Autosomal dominant Alport syndrome due to a *COL4A4* mutation with an additional *ESPN* variant detected by whole-exome analysis

Yuichiro Izumi¹ · Ami Hamaguchi¹ · Rei Miura¹ · Terumasa Nakagawa¹ · Miyuki Nakagawa¹ · Ken Saida² · Noriko Miyake² · Yu Nagayoshi¹ · Yutaka Kakizoe¹ · Taku Miyoshi¹ · Yukimasa Kohda¹ · Yohei Misumi³ · Naomichi Matsumoto² · Yukio Ando³ · Masashi Mukoyama¹

Received: 9 July 2019 / Accepted: 20 October 2019 / Published online: 1 November 2019
© Japanese Society of Nephrology 2019

Abstract

Alport syndrome (AS) is a rare hereditary disease that presents with chronic kidney disease and sensorineural hearing loss, and is diagnosed by its clinical features, pathological features on renal tissue, and mode of inheritance. We report a woman in her 20 s who exhibited persistent haematuria with normal renal function and sensorineural hearing loss. Her family members exhibited the same clinical findings among three generations and were suspected of having autosomal dominant AS (ADAS). Renal biopsy showed minor glomerular abnormalities on light microscopy and extensive thinning of the glomerular basement membrane on electron microscopy. Whole-exome analysis revealed a known *COL4A4* (type IV collagen $\alpha 4$) mutation (c. 2510 G > C; p. Gly837Ala). Two pedigrees with the same variant have been reported previously, one as ADAS and the other as autosomal recessive AS. However, these two cases exhibited no sensorineural hearing loss. The analysis in the present case revealed another missense variant in *ESPN* (Espin), an actin-bundling protein, which is a causative gene for sensorineural hearing loss. Although the pathophysiological significance of this novel missense variant needs to be clarified, computational analysis predicted that the variant creates a new phosphorylation site for protein kinase C. Our case suggests a possible association of hereditary sensorineural hearing loss with ADAS. Whole-exome analysis should be considered to diagnose hereditary and multiple-organ disorders.

Keywords Alport syndrome · Type IV collagen $\alpha 4$ (*COL4A4*) · Espin (*ESPN*) · Sensorineural hearing loss · Whole-exome analysis

Abbreviations

AS	Alport syndrome
ADAS	Autosomal dominant Alport syndrome
ARAS	Autosomal recessive Alport syndrome
XLAS	X-linked Alport syndrome

Introduction

Alport syndrome (AS) is a hereditary disease that consists of chronic kidney disease, sensorineural hearing loss, and ocular abnormalities [1]. AS is caused by mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes, which encode type IV collagen $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains, respectively. Because the glomerular basement membrane, the cochlea basement membrane, and the base of the ocular lens are constructed by a triple helix of the three type IV chains, abnormalities in the chains cause AS-specific clinical features. The occurrence frequency is approximately 1 in 5000 people. AS has three genetic modes of heredity: X-linked AS (XLAS), autosomal recessive AS (ARAS), and autosomal dominant AS (ADAS). ADAS occurs in < 5% of patients with AS. Because patients with ADAS exhibit features much milder than those with XLAS and ARAS, definitive diagnosis is difficult. Recently, a study of clinical and pathological features and the genetic background of ADAS

✉ Yuichiro Izumi
izumi_yu@kumamoto-u.ac.jp

¹ Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, 1-1-1 Honjo, Chuo-ku, Kumamoto, Kumamoto 860-8556, Japan

² Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Kanagawa, Japan

³ Department of Neurology, Kumamoto University Graduate School of Medical Sciences, 1-1-1 Honjo, Chuo-ku, Kumamoto, Kumamoto, Japan

was reported [2]. The patients with ADAS developed renal insufficiency with slower progression than patients with XLAS and ARAS. The incidence of hearing loss was quite low. Pathological studies showed normal $\alpha 5$ staining in all cases. Electron microscopy revealed that only half of the cases exhibit a thin basement membrane with no basket weave change. Considering the mild features of ADAS, there may be many undiagnosed or misdiagnosed ADAS patients.

