected in six genes. Likely pathogenic "TTC21B, c.2599C > T, p.Arg867Cys" mutation was heterozygous, likely pathogenic "SLC34A1, c.558dupC, p.Ile187Hisfs*26/c.272_292del, p.Val91_Ala97del" and pathogenic "PKHD1, c.107C > T, p.Thr36Met/c.5513A > G, p.Tyr1838Cys" mutations were both compound heterozygous, and pathogenic "FANCC, c.456 + 4A > T," "MEFV, c.2080A > G, p.Met694Val," "GJB2, c.35del, p.Gly12fs" mutations were homozygous.

Discussion

This article provides clinical and genetic data on 18 individuals from 17 Turkish families and 1 Syrian family with disease-causing mutations in COL4A3, COL4A4, and COL4A5 and coinherited genetic findings.

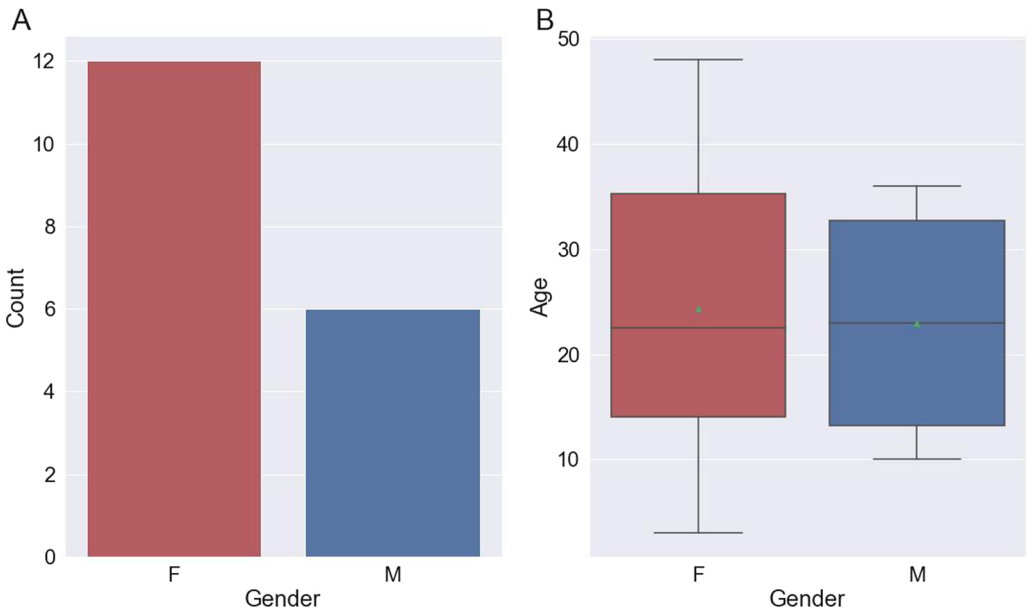

Fig. 1 Patients' characteristics. **A** Bar plot showing the number of patients in terms of gender. **B** Boxplot showing the mean (green triangle) and median (black line) age of the patients in terms of gender

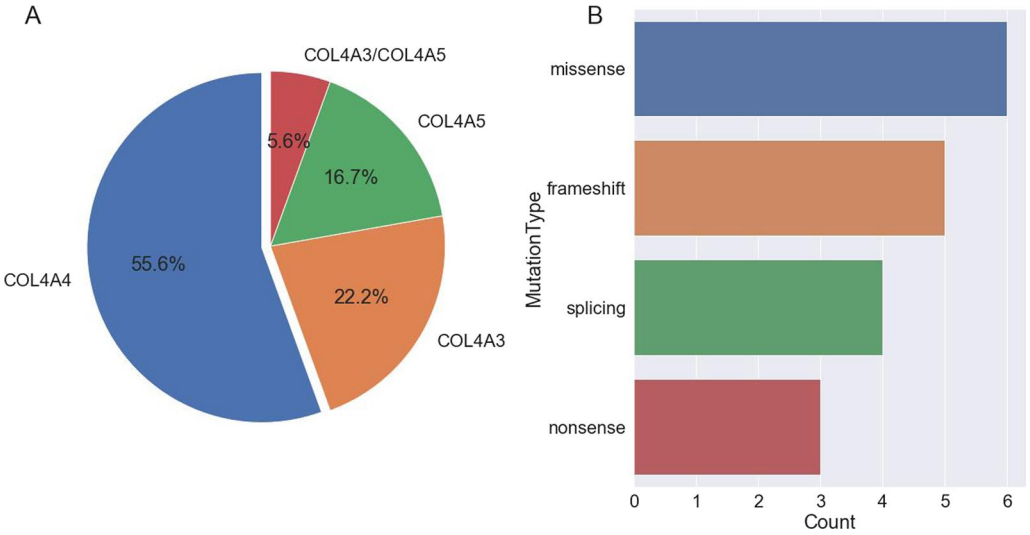

Fig. 2 Spectrum of the genes and mutation types. **A** Pie chart showing the spectrum of the mutated genes with percentages in the study. **B** Bar plot showing the mutation types in the study

