Clinical Case Report

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Novel mutations in patients with X-linked Alport syndrome

Two case reports

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Abstract

Rationale: A genotype-phenotype correlation is known to be associated with Alport syndrome (AS). Identifying novel mutations can expand the knowledge about the natural course of AS.

Patient concerns: The first patient was a-15-year-old boy detected with proteinuria during the school health check-up. The second case was a-29-year-old woman, who visited the outpatient clinic for edema.

Diagnosis: We performed targeted next-generation sequencing to identify the mutations associated with AS. Results were confirmed by Sanger sequencing and multiplex ligation-dependent probe amplification. Missense mutation (c.2332G>C, p. Gly778Arg) was identified in the first case and an exon 16 deletion was also identified in the second case.

Intervention: We treated both cases with angiotensin receptor blocker (ARB).

Outcomes: The amount of proteinuria in the first case did not change after ARB therapy, during the follow-up period (1 year). Proteinuria in the woman decreased to half of the baseline level, 1 year after treatment. Glomerular filtration rate was also maintained during the follow-up.

Conclusion: We identified novel mutations in Koreans with an X-linked AS mutation in the *COL4A5* gene and an individual phenotype. This is the first report of AS patients with a novel missense mutation and copy number variation.

Abbreviations: ACEi = angiotensin converting enzyme inhibitor, ACMG = American College of Medical Genetics and Genomics, ARB = angiotensin receptor blocker, Arg = arginine, AS = Alport syndrome, BUN = blood urea nitrogen, C3 = complement 3, C4 = complement 4, *COL4A5* = collagen type IV alpha 5 chain, Gly = glycine, MLPA = multiple ligation-dependent probe amplification, NGS = next generation sequencing.

Keywords: alport syndrome, next generation sequencing, novel mutation

1. Introduction

Alport syndrome (AS) is a hereditary nephropathy characterized by a family history of hematuria, progressive renal failure typically resulting in end-stage renal disease (ESRD), sensorineural deafness, and ocular abnormalities.[1] AS is associated with mutations in the genes encoding the α3, α4, or α5 chains of collagen IV (*COL4A3*, *COL4A4*, and *COL4A5*), which is a component of the glomerular basement membrane.[2] Patients with AS display wide phenotypic variability and incomplete penetrance; hence, genetic testing is important for clinical diagnosis and prognosis, and to support counseling. In males with X-linked AS, clinical features can be predicted from the underlying genetic mutation. However, in females, the clinical course of AS is variable, and depends not only on the mutation but also on mosaicism, the extent to which the variant is expressed in the basement membrane of the tissues.[3] This report identifies a novel mutation in a Korean family and a *de novo* mutation in a female with an X-linked AS mutation in *COL4A5*.

1.1. Case report 1

A 15-year-old boy (index case) with proteinuria and hematuria was referred to the nephrology department. He had no clinically detectable hearing loss or ocular abnormalities. There was a family history of kidney disease. His maternal grandfather died of kidney disease at 41 years of age. His maternal grandfather's younger brother underwent regular hemodialysis since 40 years of age, but the cause of his kidney failure was uncertain. His

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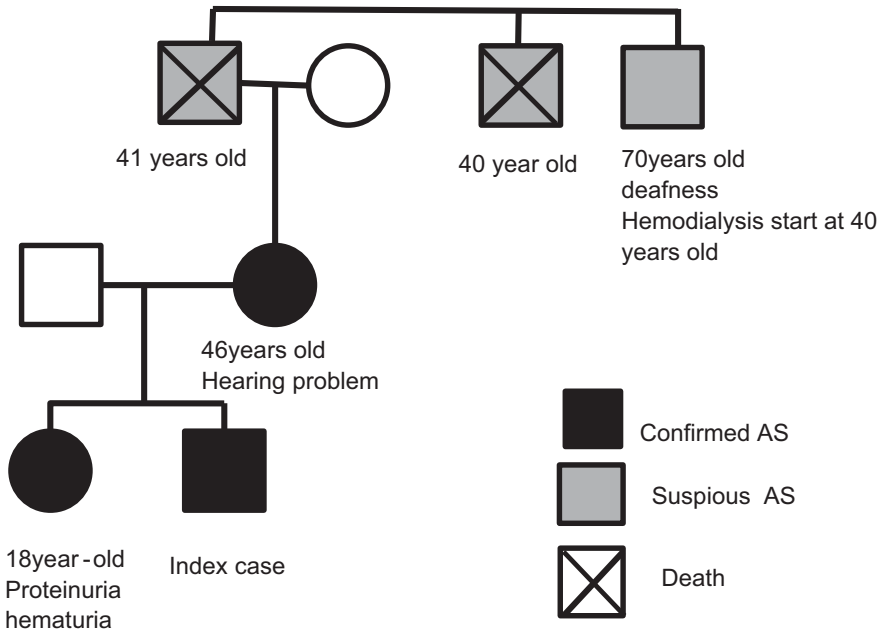