Hereditary hearing loss is genetically very heterogeneous [3]. More than 150 genes have been identified as causative genes. *ESPN*, which codes espin, a multifunctional actin cytoskeletal regulatory protein, is one of the causative genes for sensorineural hearing loss. Mutation of *ESPN* causes nonsyndromic autosomal dominant- or autosomal recessive-sensorineural hearing loss [4–6].

The diagnosis of AS is determined by a mutation or abnormal expression of type IV collagen. To detect genetic mutations, direct sequencing of type IV collagen genes is performed. Although direct sequencing of the specific gene is crucial for diagnosis, other possible mutations may be missed. As next-generation sequencing technology has progressed, whole-exome analysis has been utilized for diagnosis of hereditary syndromic diseases.

We report a case of ADAS with a pathogenic gene variant of *COL4A4* and an additional gene variant of *ESPN*, a possible causative gene for sensorineural hearing loss, which was discovered by whole-exome analysis.

Case report

A 25-year-old woman was referred to our department from the department of otorhinolaryngology in our hospital to investigate occult blood in urine. She reported having difficulty hearing since childhood. After examinations, she had been diagnosed with sensorineural hearing loss 2 years prior. Her blood pressure was 96/46 mmHg. Urine protein was negative. Urinary occult blood was 1+ in urinalysis; urine sediment showed 20–29 red blood cells/HPF. Her renal function was normal. Some of her family members exhibited occult blood in urine and subjective symptoms of mild hearing loss among three generations (Fig. 1). None had reached end-stage renal disease. Based on these findings, we suspected that she had ADAS.

To further explore a diagnosis, renal biopsy was performed. A histological study indicated minor glomerular abnormalities on light microscopy (Fig. 2a). Immunofluorescence staining for immunoglobulins showed no significant findings. Immunofluorescence staining for type IV collagen $\alpha 5$ showed normal staining (Fig. 2b, c, e). Electron microscopy revealed an extensive thin basement membrane (Fig. 2f, g).

According to the Initiative on Rare and Undiagnosed Diseases (IRUD) in Japan Agency for Medical Research and Development (AMED), her mother's and her blood samples were examined by whole-exome analysis. Their genomic DNA was extracted from peripheral blood leukocytes. Targeted enrichment was performed using

Fig. 1 Family tree showing the distribution of clinical features. The arrow indicates the proband. The proband and her mother were examined by whole-exome analysis and had the same variants. The individuals exhibiting haematuria and hearing loss are denoted in gray and black, respectively. Male and female are denoted by square and circle, respectively

Fig. 2 Morphological analysis of renal biopsy. PAS staining ($\times 400$ magnification) showed minor glomerular abnormalities (a). Immunostaining for type IV collagen $\alpha 2$ and $\alpha 5$ showed normal findings

(b–d). Electron microscopy (e with $\times 2500$ magnification and f with $\times 20,000$ magnification) showed diffuse thinning of the glomerular basement membrane. The thickness was 120–220 nm

SureSelect All Exon v6 kit (Agilent Technologies, Santa Clara, CA) and captured libraries were loaded onto an HiSeq 2500 platform (Illumina, San Diego, CA) as described previously [7]. The analysis revealed a heterozygous variant on c. 2510 G > C in *COL4A4* (NM_000092.4) with a conservative substitution of p. Gly 837 Ala. Another heterozygous variant was found on c. 439 G > A in *ESPN*

(NM_031475) with a conservative substitution of p. Ala 147 Thr. Both variants were identically detected in both the patient (III-2) and her mother (II-2) (Fig. 1). The variants were validated by Sanger sequencing method.

Her renal function has been normal with no worsening of haematuria over 5 years of observation. Her hearing loss has slowly worsened.

