Alport Syndrome: Achieving Early Diagnosis and Treatment

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Alport syndrome is a genetically and phenotypically heterogeneous disorder of glomerular, cochlear, and ocular basement membranes resulting from mutations in the collagen IV genes COL4A3, COL4A4, and COL4A5. Alport syndrome can be transmitted as an X-linked, autosomal recessive, or autosomal dominant disorder. Individuals with Alport syndrome have a significant lifetime risk for kidney failure, as well as sensorineural deafness and ocular abnormalities. The availability of effective intervention for Alport syndrome–related kidney disease makes early diagnosis crucial, but this can be impeded by the genotypic and phenotypic complexity of the disorder. This review presents an approach to enhancing early diagnosis and achieving optimal outcomes.

Introduction

The modern era of Alport syndrome can be said to have begun in the 1970s with reports of unique ultrastructural abnormalities in glomerular basement membranes of patients with the disease. These seminal observations initiated a cascade of investigation that led to the identification of collagen IV as the protein locus of Alport syndrome; the cloning and sequencing of the COL4A3, COL4A4, and COL4A5 genes; the discovery of variants in these genes in Alport syndrome families; and the generation of transgenic Alport mice. The availability of these mice led to arguably the most important recent development in Alport syndrome research: the demonstration that the natural history of Alport syndrome–related kidney disease can be ameliorated using relatively safe and inexpensive interventions. Although the interventions currently available are not curative, they can dramatically delay progression to kidney failure, especially if initiated before there is any reduction in glomerular filtration rate (GFR). Given that the progression of the Alport nephropathy can be modified by early intervention, there is an imperative for the early diagnosis of Alport syndrome. This review presents an approach to evaluating patients that aims to promote the early diagnosis and treatment of Alport syndrome.

A fundamental feature of diseases of the network of collagen type IV α3, α4, and α5 chains (the collagen IV α345 network) is phenotypic heterogeneity, resulting in a broad range of kidney outcomes and extrarenal manifestations. This heterogeneity challenges us to create a nomenclature that will provide effective guidance to a diverse group of stakeholders that includes a variety of clinicians, such as nephrologists, geneticists and genetic counselors, pathologists, otolaryngologists and audiologists, and ophthalmologists; investigators in basic and translational research in academia and the pharmaceutical industry; and patients and their families and advocacy groups. In developing this nomenclature, we must be careful to avoid erecting artificial barriers to early diagnosis and optimal care.

A working group of clinicians including nephrologists and geneticists set out to develop an inclusive classification of genetic disorders of the collagen IV α345 network to expedite the diagnosis and initiation of therapy aimed at delaying progression to kidney failure. This group proposed that the diagnosis of Alport syndrome should be applied to individuals with any genetic variant that interferes with the normal synthesis, deposition, and function of the collagen IV α345 network of basement membranes. According to this scheme, the Alport phenotype ranges from a nonprogressive kidney-limited disorder to progressive multisystem disease, and the genetic spectrum includes X-linked, autosomal recessive, autosomal dominant, and digenic inheritance (Table 1).

This approach relies primarily on the results of gene sequencing, with less emphasis on specific extrarenal signs and symptoms and kidney pathology findings than previous classification schemes. In addition, this approach changes some of the ways that we think about people with variants that affect the collagen IV α345 network.

According to this classification, women with heterozygous variants in COL4A5 are not “carriers” of X-linked Alport syndrome; rather they actually have X-linked Alport syndrome and are at risk for progressive kidney disease. Similarly, individuals with heterozygous variants in COL4A3 or COL4A4 are not only carriers of autosomal recessive Alport syndrome but they are also at risk for progressive kidney disease, that is, autosomal dominant Alport syndrome, especially if they exhibit hematuria. Patients with glomerular basement membrane thickening and a variant in COL4A3, COL4A4, or COL4A5 are classified as having Alport syndrome, with the result that “thin basement membrane nephropathy” (a description based on pathology rather than a distinct disease entity) is eliminated as a diagnosis. Finally, patients with focal segmental glomerulosclerosis on biopsy and a variant in COL4A3, COL4A4, or COL4A5 are considered to have Alport syndrome, rather than a genetic form of focal segmental glomerulosclerosis.
There are multiple advantages to this approach. Making the diagnosis of Alport syndrome establishes that an individual has a familial disease that carries the risk for progression to kidney failure, which should result in close monitoring of the patient and evaluation of at-risk relatives. Early diagnosis of Alport syndrome facilitates monitoring for the onset of albuminuria and proteinuria, the current indications for initiating treatment with angiotensin II antagonists. A diagnosis of Alport syndrome allows connection with strong patient advocacy and support groups such as the Alport Syndrome Foundation (alportsyndrome.org).

