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CASE REPORT

Alport Syndrome: De Novo Mutation in the COL4A5 Gene Converting Glycine 1205 to Valine

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Abstract

Background: Alport syndrome is a primary basement membrane disorder arising from mutations in genes encoding the type IV collagen protein family. It is a genetically heterogeneous disease with different mutations and forms of inheritance that presents with renal affection, hearing loss and eye defects. Several new mutations related to X-linked forms have been previously determined.

Methods: We report the case of a 12 years old male and his family diagnosed with Alport syndrome after genetic analysis was performed.

Result: A new mutation determining a nucleotide change c.3614G > T (p.Gly1205Val) in hemizygosis in the COL4A5 gene was found. This molecular defect has not been previously described.

Conclusion: Molecular biology has helped us to comprehend the mechanisms of pathophysiology in Alport syndrome. Genetic analysis provides the only conclusive diagnosis of the disorder at the moment. Our contribution with a new mutation further supports the need of more sophisticated molecular methods to increase the mutation detection rates with lower costs and less time.

Keywords: Alport syndrome, de novo mutation, X-linked inheritance, COL4A5 gene, lyonization

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Introduction

Alport syndrome is an inherited progressive form of glomerular disease that is often associated with sensorineural hearing loss and ocular defects. The primary abnormality arises from mutations in genes encoding the type IV collagen protein family. This syndrome is a genetically heterogeneous disease having different mutations and forms of inheritance: X-linked (mutations in COL4A5 gene), autosomal recessive (homozygous or compound heterozygous mutations in COL4A3 or COL4A4 genes) and autosomal dominant (heterozygous mutations in COL4A3 or COL4A4 genes). Nephropathy presents with microscopic or gross hematuria, proteinuria and possible progression to terminal renal failure. Hearing loss is not congenital and progresses with renal insufficiency while eye disorders usually appear late in the disease. Clinical heterogeneity and severity and progression of the disease depend on its type of mutation and mode of transmission. Diagnosis can be performed using clinical criteria, biopsy and/or genetic analysis. Despite the high complexity and cost of the molecular studies, they provide the only conclusive diagnosis and are becoming the diagnostic procedure of choice. Follow-up of the patients with renal function monitoring and otolaryngologic and ophthalmologic examination must be the rule. Although angiotensin blockade diminish the rate of glomerulosclerosis and renal disease progression, prognosis to terminal uremia is variable.[1] We report the case of a 12 years old male and his family diagnosed with Alport syndrome after genetic analysis was performed. A new mutation determining the amino acid change of glycine to valine at position 1205 of the protein codified by the COL4A5 gene was found. This molecular defect has not been previously described.

Case Report

A 12 years old male (patient A in Fig. 1) with microhematuria and proteinuria was referred to our hospital at the age of 9 years. He was a full term baby delivered by Caesarean section due to macrosomia (birth weight 4380 grams). He had no history of dietary or developmental issues and he had received the routine vaccinations. He had a unique Schönlein-Henoch episode at 4 years old with skin affection and microhematuria, and he suffered a self-limited episode of gross hematuria due to an upper respiratory tract infection at the age of 7 years.

During his follow-up he had persistent proteinuria and microhematuria with elevation of blood pressure till the percentile 95 for his age and height. Several 24-hour ambulatory blood pressure monitoring (ABPM) showed hypertension with a non-dipper status. He started treatment with an angiotensin-converting enzyme inhibitor (ACE inhibitor) at 10 years old, changing to a combined therapy with an ACE inhibitor and an angiotensin receptor blocker (ARB) after one year.

