CASE REPORT

A NOVEL COL4A4 GENE VARIANT (C.1856>A): FROM A FOCAL SEGMENTAL GLOMERULOSCLEROSIS CASE TO A FAMILY WITH ALPORT SYNDROME

UNA NUEVA VARIANTE DEL GEN COL4A4 (C.1856>A): DE UN CASO DE GLOMÉRULOESCLEROSIS FOCAL Y SEGMENTARIA A UNA FAMILIA CON SÍNDROME DE ALPORT

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ABSTRACT

Alport syndrome, also known as hereditary nephritis, is an inherited progressive form of glomerular disease that is often associated with sensorineural hearing loss and ocular abnormalities. It is caused by mutations in genes encoding several members of type IV colagen proteins primarily found in basement membranes. Genetic analyses of affected families have identified four different modes of transmission in patients with Alport syndrome. X-linked form of the syndrome arises from mutations of COL4A5 and COL4A6 on chromosome X, whereas autosomal forms result from genetic defects in either the COL4A3 or COL4A4 genes at chromosome 2q35-37. Digenic forms include patients with coexisting mutations in COL4A3, COL4A4, and COL4A5.

KEYWORDS: Alport syndrome; COL4A4 mutations; focal segmental glomerulosclerosis

RESUMEN

El síndrome de Alport (SA), también conocido como nefritis hereditaria, es una forma progresiva hereditaria de enfermedad glomerular que a menudo se asocia con pérdida auditiva neurosensorial y anomalías oculares. Es causada por mutaciones en los genes que codifican varios miembros de las proteínas de colágeno del tipo IV, que se hallan en las membranas basales principalmente. Los análisis genéticos de las familias afectadas han identificado cuatro modos diferentes de transmisión en pacientes con síndrome de Alport. La forma del síndrome ligada al X surge a partir de mutaciones de COL4A5 y COL4A6 en el cromosoma X, mientras que las formas autosómicas resultan de defectos genéticos tanto en el gen COL4A3 como en el COL4A4, en el cromosoma 2q35-37. Las formas digénicas incluyen pacientes con mutaciones coexistentes en COL4A3, COL4A4 y COL4A5. El resultado clínico a largo plazo en pacientes con SA con mutaciones heterocigotas

de COL4A3/A4 es generalmente impredecible. La glomeruloesclerosis focal y segmentaria suele desarrollarse en el SA clásico en etapas posteriores y se presenta predominantemente con proteinuria asociada con hematuria. En el caso índice presentado en este informe, a un hombre de 26 años se le realizó una biopsia de riñón debido a una proteinuria nefrótica y una hematuria microscópica acompañada de una función renal alterada. Se le diagnosticó glomeruloesclerosis focal y segmentaria. Debido a que tenía una pérdida auditiva progresiva desde el inicio del estudio, se le realizó un estudio genético de mutaciones en los genes COL4A3 y COL4A4. Se detectó una nueva mutación en el gen COL4A4 (c.1804-7T> C). Debido a que sus padres tenían un matrimonio consanguíneo, el resto de la familia fue sometida a estudio para la misma variante. Sus padres y su hermana fueron heterocigotos y homocigota para la misma variante, respectivamente. En este estudio, se demostró la existencia de una familia con síndrome de Alport con una nueva mutación en el gen COL4A4 (c.1856G> A) que, según sabemos, es el primer caso reportado.