Expedited diagnosis is important in adults and children. The higher the GFR when treatment is initiated, the greater the delay in progression to kidney failure. In addition, diagnosis in adults, even those who have already advanced to kidney failure, creates the opportunity to establish the diagnosis in related adults and children who can benefit from intervention.

There are also obstacles to this approach. In the United States, insurance coverage for gene sequencing is variable and the cost may exceed a family’s financial means. Some variants in COL4A3, COL4A4, and COL4A5 will go undetected by current sequencing methods. People may fear a return to the pre-Affordable Care Act era, when those with pre-existing conditions could be denied health insurance or charged enormous premiums. Nevertheless, nephrologists should take every opportunity to diagnose, monitor, and treat Alport syndrome before kidney function begins to decline.

What Is Alport Syndrome?

The consensus definition of Alport syndrome has evolved during the past several decades. In the 1980s, deafness was considered essential for the diagnosis; one study stated “Since Alport emphasized that deafness was an integral part of the syndrome, we suggest that the eponym Alport’s syndrome should be reserved for patients affected with the same disease as Alport’s family.”

Table 1. Alport Syndrome Classification

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Affected Gene(s)</th>
<th>Genetic State</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Linked</td>
<td>COL4A5</td>
<td>Hemizygous (males)</td>
</tr>
<tr>
<td>Autosomal</td>
<td>COL4A3 or COL4A4</td>
<td>Recessive (homozygous or compound heterozygous)</td>
</tr>
<tr>
<td>Digenic</td>
<td>COL4A3, COL4A4, and COL4A5</td>
<td>COL4A3 &amp; COL4A4 variants in trans (recessive)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variants in COL4A5 and in COL4A3 or COL4A4 (non-Mendelian)</td>
</tr>
<tr>
<td>Suspected</td>
<td>—</td>
<td>Clinical, pedigree, tissue data are highly suggestive of Alport syndrome but genetic data are not confirmatory</td>
</tr>
</tbody>
</table>

with Alport syndrome were characterized as developing kidney failure in their teens or early twenties. This narrow definition of the Alport phenotype facilitated genetic linkage studies that mapped the first known Alport locus to the long arm of the X chromosome and the sequencing studies that identified COL4A5 as the locus for X-linked Alport syndrome. However, subsequent studies established a much broader phenotypic spectrum for males with COL4A5 variants, with kidney failure and deafness frequently delayed until age 40 to 60 years. This is a familiar paradigm for genetic diseases when the locus is unknown: initial studies use a constricted phenotypic definition to minimize potential genetic heterogeneity and then when a gene is implicated, heterogeneity of phenotypes associated with variants in that gene is revealed.

The identification of COL4A5 as the locus for X-linked Alport syndrome was rapidly followed by the cloning and sequencing of COL4A3 and COL4A4 and the discovery of homozygous and compound heterozygous variants in these genes in patients with autosomal recessive Alport syndrome. Eventually heterozygous variants in these genes were found in families with autosomal dominant transmission of a nephropathy with characteristic ultrastructural features of Alport syndrome, firmly establishing the existence of an autosomal dominant form of Alport syndrome, a hypothesis that had been advanced by some groups but questioned by others. Although the precise proportion of Alport cases attributable to the autosomal dominant form has not been defined, it appears to account for a substantially greater fraction of affected individuals than previously understood.