Figure 1. The pedigree of the first case. AS = Alport syndrome.

maternal grandfather and mother, both, had hearing loss, and his sister also had a history of proteinuria and hematuria (Fig. 1). Written informed consent was obtained from the patients and their family members for publication of the case details.

He was admitted to the hospital for a renal biopsy. On admission, the physical examination revealed a blood pressure of 110/60 mm Hg with no peripheral edema. Twenty four-hour urine protein was 3819 mg. Microscopic urinary analysis showed urine sediment containing >100 red cells per high-power field. His blood urea nitrogen (BUN) level was 14.8 mg/dl, serum creatinine (Cr) level was 0.89 mg/dl, estimated glomerular filtration rate (eGFR) by chronic kidney disease epidemiology collaboration (CKD-EPI) equation was 128.3 ml/minute, total serum protein was 5.6 g/dl, and albumin was 3.8 g/dl. Serum C3 and C4 levels were 99 mg/dl and 32 mg/dl, respectively. Antinuclear antibodies were negative. Renal ultrasonography was unremarkable (Table 1)

Renal biopsy revealed glomerular basement membranes with irregular thickness and wrinkling. No significant electron dense deposits were present. A novel heterozygous missense variant of *COL4A5* gene (NM_00495.4, c.2332G>C, p.(Gly778Arg)) was detected by next-generation sequencing (NGS) for a glomerular disease panel targeting 30 associated genes. The variant was interpreted as "likely pathogenic," based on the criteria defined by the American College of Medical Genetics and Genomics (ACMG)[4] and the index case, his sister and her mother were confirmed by Sanger sequencing. All cases received angiotensin receptor blocker (ARB) after confirmation of AS. One year after treatment, eGFR by CKD-EPI of the index case was 107.8 ml/minute and 24-hour proteinuria was 3957.8 mg. He had no hearing or visual abnormality.

1.2. Case report 2

The patient was a 29-year-old female with proteinuria. She visited the nephrology clinic with complains of generalized edema and foamy urine since the last 1 year. She had no family history of proteinuria, hematuria or renal dysfunction, deafness, or ocular abnormalities. Her blood pressure at the first visit was 110/60 mm Hg. Urinalysis showed 2+ proteinuria and 1+ hematuria. Twenty four-hour urine protein was 1006 mg. Her BUN level was 11.7 mg/dl, serum Cr level was 0.68 mg/dl, eGFR by CKD-EPI was 119.4 ml/minute, serum cystatin-C level was 0.97 mg/L, serum total protein level was 7.0 g/dl, and albumin level was 4.5 g/dl. Anti-ds-DNA was 3.52 IU/ml. Renal ultrasonography was unremarkable. Her mother and father had no urinary abnormalities or hearing problems (Table 2).

Renal biopsy showed glomerular basement membrane with irregular thickness and wrinkling. A heterozygous exon 39 deletion of *COL4A5* gene (NM_00495.4, c.2332G>C, p.(Gly778Arg)) was

Table 1

Clinical information and genotypes of AS patient in case 1 and his family.

Individual	Age	Gender	Affect or not	Hypertension	Proteinuria	Sensorineural hearing loss	Ocular involvement	Age of ESRD
Index case	15	M	+	−	+	−	−	
Sister	18	F	+	−	+	−	−	
mother	46	F	+	−	−	+	−	
Maternal grandfather's younger brother	70	M	+	+	NA	+	−	41

ESRD = end stage renal disease.

Table 2

Clinical information and genotypes of the female patient from case 2 of Alport syndrome and her family.

Individual	Age	Gender	Affect or not	Hypertension	Proteinuria	Sensorineural hearing loss	Ocular envovlemen	Age of ESRD
Patient	29	F	+	−	+	−	−	
Mother	52	F	−	−	−	−	−	
Father	56	M	−	−	−	−	−	

ESRD = end stage renal disese.

detected by copy number analysis through NGS, which was then confirmed by multiple ligation-dependent probe amplification (MLPA) using P192-B3 ALPORT probemix-2 (MRC-Holland, Amsterdam, The Netherlands). She received ARB after the diagnosis of AS. One year after treatment, her eGFR by CKD-EPI did not change (116. 5 ml/minute) and 24-hour proteinuria decreased (414 mg/day). She had also no visual or hearing difficulty.

