Pathology versus molecular genetics: (re)defining the spectrum of Alport syndrome

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Abstract

Next generation sequencing applied to families with glomerular disease has been instrumental in identifying new genes and pathways involved in podocyte homeostasis. Malone et al. performed sequencing on 70 families with FSGS and discovered that 10% had variants in surprising “old” genes, COL4A3 and COL4A4, which are involved in Alport syndrome and thin basement membrane nephropathy. These data show that a subset of renal manifestations associated with COL4A3 or COL4A4 variants cannot be distinguished from FSGS by clinical data or by histopathology. Thus, a diagnosis of FSGS may sometimes fall within the spectrum of Alport syndrome.

Keywords

Alport syndrome; glomerular disease; genetics; FSGS

Alport syndrome is one of the best characterized genetic diseases that affect the kidney, in terms of its presentation, pathologic features, and molecular genetics. The syndrome includes hematuria that usually begins in childhood, with eventual progression to proteinuria and end-stage renal disease (ESRD). Ultrastructural analysis reveals a thickened, split glomerular basement membrane (GBM) with a basket weave-like appearance that is considered diagnostic/pathognomic for Alport syndrome. The renal manifestations are often accompanied by sensorineural deafness and distinct defects in the lens capsule and in the retina. Alport syndrome is caused by mutations that affect the major type IV collagen network of the GBM, a network also present in the inner ear, in the lens, and in the retina. This network contains the collagen α3, α4, and α5(IV) chains, which form an obligate heterotrimer. These chains are encoded, respectively, by the autosomal COL4A3 and COL4A4 genes and the X-linked COL4A5 gene.

When Karl Tryggvason and colleagues published in 1990 that the X-linked form of Alport syndrome is caused by mutations in the COL4A5 gene, it was the first report of the genetic basis for a kidney disease. (This feat predated by several years the discoveries of genes for polycystic kidney disease, for congenital nephrotic syndrome of the Finnish type, and for...
other forms of steroid resistant nephrotic syndrome.) Homozygous and compound heterozygous mutations in \textit{COL4A3} and \textit{COL4A4} were soon thereafter found in patients with autosomal recessive Alport syndrome. Together with the \textit{COL4A5} finding, this established the genetic bases for the most common forms of Alport syndrome, which show either classic X-linked or autosomal recessive inheritance.\textsuperscript{1}

But not all cases of kidney disease with the features of Alport syndrome and verified \textit{COL4} mutations can be so easily categorized into these two simple inheritance patterns. Even before the implication of the three collagen IV genes in disease pathogenesis, there were cases of Alport syndrome in the literature described as autosomal dominant and included father to son transmission. This would seem to both rule out X-linkage and argue against a purely autosomal recessive mode of inheritance.\textsuperscript{3} The realization that “benign” familial hematuria, also known as thin basement membrane nephropathy, could be present in families affected by what appeared to be autosomal recessive Alport syndrome suggested a connection between the two conditions.\textsuperscript{4} Indeed, the finding of heterozygous mutations in \textit{COL4A3} and \textit{COL4A4} in association with both thin basement membrane disease and with what has been described as Alport syndrome\textsuperscript{5–8} cemented the molecular genetic relationship between these two conditions. However, these findings did not dissolve the aura of complexity surrounding the different modes of inheritance in Alport syndrome.

Borrowing a term used by others, the various clinically distinct but GBM-related manifestations of \textit{COL4A3/A4/A5} mutations might best be referred to overall as comprising the spectrum of Alport syndrome. This would encompass what manifests at one extreme as truly benign familial hematuria (with no consequential impact on kidney function), to the other extreme—classic Alport syndrome with no detectable collagen \(\alpha_3\alpha_4\alpha_5(IV)\) in the GBM, eventual ESRD and hearing and eye defects—as well as everything in between (Fig. 1). Given that a mutation causing benign hematuria in one individual can cause progressive kidney disease in another individual, classifying these conditions at different sites within the continuous spectrum of Alport syndrome should be beneficial in informing clinicians as well as patients and their families that the risk of kidney disease is real and should be carefully considered.

