NEW UNDERSTANDING OF **ALPORT SYNDROME:**

ETIOLOGY AND RECOMMENDED RECLASSIFICATION

REATA PHARMACEUTICALS

ETIOLOGY OF ALPORT SYNDROME AND RECOMMENDED RECLASSIFICATION

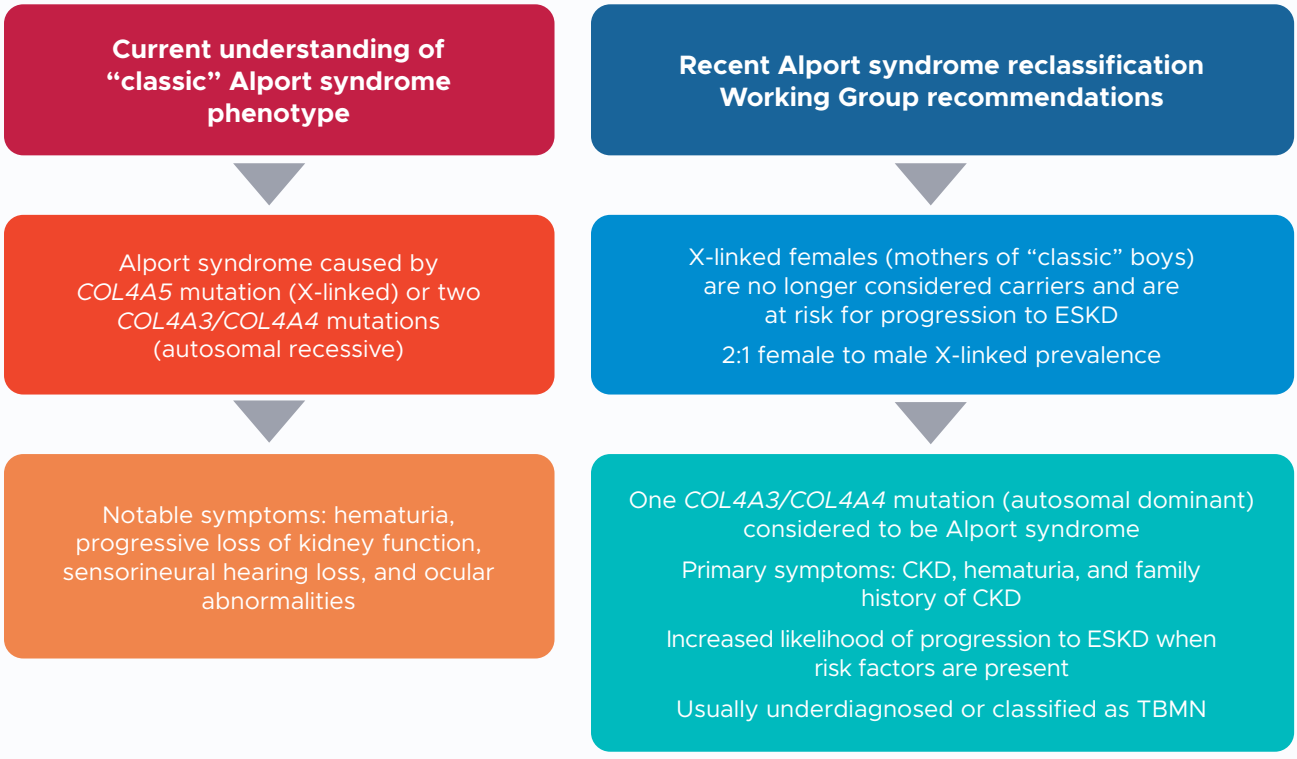
Introduction

Among monogenetic causes of chronic kidney disease (CKD), the *COL4A3*, *COL4A4*, and *COL4A5* mutations associated with Alport syndrome are more common than previously reported. Recent Alport Syndrome Classification Working Group recommendations for the reclassification of Alport syndrome consolidate thin basement membrane nephropathy (TBMN) and what we currently understand as Alport syndrome into a broader definition of Alport disease related to inherited disorders of *COL4A3*, *COL4A4*, and *COL4A5*. These recommendations can minimize diagnostic confusion, promote easier and earlier diagnosis, and facilitate lifelong surveillance and treatment.

