Autosomal dominant Alport syndrome: Applying the Alport Variant Collaborative guidelines in the real-world scenario

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NefroPlus 2023;15(2):79-82

ABSTRACT

The recent awareness that individuals displaying pathogenic heterozygous COL4A3 *or* COL4A4 *variants are at greater risk of kidney failure has led to the broadening of phenotype imputed to* COL4A3-COL4A4 *gene variants. In this work, we present the clinical and genotype findings of two families displaying heterozygous variants in* COL4A3 *that highlight the role of applying the Alport Variant Collaborative guidelines in real-world practice.*

Keywords: Alport syndrome. COL4A3-COL4A4 genes. Genetic diseases.

INTRODUCTION

Reclassification of Alport Syndrome (AS) in order to accommodate autosomal patterns associated with *COL4A3* and *COL4A4* genes[1,2] led to a reassessment in the incidence of Autosomal Dominant AS (ADAS) forms[3-8], with up to 1% of the general population bearing a heterozygous variant in any of these genes[9]. In fact, X-linked to ADAS proportion now appears to affect the population in a ratio of 5:95[8]. The Chandos House meeting on the Alport Variant Collaborative (AVC) broadened the screening for variants in *COL4A3-5* in cases of persistent proteinuria, steroid resistant nephrotic syndrome, focal segmental glomerulosclerosis (FSGS), familial forms of IgA nephropathy as well chronic kidney disease of unknown etiology[1]. Alternative taxonomies had already been proposed for these extended phenotypes, as exemplified by the collagen type IV related nephropathies terminology[10]. Mostly important, the AVC redefined American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) criteria to include position 1 Gly in the Gly-X-Y repeats in the intermediate collagenous domains as "mutational hotspots".

It is the goal of our work to assess the implications of these amendments in the real-world scenario. Within our kidney genetics clinic cohort[11] we identified two probands displaying heterozygous variants in *COL4A3* and reviewed the pedigrees' genotypes and phenotypes (pedigrees A and B – fig. 1). This report also describes the histopathologic findings of a diabetic nephropathy superimposed on non-specific findings of FSGS found in a first biopsy performed 20 years earlier, highlighting the role of cascade testing in family members in ADAS and the potential to mask ADAS diagnosis.

CASE REPORT

Index case for family A is a Caucasian female (A1) with persistent isolated hematuria with occasional bouts of macroscopic hematuria, first reported at the age of 24. When evaluated 24 years later, proteinuria was distinctly absent, serum creatinine was 1.1 mg/dL and there was neither hearing nor visual impairment. By the age of 53, renal function first deteriorated and she underwent a kid-

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Revisión por expertos bajo la responsabilidad de la Sociedad Española de Nefrología.