The complexity of Alport genotype-phenotype relationships does not end there. Females heterozygous for COL4A5 variants were long considered to be carriers of X-linked Alport syndrome but they are clearly at risk for kidney failure and deafness. Some people with heterozygous variants in COL4A3 or COL4A4 are asymptomatic and many have only isolated microhematuria, at least at the time of study, with or without demonstration of thin glomerular basement membranes. Ocular manifestations of Alport syndrome such as anterior lenticonus and retinopathy are dependent on collagen type IV genotype, age, and sex. These signs are extremely useful diagnostically but are frequently absent or go undetected.

And there is more. Two forms of “digenic” inheritance in Alport families have been reported. Families may transmit variants in 2 of the Alport loci (COL4A3, COL4A4, or COL4A5), potentially resulting in non-Mendelian inheritance patterns and phenotypic heterogeneity within the family. Or families may transmit a COL4A4 variant along with a variant in a noncollagen gene, potentially resulting in a more severe phenotype.

How do we organize this genetic and phenotypic heterogeneity into a coherent classification scheme that is accessible and useful to patients and families, the clinicians who care for them, and investigators developing novel treatments for Alport syndrome while also facilitating
expedited diagnosis and treatment? There are significant advantages associated with an inclusive classification that organizes the genetic disorders of the collagen IV α345 network under a single tent, rather than attempting to arbitrarily divide these disorders into discrete entities by phenotype (Table 1). Furthermore, classification of these disorders as forms of Alport syndrome provides a “home” for patients and families and clear points of contact for clinicians and investigators.

**Achieving Early Diagnosis of Alport Syndrome**

Urinalysis is an extremely effective method of screening for Alport syndrome. All males with X-linked Alport syndrome, as well as all males and females with autosomal recessive Alport syndrome, have persistent microhematuria. In females who are heterozygous for X-linked Alport syndrome, the likelihood of microhematuria is 95%, although it may be intermittent. Some individuals with heterozygous variants in COL4A3 or COL4A4 are asymptomatic; the risk for developing chronic kidney disease (CKD) in these individuals is uncertain but is probably lower than for those with microhematuria. Consequently, screening urinalyses of at-risk family members should be carried out whenever the diagnosis of Alport syndrome is made.

Alport syndrome should be in the differential diagnosis of patients with persistent glomerular hematuria, whether identified by routine urinalysis, presentation with gross hematuria, or discovery of microhematuria during evaluation for possible urinary tract infection or other urinary tract disorders. The absence of a family history of hematuria or CKD does not rule out Alport syndrome. Approximately 12% of children with X-linked Alport syndrome have de novo variants, and a negative family history of kidney disease is common in patients with autosomal recessive Alport syndrome. Furthermore, families may be unaware that relatives have hematuria or CKD, or CKD may have been attributed to aging or other disorders such as diabetes. Extrarenal manifestations such as sensorineural deafness and ocular anomalies are influenced by age, genotype, and, in X-linked Alport syndrome, by sex, and therefore their absence does not rule out Alport syndrome. Hearing loss is unusual in autosomal dominant Alport syndrome, affecting 4% to 13% of individuals, as are ocular lesions.

Although routine examination of urine in the school-age population would enhance the diagnosis of Alport syndrome, children in the United States do not undergo routine screening urinalysis. The recommendation of the American Academy of Pediatrics is that screening urinalysis should be limited to children “who are at high risk for chronic kidney disease, such as those with a history of chronic kidney disease, acute kidney injury, congenital anomalies of the urinary tract, acute nephritis, hypertension, active systemic disease, prematurity, intrauterine growth retardation or a family history of genetic renal disease.” This recommendation is based on studies showing that routine screening urinalysis is associated with a high false-positive/transient abnormality rate and a low yield of detection of treatable problems in the healthy pediatric population, creating a relatively poor cost-benefit relationship. Although the true prevalence of Alport syndrome in the United States is uncertain, it is likely that screening urinalyses in the school-age population would identify a significant number of affected children. Would the early identification and treatment of affected children result in a benefit greater than the economic and emotional costs associated with false-positive urinalyses? This should remain an open question to be revisited as we learn more about the epidemiology of Alport syndrome and the efficacy of early intervention.