Table 1 Allele frequency and in silico prediction scores of COL4A4 and ESPN variants

Gene	Variant	SIFT	PolyPhen2	CADD	MAF (gnomAD)	Diseases
<i>COL4A4</i>	c.2510G>C: p.Gly837Ala	0	1	23.5	0.000008	Alport syndrome
<i>ESPN</i>	c.439G>A: p.Ala147Thr	0.074	1	33	0.000016	Hearing loss

SIFT sorting intolerant from tolerant, *PolyPhen2* polymorphism phenotyping v2, *CADD* combined annotation-dependent depletion, *MAF* minor allele frequency, *gnomAD* The Genome Aggregation Database

Discussion

We presented a case that exhibited persistent haematuria and sensorineural hearing loss with autosomal dominant inheritance. After histological analysis and whole-exome analysis were performed, the patient was diagnosed with ADAS with a *COL4A4* mutation. Whole-exome analysis provided another variant of *ESPN* that is possibly involved in sensorineural hearing loss, although the pathophysiological significance of the variant needs to be verified. Minor allele frequency of both variants detected in *COL4A4* and *ESPN* was extremely rare, and the scores for SIFT (Sorting Intolerant From Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), and CADD (Combined Annotation Dependent Depletion) indicate that both variants detected in *COL4A4* and *ESPN* were located on conserved sequences and potentially cause deleterious effects on their functions [8–10] (Table 1).

We reviewed the literature of retrospective cases or case studies of patients with AS who were genetically diagnosed. We found two pedigrees with the same variant of *COL4A4* as the variant in our case [2, 11]. One case exhibited haematuria with no proteinuria or hearing loss. Pathological features were minimal glomerular abnormalities, thin basement membranes, and basket weave changes. Another patient was reported as having ARAS and had an additional variant on c. 3151 G>C with the substitution of p. Gly 1051 Arg, exhibiting haematuria and proteinuria with no hearing loss. Pathological studies revealed positive staining of type IV collagen $\alpha 5$ on the glomerular basement membrane and basket weave changes on electron microscopy (Table 2). As previously reported, half of the variants in *COL4A3* and *COL4A4* detected in patients with ADAS were also reported as causative mutations of ARAS, suggesting the existence of secondary factors that contribute to the phenotype of patients with ADAS [2].

By applying whole-exome analysis, we confirmed the diagnosis of ADAS for the present case. In addition, we found another variant of *ESPN*, which may have caused sensorineural hearing loss in our case. Espin, coded by

ESPN, is a multifunctional actin cytoskeletal regulatory protein. It is involved in regulating the organization, dimension, dynamics, and signalling capabilities of actin-filament-rich microvilli in mechanosensory and chemosensory cells. Three papers have reported that mutations located on 585–854 in the C-terminal region of espin were associated with familial cases of sensorineural hearing loss [4–6]. The variant found in our case caused the replacement of alanine with threonine on 147 (Ala147Thr), which is located in the ankyrin-repeat domain. The ankyrin-repeat domain in espin has been reported to regulate its binding to actin filaments [12]. Thus far, the pathological significance of the variant in the present case is unclear. Computational analysis using NetPhos 3.1 Server and KinasePhos predicted that the variant creates a new phosphorylation site for protein kinase C (PKC) with a high possibility [13, 14]. NetPhos 3.1 server predicts serine, threonine, or tyrosine phosphorylation site of given protein sequences. A value of the prediction score in the range of 0.5–1.0 indicates positive prediction. In the analysis, substitution of p. Ala 147 Thr in espin created new phosphorylation site for PKC with 0.879 of prediction score. KinasePhos is a program incorporating Profile Hidden Markov Model that predicts phosphorylation site of given protein sequences. By applying sequences for espin with a substitution of p. Ala 147 Thr, new phosphorylation site for PKC was created with 90% specificity. Further in vitro and in vivo studies are required.

In conclusion, we report a case with ADAS that was diagnosed by whole-exome analysis, which provided precise information about the mutation in *COL4A4* and further information about a novel gene variant of *ESPN* that may be involved in the features observed in our case. When a suspicious hereditary disease exists, direct sequencing of the gene is usually performed. Although direct sequencing of the specific gene is crucial for diagnosis, other possible mutations may be missed. Whole-exome analysis should be considered as a method to diagnose hereditary and multiple-organ disorders.