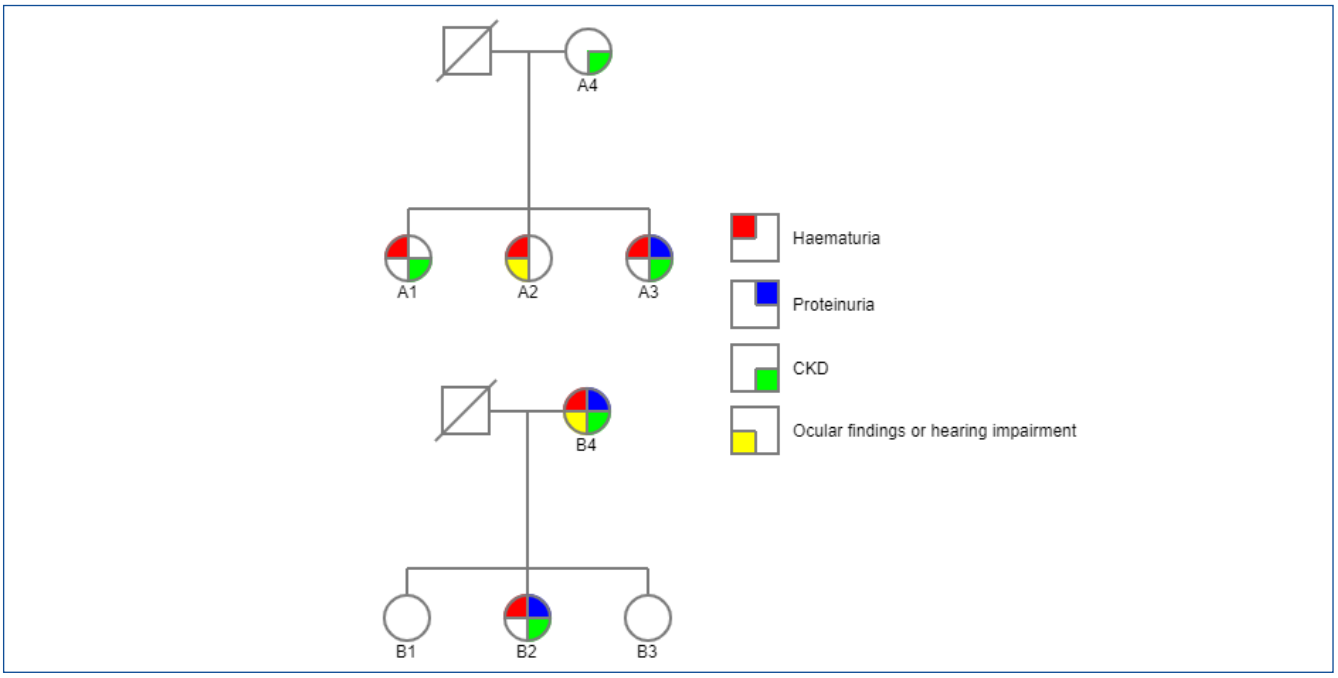

Figure 1. Genograms of family A and B.

ney biopsy. The specimen had normal appearance on light microscopy (LM) and immunohistochemistry (IHC), while electron microscopy (EM) showed thin glomerular basement membrane (GBM) (values systematically < 200 nm, with extremely thin segments (< 100 nm)), apart others segments with some signs of repair and shrinking, and podocyte foot process enfacement (fig. 2A). At that time, diagnosis of thin basement membrane nephropathy was made. On follow-up, and by the age of 65, she had developed chronic kidney disease (CKD) G3-A1 and systemic arterial hypertension (HTN) secondary to CKD. The patient's youngest sister (A3) was evaluated at the age of 47, having CKD G3-A3, hematuria and proteinuria > 300 mg/g. At that time, she presented with high blood pressure and a diagnosis of HTN was made. She also lacked hearing impairment or clinically relevant ocular abnormalities. A kidney biopsy was performed but the specimen failed to identify significant changes. The third sister (A2) similarly had persistent isolated hematuria since an early age, without proteinuria or CKD; she had, though, a diagnosis of asymmetrical astigmatism, a finding that falls within the spectrum of AS ocular manifestations. Their mother (A4) was referred to a nephrology consultant for further investigation, but she already had advanced CKD G5-A1, and dialysis was initiated at the age of 84.

Whole exome sequencing was performed in the *index* patient and a *novel* variant c.1603G>A p.(Gly535Arg) was identified in heterozygosity, located in exon 25 of the *COL4A3* gene. Cascade testing revealed that A2 and A3 displayed the same missense variant in heterozygosity. This variant affects a Gly 1 position within the intermediate collagenous domain Gly-X-Y repeats that is absent from the population GnomAD, ClinVar and the Genomics England 100,000 Genomes Project (100kGP) project (PM2) databases, found to segregate with the phenotype (PP1), occurring in a gene where missense variants are a common mechanism of disease (PP2) and with *in silico* prediction of being deleterious (PP3). It was, however, the recent redefinition of Gly-1 missense as mutational "hot spot" (PM1), that scored this variant as likely pathogenic.