What are the proper roles of tissue biopsy and genetic testing in the evaluation of individuals with persistent glomerular hematuria? As argued in this review, there is potentially a very large benefit associated with early diagnosis of Alport syndrome. The value of this benefit must be weighed against the risks of kidney biopsy and the costs, financial and otherwise, of genetic testing. In some cases, the diagnostic benefit will not accrue principally to the individual undergoing biopsy or genetic testing, for example, in the case of a patient with CKD or kidney failure, but primarily to the patient’s children or relatives.

In the pediatric population, the major causes of persistent isolated glomerular hematuria with normal serum complement levels include immunoglobulin A nephropathy (IgAN) and the conditions associated with variants in the COL4A3, COL4A4, and COL4A5 genes, which, as described, can be grouped together as Alport syndrome. These diagnoses have different implications. Children with...
IgAN who present with isolated hematuria frequently have static disease for years, whereas children with Alport syndrome have a significant risk for progression to overt proteinuria, CKD, and kidney failure, a risk that can be modified with early intervention. Unlike IgAN, Alport syndrome is frequently associated with important extra-renal problems and commonly affects related individuals. Children with Alport syndrome frequently develop sensorineural deafness that can be identified and managed before it becomes clinically symptomatic. Finally, a diagnosis of Alport syndrome establishes risks for the child’s parents, siblings, and other relatives. For these reasons, it is crucial to establish the diagnosis of Alport syndrome whenever possible and as early as possible. Figure 1 describes one possible approach.

The approach suggested in Fig 1 can also apply to adults with persistent glomerular hematuria. A diagnosis of Alport syndrome in an adult is associated with a modifiable risk for CKD and kidney failure, extrarenal complications, and potentially affected relatives. Although adult patients with isolated hematuria of glomerular origin may not require a kidney biopsy, they deserve a specific diagnosis, which may be provided by genetic testing. As in children, the diagnosis of Alport syndrome in adults should ideally be made before the onset of overt proteinuria and certainly before GFR begins to decline.

Tissue studies remain important in the diagnostic evaluation of patients with persistent glomerular hematuria despite the increasing availability of genetic testing. Assessment of kidney biopsy specimens should include electron microscopy, as well as light microscopy and routine immunofluorescence. When routine immunofluorescence shows negative or nonspecific immunoreactant deposition, collagen IV immunostaining can provide both diagnostic and prognostic information that is useful even if electron microscopy cannot be performed. Skin biopsy with collagen IV immunostaining is a possible alternative to kidney biopsy for the diagnosis of X-linked Alport syndrome but cannot exclude IgAN or identify autosomal forms of Alport syndrome.

It is important to avoid minimizing the limitations associated with genetic testing (Box 2). Laboratories generally use standard terminology to describe sequence variants—pathogenic, likely pathogenic, uncertain significance, likely benign, and benign—based on specific types of evidence, including population data, computational data, functional data, and segregation data, but it is possible that a substantial proportion of nephrologists are uncomfortable interpreting these reports and discussing

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**Box 2. Genetic Testing for Alport Syndrome: Advantages and Limitations**

**Advantages**
- Confirmation of diagnosis
- Genotype has implications for:
  - Prognosis and monitoring
  - Identification of at-risk relatives
  - Predicting risk for recurrence in future pregnancies
  - Personalized treatment (future)

**Limitations**
- ~10% of patients with clinically and pathologically confirmed Alport syndrome have mutations that are not identified by NGS or WES
- Significance of some variants will be uncertain
- Insurance coverage is variable
- Access to genetic counseling is variable

Abbreviations: NGS, next-generation sequencing; WES, whole-exome sequencing.

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**Figure 1.** An approach to the diagnosis of individuals with persistent glomerular hematuria using kidney biopsy and/or genetic testing. Kidney biopsy should always include routine transmission electron microscopy (TEM). When TEM shows glomerular basement membrane changes suggestive of Alport syndrome, immunofluorescence studies of collagen IV α chain expression can provide useful diagnostic and prognostic information. Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; VUS, variant of uncertain significance.
the results with their patients. In the United States, patients’ access to genetic counseling services is often limited, and they may have variable and often inadequate insurance coverage. Despite these issues, the opportunities that genetic testing offers for meaningful interventions should be pursued when possible.

