DE NOVO BIOPSY-NEGATIVE ALPORT SYNDROME: A NOVEL MUTATION VARIANT

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Abstract:

Alport syndrome (AS) is an inherited disease caused by a mutation in type IV collagen genes, including the COL4A3 COL4A4 and COL4A5 genes. X-linked Alport syndrome associated with a mutation in COL4A5 gene is the most commonly observed type of AS. The clinical symptoms of AS include renal, hearing and vision impairments. The diagnosis is based on the manifestation of these symptoms and either specific kidney biopsy lesions or positive genetic tests. We present a case of a de novo biopsy negative Alport syndrome. The patient's diagnosis was delayed because the biopsy did not show specific findings for AS. Genetic tests (Next-Gen Sequencing, NGS) made it possible to establish the final correct diagnosis. A c.2441G>A variant of COL4A5 gene detected in probant has not been described in the literature yet. In the past years a significance of NGS in AS diagnosis has increased. The new pathogenic variants are still being reported including de novo mutations having an incidence rate of around 12%. Early diagnosis is crucial for the effective treatment of patients with AS.

Keywords: Alport syndrome, molecular pathology, whole exome sequencing, chronic renal insufficiency, genetic testing.

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Introduction

Alport syndrome (AS) is a hereditary disease in which collagen network defects in the basement membranes cause glomerular, cochlear and ocular abnormalities that manifest clinically as kidney failure, hearing and vision impairments [1]. It is described that Alport syndrome is most frequently associated with an X-linked transmission, with mutation being seen in the COL4A5 gene, while pathogenic mutations in COL4A3 and COL4A4 can cause Alport syndrome via an autosomal transmission with either a recessive or dominant inheritance [2]. The phenotype of AS has been observed to vary depending on the mutation, ranging from a nonprogressive disorder limited to the kidneys to a progressive one with multisystem involvement [3]. The most common renal symptom of AS is persistent haematuria, observed in all male patients. Other clinical features include proteinuria in early childhood, hearing loss in late childhood and renal failure before 40 years of age in 90% of males [4]. To diagnose AS a patient's suggestive clinical picture and either sufficient kidney biopsy findings or a positive genetic test are needed [5]. Sometimes, however, especially in early stages of AS the biopsy results are not specific, which can be misleading [4]. Before the widespread utilization of Next-Generation Sequencing (NGS), genetic tests were expensive and inaccessible. We describe the case of a de novo, biopsy-negative Alport Syndrome with a novel mutation variant that was successfully diagnosed because of NGS.

Case report

A 39-year old man with a history of chronic kidney disease, microhaematuria, proteinuria and hearing impairment was admitted in 2018 to the nephrology clinic in order to modify his treatment. He underwent a kidney biopsy in 2006, in which focal segmental glomerulosclerosis was discovered, with no findings suggestive of AS. Long-term immunosuppressive therapy (cyclosporine and oral steroids) introduced after the biopsy resulted in clinical remission, however a relapse of proteinuria was observed. On admission the patient denied dysuria and fever, but reported swelling of the lower limbs. His cardiovascular parameters were stable, with a heart rate of 80 BPM and blood pressure of 145/90 mmHg. Laboratory studies showed elevated levels of creatinine (2,3 mg/dl) and urea (82 mg/dl), and the estimated glomerular filtration rate was 34 ml/min (using short MDRD formula). Urinalysis revealed proteinuria (100 mg/dl) and microhaematuria. In a 24-hour urine collection 3043 mg of protein was detected. Methylprednisolone pulse therapy, with three 500 mg intravenous doses administered every

3 months, was introduced and subsequently the patient was treated with oral steroids, with a starting dose of 30 mg Prednisone every two days. After 3 months the dose was adjusted to 20 mg every two days. Two months later the dose was decreased to 15 mg every two days, but after the next two months eventually changed to 15 mg daily. The patient was observed over the following months, his renal function parameters were stable with no significant deterioration and onset of new symptoms. In 2020, considering the patient's medical history and his symptoms, as well as the accessibility of genetic testing the decision about returning to the diagnostic process of Alport syndrome was made, despite a non-suggestive family history of kidney disease. The previous kidney biopsy did not show the findings characteristic for AS, so Whole Exome Sequencing (WES) of COL4A3-5 genes in the patient, his parents and his daughter was conducted. A ClinVar database, which includes the information about pathogenic variants of the COL4A5 gene was applied. The analysis exposed the presence of a c.2441G>A variant of COL4A5 gene in the probant, whereas his parents were shown to exhibit no such mutation. The absence of the c.2441G>A variant in COL4A5 gene in the patient's parents indicates de novo mutation. This mutation was also detected in the patient's daughter, who has recently developed haematuria. What is noteworthy, the c.2442G>A variant has not been described in literature, however the ClinVar database classifies it as possibly pathogenic – of unknown clinical significance.