In family B, the Caucasian female proband was 35 years old (B2) when referred for kidney biopsy because of non-nephrotic proteinuria (~2000 mg/g) and hematuria, the later detected at the age of 25. The biopsy specimen showed podocyte hypertrophy by LM (fig. 2B), together with significant interstitial fibrosis; IHC revealed faint C3 and IgM deposits in the glomeruli in a segmental pattern and normal expression of collagen α3-5(IV) chains. A diagnosis of FSGS was made given these findings. She was lost for follow-up for the ensuing 3 years and reached kidney failure by the age of 42. The patient's mother (B4) also had a kidney biopsy performed at the age of 49, due to non-nephrotic proteinuria and hematuria. Histological findings were rather non-specific, with only slight mesangial proliferation on LM. She later developed hearing impairment although no diagnosis of sensorineural hearing loss was made, HTN e type 2 diabetes mellitus (T2D). By the age of 69 she developed nephrotic syndrome, and a second kidney biopsy was performed, revealing the presence of diabetic nephropathy lesions.

The proband was screened for variants in the *COL4A3-5* genes by next generation sequencing multigene panel targeted for hematuric nephropathies, developed *in-house*. The heterozygous variant c.2657-1G>T was identified in intron 32 of *COL4A3* gene in proband and mother, but not in the asymptomatic sisters (B1 and B3). It concerns a *consensus* splice site (LOF) (PVS1), it is absent from the population GnomAD, ClinVar and the 100kGP (PM2) databases, segregates with the phenotype (PP1) and, as such, scored as pathogenic.

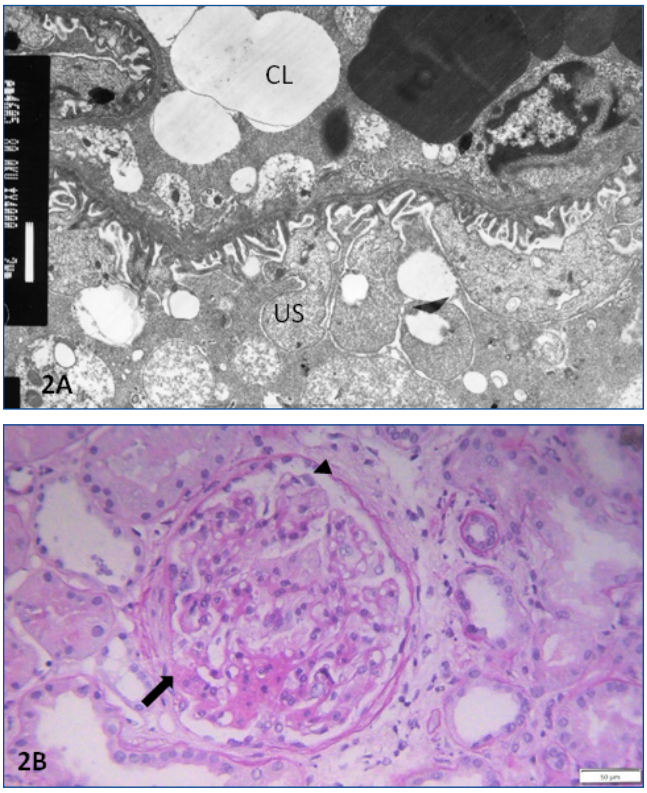

Figure 2. Representative histopathological findings in affected probands. (A) Proband A1: electron microscopy showing extremely thin GBM (62 nm) (CL, capillary lumen; US, urinary space). (B) Proband B2: light microscopy showing glomerulus with partial tuft effacement, with segmental sclerosis and synechia to Bowman capsule (arrow); podocyte hypertrophy (arrowhead).

DISCUSSION AND CONCLUSION

We report two families with CKD manifestations (hematuria and proteinuria) and displaying significant intra and interfamilial phenotype variability whose cause remained unknown, even after kidney biopsies were performed in several affected members. It was the identification of pathogenic variants in the *COL4A3* gene that established the diagnosis of ADAS. The affected individuals were all female and proteinuria was not a universal finding, even in patients with advanced CKD. Although in X-linked AS female patients proteinuria's role as a marker of disease progression has been questioned[12], in ADAS, it is reported to be associated with disease progression regardless of gender[13,14]. Our findings, however, are contradictory.

Allied with clinical suspicion, genetic testing is the most sensitive diagnostic tool to use in patients with suspected AS; it can detect pathogenic/likely pathogenic variants of the *COL4A* genes in up to 90% of cases[2,15]. The Chandos House meeting on the AVC broadened the screening for variants in *COL4A3-COL4A5* and as such, *COL4A3* and *COL4A4* pathogenic/likely pathogenic variants are expected to be increasingly detected. The risk of CKD in patients with ADAS can range from less than 1% to 20%, with non-genetic risk factors such as proteinuria, hearing impairment and histological findings of FSGS and GBM thickening or lamellation being associated with greater disease progression risk[8,13,14].