Treatment of Alport Kidney Disease

Alport nephropathy progresses through a consistent set of milestones, beginning with isolated hematuria, followed by moderate albuminuria, severe proteinuria, and decline in GFR, in that order. The interval between milestones varies from patient to patient and is influenced primarily by sex and COL4A genotype. The spectrum of progression rate is very broad, ranging from very rapid, requiring kidney replacement therapy in adolescence or early adulthood, to very slow with death at an advanced age with normal kidney function.

Currently, the goal of therapy of Alport kidney disease is to safely lengthen the intervals between the milestones of progression to the greatest extent possible. Data from experimental and human research indicate that this goal can be accomplished effectively with angiotensin-converting enzyme (ACE) inhibitor treatment, achieving optimal results when initiated before GFR begins to decline. According to a recent consensus statement, treatment should be initiated in all patients with overt proteinuria, aiming for the maximal recommended dose (based on a maximal ramipril dosage of 6 mg/m² per day in children) that the patient will tolerate. Moderate albuminuria is an indication for initiation of treatment in male patients with COL4A5 variants predictive of rapid progression to kidney failure and in male and female patients with autosomal recessive Alport syndrome. In individuals who have moderate albuminuria and in whom progression is likely to be relatively gradual (males with COL4A5 missense variants, females with heterozygous COL4A5 variants, and males and females with heterozygous COL4A3 and COL4A4 variants), the risks of ACE-inhibitor treatment, including fetal injury, must be balanced against the potential benefit.

It is unclear how COL4A genotype may influence the response to ACE-inhibitor therapy. It is entirely conceivable that those with relatively favorable genotypes (eg, men with COL4A5 missense variants, women with heterozygous COL4A5 variants, and those with heterozygous COL4A3 and COL4A4 variants) will experience the best treatment outcome: avoiding kidney failure. These are precisely the patients who are most likely to be undiagnosed or labeled as “benign familial hematuria” or “thin basement membrane nephropathy” because of their relative paucity of symptoms, thereby losing the opportunity for early intervention.

Could better outcomes be achieved if ACE-inhibitor therapy is initiated before the onset of overt proteinuria? This question is being examined by the EARLY-PROTECT trial, in which children with Alport syndrome who have isolated hematuria or hematuria and moderate albuminuria are randomly assigned to placebo or ramipril treatment. Treatment recommendations are likely to change if this trial shows that ramipril therapy delays progression from isolated hematuria to hematuria and moderate albuminuria and/or moderate albuminuria to overt proteinuria.

In addition to the EARLY-PROTECT trial, two randomized trials of novel therapies for Alport kidney disease are in progress (see disclosure information at end of article). The CARDINAL trial (ClinicalTrials.gov Identifier NCT03019185) is a phase 2/3 trial examining the safety, tolerability, and efficacy of bardoxylone methyl (an activator of the transcription factor Nrf2), using the increase in estimated GFR from baseline to 48 weeks of treatment as the primary outcome. The HERA trial (ClinicalTrials.gov Identifier NCT02855268) is a phase 2 trial of the anti-microRNA 21 agent SAR339375, with adverse events and annualized change in estimated GFR as primary outcomes. These trials are discussed in greater detail in two excellent recent reviews.

The need for investigation of curative treatments must be balanced against the generally very successful outcomes of kidney transplantation for kidney failure due to Alport syndrome, which should inform consideration of the risks for adverse events. Patient-focused outcomes should be incorporated into trial design.

Case Vignettes

The following clinical scenarios illustrate some of the features, diagnostic considerations, and treatment opportunities discussed in the previous sections.