Reclassifying Alport Syndrome

Alport syndrome is a multisystem disease with a genetic spectrum that includes X-linked, autosomal, and digenic inheritance.[1] Alport syndrome occurs as a result of mutations in the genes *COL4A3*, *COL4A4*, and *COL4A5*, which code for the α3, α4, and α5 chains of collagen IV, respectively.[2] Based on recent recommendations of the Alport Syndrome Classification Working Group, Alport syndrome has been reclassified to allow for incorporation of patients with CKD, hematuria, thin glomerular basement membranes (GBMs), and heterozygous mutations in *COL4A3* or *COL4A4* into autosomal Alport syndrome, consolidating the diagnosis of TBMN into a broader classification of Alport syndrome (**Figure 1** and **Table 1**).[1] Alport syndrome and TBMN have a common molecular basis arising as a result of *COL4A3*, *COL4A4*, or *COL4A5* gene mutations.[1] This shared molecular etiology is heterogeneously expressed at the histologic and clinical levels, defying categorization into 2 distinct disorders.[1]

Figure 1. Emerging Insights Into the Definitions and Subtypes of Alport Syndrome[1,3,4]



ESKD, end stage kidney disease.

Table 1. Alport Syndrome Working Group Classification System for *COL4A3*, *COL4A4*, and *COL4A5* Disorders[1]

New Classification Scheme Categorizes Genetic Diseases of *COL4A3*, *COL4A4*, and *COL4A5* Into 3 Types of Alport Syndrome: X-linked, Autosomal, and Digenic[1]

Inheritance	Affected Gene(s)	Allelic State	Mutation Phenotype
X-linked	*COL4A5*	Hemizygous (male subjects)	N/A
		Heterozygous (female subjects)	N/A
Autosomal	*COL4A3* or *COL4A4*	Homozygous or compound heterozygous	Recessive
		Heterozygous	Dominant
Digenic	*COL4A3*, *COL4A4*, and *COL4A5*	Variable	

N/A, not applicable.

The Alport Syndrome Classification Working Group recommendations can minimize diagnostic confusion, promote easier and earlier diagnosis, and facilitate lifelong surveillance and treatment of Alport syndrome.[1]

The Alport Syndrome Classification Working Group has made the following recommendations[1]:

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, consolidating the diagnosis of TBMN into a broader classification of Alport syndrome

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 kidney disease

Mutations in Type IV Collagen Cause Alport Syndrome

About 50% of the GBM is comprised of type IV collagen,[2] and 6 genetically distinct collagen IV α chains exist—α1 through α6—which assemble to form 3 different collagen heterotrimers; α1α1α2, α3α4α5, and α5α5α6.[2] *COL4A3*, *COL4A4*, and *COL4A5* gene mutations affect the synthesis, assembly, deposition, or function of collagen IV, and lead to a dysfunctional GBM (**Figure 2**).[1,2]

Figure 2. *COL4A3*, *COL4A4*, or *COL4A5* Mutations Cause a Dysfunctional GBM

Normal | Thin Basement Membrane Lesion | Alport Syndrome

Images used with permission from UNC Kidney Center. www.unckidneycenter.org. Accessed April 20, 2020.

Mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes that cause Alport syndrome are associated with a complex array of transmission patterns.[1]

Alport syndrome caused by mutations in the *COL4A3*, *COL4A4*, or *COL4A5* genes are subject to one of the following[1]:

X-linked transmission, when the mutation is located in the *COL4A5* gene[1]

Autosomal transmission, when the mutation or mutations are located in the *COL4A3* or *COL4A4* genes.[1] Mutations in both alleles of *COL4A3* or *COL4A4* cause autosomal recessive Alport syndrome, while heterozygous mutations in *COL4A3* and *COL4A4* are transmitted in an autosomal dominant fashion[1]

Digenic transmission, when a combination of 2 mutations is located in different genes.[1] Coexisting mutations in *COL4A3*, *COL4A4*, or *COL4A5* have been reported to cause an Alport syndrome phenotype with digenic inheritance[5]

Patients With Alport Syndrome Are at Risk for Development of ESKD

The estimated risk of ESKD in Alport syndrome varies with the mode of inheritance. For X-linked Alport syndrome, the clinical phenotype in women differs from that in men who have a more severe presentation and universally develop ESKD: X-linked women have up to a 25% risk for ESKD, whereas X-linked men have a 100% risk.[1] Under the reclassification scheme, women with X-linked inheritance, previously seen only as carriers, would be diagnosed with Alport syndrome and considered to have an appreciable risk for disease progression.[1]

