The Interface of Genetics with Pathology in Alport Nephritis

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Alport syndrome (AS) is a rare disorder characterized by deafness, ocular abnormalities, and progressive renal failure. One of the challenges in diagnosing, managing, and treating patients with AS is the considerable variability in disease presentation, pathology, and multisystem involvement.1 Autosomal dominant, recessive, and X-linked modes of inheritance have been described. Diagnosis of AS can be relatively straightforward in patients with typical clinical presentation, family history, or characteristic pathology such as abnormal immunostaining patterns for COL4α3–5 chains and basement membrane abnormalities on electron microscopy (EM).2 On the other hand, diagnosis of AS can be quite challenging when the following occur: (1) the clinical presentation is atypical, (2) there is extreme variability in histologic findings, (3) there is an unexpected immunostaining pattern, (4) the family history is unavailable, (5) glomerular lesions such as FSGS are the only finding, (6) other renal diseases that may confound pathologic findings are present, or (7) biopsy tissue is inadequate. Histologic features of thin basement membrane disease may be seen in the early presentation of AS and may pose a diagnostic dilemma. In individuals with autosomal recessive AS, heterozygous parent carriers may have subclinical disease with segmental abnormalities in the glomerular basement membrane (GBM) or on immunostaining. Furthermore, histopathology does not provide insights into the variability of clinical features, age of disease onset, or disease severity. Genotyping patients for mutations in COL4α-chains aids in accurately diagnosing AS and also helps in gleaning insights into disease severity; therefore, it is considered the gold standard for diagnosis of AS.

Renal disease is due to abnormalities in collagen IV in the GBM. Formation of a functional heterotrimeric complex between three proteins encoded by the COL4A3, COL4A4, and COL4A5 genes is critical for the normal function of the adult GBM. Mutations in COL4A3 and COL4A4 are believed to be the primary cause of recessive AS (approximately 15%), whereas mutations in COL4A5 are the primary cause of dominant AS (approximately 85%). In the last 2 decades, mutational screening for these genes has revealed that structural changes such as large deletions or nonsense mutations in COL4A5 are associated with early disease onset and the worst prognosis, whereas missense mutations are associated with less severe disease.5

In this issue of JASN, Storey et al.6 report novel mutations of autosomal recessive AS that were not previously described; several individuals were identified with two COL4A3 or COL4A4 mutations. Storey et al. found that 20% of their cohort of 205 patients with suspected AS or thin basement membrane disease had two pathogenic mutations in COL4A3 or COL4A4. The median age of ESRD in these patients was 22.5 years. Renal function was normal in approximately 35% of these patients. The majority of the patients had characteristic EM abnormalities in the GBM, with <5% showing normal or thin basement membrane on pathologic examination. Their analyses found 39 novel variants in the COL4A3 or COL4A4 genes. Although most of the mutations occurred as compound heterozygous, 12 were homozygous. With the increased sensitivity of the sequencing method used (>99%), the cohort is reported to be the largest to identify two mutations in autosomal recessive AS. Nonsense mutations or mutations leading to a stop codon were associated with early onset renal failure. Although direct sequencing clearly increased methodologic sensitivity, other structural changes that may be concurrently present may have been missed or, as the authors acknowledge, even individuals with single mutations may not have been detected with their sequencing approach. These results establish that the type of mutation in recessive AS correlated with outcome in a manner similar to that seen in COL4A5 mutations in X-linked dominant AS, in which nonsense or frame shift changes cause early onset renal disease. These findings have implications for the significance of genetic testing in AS in regard to advice and care provided to patients.

The renal defects in AS are due to inability of the mutant COL4α-chains to form normal α3-α4-α5 heterotrimeric complexes in the basement membrane of the glomeruli, thus compromising the filtration barrier. Several laboratories have sequenced COL4 family genes in patients with AS and found mutations that span the entire coding region, with no particular hot spots, as well as deletions in exons and noncoding regions. Molecular and informatics analyses suggested that the mutations in the conserved glycine-rich regions or in the NC1 carboxy terminus of the involved proteins are deleterious. In X-linked AS, an important correlation was that large structural changes such as deletions, duplications, frame shifts, or...
splice-site or nonsense mutations had a more severe effect on early onset and progression to ESRD, whereas missense mutations were associated with late onset, incomplete penetrance, or slow progression to ESRD. Although genotyping for mutations in COL4A genes is undoubtedly valuable, there are also several challenges. With 51 exons in COL4A5, 48 in COL4A4, and 52 in COL4A3, the large size of COL4 family genes renders widely used traditional Sanger or denaturing high-performance liquid chromatography sequencing costly and inefficient. Selective genotyping for COL4A5 is reasonable for typical X-linked AS in which the family history suggests X-linked dominant inheritance, whereas these molecular approaches can significantly add to costs in atypical or autosomal recessive cases. In fact, in many instances reported in the literature, only portions of certain regions, such as the NC1 domain, were sequenced. This can lead to missing additional or true causative mutations because regions harboring these mutations were not sequenced and true genetic mechanisms may have been missed. It is also being increasingly reported that two mutations in different COL4A genes may be present in an affected individual. These reasons, in addition to the rarity of autosomal recessive AS, have prevented more detailed data on the types of mutations and their clinical or pathologic correlations in autosomal recessive AS. One of the gaps in published autosomal recessive AS studies has been the limited data on compound heterozygous or homozygous mutations in the affected kindred, which poses a challenge in distinguishing AS from cases of thin basement membrane disease and providing genotype-phenotype correlations.