AS is inherited as X-linked (XLAS), autosomal recessive (ARAS), and autosomal dominant (ADAS). AS is characterized by impaired synthesis and deposition of collagen IV alpha3/4/5 networks in the BMs of the glomerulus, cochlea, and eye [7]. Hematuria is the most common symptom of AS, and males are affected more often than women [14]. Interestingly, we had more female patients, and COL4A4 was the most common mutated gene in our study, which differs from the literature [9].

The clinical manifestations of ADAS patients vary from asymptomatic to renal failure, with no association with the causative gene or variant type. Due to the many phenotypes associated with ADAS, it is underdiagnosed in clinical practice [5, 15]. Few individuals with heterozygous COL4A3 or COL4A4 mutations develop ESRD, hearing loss, or a lamellated GBM. Hence, there is no consensus regarding the term ADAS. Nonetheless, the prognosis of patients with ADAS is not always favorable, and a small but unexpected proportion of them suffer renal failure. Given that most other family members with similar mutations maintain normal renal function throughout their lifetimes, there is no obvious explanation for this. This discrepancy demonstrates that there are other causes of renal failure for these patients [16]. Patients with ADAS have a slower rate of progression to ESRD and are less likely to have extrarenal symptoms than patients with XLAS. ESRD and SNHL often manifest in later adulthood, and ocular involvement is infrequent [2, 3].

Fallerini et al. [13] and Mencarelli et al. [12] proposed digenic inheritance in AS, which could explain the variable expressivity of AS. Patients with heterozygous mutations in two distinct collagen IV genes suffer renal failure later than those with XLAS or ARAS but earlier than those with ADAS [12]. In patient 10, both heterozygous likely pathogenic "COL4A3, c.3536del, p.Pro1179Glnfs*42" and "COL4A5, c.3190_3192dup, p.Asp1064dup" mutations were detected. Her mother had "COL4A5, c.3190_3192dup, p.Asp1064dup" mutation, and her father had the "COL4A3, c.3536del, p.Pro1179Glnfs*42" mutation. The patient had proteinuria, hematuria, focal segmental glomerulosclerosis (FSGS), interstitial fibrosis, tubular atrophy, and hypertension. The COL4A3/COL4A5 combination has been reported with different mutations [13]. This case provides more evidence of digenic inheritance in AS.

Modifiers play an essential role in AS. The NPHS2, LAMA5, LAMB2, APO1E, CFHR5, ACTN4, PODXL, WT1, TRPC6, CD2AP, and INF2 genes have been reported to be modifiers [16]. In our study, coinherited genetic mutations were detected in six genes, including TTC21B (responsible for Nephronophthisis 12 (MIM: 613820) autosomal dominant and recessive and Short-rib thoracic dysplasia 4 with or without polydactyly (MIM: 613819), autosomal recessive), SLC34A1 (responsible for Fanconi renotubular syndrome 2 (MIM: 613388), autosomal recessive, hypercalcemia, infantile, 2 (MIM: 616963), autosomal recessive, nephrolithiasis/osteoporosis, hypophosphatemic, 1 (MIM: 612286), autosomal dominant), PKHD1 (responsible from polycystic kidney disease 4, with or without hepatic disease (MIM: 263200), autosomal recessive), FANCC (responsible for Fanconi anemia, complementation group C (MIM: 227645), autosomal recessive), and MEFV (responsible for familial

Mediterranean fever (MIM: 134610, 249100), autosomal recessive and dominant), GJB2 (responsible for Deafness (MIM: 601544, 220290), autosomal dominant and recessive).