One would presume that the advent of next generation sequencing to aid in the diagnosis of human kidney disease, whether sporadic or familial, would provide additional insights into how best to systematize the spectrum of Alport syndrome and other diseases that affect the kidney. However, as revealed in a paper published in this issue of \textit{Kidney International},\textsuperscript{9} a genetic analysis of patients with proteinuria and hematuria and a diagnosis of hereditary focal segmental glomerulosclerosis (FSGS) has made the spectrum of Alport syndrome a bit more complicated.

Malone et al. studied a cohort of 70 families with a pathological diagnosis of familial FSGS of unknown cause. Whole exome sequencing of one family (the index family) with three affected siblings revealed no mutations in any known FSGS genes, but compound heterozygosity was found for \textit{COL4A3}. Each of the \textit{COL4A3} alleles contained a truncating mutation, which together would be expected to cause typical Alport syndrome with lack of the collagen \(\alpha_3\alpha_4\alpha_5(IV)\) network. Unfortunately, electron microscopy was not available for
this family, so the ultrastructure of the GBM remains unknown, but it likely would have indicated Alport syndrome. But in any event, the finding of COL4A3 mutations in this FSGS family spurred the investigators to look for variants in COL4A3 and COL4A4 in the other 69 families, either using next generation or direct sequencing. Six additional rare or novel variants—three glycine substitutions, two other missense, and one truncating—that segregated with FSGS were discovered. Interestingly, each of these was heterozygous, and no mutations in known FSGS-associated genes were found.

Because the pathogenesis of Alport syndrome involves defects in the GBM that eventually lead to foot process effacement and GBM scarring, secondary FSGS is often observed in later stage biopsies. What is interesting about the cases reported here is that nephrotic range proteinuria and FSGS lesions were found in an 8-year old compound heterozygote from the index family, and also in heterozygotes from other families who were not diagnosed until they were well into adulthood. Together these families made up 10% of the 70 familial FSGS families that were studied, an unexpectedly high fraction. These findings raise several questions and provide important lessons for pathologists and nephrologists.

As with any genetic study, questions arise as to whether the variants discovered in patients are actually pathogenic. The truncating COL4A3 mutations in the index family are undoubtedly pathogenic, but the effects of the heterozygous changes in the other families are less clear. The truncating COL4A4 mutation S969X is a null mutation and would indubitably cause Alport syndrome if homozygous, but is it causing hematuria (in children), FSGS, and proteinuria in this family? Based on what is known about the spectrum of Alport syndrome, the answer is likely yes, especially because hematuria was present in five family members and was manifested in a dominantly inherited fashion. That one of the five developed proteinuria and a diagnosis of FSGS at age 20 suggests a confounding factor, perhaps genetic or environmental, in that individual. The heterozygous missense mutations found in the remaining four families were associated with much later diagnoses, suggesting that if pathogenic, they are less severe. Three of these are collagenous domain glycine substitutions, which are frequently associated with disease because the proper formation (and/or maintenance) of collagen triple helices absolutely requires a glycine at every third position. However, there are many cases of heterozygous COL4A3 and COL4A4 glycine substitutions that do not cause disease, but, as with truncating mutations, they do confer Alport syndrome carrier status. The final two variants, COL4A3-L1474P and -R1661C, affect the COOH-terminal noncollagenous domain (NC1) and are more difficult to justify as pathogenic, though the affected amino acids are conserved in mouse, zebrafish, and chicken. Moreover, two members of each family were diagnosed with FSGS, and one individual with the latter mutation exhibited proteinuria and developed hearing abnormalities, consistent with the spectrum of Alport syndrome.

Whether or not the reported heterozygous variants alone are sufficient to cause disease or are only partially penetrant (as some seem to be), the main message of Malone et al.’s paper is that a diagnosis of FSGS based on proteinuria and glomerular pathology can be associated with type IV collagen mutations (at a rate of 10% in their families), so the relevant COL4 genes should be considered in any sequencing-based approach to define pathogenesis in familial FSGS. It is sometimes forgotten that FSGS is a descriptive pathological diagnosis
rather than its own disease entity. As such, based on these new data it should be included within the spectrum of Alport syndrome as an uncommon but real potential manifestation of COL4A3/A4/A5 defects.

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Figure 1.
The spectrum of Alport syndrome, from benign familial hematuria at one extreme and early onset ESRD with hearing and eye defects at the other extreme. Other manifestations of COL4A3, COL4A4, and COL4A5 mutations, which can vary even within a family, fall between the extremes in what are likely overlapping positions.