The study by Storey et al. provides new insights into the recessive form of AS and further reinforces the increased need for AS genotyping and clinical correlations to understand the molecular and genetic basis for variable expressivity and incomplete penetrance associated with this disorder. Next-generation sequencing (NGS) methods offer the opportunity to use scalable approaches to sequence multiple individuals and many genes at the same time. This method is preferable in lieu of one gene at a time, especially in atypical cases or where more than one disease may be present. Recent studies report leveraging the power of NGS and implementing it to genotyping patients for COL4A3, COL4A4, and COL4A5 mutations. Artuso et al. generated amplicons spanning the COL4A3 gene and used NGS to identify mutations in this gene in multiple affected individuals. A recent study by Chatterjee et al. implemented a targeted custom exome capture approach to find a potential genetic cause in a patient with complex presentation in which AS was not initially suspected and the patient also had a congenital renal anomaly. They simultaneously tested coding regions of 292 genes that included glomerular and developmental genes and found compound heterozygous COL4A3 mutations and a mutation in SALL2 that may be pathogenic for the renal anomaly. They further scaled their assay to candidate glomerular genes in another set of patients with known or unclear cases of AS and were able to detect COL4A3, COL4A4, or COL4A5 mutations in several of the cases. These scalable NGS methods offer a highly efficient approach that also allows secondary target discovery or genes that may interact with the COL4A genes and may affect the phenotype. Using scalable targeted panels based on clinicopathologic information can be more interpretable and economical than whole exome or whole genome sequencing as a first-line approach in these cases.

Although newer genomic technologies are rapidly evolving and may provide new insights into disease pathogenesis, diagnosis, genetics, or prognosis or explain histopathologic findings, the mechanisms that lead to glomerulosclerosis and progressive renal disease in AS are still debated. For example, Kruegel et al. proposed that podocyte receptors can recognize the mutated COL4 leading to upregulation of podocyte profibrotic factors such as TGF-β, connective tissue growth factor, and matrix metalloproteinases 2 and 9. Repair mechanisms are initiated in the podocyte; however, such attempts fail because COL4 mutations prevent formation of an intact GBM. Other studies in mice with AS show that the defective GBM leads to glomerular capillary loop rupture, crescent formation, and eventual glomerulosclerosis. A better understanding of progressive disease in AS may lead to novel therapeutic agents specifically targeting the podocyte that may be added to the current AS therapeutic regimens.

The large amount of data generated by genomic analyses poses a set of challenges. The most significant challenges are obtaining false positive results and knowing the pathologic significance of the discovered mutations. Are the changes identified functionally deleterious, neutral, or advantageous? Thus, validation is needed when possible until data for pathogenicity of the identified mutations are available. Clinico-pathologic correlations must be made with genomic data. For example, if a suspect mutation is discovered, absence of COL4A chains in the biopsy will substantiate the diagnosis, and phenotyping of extrarenal abnormalities should be carefully done to detect subclinical organ involvement. Segmental immunostaining patterns may suggest milder disease, and their correlation with the types of mutations identified not only in COL4A genes but other yet to be discovered modifiers of COL4A nephropathies will ultimately lead to more robust databases in which genotype-phenotype correlations can be more accurately made. With the power of sequencing, the burden also increases to ultimately improve ways to assess the functional effect of identified mutations. This will be extremely useful in assessing the effect of missense mutations when the immunostaining is not altered or unequivocal. In this regard, there has been a general lack of bioassays that can rapidly evaluate function, and efforts to develop these bioassays will significantly enhance our ability to interpret and act on the genotype and the resulting phenotype. A collaborative approach among biologists, pathologists, nephrologists, genetic counselors, and genome scientists to interpret the significance of AS-causing mutations on an individual's clinical
course, the underlying pathology, and potential interventions will provide better patient management and understanding of this disease.

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DISCLOSURES

None.

REFERENCES