In Patient 1, since the COL4A4 mutation was heterozygous, the likely pathogenic "TTC21B, c.2599C > T, p.Arg867Cys" mutation may be responsible for the early onset of clinical symptoms and renal failure both in herself and her mother. Patient 4 was only three years old. At very early ages, hematuria with a heterozygous COL4A3 mutation is not expected. Likely pathogenic "SLC34A1, c.558dupC, p.Ile187Hisfs*26/c.272_292del, p.Val91_Ala97del" and pathogenic "PKHD1, c.107C > T, p.Thr36Met/c.5513A > G, p.Tyr1838Cys" mutations could be the reason patient 4 suffered from bilateral renal cysts and aggravated hematuria. Patient 14 had heterozygous splicing COL4A4 mutation and some dysmorphic features. Her mother had the same COL4A4 mutation and had no clinical symptoms. Pathogenic homozygous "FANCC, c.456 + 4A > T" mutation could explain not only the dysmorphic features but also the finding of AS. Patient 15 had renal failure at a very early age. Homozygous MEFV M694V mutation along with a frameshift homozygous COL4A3 were detected. MEFV mutation could explain the patient's fever, abdominal pain attacks, aggravated renal failure, and AS. Although parents were carriers of both mutations, they had no clinical findings.

Patient 16 had hematuria starting at age six and bilateral sensorineural hearing loss. She had a heterozygous missense COL4A4 mutation, which replaced glycine with arginine. It has been proposed that mutations cause different degrees of pathogenicity: more severe mutations, such as frame deletions, stop codons, and those resulting in chain termination, result in more severe phenotypes, for example, worse renal function, along with increased hearing and vision alterations. In contrast, missense mutations with glycine replacement are related to a less aggressive form of the disease [2]. The "GJB2, c.35del, p.Gly12fs" homozygous pathogenic variant led to bilateral severe sensorineural hearing loss and clinical signs at an earlier age, which were more severe than expected. The parents had no clinical symptoms.

There is still some confusion about AS, including phenotypic variability. Patients with the same mutations showed different features, from mild to severe. Some of our patients had a more severe phenotype compared to their parents or siblings, although they had the same mutation. Clinical variability may be attributed to several factors, such as different expressivity, incomplete penetrance, and the impact of mutant alleles on wild-type proteins. Other genes may be involved in the phenotype variability, acting as disease modifiers. The high familial variability suggests that genetic modifiers, as well as epigenetic or environmental variables, may play a role. Therefore, comprehensive sequencing analysis by NGS should be the primary strategy for gene screening in AS.

Another issue is that it is not easy to distinguish ADAS from ARAS. Some mutations exhibit a dominant and recessive inheritance pattern, complicating our understanding of the genotype–phenotype correlation and mode of inheritance. This was also evident in our patients. Even after testing healthy parents and siblings, it was not easy to diagnose ADAS or ARAS based on the clinical findings. Patients with ADAS exhibit a broad range of clinical manifestations, from asymptomatic to renal failure, with no evident correlation to the causal gene or variant type. Due to the diverse phenotypes associated with ADAS, it is underdiagnosed in clinical practice [15, 17]. Establishing the mode of inheritance is critical for genetic counseling, identifying other at-risk family members, assessing the status of potential kidney donors, and for prenatal and preimplantation genetic diagnosis. A proper ADAS diagnosis will lead to genetic counseling for families, avoidance of kidney biopsies, and more effective treatment strategies.

The third problem is the difficulty in predicting the phenotype and prognosis based on mutations. Patients with non-functional early truncating mutations would be expected to have severe clinical manifestations, whereas missense mutations with glycine substitution are associated with a less aggressive form of the illness [2]. According to this presumption, most of our patients should have had a severe phenotype; however, they had milder phenotypes. The relationship between genetic abnormalities, disease etiology, and clinical findings remains unknown. Founder and recurrent mutations are not frequent in AS, making it harder to predict the effect of the mutations.

Conclusions

This research revealed many novel AS mutations and demonstrated robust genotype–phenotype heterogeneity in the disease. Notably, the results indicated a high prevalence of ADAS in patients with AS and presented empirical evidence showing that the clinical use of comprehensive NGS methodologies can enhance our understanding of the complicated forms of AS. Fifteen new variants were added to the growing database of AS-associated mutations. The identification of a number of new disease-causing mutations within diverse populations improves our understanding of genotype–phenotype correlations, potentially leading to new

therapeutic strategies. Further functional studies are needed to investigate the effects of mutations on AS in relation to modifiers.

Ethical publication statement
We confirm that we have read the journal's position on issues involved in ethical publication, and we affirm that this report is consistent with those guidelines. The study was approved (Number: 140/09) by the Ethics Committee of the University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, and informed consent was obtained from the patients or their parents (mentioned within "Patients"). The manuscript has been edited by Scribendi Academic Editing Services (Order #900306) and Grammarly software (Grammarly, Inc., CA, USA).