On physical examination he appeared healthy, with normal weight and height for his age, and

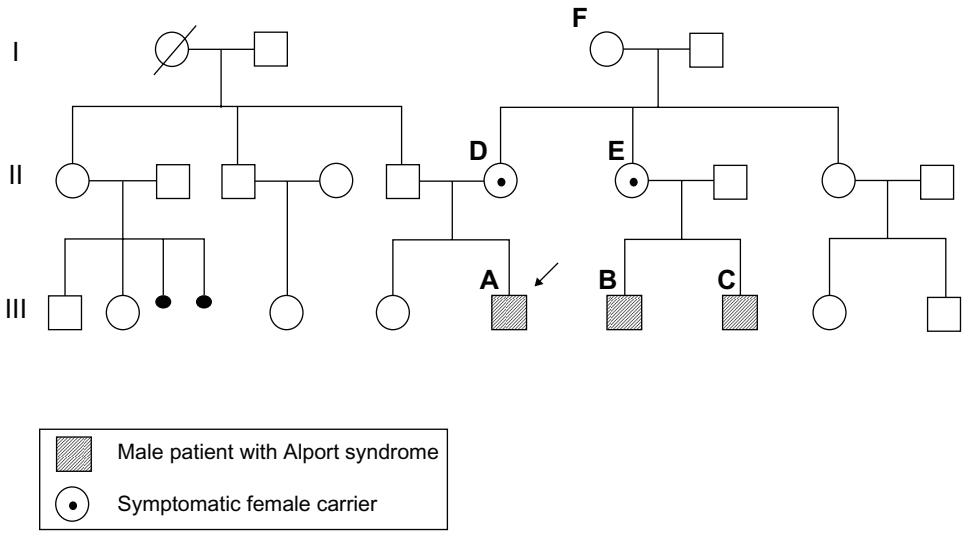

Figure 1. Pedigree Alport Syndrome X-linked.

without any remarkable findings. Blood laboratory results (biochemistry with blood urea nitrogen and serum creatinin, immunoglobulins, complement quantification, autoantibodies, arterial blood gas and complete blood count) were always within normal limits. Urine sediments and 24-hour urinalysis showed microhematuria and microalbuminuria, while hypercalciuria and other urinary metabolic disturbances were ruled out. Several abdominal ultrasounds showed kidneys of normal size and echostructure without Doppler abnormalities.

Although initial ophthalmology and otolaryngology studies showed no abnormalities, a sensorineural hearing loss was detected at the age of 12 years, which progressed to the need for a hearing-aid nowadays.

At the moment, his renal function and blood pressure remain stable but both proteinuria and microhematuria persist.

Family history (Fig. 1) has a 46 years old father and a 16 years old sister without nephropathy or deafness. The 46 years old mother (patient D) has had a progressive sensorineural hearing loss since youth that was attributed to another cause. After diagnosing her son she was reported to nephrology, however no abnormalities appeared until the last year when she was diagnosed with microalbuminuria and initiated treatment with ACE inhibitors.

In the maternal family (Fig. 1), the patient's grandmother (patient F), aunt (patient E) and her two sons (patients B and C) are also affected. Patient B is a 22 years old male with severe proteinuria and hematuria since the age of 7 years. Renal biopsy was performed at the age of 8 years showing pathological changes related to Alport syndrome. He was then treated with an ACE inhibitor and an ARB. At the moment, despite the treatment he has hypertension and a severe renal failure needing dialysis and waiting for a renal transplantation. On the other hand, he also suffers from bilateral cataracts and a sensorineural deafness treated with hearing-aids. Patient C is a 14 years old male with proteinuria and microhematuria since the age of 18 months. He has been followed-up by nephrology receiving treatment with an ACE inhibitor and an ARB. Although his renal function remains stable, he has a bilateral sensorineural hearing loss treated with a hearing-aid. Patient E is a 46 years old female reported to nephrology after her two son's diagnosis. A progressive unilateral deafness was diagnosed several years ago, but her renal impairment developed later with intermittent proteinuria and microhematuria treated with an ARB. Patient F is a 76 years old female with an idiopathic chronic renal failure (stage II) under antihypertensive therapy and a unilateral cataract which needed surgery some years ago.