PALABRAS CLAVE: síndrome de Alport; mutaciones COL4A4; glomeruloesclerosis focal segmentaria

INTRODUCTION

Alport syndrome (AS) is characterized by hematuria and progressive renal impairment with proteinuria, often in association with sensorineural hearing loss, anterior lenticonus and retinopathy. Biopsy appearances are often non-diagnostic with histological findings of various degrees of FSGS, and electron microscopical findings of thinning and thickening of the glomerular basement membrane (GBM), splitting and lamellation, which are neither sensitive nor specific as well. COL4A5 mutations with X-linked inheritance are responsible for ~85% of AS. Homozygous or compound heterozygous COL4A3 and COL4A4 mutations cause autosomal recessive AS.[1,2]

The histological pattern of FSGS reflects a common pathway for a number of distinct entities and the term 'primary FSGS' is reserved for cases caused by primary podocyte injury, usually associated with the presence of a circulating permeability factor.[3] Common secondary causes of FSGS include adaptive responses to congenital or acquired reduction of functioning nephrons, viral infections, obesity, drug toxicity or mutations to well-characterized genes.[4] Mutations in genes specific to the function and structure of podocytes, and in COL4A gene responsible for AS or thin basement membrane nephropathy (TBMN) have been reported to cause familial FSGS.[1,3,5-8]

Recently it has been suggested that COL4 associated TBMN, AS, and familial or sporadic FSGS should be considered as collagen type IV kidney disease subtypes that represent different phases of disease progression.[9] Pathogenic variants in COL4A genes are related to a spectrum of disorders with heterogeneous clinical manifestations ranging from no symptoms to microscopic hematuria, microscopic hematuria with proteinuria, and end stage renal disease (ESRD).[5-6,10] Genetic association studies have implicated a role for genetic modifiers and environmental factors leading to progression from isolated microscopic hematuria to severe proteinuria and ESRD.[3] The question in the genetic study of spectrum of COL4A mutations in AS arise as to whether the variants discovered in patients are actually pathogenic.[11]

In this study we identified a novel disease-causing variant, c.1856G>A, in the COL4A4 gene in a family with signs of AS.

CASES

Index case: A 26-year-old man admitted to the outpatient clinic with gradually increasing pedal edema. He had a history of consanguinity. On physical examination blood pressure was high (150/95 mmHg), and bilateral pretibial edema was present. He suffered from hearing loss since childhood, and wore hearing device. He had renal impairment with microscopic hematuria and nephrotic range proteinuria **(Table 1)**.

Table 1. Clinical and genetic data of the family

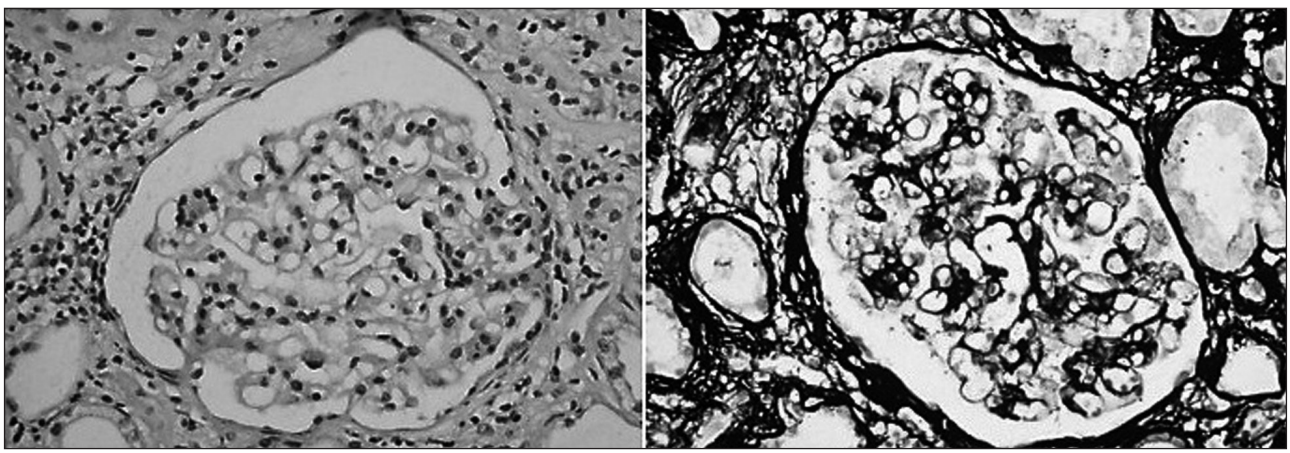
	Index case	Sister	Mother	Father
Sex	M	F	F	M
Age (years)	26	23	49	51
Creatinine (mg/dl)	1.5	1.0	0.8	NA
Microscopic hematuria	Yes	Yes	Yes	Yes
Proteinuria (gr/day)	3.3	5.1	1.7	++
Audiological exam	Sensorineural hearing loss	Sensorineural hearing loss	Sensorineural hearing loss	NA
Ophtalmological exam	Normal	Normal	Normal	NA
Genetic analysis	COL4A4 (c.1804-7T>C) homozygote	COL4A4 (c.1804-7T>C) homozygote	COL4A4 (c.1804-7T>C) heterozygote	COL4A4 (c.1804-7T>C) heterozygote