Author contributions
IS designed the research, analyzed, and interpreted the results (including the coding part with Python version 3.10.6), wrote the manuscript, and approved the final manuscript. NK collected the data, analyzed, and interpreted the results, and reviewed the manuscript. HS collected the data, analyzed, and interpreted the results, reviewed the manuscript, and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The current study's data are available from the corresponding author upon reasonable request.

Code availability
The code of the current study is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved (Number: 140/09) by the Ethics Committee of the University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, and informed consent was obtained from the patients or their parents (mentioned within "Patients").

Consent for publication
Informed consent for publication was obtained from the patients or their parents.

Competing interests
The authors declare that they have no competing interests.

Received: 15 December 2022 Accepted: 5 October 2023
Published online: 13 October 2023

References

1. Savige J, Harraka P (2021) Pathogenic variants in the genes affected in Alport syndrome (COL4A3–COL4A5) and their association with other kidney conditions: a review. Am J Kidney Dis 78(6):857–864
2. Pedrosa AL, Bitencourt L, Paranhos RM, Leitáo CA, Ferreira GC, Simões e Silva AC (2021) Alport syndrome: a comprehensive review on genetics, pathophysiology, histology, clinical and therapeutic perspectives. Curr Med Chem 28(27):5602–5624
3. Kashtan C (2017) Alport syndrome: facts and opinions. F1000Research 6:50
4. Morinière V, Dahan K, Hilbert P, Lison M, Lebbah S, Topa A et al (2014) Improving mutation screening in familial hematuric nephropathies through next generation sequencing. J Am Soc Nephrol 25(12):2740–2751
5. Furlano M, Martínez V, Pybus M, Arce Y, Crespí J, Venegas MP et al (2021) Clinical and genetic features of autosomal dominant Alport syndrome: a cohort study. Am J Kidney Dis 78(4):560-570.e1
6. Kashtan CE, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K et al (2018) Alport syndrome: a unified classification of genetic disorders of collagen IV α345: a position paper of the Alport Syndrome Classification Working Group. Kidney Int 93(5):1045–1051
7. Delimont D, Dufek BM, Meehan DT, Zallocchi M, Gratton MA, Phillips G et al (2014) Laminin α2-mediated focal adhesion kinase activation triggers Alport glomerular pathogenesis. PLoS ONE 9(6):e99083
8. Zhang X, Zhang YQ, Zhang YM, Gu HB, Chen Z, Ren L et al (2018) X-linked Alport syndrome: auditory pathogenic variant features and further genotype-phenotype correlations in female patients. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 32(16):1232–1237
9. Avner ED, Harmon WE, Niaudet P, Yoshikawa N, Emma F, Goldstein SL (2015) Pediatric nephrology, 7th edn. Springer, Heidelberg
10. Kajimoto Y, Endo Y, Terasaki M, Kunugi S, Igarashi T, Mii A et al (2019) Pathologic glomerular characteristics and glomerular basement membrane alterations in biopsy-proven thin basement membrane nephropathy. Clin Exp Nephrol 23(5):638–649
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424
12. Mencarelli MA, Heidet L, Storey H, Van Geel M, Knebelmann B, Fallerini C et al (2015) Evidence of digenic inheritance in alport syndrome. J Med Genet 52(3):163–174
13. Fallerini C, Baldassarri M, Trevisson E, Morbidoni V, La Manna A, Lazzarin R et al (2017) Alport syndrome: impact of digenic inheritance in patients management. Clin Genet 92(1):34–44
14. Savige J (2020) Alport syndrome: deducing the mode of inheritance from the presence of haematuria in family members. Pediatr Nephrol 35(1):59–66
15. Kamiyoshi N, Nozu K, Fu XJ, Morisada N, Nozu Y, Ye MJ et al (2016) Genetic, clinical, and pathologic backgrounds of patients with autosomal dominant alport syndrome. Clin J Am Soc Nephrol 11(8):1441–1449
16. Savige J, Ariani F, Mari F, Bruttini M, Renieri A, Gross O et al (2019) Expert consensus guidelines for the genetic diagnosis of Alport syndrome. Pediatr Nephrol 34(7):1175–1189
17. Stokman MF, Renkema KY, Giles RH, Schaefer F, Knoers NVAM, Van Eerde AM (2016) The expanding phenotypic spectra of kidney diseases: insights from genetic studies. Nat Rev Nephrol 12(8):472–483

Publisher's Note