Genetic analysis was performed in the family, due to the clinical findings and the family history (Fig. 2). Molecular study was conducted after informed consent. DNA from peripheral blood of the probands was isolated using the High Pure PCR Template Preparation Extraction Kit (Roche, Basel, Switzerland) following the instructions of the manufacturer. Intronic primer pairs for each of the 51 exons making up the COL4A5 transcript (Genbank accession number NM_033380) were designed from the genomic sequence and used for PCR and sequencing. Each PCR amplification was carried out in a reaction volume of 50 µL, containing 5 µL of 10X PCR buffer (100 mM Tris-HCl, 500 mM KCl), 1 µL of 10 mM dNTPs, 1 µL of each 20 µL forward and reverse primers, 1 U of Taq DNA polymerase GOLD (Applied Biosystems, Foster City, CA), and 1 µL of the DNA extracted from peripheral blood. All amplicons were purified and bidirectionally sequenced using ABI BigDye Terminator v3.0 chemistry on an ABI 3730xl automated DNA sequencer (Applied Biosystems, USA). Bioinformatics analysis for mutations scanning was performed with program SeqScape v2.5 (Applied Biosystems, USA) employing NM_033380 as a reference sequence. Sequence analysis revealed a G to T substitution at position 3614 of COL4A5 mRNA in hemizygosis (c.3614G > T), which changes glycine 1205 to valine (p.Gly1205Val) and thereby interrupts the Gly-X-Y repeat sequence α5 triple helix. No other sequence variants were identified in the entire coding region of COL4A5 gene (Fig. 3).

The mutation was positive on the patient, mother, maternal grandmother, aunt and two cousins, but negative on the father and sister's analysis.

Discussion

Epidemiology

The prevalence of Alport syndrome in the general population is 1:10.000–1:50.000.[1,2] It causes 1%–2%

of the end stage renal disease (ESRD) in adults, depending on the series,[1] and has race or geography diversity, being described worldwide.

Etiology and pathophysiology

Basement membranes are a supporting structure of epithelial cells whose composition varies by tissue type and stage of development. Their main components are type IV collagen, laminin, elastin and proteoglycans. Alport syndrome is an alteration in the development of basement membranes due to mutations in collagen type IV.

The family of the collagen type IV protein consists of six isotypes, designated by α1 (IV)–α6 (IV). Each chain is encoded by a different gene (COL4A1–COL4A6), which are located in pairs on three different chromosomes. α1–α2 chains are encoded by the genes COL4A1 and COL4A2 (chromosome 13), α3-α4 chains by the genes COL4A3 and COL4A4 (chromosome 2) and α5–α6 chains are encoded by the genes COL4A5 and COL4A6 (chromosome Xq22). These structures form three triple helical protomers (α-1-1-2, α-3-4-5 and α-5-5-6) that further organizes into collagen chains.[1,2]

The six genes are divided into two groups: the α1-like (chains α1, α3 and α5) and α2-like (chains α2, α4 and α6). Each gene encoding an α1-like chain is paired with a gene encoding an α2-like chain. They are transcribed in opposite directions and share regulatory sequences with each other. The 5′ end of each gene encodes a signal peptide and the last 5 exons of the 3′ encodes a carboxy-terminal domain (NC1). This domain has 12 cysteine residues that create disulfide bonds to link α chains together. If a mutation replaces one of these cysteine residues with another amino acid, the formation of the triple helix will be affected.[2]

Chains α1 (IV) and α2 (IV) are expressed in all basement membranes, but the chains α3 (IV) and α4 (IV) are specific to the glomerular basement membrane (GBM), Bowman's capsule, distal tubule basement membrane, Lens capsule, Descemet membrane, Bruch's membrane and several basement membranes of the cochlea. All basement membranes expressing α3 chains (IV) and α4 (IV) also express the α5 chain (IV), which is present in the epidermal basement membrane (EBM).