Table 2 Clinical and pathological findings of the cases with the same variant of *COL4A4* as the variant in present case

Authors	Year	Age	Sex	Urinary protein creatinine ratio (g/gCr)	eGFR (ml/min/1.73m ²)	Hearing loss	Renal pathological findings			Additional variants
							Light microscopy	Electron microscopy	α5-staining	
Izumi et al. (present report)	2019	25	F	0.04	121.5	Mild	MGA	TBM	Positive	c. 439 G>A: p. Ala 147 Thr in <i>ESPN</i>
Kamiyoshi et al. [2]	2016	14	F	0.04	131.1	–	MGA	TBM, BWC	Positive	
Oka et al. [11]	2014	28	F	2.9	120.8	–	N.D	BWC	Positive	c.3151G>C: p.Gly1051Arg in <i>COL4A4</i>

F female, MGA minor glomerular abnormality, TBM thin basement membrane, BWC basket weave change, N.D. not determined

Acknowledgements We thank the patient and her family for taking part in this study.

Author contributions YI designed and YI, YM, and YA organized the study. YI and TM cared for the patient. TN, MN, and YK performed renal biopsy and prepared the samples. YK provided pathological diagnoses. KS, NM, and NM performed whole-exome analysis and Sanger sequencing. YI and KS performed computational analysis. YI, AH, RM, YN, and MM reviewed the literature and interpreted the data obtained from all procedures. YI and MM wrote the manuscript, which was edited by all other authors. All authors have read and approved the manuscript.

Funding This research received no specific grant from any funding agency.

Data availability The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

Ethics approval All procedures performed in the studies were in accordance with the Ethical Committee of Kumamoto University Graduate School of Medical Sciences.

Consent for publication The patients provided written informed consent to participate in this study. The patients provided written informed consent to publish this case report, including case description, medical data, and images, maintaining anonymity.

References

- Nozu K, Nakanishi K, Abe Y, Udagawa T, Okada S, Okamoto T, et al. A review of clinical characteristics and genetic backgrounds in Alport syndrome. *Clin Exp Nephrol*. 2019;23:158–68.
- Kamiyoshi N, Nozu K, Fu XJ, Morisada N, Nozu Y, Ye MJ, et al. Genetic, clinical, and pathologic backgrounds of patients with autosomal dominant Alport syndrome. *Clin J Am Soc Nephrol*. 2016;11:1441–9.
- Kremer H. Hereditary hearing loss; about the known and the unknown. *Hear Res*. 2019;376:58–68.
- Donaudy F, Zheng L, Ficarella R, Ballana E, Carella M, Melchionda S, et al. Espin gene (*ESPN*) mutations associated with autosomal dominant hearing loss cause defects in microvillar elongation or organisation. *J Med Genet*. 2006;43:157–61.
- Naz S, Giffith AJ, Riazuddin S, Hampton LL, Battey JF Jr, Khan SN, et al. Mutations of *ESPN* cause autosomal recessive deafness and vestibular dysfunction. *J Med Genet*. 2004;41:591–5.
- Boulouiz R, Li Y, Soualhine H, Abidi O, Chafik A, Nürnberg G, et al. A novel mutation in the Espin gene causes autosomal recessive nonsyndromic hearing loss but no apparent vestibular dysfunction in Moroccan family. *Am J Med Genet A*. 2008;23:3086–9.
- Saida K, Kim CA, Ceroni JRM, Bertola DR, Honjo RS, Mitsuhashi S, et al. Hemorrhagic stroke and renovascular hypertension

- with Grange syndrome arising from a novel pathogenic variant in *YY1AP1*. *J Hum Genet*. 2019;64:885–90.
8. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003;31:3812–4.
 9. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46:310–5.
 10. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–9.
 11. Oka M, Nozu K, Kaito H, Fu XJ, Nakanishi K, Hashimura Y, et al. Natural history of genetically proven autosomal recessive Alport syndrome. *Pediatr Nephrol*. 2014;29:1535–44.
 12. Zheng L, Beeler DM, Bartles JR. Characterization and regulation an additional actin-filament-binding site in large isoforms of the stereocilia actin-bundling protein *espin*. *J Cell Sci*. 2014;127:1306–17.
 13. Blom N, Sicheritz-Ponten T, Gupta R, Gammeltoft S, Brunak S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. *Proteomics*. 2004;4:1633–49.
 14. Huang HD, Lee TY, Tzeng SW, Horng JT. KinasePhos: a web tool for identifying protein kinase-specific phosphorylation sites. *Nucleic Acids Res*. 2005;33:W226–W229.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.