Visual examination was normal. The renal biopsy revealed histological findings of FSGS as shown in **Figure 1**. Audiometric examination revealed bilateral moderately severe sensorineural hearing loss **(Figure 2)**.

Figure 1. Focal glomerulosclerosis (index case)

Left: Adhesions of glomerular basement membrane to Browman's capsule (H&E, x40)

Right: Normal glomerular appearance (PAMS, x40)



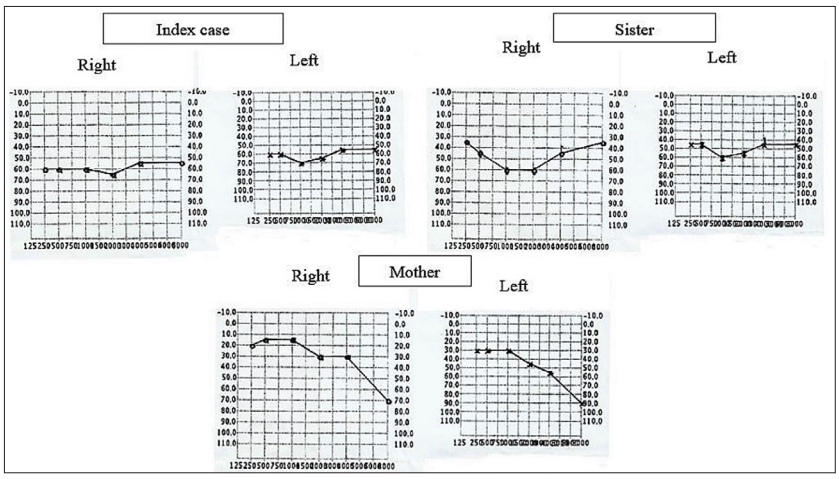


Figure 2. Audiometric measurements of the family demostrating moderate to severe sensorineural hearing loss

The findings led us to suspect AS. Consequently the genetic sequencing of COL4 genes revealed homozygote COL4A4 variant c.1856G>A in the case **(Figure 3 and 4)**. The family tree indicating the variants of each family member was shown in **Figure 5**.