The absence of these three chains (α3 (IV), α4 (IV) and α5 (IV) chains) is a marker of the syndrome, but normal expression in the GBM does not exclude the disease. A mutation in any of them can cause the absence of the other two. This implies a gradual occupation of the chains α1 (IV) and α2 (IV) in the GBM, simulating a fetal distribution (isotype switching), which confers a higher susceptibility to proteolytic attack by collagenases and cathepsins. The new distribution will gradually deteriorate a previously healthy kidney.[3,4]

There are three types of inheritance related to the pathology that determine different clinical patterns. X-linked inheritance (mutations in COL4A5 gene), autosomal recessive (homozygous or compound heterozygous mutations in the COL4A3 or COL4A4 genes) and autosomal dominant (heterozygous mutations of COL4A3 or COL4A4 genes).

X-linked inheritance

It occurs in 80%–85% of the patients and is due to mutations in the COL4A5 gene. It was first described in 1927 by A. Cecil Alport in a family with renal failure and deafness in which men died with uremia and women lived healthy ("Hereditary familial congenital hemorrhagic nephritis").[2,5]

More than 300 different mutations have been described.[6–8] They are distributed throughout the surface of the COL4A5 gene, and are private (very few families share the same mutation). 10%–15% are "de novo" mutations in parent's gametes. The most frequent types of mutations are nonsense and frame-shift (40%), missense (35%), in-frame splicing (10%) and large gene rearrangements (5%–15%).

Many authors have described a correlation between the genotype and the phenotype of the patients. It seems that large rearrangements of genes and nonsense, frame-shift and splicing mutations are associated with terminal renal failure, deafness and ocular lesions at earlier stages of life.[9,10]

Furthermore, the pattern of X-linked inheritance implies that men develop the disease while women are heterozygous carriers. In men, symptoms appear earlier and progression to ESRD is faster. Women have different degrees of clinical involvement. Almost all carriers have microhematuria but only a significant minority develops renal failure. This clinical variability seems to be related to lyonization (inactivation of one of the two X chromosomes in every cell during embryonic development, by which

one-half of the cells will express the mutant X gene and the remaining cells the normal COL4A5 gene). Consequently, women with severe clinical symptoms will express greater proportion of the X chromosome with the mutant gene.[11,12]

Autosomal recessive inheritance

It arises from homozygous or compound heterozygous mutations in COL4A3 or COL4A4 genes and affects both men and women with the same intensity. It was first described in 1985 being the second form of inheritance in frequency (15%). The most common mutations are nonsense and frame-shift type. The prevalence and clinical pattern in carriers has not been determined yet, although hematuria seems to be a common symptom. Consanguinity, no family history or women affection should make us suspect this inheritance.[2]

Autosomal dominant inheritance

It was first described in 1997 and occurs in 5% of patients. Heterozygous mutations in COL4A3 and COL4A4 genes are the molecular basis. The most common mutations are splicing and missense type. Some authors have described slower progression to ESRD in this group. It should be suspected when there is a male to male transmission.[2]

Clinical characteristics

Alport syndrome is characterized by a progressive inherited nephropathy that may be associated with hearing loss, eye damage and/or leiomyomatosis.[13]

Nephropathy is the most common disorder. Patients have persistent microscopic hematuria and/or episodic macroscopic hematuria associated with upper respiratory tract infections. It begins in childhood and progressively deteriorates. In X-linked forms, microhematuria is often intermittent in women, whereas in the recessive forms is persistent in both men and women. It usually associates proteinuria that increases with age but rarely progresses to nephrotic syndrome. It is common in men but intermittent in women. High blood pressure increases with the age and the degree of nephropathy.[2]

Hearing loss is prevalent in 80% of men and 45% of women. It affects high frequencies and progresses over time to frequencies in the range of conversational speech. It appears in childhood (never congenital) and the rate of deafness is similar to the progression of renal insufficiency. In X-linked forms, it begins in