2. Discussion

Novel mutations have been identified in the *COL4A5* gene in patients with AS. More than 700 disease-causing mutations have already been reported in such patients.[3,5] Data on such mutations can help explain the natural course of AS in patients.

AS is inherited in various ways and the phenotypes are varied. Mosaicism is a well-known phenomenon observed in several Mendelian diseases. This phenomenon produces varied phenotypes in AS patients. Generally, the phenotype associated with glycine XY involving exon 21 to 47 has been reported as moderate to severe. Male patients with this mutation typically reach ESRD by the age of 26 years; 65% of them experience hearing loss, and 30% display ocular lesions.[6] However, in a previous case, a patient with a mutation at this same location (2332G>A, p.Gly778Ser) did not reach ESRD until the age of 33 years.[7] This report is consistent with the phenotype of the patient in Case 1. This patient's family history shows that his grandfather passed away due to kidney disease at 41 years of age and the younger brother of the grandfather was initiated on hemodialysis at 40 years of age. Therefore, the decline in renal function of the index case can be expected to progress slowly. Moreover, no ocular abnormalities developed in his grandfather. Ocular lesions occur in 44% of patients with X-linked AS.[3] No ocular lesion are expected in the index case. Women in the family show different phenotypes. Intrafamilial variability of the disease in girls and women is well known.[2] Such heterogeneity was reported in the family studied by Alport.[1]

In the second case, parents of the patient had no history of renal, ocular, or auditory symptoms of AS. This index case might have been caused by a *de novo* mutation. *De novo* mutation can account for 15% of cases of male X linked AS.[2] However, the incidence of *de novo* mutation in females with AS is uncertain. Among female patients with AS, 5% were reported to be completely asymptomatic carriers with normal urine analysis[3]; thus, the prevalence of female AS mutation is likely underestimated. In the index case, kidney function was preserved and extrarenal manifestations of AS were not observed. The occurrence of proteinuria and its progressive increase indicates a poor prognosis in females with AS.[5]

Further, the risk for developing ESRD is higher in females with hearing loss.[3] This case initially presented with moderate proteinuria without hearing difficulties. Her renal prognosis is still uncertain. Deletion of the gene in males could cause severe AS.[3,5] Since male patients with this mutation have not be identified, the phenotype induced is not known. A study using angiotensin converting enzyme inhibitor (ACEi) to treat Samoyed dogs with X-linked hereditary nephritis has been published. This study suggested that ACEi treatment was beneficial in improving renal function and survival.[8] Another study suggested that an ARB is effective in treating AS. In mice, ARB showed a renoprotective effect including inhibiting the progression of glomerulopathy and interstitial fibrosis. In this respect ARB is superior to calcium antagonists.[9,10] Clinical studies also suggested a beneficial effect of ACEi for treating AS patients.[11–13] Based on these reports, we prescribed ARB in both cases, for reduction of proteinuria. However, no significant reduction in proteinuria was seen after up to 1 year of treatment. Long-term follow-up is necessary to fully evaluate the efficacy of ARB in treating AS.

Genetic testing is now the "gold standard" for the diagnosis of AS and for confirming inheritance. Next-generation sequencing (NGS) technology is used to substantially improve the coverage of X-chromosome coding sequences.[14] NGS can improve the diagnosis of AS by providing molecular confirmation of *COL4A5*, *COL4A3*, or *COL4A4* mutations, which might be single nucleotide variations, small deletions or insertions, or copy number variations. This technology reduces the cost of genetic testing for inherited diseases.[15,16] Since renal biopsy is invasive, NGS could be a more economical alternative for the diagnosis of AS. In particular, this could be the first option for juveniles or children with proteinuria or hematuria, with a family history of kidney disease.

Genetic counseling based on genotype-phenotype studies is important in AS. Male AS can be counseled about the expected long-term prognosis. However, the absence of genotype-phenotype correlation and intra-familial clinical variability make it impossible to predict the clinical course in girls and women.[3,17] Middle-aged women with asymptomatic hematuria have a very small risk of developing late renal failure.[17] Kidney donation to the affected male sibling or son might need reconsideration due to the risk on the long-term health of the female carrier.

In summary, this report is the first to show that a new mutation in the *COL4A5* gene can cause AS. In addition, the study indicates that NGS can be a useful tool for screening of the inherited disease.

Author contributions

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