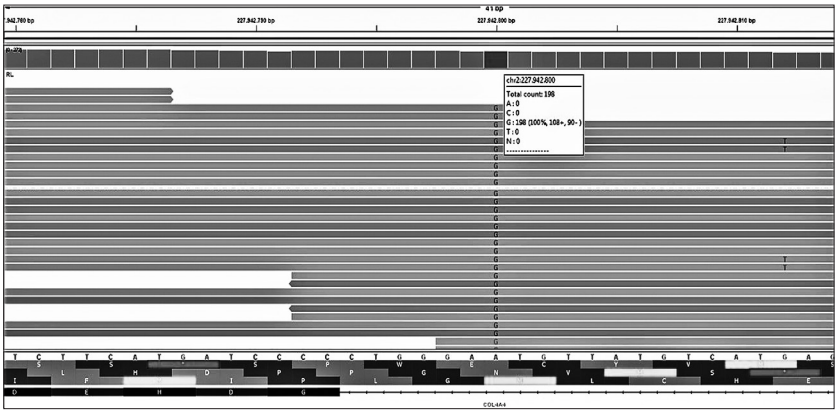


Figure 3. COL4A4 variants in the family

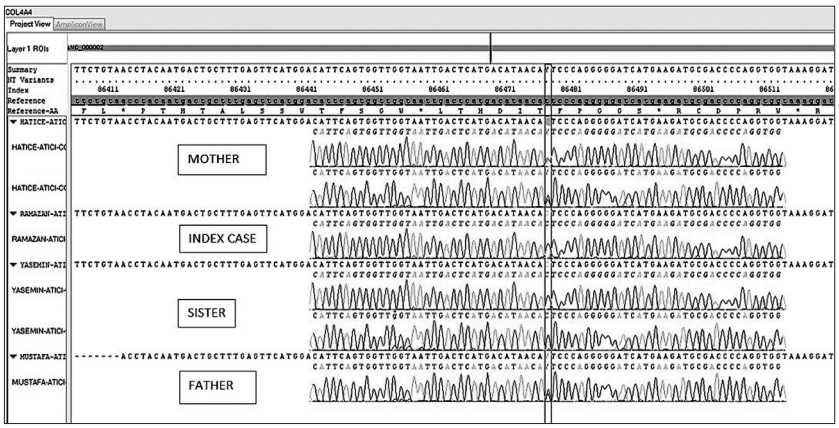


Figure 4. Next generation sequence analysis of the index case

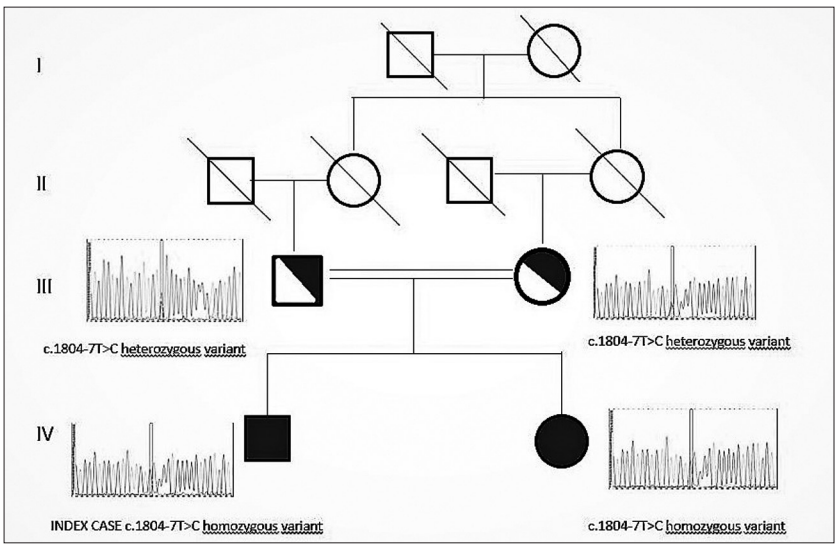


Figure 5. Family tree showing the variant mutations of each member

The mother was a 49-year old woman who had no clinical symptoms. She had microscopic hematuria, and sensorineural hearing loss without renal and ophtalmological impairments. She was found to carry heterozygote mutation in the same gene.

The sister who was 23-year old woman did not show any significant symptoms apart

from moderate sensorineural hearing loss since childhood. Further evaluation revealed bilateral sensorineural hearing loss, microscopic hematuria, and nephrotic proteinuria. She was found to have homozygote mutation.

The father refused any evaluation other than dipstick urinalysis and genetic sequencing. The urinalysis showed hematuria and proteinuria. The genetic sequencing revealed heterozygote inheritance of the same mutation.

METHODOLOGY FOR THE GENETIC ANALYSIS

Isolation of genomic DNA

Blood samples were obtained and genomic DNA was isolated from cases. DNA was isolated from a 200-µL blood sample using the MagPurix Blood DNA Extraction Kit with a Zinexts MagPurix system (Zinexts Life Science Corp., New Taipei City, Taiwan) according to the manufacturer's protocol.