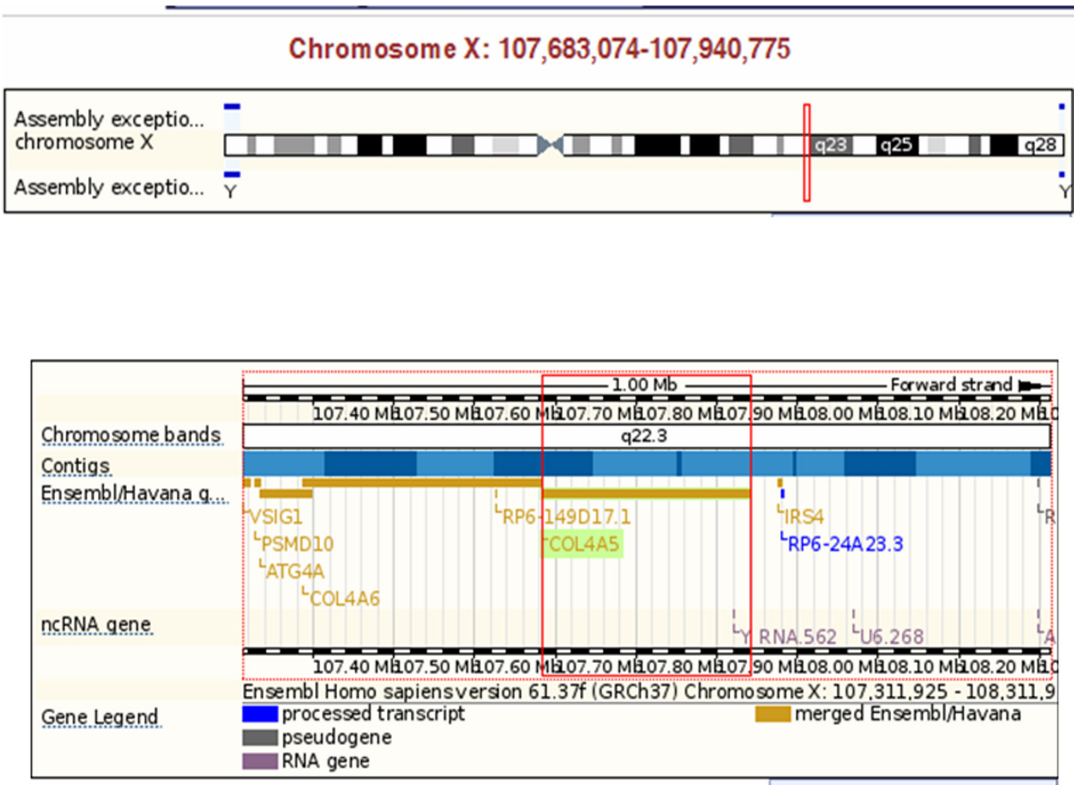

Figure 2. Alport syndrome X-linked. COL4A5 gene.

Figure 3. Alport syndrome X-linked. COL4A5 gene.
Note: Electrophoregram shows G to T substitution at position 3614 of the COL4A5 gene.

the first decade in males but is usually asymptomatic and only diagnosed by audiometry in women.[2]

Eye defects have been reported in 15%–30% of the patients. These disorders affect the lens, retina and cornea: lenticonus, subcapsular cataracts, myopia, abnormal retinal pigmentation, corneal erosions and perimacular flecks. Anterior lenticonus is pathognomonic for the disease. These eye injuries often occur in families that have previously developed bilateral sensory neural hearing loss.[14]

Also, leiomyomatosis has been described in X-linked forms with deletions of 5′ ends of COL4A5 and COL4A6. This disorder affects the esophageal or bronchial tissue causing retrosternal pain, dysphagia, postprandial vomiting, recurrent bronchitis, dyspnea, cough, etc. Women can have genital leiomyomas, clitoris hypertrophy and involvement of the labia majora and uterus.[15]

Diagnosis

The disease should be suspected in patients with the previous symptoms and direct family history, although "de novo" mutations and autosomal recessive inheritance may have no family affection. The presence of an extrarenal abnormality raises the index of suspicion, although its absence does not rule it out. The diagnosis can be performed using clinical criteria, biopsy and genetic analysis.