Among persons with autosomal recessive inheritance, both sexes experience a 100% risk for progression to ESKD.[1] In patients with heterozygous *COL4A3* or *COL4A4* variants, isolated hematuria has traditionally been associated with thin GBMs and less severe kidney outcomes; however, patients with GBM thinning, including those with autosomal dominant Alport syndrome, can also exhibit progressive kidney disease.[1] Next generation sequencing has demonstrated that autosomal dominant Alport syndrome is more prevalent than previously reported.[6] Both males and females with autosomal dominant mutations may be at risk for ESKD; those with other risk factors for progression have an estimated ≥20% lifetime risk of ESKD, while those with no risk factors have a <1% lifetime risk (**Table 2**).[1]

Table 2. Estimated Risk of ESKD in Patients With Alport Syndrome[1]

	X-Linked		Autosomal Recessive	Autosomal Dominant
	Males	Females	Males & Females	Males & Females
Estimated Risk of ESKD	100% risk of ESKD	25% risk of ESKD	100% risk of ESKD	20% risk of ESKD

SUMMARY

In summary, the Alport Syndrome Classification Working Group recently recommended the reclassification of Alport syndrome based on a common molecular etiology (abnormal *COL4A3*, *COL4A4*, or *COL4A5*), and to minimize diagnostic confusion, promote easier and earlier diagnosis, and facilitate lifelong surveillance and treatment of Alport syndrome.[1] These recommendations include simplifying the diagnostic criteria by incorporating patients with hematuria, thin GBMs, and heterozygous mutations in *COL4A3* or *COL4A4* into autosomal Alport syndrome, which would consolidate the diagnosis of TBMN into a broader classification of Alport syndrome.[1]

ACRONYMS

CKD, chronic kidney disease
ESKD, end-stage kidney disease
GBM, glomerular basement membrane
N/A, not applicable
TBMN, thin basement membrane nephropathy

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NEW UNDERSTANDING OF **ALPORT SYNDROME:**

DIAGNOSTIC CONSIDERATIONS

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DIAGNOSIS OF ALPORT SYNDROME

Introduction

Alport syndrome affects approximately 30,000-60,000 people in the United States (US).[1,2] More than 1% of patients receiving renal replacement therapy have Alport syndrome.[3] However, many at-risk women as well as men who present later in life with hematuria but without hearing loss may be misclassified, which contributes to the possible underestimation of Alport syndrome due to patients never being tested.[4,5]

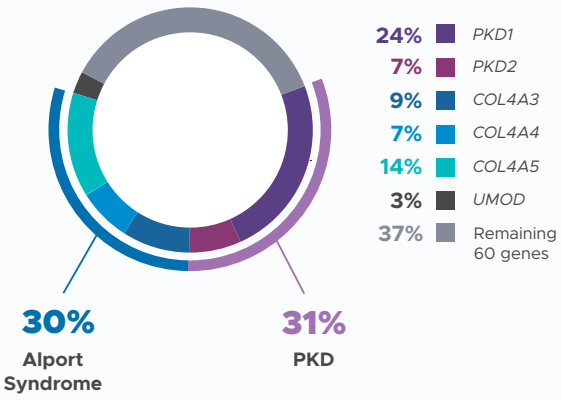
Alport Syndrome Accounts for 30% of Patients With a Genetic Cause of Chronic Kidney Disease (CKD)[6]

In a recent exome sequencing analysis of patients with CKD, Alport syndrome and autosomal dominant polycystic kidney disease (ADPKD) were the most prevalent monogenetic causes of CKD detected (**Figure**).[7] Notably, 56 of the 91 patients (62%) identified by mutational screening as having Alport syndrome did not have a clinical diagnosis of Alport syndrome.[7]