Next generation sequence (NGS) analysis

For the molecular genetic evaluation, the Custom Target Capture ALPORT NGS Panel (Celemix, Inc. Seoul, Korea) that was covering all the coding regions and, in the exon, -intron boundaries of the COL4A3, COL4A4 and COL4A5 genes was used. After the DNA isolation, the library preparation was done according to manufacturer's protocol. NGS was performed on an Illumina MiSeq NGS System (Illumina Inc., San Diego, CA, USA) using the MiSeq Reagent Kit v3 600 cycle (Illumina Inc., San Diego, CA, USA). The raw data was analyzed using 'SEQ' variant analysis software (Genomize, Istanbul, Turkey) according to the reference genome of GRCh37(h19). In addition, it was evaluated by using the Integrative Genomics Viewer (IGV) software (Broad Institute, and the Regents of the University of California, CA, USA).

Confirmation studies using Sanger sequencing on an ABI PRISM 3500 DNA analyzer (Applied Biosystems, Foster City, CA, USA) were performed for the detected variant in the family.

The variant, c.1804-7T>C, has not been found in any of the databases looked for (Ensembl, ClinVar).

DISCUSSION

This is a first report of a novel disease-causing COL4A4 variant c.1804-7T>C in a Turkish family with AS. The index case presented with a 24-hour proteinuria of 3.3 gr and further evaluation by renal biopsy revealed FSGS. A young man with a history of consanguinity and sensorineural hearing loss since childhood, and FSGS on renal biopsy finding prompted us a familial cause of FSGS, i.e., AS.

FSGS accounts for approximately 20% of patients with nephrotic syndrome in children and 40% of such cases in adults.[12] It can be classified as primary and secondary FSGS, and up to 18% of primary FSGS cases is attributed to genetic mutations primarily of the constituents of podocytes, and of the COL4A gene.[1,7,13] Mutations in the COL4A3/COL4A4 gene produce abnormal a3/a4(IV) chain, which incorporates improperly into the triple helix of type IV collagen, and leads to destabilization of the molecular superstructure of the glomerular basement membrane. More than 330 different mutations for the COL4A3/COL4A4 gene have been reported both in AS and TBMN.[13-15] Several reports have linked FSGS with a disease-causing mutations of COL4A3/ COL4A4 gene, and FSGS can be secondary to TBMN and AS, presenting as the only pathological lesion process in these diseases.[1,3,6-7,10,13] A study in a four-generation Chinese families with FSGS identified a novel missense mutation, c.1856G>A (p.Gly619Asp) in the COL4A4.[13] Papazachariou et al. studied 24 Greek families presenting with glomerular familial microscopic hematuria and identified 15 disease-causing mutations, 9 of them novel, in 17 families. In their study they showed that members of five families inherited classical AS with hemizygous X-linked COL4A5 mutations, and even more patients developed later-onset Alport-related nephropathy with heterozygous COL4A3/A4 mutations.[10]

It has been suggested that multiple

heterozygous mutations of the same gene can be observed in patients with familial FSGS, TBMN and AS, and because of the clinical overlap of symptoms, this group of disorders may be better classified as subtypes of collagen IV nephropathies, caused by collagen IV abnormalities.[3,9,13]

In our report diagnostic biopsy of the index case demonstrated the typical findings of focal segmental sclerosis on LM. As we lack electron microscopic (EM) facility in our center we could not evaluate the biopsy for EM findings. Audiological examination of members of the family revealed varying degrees of sensorineural hearing loss that was more suggestive of the diagnosis of AS.

In conclusion, a novel disease-causing mutation, c.1804-7T>C, in the COL4A4 gene, was identified in a Turkish family with AS. To our knowledge, this is the first report of c.1804-7T>C in the COL4A4 gene.

Conflicto de intereses: Los autores declaran no poseer ningún interés comercial o asociativo que presente un conflicto de intereses con el trabajo presentado.

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