Diagnostic criteria

Some authors have published clinical and laboratory criteria to optimize initial diagnosis (must meet at least 2 criteria or at least 4 if no family history).[16]

1. Family history of nephritis or idiopathic hematuria in a first degree relative of the index patient or a male family member related to him through generations of women.
2. Persistent hematuria after ruling out other hereditary kidney disease as thin basement membrane disease, polycystic kidney disease or IgA nephropathy.
3. Bilateral sensorineural hearing loss absent in childhood that develops before the age of 30 years.
4. Mutation in COL4A3, COL4A4 or COL4A5.
5. Immunohistochemical evidence of partial or total absence of Alport epitope in the GBM, EBM, or both.
6. Ultrastructural abnormalities (thickening, thinning and lamellation) in the GBM.
7. Typical eye lesions (lenticonus, posterior subcapsular cataract, posterior polymorphous dystrophy and retinal flecks).

8. Gradual progression to ESRD in the index or at least in two members of the family.
9. Diffuse leiomyomatosis of the esophagus, female genitals, or both.

Sometimes these criteria may not be met since many of them or family involvement can appear later. Some authors have recommended genetic analysis in any patient meeting at least two clinical diagnostic criteria.[17]

Biopsy

The next step relies on pathologic evidence. Renal and/or skin biopsy determines the expression of type IV collagen chains. It is recommended to be performed in both tissues because there are X-linked cases with several degrees of inactivation of the X chromosome that develop different results in biopsies. Optical microscopy is nonspecific and often shows increased cellularity in the mesangium in early stages or glomerulosclerosis, tubular atrophy and interstitial fibrosis in late stages. Immunofluorescence detects partial or total absence of α3 chains (IV), α4 (IV) or α5 (IV) in GBM and/or EBM depending on the type of disorder. Electron microscopy shows variable areas of thinning and thickening of the GBM with lamellation of the lamina densa circumscribing areas containing electron-dense granules. The ultrastructural pattern correlates, but not strictly, with clinical severity and magnitude of mutation.

Skin biopsy is less invasive than renal biopsy and should be performed first. The absence of α5 or its discontinuous pattern in the EBM is pathognomonic of X-linked forms. Normal staining does not exclude the diagnosis. In the renal biopsy, diffuse thinning, thickening and splitting of the GBM, suggest the disorder. Normal ultrastructure of the GBM makes the diagnosis very unlikely but does not exclude it.[18]

Genetic analysis

The study of genetic mutations is the mainstay. It should be performed in the patient and first-degree relatives. The genetic heterogeneity of the disease, the large size of type IV collagen genes (50 exons) and the random distribution of mutations, make it difficult in many cases. New molecular techniques have been developed in the last decades,[19,20] and databases with genes sequences have been reported.[21,22] The clinical heterogeneity of the disease depends on its type of mutation and mode of transmission.

Follow-up

Monitoring should be performed during follow-up. Urine and blood renal function must be achieved to control the levels of hematuria or proteinuria. Blood pressure should be closely monitored. Abdominal and renal ultrasound with Doppler should be done to rule out stone disease, cystic dysplasia, and other structural or vascular disorders. All children with a history suggestive of Alport syndrome should undergo audiometry to confirm the diagnosis, as well as periodic monitoring. Ophthalmologic examination is important for the early detection and monitoring of anterior lenticonus, perimacular flecks, and other eye lesions. This clinical workup should include the screening of first-degree relatives.