Discussion

Renal biopsy findings suggestive of Alport syndrome have been described to vary in patients with different models of inheritance, with male X-linked AS and autosomal recessive AS cases often showing nephritis progression and lesions specific to AS, and female X-linked AS and autosomal dominant AS cases linked to electron microscopy findings, frequently limited to thinned glomerular basement membranes (GBM) [4]. AS-characteristic lesions found in fully-developed AS include irregular, focal alternation of thinning and thickening of the GBM with lamination and splitting of the lamina densa [6]. In a study from 2014, which described 7 families with genetically-confirmed Alport syndrome mistakenly diagnosed for hereditary focal segmental glomerulosclerosis (FSGS) after kidney biopsies, four families had biopsy findings not suggestive of AS, despite showing specific symptoms [7]. These families had mutations in the COL4A3 and COL4A4 genes, not COL4A5, yet had a similar diagnostic pathway to our patient - they were diagnosed with FSGS after an electron biopsy. That study also highlights the importance of NGS in evaluating renal phenotypes of AS. In the newest consensus regarding the diagnostic process of AS, molecular studies in the form of comprehensive parallel testing of COL4A3, COL4A4 and COL4A5 genes are suggested as the primary, most accurate option, while kidney biopsy is regarded as not necessary in causative cases, especially considering the risk of such a procedure [8]. There are two options for molecular genetic testing. The recommended approach includes using a multigene panel, which identifies genes most frequently associated with specific phenotype or focuses on the particular genes chosen for analysis [9]. A multigene panel is the most effective in finding the mutation responsible for the disease. Sequence analysis and other non-sequencing-based tests are techniques used in a panel. The second option for finding the pathogenic variants in genes is comprehensive genomic testing using exome sequencing or genome sequencing. This method does not require to choose which genes are most likely to be the cause of the medical condition and is used commonly in individuals with atypical symptoms. A potentially pathogenic variant may be connected with the development of a particular disease, but proving this relation is not possible due to lack of data at the moment of its discovery. Confirming the link between a variant and the condition requires additional tests and scientific evidence – it cannot be said that further research will disprove the clinical significance of the finding. As of now, over 3,000 pathogenic variants of the COL4A3, COL4A4 and COL4A5 genes have been reported [10]. The general consensus in literature is that numerous mutations of aforementioned genes are still to be described and have their phenotype and clinical significance determined to ensure adequate introduction of treatment and assessment of prognosis [11]. The exact prevalence of pathogenic variants is unknown, however a recent population-based study using the data from the gnomAD cohort has shown a frequency of 1/2320 individuals with an incidence of a predicted pathogenic mutation in the COL4A5 gene, while 1/106 individuals had a heterozygous predicted pathogenic mutation in the COL4A3 or COL4A4 genes, and 1/88,866 subjects that had two heterozygous predicted pathogenic mutations [12]. The variant of our patient - the c.2441G>A variant of the COL4A5 gene - prior to his genetic test had not been described in literature, and was considered to be of unknown clinical significance, or potentially pathogenic. Since the patient's daughter exhibits symptoms of Alport syndrome, it is possible to presume that this variant is among the pathogenic ones. One study of 195 families with COL4A5 mutations described the incidence of de novo mutations in this gene as approximately 12% [13], so it is possible that even the cases with negative family history or biopsy, but exhibiting clinical symptoms of AS, should be assessed for de novo mutations. There are articles that demonstrate a need for widely accessible and cost-effective molecular diagnostic methods, since the significance of genetic testing in AS diagnosis has been increasing over the recent years [14]. New, emerging variants are being discovered [15] – confirming their relationship with pathogenicity and plausible clinical implications or phenotype variations are important to develop knowledge of this condition and its future management.

The current management of renal AS manifestations targets mainly the renin-angiotensin-aldosterone system, using ACE-inhibitors to slow down the progression of the disease, which are especially effective in delaying end-stage disease in males with X-linked AS, even if introduced before the onset of proteinuria [5], but novel treatment options are pursued as well [16]. With the recent FDA Complete Response Letter rejecting the New Drug Application for bardoxolone for AS treatment due to unsatisfactory performance [17] the USA registration process of one of the more promising AS drugs was effectively closed. Other emerging therapeutic options employ genetics and include nonsense read-through therapy and exon skipping therapy [4]. A podocyte-lineage cell genetic therapy using Clustered Regularly Interspaced

Short Palindromic Repeats (CRISPR) is being studied in pre-clinical models with COL4A3 and COL4A5 genes [18]. Genetic therapies are described to be the target of future therapeutic interventions and are a promising option whose importance can be greatly increased.

The advancements reached in availability and quality of genetic testing can improve the patient's chance of quick and accurate diagnosis, enabling introduction of adequate management in early stages of the disease, which has been shown to delay the onset of end-stage disease and improve prognosis [8, 19].

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