Alport syndrome: a unified classification of genetic disorders of collagen IV α345: a position paper of the Alport Syndrome Classification Working Group

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Mutations in the genes *COL4A3, COL4A4*, and *COL4A5* affect the synthesis, assembly, deposition, or function of the collagen IV α345 molecule, the major collagenous constituent of the mature mammalian glomerular basement membrane. These mutations are associated with a spectrum of nephropathy, from microscopic hematuria to progressive renal disease leading to ESRD, and with extrarenal manifestations such as sensorineural deafness and ocular anomalies. The existing nomenclature for these conditions is confusing and can delay institution of appropriate nephroprotective therapy. Herein we propose a new classification of genetic disorders of the collagen IV α345 molecule with the goal of improving renal outcomes through regular monitoring and early treatment.

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Inherited forms of glomerular hematuria include disorders of basement membrane collagen (Alport syndrome, thin basement membrane nephropathy/benign familial hematuria, hereditary angiopathy with nephropathy, aneurysms, and muscle cramps syndrome), complement (C3 glomerulopathy), and the podocyte cytoskeleton (Epstein, Fechtner, Sebastian, and May Hegglin syndromes). Two of these disorders—Alport syndrome and a milder disorder previously known as benign familial hematuria or thin basement membrane nephropathy—have a common molecular basis. These conditions arise as a result of mutations in genes *COL4A3*, *COL4A4*, and *COL4A5* that affect the synthesis, assembly, deposition, or function (or a combination of these) of the collagen IV α345 molecule, the major collagenous constituent of the mature mammalian glomerular basement membrane (GBM). This shared molecular etiology is heterogeneously expressed at the histologic and clinical levels, producing a complex array of disease phenotypes and transmission patterns that defies categorization into 2 distinct disorders. The need for a new classification system that would promote easier and earlier diagnosis and proper lifelong surveillance and treatment of Alport syndrome was discussed at the 2015 International Workshop on Alport Syndrome.[1]

It has been proposed that Alport syndrome and benign familial hematuria/thin basement membrane nephropathy be classified as forms of collagen IV–related renal disease,[2] but this approach has not been generally adopted. As the use of next generation and whole exome sequencing in the evaluation of familial hematuria,[3,4] familial proteinuria, and focal segmental glomerulosclerosis[5–8] becomes more frequent, increased numbers of patients with mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes will be identified and standardization of disease classification will be needed to minimize diagnostic confusion. Herein we propose the classification of all disorders arising from

abnormalities of the collagen IV α345 molecule as forms of Alport syndrome. We choose to modify existing terminology rather than attempt to create a novel nomenclature. In this revised nomenclature, Alport syndrome encompasses a phenotypic spectrum extending from nonprogressive, renal-limited disease to progressive, multisystem disease and a genetic spectrum that includes X-linked, autosomal, and digenic inheritance. The results of this classification scheme are as follow: (i) a simplified diagnostic terminology that aims to improve early diagnosis and treatment of Alport syndrome; (ii) clarification that thinning of GBMs is a lesion description rather than a diagnosis; (iii) incorporation of patients with hematuria, thin GBMs, and heterozygous mutations in *COL4A3* or *COL4A4* into autosomal Alport syndrome, eliminating thin basement membrane nephropathy as a diagnostic entity; and (iv) the recognition of X-chromosomal female subjects and microhematuric autosomal heterozygotes as patients with Alport syndrome in whom there is a significant and not negligible risk of progressive renal disease.