Prognosis

The prognosis of patients depends on the type of mutation and the inheritance pattern. The evolution to terminal renal failure is more common in patients with large gene rearrangements and nonsense, frame-shift and splicing mutations. In X-linked forms, this situation is common in males, but variable in women, depending on the pattern of X chromosome inactivation. Risk factors for chronic renal insufficiency in female carriers include episodic gross hematuria in childhood, heavy proteinuria, sensorineural deafness, and the presence of the characteristic lamination of the GBM on renal biopsy.[1,2]

Treatment

There is no specific treatment for Alport syndrome currently available. The use of angiotensin blockade may diminish the rate of glomerulosclerosis and disease progression in patients with Alport syndrome. They may reduce protein excretion and stabilize renal function. Angiotensin blockade with an ACE inhibitor or ARB[23,24] is a reasonable intervention in any hypertensive patient and should be considered in normotensive patients with evidence of progressive disease or proteinuria. In case of nephrotic syndrome different protocols of immunosuppressants are used. Renal transplantation is indicated in cases of terminal uremia.[25]

Conclusions

The investigation of this family with Alport syndrome supports previous descriptions in the literature. The presence of "de novo" mutations related to X-linked forms has been previously described.[6–8] The pattern of inheritance in the family must be X-linked, since the mutation is located in the COL4A5 gene, the maternal grandmother, patient's mother and aunt are the paucisymptomatic carriers and the child and two cousins have initiated the disease in childhood and their evolution seems to be fast and progressive.

This nucleotide substitution of glycine to valine at position 1205 of the protein has not been previously described as a mutation associated with this syndrome. It might be associated with increased deafness since the incidence of hearing loss is particularly high among the males (index case and his cousins), and the mother and aunt have developed deafness prior to kidney disease. Furthermore, both mother and aunt initiated the hearing impairment in youth and a progressive nephropathy in early adulthood, which suggest lyonization with inactivation of the majority of the X chromosomes with the normal COL4A5 allele, as the responsible for the severity of the disease in both carriers.

On the other hand, clinical criteria may not be an useful tool in diagnosing some patients. As in our family, some symptoms may appear late in the progression of the disease and familial involvement may be delayed. The presence of deafness in a first-degree relative of a patient with a nephropathy must keep in mind our diagnosis. Consequently, otolaryngologic and ophthalmologic examinations must be achieved for early detection and progression of the disease.

Although many authors have described biopsy as a helpful tool for diagnosis, we decided to perform genetic studies. Molecular testing may become the diagnostic procedure of choice because it is noninvasive and can be extremely accurate. It provides more prognostic data than biopsy, since the rate of progression of renal disease may be dependent upon the underlying specific mutation. Genetic analysis may be beneficial for prenatal diagnosis, genetic counseling, diagnosis in an asymptomatic family member and confirmation of the disorder in patients with no type of inheritance previously described. The development of future technological improvements such as the microarray technology, may improve the yield of mutation screening nowadays.

The investigation on Alport's syndrome exhibits the impact of molecular biology on our comprehension and classification of renal diseases. The description of its relation with genes encoding several members of the type IV collagen protein family and the three varieties of inheritance have provided a way to understand the pathophysiology of the syndrome and its clinical characteristics. The value of clinical and laboratory findings, family history, pattern for α (IV) chains in kidney and skin basement membranes and genetic techniques may help us to elucidate the syndrome. Genetic analysis provides the only conclusive diagnosis of the disorder at the moment. Further elucidation of the molecular basis of the disease will undoubtedly widen our understanding of hereditary nephropathies. More sophisticated molecular methods are needed to increase mutation detection rates with lower costs and less time.

Author Contributions

Conceived and designed the experiments: CAL, SRL, BGF, FSS, SSG. Analysed the data: PAM, CAL, SRL, BGF, FSS, SSG. Wrote the first draft of the manuscript: PAM. Contributed to the writing of the manuscript: CAL, SRL, BGF. Agree with manuscript results and conclusions: PAM, CAL, SRL, BGF, FSS, SSG. Jointly developed the structure and arguments for the paper: PAM. Made critical revisions and approved final version: PAM.

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Competing Interests

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