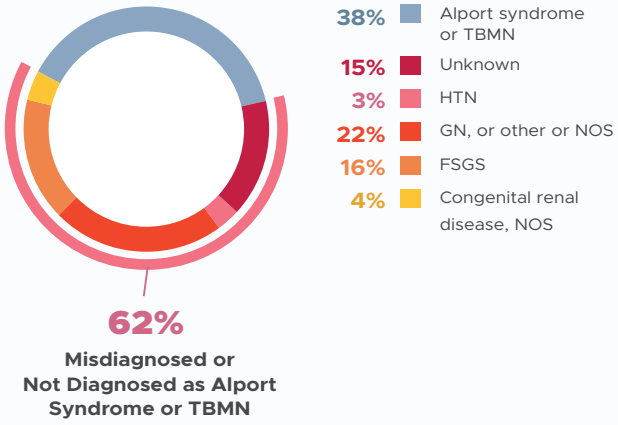
Figure. ***COL4A3*, *COL4A4*, and *COL4A5* Mutations Represent 30% of Patients With a Genetic Cause of CKD[7]**

In a recent exome sequencing analysis of 3315 patients with CKD, ~10% (307/3315) had a genetic cause.

Abnormalities in *COL4A3*, *COL4A4*, and *COL4A5* Represent 30% of Patients With a Genetic Finding



Of *COL4A3*, *COL4A4*, and *COL4A5* Mutations, 62% Were Misdiagnosed or Not Diagnosed as Having Alport Syndrome or TBMN



FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; HTN, hypertensive nephropathy; NOS, not otherwise specified; PKD, polycystic kidney disease; TBMN, thin basement membrane nephropathy.

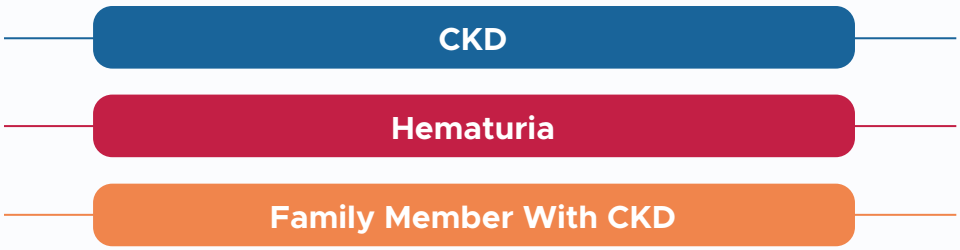
Autosomal Dominant Alport Syndrome Is More Prevalent Than Previously Reported

In a study of 101 unrelated patients (average age 35 years) suspected of having Alport syndrome with hematuric nephropathy, a positive family history of CKD, and no evidence of end-stage kidney disease (ESKD), sequencing for *COL4A3*, *COL4A4*, and *COL4A5* gene mutations identified pathogenic variants in 80% (81/101) of patients.[8] The autosomal dominant inheritance pattern was more common than previously thought, representing 20% (22/1010) of the patients, and this inheritance pattern was noted to not fully capture the risk of progressive CKD.[8] These study findings, which to some extent are contrary to previously published evidence, highlight the ongoing need to minimize diagnostic confusion, and promote easier and earlier diagnosis, which may be facilitated by the recent Alport Syndrome Classification Working Group recommendations.[4,8]

Characteristics of Patients Who Should Be Screened for Alport Syndrome Mutations

Alport syndrome should be suspected in the presence of characteristic clinical features including CKD, hematuria, a family history of CKD, and no other obvious cause.[4,9]

3 Key Features to Identify Potential Patients With Alport Syndrome



ALPORT SYNDROME CLINICAL CASE PRESENTATIONS

The inheritance patterns of Alport syndrome include X-linked, autosomal dominant, or autosomal recessive and can occur in both male and female patients. The following 3 demonstrative cases illustrate how the application of molecular genetics to clinical practice can generate a proper diagnosis for patients and their families with kidney disease.

Case 1: Autosomal Dominant Alport Syndrome

This patient has autosomal dominant Alport syndrome that has progressed to ESKD. A prior kidney biopsy revealed thinned glomerular basement membranes (GBMs), which traditionally was determined to be TBMN but is now considered to be autosomal dominant Alport syndrome.[4] A subsequent genetic test confirmed that she has autosomal dominant Alport syndrome. This patient also appears to have an undiagnosed family history of Alport syndrome, as her parents have CKD that has not progressed to ESKD (stage 3 CKD), and her aunt has hematuria. This finding highlights that the phenotype observed in patients with Alport syndrome, even among relatives, can be variable. The patient's children are being screened and followed-up given the high likelihood of them inheriting Alport syndrome.