In standardizing the nomenclature for these disorders, we hope to ensure early diagnosis, genetic counseling, and appropriate monitoring and management of patients with genetic abnormalities affecting the collagen IV α345 molecule. We stress that all disorders of the collagen IV α345 molecule present a risk for development of progressive renal disease that can be delayed or possibly prevented by timely therapy. Although recent studies have demonstrated that early initiation of therapy with angiotensin-converting enzyme inhibitors delays progression of Alport syndrome to end-stage renal disease (ESRD),[9,10] leading to expert recommendations promoting renin-angiotensin-aldosterone system (RAAS) blockade in proteinuric Alport patients,[11,12] management guidelines are inconsistently applied. For example, only 66% of participants in the ATHENA Natural History Study in Alport Syndrome Patients with declining renal function were receiving RAAS blockade.[13] A prospective study of RAAS blockade in Alport patients with heterozygous mutations found a mean time from appearance of the first symptom to diagnosis of 8.1 ± 14.2 years; at the time of starting RAAS blockade, 5.4% of patients had an estimated glomerular filtration rate <60 ml/min and 67.6% had proteinuria.[14] These observations support the need to ameliorate the phenotypic complexity that may lead to late diagnosis and therapeutic intervention, resulting potentially in preventable ESRD in older patients. We expect that this new classification will improve the prognosis of patients presently diagnosed as benign familial hematuria or thin basement membrane nephropathy but not followed or treated or both due to the erroneous assumption of complete benignity. To us this benefit significantly outweighs the possible harm of classifying a patient with a benign prognosis as having a potentially progressive disorder.

Genetic etiologies and clinical effects of abnormal collagen IV α345 (a brief synopsis)

Collagen IV α345 molecules associate in networks that interact with laminin-521, agrin, nidogen, and other proteins to form mature GBMs.[15] Collagen IV α345 molecules are heterotrimeric moieties created by the specific association of α3, α4, and α5 collagen IV chains in a 1:1:1 ratio. These chains are encoded by the genes *COL4A3, COL4A4,* and *COL4A5,* respectively. The *COL4A3* and *COL4A4* genes reside on chromosome 2 while the *COL4A5* gene is located on the X chromosome. Disease-causing mutations in these genes may alter the synthesis, assembly, deposition, or function (or a combination of these) of α345(IV) molecules. The diseases caused by mutations in these genes are transmitted in an X-linked manner in the case of *COL4A5* mutations, as autosomal disorders when the mutation or mutations are located in the *COL4A3* or *COL4A4* genes or as digenic inheritance when a combination of 2 mutations in different genes occurs.[16]

COL4A5 mutations

Numerous studies of X-linked and autosomal Alport syndrome in mice, dogs, and humans have documented abnormalities of α345(IV) in basement membranes. *COL4A5* mutations that interfere with the synthesis of the α5(IV) chain, such as deletions, frame shift mutations, and nonsense mutations, are associated with loss of expression of α345(IV) in basement membranes and a severe disease phenotype in hemizygous male subjects,[17,18] while the effects of splicing and missense mutations on α345(IV) expression and clinical phenotype are more variable.[17] In X-linked Alport syndrome, renal disease is frequently associated with progressive sensorineural deafness and specific ocular lesions such as perimacular flecks and lenticonus.

COL4A3 and *COL4A4* mutations

Mutations in both alleles of *COL4A3* or *COL4A4* cause autosomal recessive Alport syndrome and include deletion, nonsense, frame shift, splicing, and missense alterations. Genotype-phenotype correlations for autosomal recessive Alport syndrome are less robust than those for X-linked Alport syndrome, but lack of expression of the α345(IV) network in basement membranes is associated with a severe phenotype.[19,20] As in X-linked Alport syndrome, sensorineural deafness and ocular lesions frequently accompany renal disease in patients with autosomal recessive Alport syndrome. Autosomal recessive inheritance may be suggested by similar severity of disease in male and female siblings, parental consanguinity, or microscopic hematuria in the father or in both parents of a severely affected boy.

Heterozygous mutations in *COL4A3* and *COL4A4* are associated with a spectrum of phenotypes ranging from complete absence of detectable symptomatology through isolated, asymptomatic hematuria to progressive renal disease, sensorineural deafness, and ocular abnormalities (autosomal dominant Alport syndrome).[2] Within a family, the phenotype