There are several published studies regarding the phenotypes of patients with ADAS families[5,16]. In a systematic review carried out by Matthaiou et al.[17], which included 777 patients (256 families) with variants in *COL4A* genes, 95% had hematuria, 46% had proteinuria and 29% had CKD; 15% progressed to kidney failure (mean age 53 years). Prevalence of hearing impairment and ocular abnormalities was 16% and <5%, respectively. Of the 174 patients that underwent kidney biopsy and in which EM was performed, 81% had thin GBM; 35% had a pattern of FSGS. In this sample, *COL4A3* pathogenic variants were frequently detected (53.5% of cases), with missense variants associated with Gly substitution the most common finding.

In the 3 patients of family A harboring the *COL4A3* heterozygous variant c.1603G>A p.(Gly535Arg), all had hematuria and HTN, one had proteinuria and two had CKD; one patient had asymmetrical astigmatism. None had hearing impairment. These findings are consistent with the incomplete penetrance and expressivity associated with ADAS[2]. In this family a *novel* missense variant located in the collagenous domain of *COL4A3* with consequent alteration of a Gly for an Arg at position 535 was described. Missense substitutions, in particular those affecting Gly, are among the variants most frequently found in AS patients[1,2]. The AVC consensus group recently redefined the ACMG/AMP criteria with the Gly residues at position 1 (of the Gly-X-Y repeat) of intermediate collagenous domains at these sites being considered "mutational hotspots" and equivalent to a functional domain[1]. Since the exchange of this amino acid for another can result in the formation of an abnormal collagen α-3(IV) chain, it interferes with the correct formation of the triple helix along with the remaining chains, making the molecule more prone to degradation[1,7,12,18-20]. In our case, the above-mentioned variant was initially reported as a variant of unknown significance. However, following the AVC consensus, it was reclassified as pathogenic attesting the importance of the continuous reassessment of the ACMG guidelines.

In family B, the heterozygotic pathogenic variant c.2657-1G>T in intron 32 of *COL4A3* gene, previously described[10], affects mRNA splicing. Changes in splicing, although rarer and more difficult to predict than missense mutations[21], are described in up to 10% of cases[1]. In this family patient B2 presented persistent hematuria, proteinuria and kidney failure at a young age, while her mother B4 had preserved kidney function until older age. This phenotypic variability demonstrates that not only the *COL4A3* genotype, but other genetic, epigenetic and environmental factors contribute to the different intrafamilial clinical manifestations observed[17,21]. In patient B2, a pattern of FSGS was identified. Up to 40% of individuals with proteinuria and *COL4A3/COL4A4* variants may be diagnosed with FSGS[1,5,9], usually secondary to pathological alterations of the GBM, due to the incorporation of abnormal collagen chains with loss of podocytes and consequent compensatory hyperfiltration[9,13]. These changes can be potentiated by exposure of the collagen IV α-chains to advanced glycation end-products as seen in diabetic patients and contribute to progression of CKD[9,22].

It is debatable whether kidney biopsy will in the future still have a role in the diagnosis of AS. Although it can be useful in ruling out coexisting glomerular disease[5], it can also be misleading. If only the renal histopathology findings of a diabetic nephropathy superimposed on non-specific findings detected in a specimen collected 20 years earlier were considered for the diagnosis of individual B4, the true nature of the familial CKD would have been missed. The typical description of GBM lamellation or abnormal collagen IV composition, that are observed in X-linked and recessive AS, are less frequent in ADAS, emphasizing the role of genetic testing in the real-world scenario of unknown CKD cases, in whom up to 10% may have variants in the *COL4A3-COL4A5* genes[4,9].

Statement of ethics

Written informed consent was obtained from the patients for publication of this case report and any accompanying images. Ethics approval was not required.

Funding

None.

Conflict of interest

The authors have no conflicts of interest to disclose.

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