Case 2: Autosomal Recessive Alport Syndrome

This patient, an 18-year-old woman, has autosomal recessive Alport syndrome, but her parents do not have clinical evidence of kidney disease at this point. Note that the lack of family history could have increased the chances of a misdiagnosis. Upon diagnosis of the patient, her immediate family members underwent genetic testing and both parents and her 3 siblings were positive for a *COL4A* variant. The patient's parents would be expected to each have a *COL4A* variant considering that autosomal recessive Alport syndrome requires inheritance of a variant from each parent. The patient's parents and 3 siblings are now being monitored regularly for signs of CKD.

Case 1

52-year-old Woman

Presentation:
- Middle Eastern woman
- SCr 2.5 (eGFR 30)
- Has 2 children and 2 siblings

Comorbidities:
- Obesity, hypertension, type 2 diabetes mellitus

Family History:
- Both mother and father have CKD (stage 3)
- Aunt in the Middle East always had blood in urine

Diagnosis:
- Patient was screened for Alport syndrome
- Genetic confirmation of autosomal dominant Alport syndrome

Clinical Follow-up:
- Progressed to kidney transplant
- Two children now being screened and followed-up

Case 2

18-year-old Woman

Presentation:
- Smart Watch indicated abnormal heart rhythm
- Presents to ER with SCr 2.5
- History of post-strep glomerulonephritis
- Has 3 siblings
- Kidney biopsy shows advanced IFTA and electron microscopy suggests Alport syndrome

Family History:
- No clinical evidence of kidney disease

Diagnosis:
- Patient was screened for Alport syndrome
- Genetic confirmation of autosomal recessive Alport syndrome

Clinical Follow-up:
- Parents and 3 siblings also underwent genetic testing and were positive for a *COL4A* variant; they are being monitored regularly for signs of CKD

eGFR, estimated glomerular filtration rate; ER, emergency room; IFTA, interstitial fibrosis and tubular atrophy; SCr, serum creatinine.

Case 3: X-Linked Alport Syndrome

This patient was previously misdiagnosed as having FSGS based on a kidney biopsy. His mother had a history of CKD, which progressed to ESKD in her 30's requiring a kidney transplant. Genetic testing ultimately revealed he had a pathogenic mutation in *COL4A5*, confirming X-linked Alport syndrome. The patient eventually developed progressive kidney impairment associated with persistent proteinuria. If genetic testing was performed earlier, kidney biopsy could have been avoided and earlier detection and management of the patient's hypertension and proteinuria may have slowed the rate of decline of his kidney function.

Case 3

28-year-old Man

Presentation:

- Hematuria
- Nephrotic-range proteinuria
- Normal GFR
- Prior diagnosis of FSGS based on a kidney biopsy

Comorbidities:

- Hypertension
- Sensorineural deafness

Family History:

- Mother underwent a kidney transplant due to ESKD

Diagnosis:

- Genetic confirmation of X-linked Alport syndrome for both mother and son

Clinical Follow-up:

- Developed progressive kidney impairment associated with persistent proteinuria

GFR, glomerular filtration rate.
Cases are provided for demonstrative purposes only.

ACRONYMS

ADPKD, autosomal dominant polycystic kidney disease
CKD, chronic kidney disease
eGFR, estimated glomerular filtration rate
ER, emergency room
ESKD, end-stage kidney disease
FSGS, focal segmental glomerulosclerosis
GBM, glomerular basement membrane
GFR, glomerular filtration rate
GN, glomerulonephritis
HTN, hypertensive nephropathy
IFTA, interstitial fibrosis and tubular atrophy
NOS, not otherwise specified
PKD, polycystic kidney disease
SCr, serum creatinine
TBMN, thin basement membrane nephropathy
US, United States

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NEW UNDERSTANDING OF **ALPORT SYNDROME:**

EMERGING EVIDENCE ON THE ROLE OF CHRONIC INFLAMMATION

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ALL FORMS OF CHRONIC KIDNEY DISEASE (CKD) ARE CHARACTERIZED BY PROGRESSIVE LOSS OF GLOMERULAR FILTRATION RATE (GFR)