Case 1

A 4-year-old boy is evaluated for fever. Urinalysis shows 50 to 75 red blood cells per high-power field but urine culture is negative. Repeat urinalysis several weeks later again shows hematuria and he is referred to a pediatric nephrologist. His mother states that she has had blood in her urine since childhood but has been told that she likely has a benign disorder. She has not received a specific diagnosis or genetic counseling. There is no other known family history of hematuria or kidney disease.

Because of this family history, the family is referred to a geneticist. The child and his mother are found to have X-linked Alport syndrome due to a splicing variant in COL4A5. The mother is referred to her primary provider for further evaluation and found to have proteinuria (urinary protein-creatinine ratio of 1.5 mg/mg) and CKD stage 2 with a serum creatinine level of 1.4 mg/dL.

This case illustrates several points:

1. Families with Alport syndrome are often small, making it difficult to predict inheritance or prognosis from examination of the family pedigree.
2. Familial hematuria may be undiagnosed or treated due to misconceptions about its prognosis.
3. Lack of a diagnosis can result in a missed opportunity for early intervention.

Persistent hematuria is the earliest manifestation of a potentially progressive but treatable nephropathy, Alport syndrome. Therefore, disease classifications and diagnostic algorithms should be oriented to early diagnosis and intervention.

Case 2

A 12-year-old girl is referred to pediatric nephrology for evaluation of hematuria initially discovered during evaluation of abdominal pain. Ultrasound of the abdomen obtained as part of her workup for abdominal pain did not show urinary tract abnormalities. Family history for hematuria or kidney disease was negative, but the family is no longer in contact with the child’s father. Diagnostic evaluation shows normal blood pressure, estimated GFR, urinary protein excretion, and serum complement levels.

Urinalyses over the course of the next 6 months show persistence of hematuria. The pediatric nephrolgist elects to perform a kidney biopsy, which shows no light microscopic abnormalities and negative routine immunofluorescence studies. Electron microscopy shows uniformly thin glomerular basement membranes with an average width of 190 nm, without duplication, lamellation, or electron-dense deposits. Genetic testing shows a unique heterozygous missense variant in COL4A3.

This case raises several questions:

1. What is the diagnosis, Alport syndrome or thin basement membrane nephropathy?
2. What should the patient and family be advised regarding her prognosis?
3. How should she be monitored prospectively?
4. When is intervention appropriate?

Regardless of the name given to this child’s condition, she has a risk for progression to CKD that is difficult to precisely predict. A diagnosis of Alport syndrome conveys the reality of this risk and the need for regular follow-up by a primary provider or nephrologist. A diagnosis of Alport syndrome provides her family with sources of information and support geared to the Alport community.

Conclusions

It is now clear that a variant in COL4A3, COL4A4, and COL4A5 is a risk factor for CKD. Whether one thinks of people with these variants as having a single disease with a spectrum of phenotypes (Alport syndrome) or as having distinct disorders (Alport syndrome or thin basement membrane nephropathy), it is crucial that they have regular follow-up by a primary provider or nephrologist and initiation of ACE-inhibitor therapy when appropriate. It is likewise crucial that clinicians attempt to establish a definitive diagnosis in people with persistent glomerular hematuria. Individuals diagnosed with Alport syndrome or found to have COL4A variants should be informed by clinicians about Alport syndrome registries to help further our understanding of the disease and responses to intervention.

Article Information

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Support: This review was produced without any direct financial support.

Financial Disclosure: Dr Kashtan is a site investigator for the CARDINAL trial, sponsored by Reata Pharmaceuticals, and the HERA trial, sponsored by Sanofi-Genzyme. He has received research funding from the Novartis Institute for Biomedical Research; has recent or current consulting relationships with Daiichi-Sankyo, Ono Pharmaceuticals, and Retrophin; and is the Executive Director of the Alport Syndrome Treatments and Outcomes Registry (ASTOR; alportregistry.org), which is supported by the Alport Syndrome Foundation (alportsyndrome.org) and private donations. None of the entities listed had a role in defining the content of this review.

Peer Review: Received January 2, 2020, in response to an invitation from the journal. Evaluated by 3 external peer reviewers, with direct editorial input from an Associate Editor and a Deputy Editor. Accepted in revised form March 24, 2020.

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