Introduction

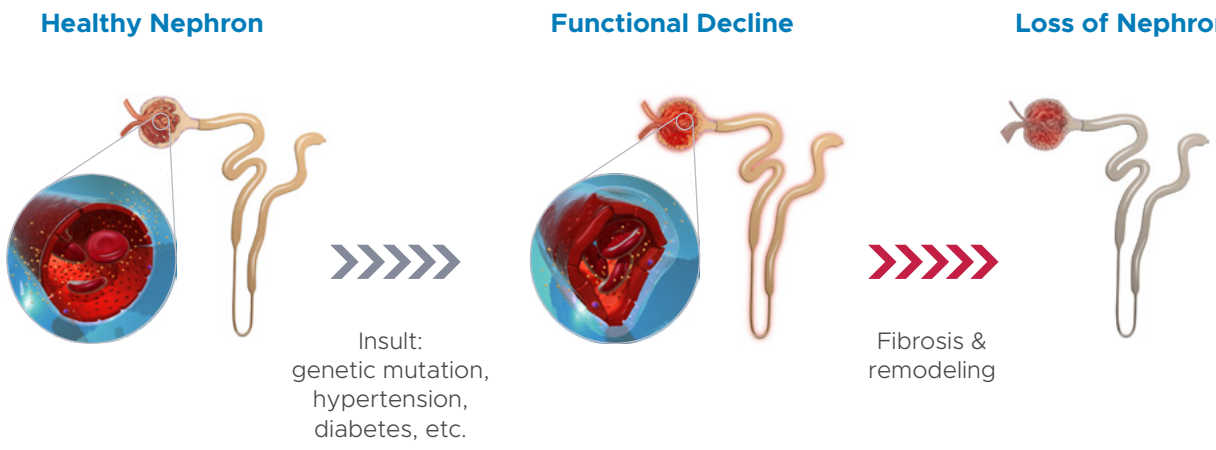
There are currently no approved therapies for Alport syndrome, and patients are typically prescribed the current standard of care for patients with CKD, renin-angiotensin-aldosterone system (RAAS) inhibitors. As is common in patients with other causes of CKD, patients with Alport syndrome often show progressive loss of kidney function despite treatment with RAAS inhibitors. As a result, the incidence of end-stage kidney disease (ESKD) in the United States (US) remains unacceptably high and additional pathways and mechanisms involved in the progression of CKD need to be characterized and hopefully targeted by novel therapies.

The inflammatory mechanism is the final common pathway of CKD progression irrespective of disease etiology and it is being increasingly recognized as an important factor in the pathophysiology and progression of CKD, including Alport syndrome. Exposure of the kidney to harmful stimuli causes an acute decline in kidney function and subsequently a persistent inflammatory reaction among resident kidney cells, ultimately leading to progressive CKD. Chronic inflammation in CKD is a multifactorial process involving many proinflammatory mediators including initiating, reinforcing, signaling, and transcription factors. These mediators promote remodeling, fibrosis, and acute and chronic loss of kidney function in CKD.

Nuclear factor erythroid 2–related factor 2 (Nrf2) is a key regulator of the anti-inflammatory pathway in CKD, and its activation can reduce the inflammatory cascade, prevent fibrosis, and restore kidney function, making Nrf2 an attractive target for novel therapies.

Patients with Alport syndrome are at risk of developing CKD.[1] All forms of CKD are characterized by progressive loss of GFR, with patients requiring dialysis or transplant once the GFR falls below ~15 mL/min/1.73 m^2.[2,3] Kidney cell insults caused by specific genetic mutations (eg, *COL4A* gene mutations), hypertension, or diseases such as diabetes, initially cause glomerular injury, which leads to impaired function and eventually to nephron loss (**Figure 1**).[2,4-6] Decline in GFR is consistently associated with chronic oxidative stress, metabolic impairment and inflammation-mediated fibrosis and remodeling.[5,6]

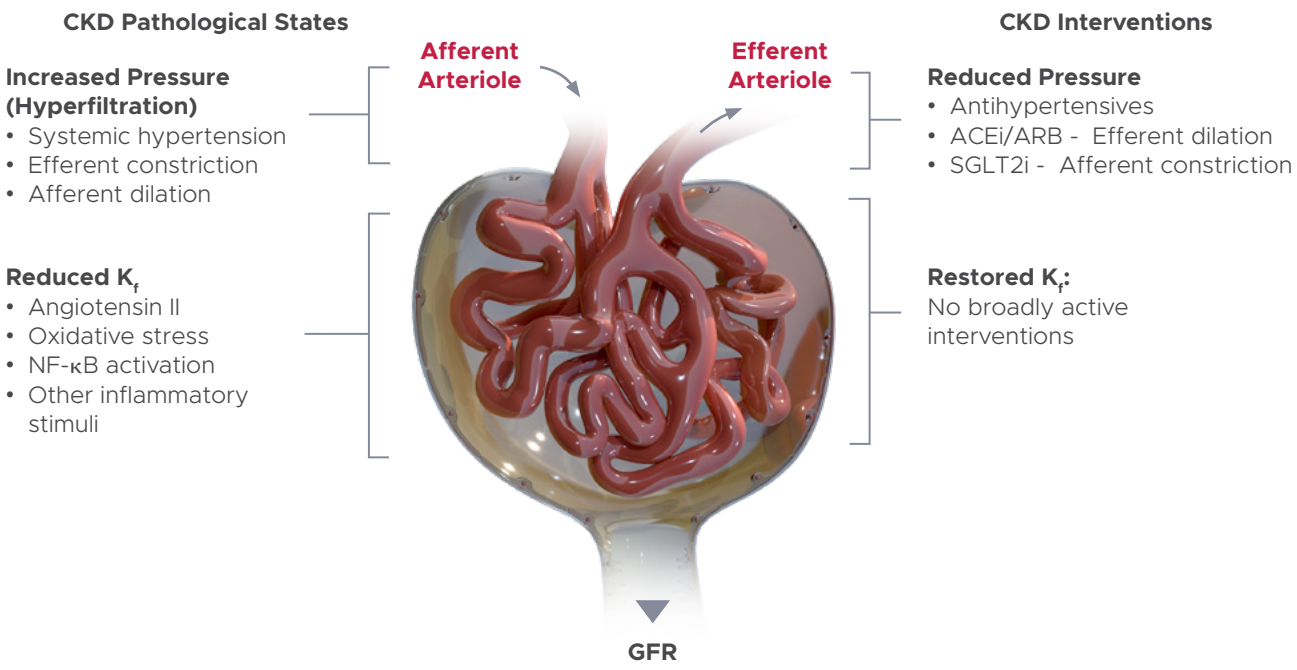
Figure 1. Loss of GFR Due to Impaired Function of Nephrons and Nephron Loss[2,4-6]



GFR is determined by both the net glomerular filtration pressure, as well as the capillary surface area and its permeability. Hyperfiltration results from persistent changes in efferent and afferent arteriolar resistance and changes in systemic arterial pressure, which increases mean net glomerular capillary hydraulic pressure.[6] Note that GFR is directly proportional to the pressure gradient in the glomerulus, so changes in intraglomerular pressure will change GFR.[7] A combination of hemodynamic, vasoactive, tubular, growth promoting and metabolic/systemic factors including increased blood pressure (BP) contribute to the pathogenesis of hyperfiltration.[6] Vasoactive factors implicated in the regulation of glomerular arteriole tone include the RAAS, the nitric oxide (NO) system, and cyclo-oxygenase 2 (COX-2) derived prostanoids.[6]

Increased hydrostatic pressure (hyperfiltration) is harmful and targeted by a variety of antihypertensive agents (**Figure 2**).[2,6] RAAS inhibitors including angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) reduce intraglomerular pressure by blocking the vasoconstrictive effect of angiotensin II on the efferent arteriole.[2,6] The initial decrease in GFR associated with angiotensin II inhibition has been associated with a reduced rate of long-term decline in kidney function.[8] However, ACEi, ARBs, and even a combination of the 2 classes appear to only offer partial kidney protection.[6] Angiotensin II and aldosterone breakthrough due to activation of compensatory pathways may be important mechanisms to explain limited efficacy.[6] Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are antihyperglycemic drugs that also favorably affect kidney hemodynamics.[2,6] Like RAAS inhibitors, SGLT2i initially cause a reduction in estimated glomerular filtration rate (eGFR), acting through the tubulo-glomerular feedback mechanism to cause constriction of the afferent arteriole, thereby reducing intraglomerular pressure.[2,6]

Figure 2. CKD Pathological States and Targeted Interventions[2,5,6]



K_f, filtration coefficient; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

[Placeholder for permission credit line.]

The filtration coefficient—the glomerular surface area and permeability of the filtration barrier denoted by K_f—contributes substantively to GFR, is controlled dynamically by endothelial cells, mesangial cells, and podocytes, and is reduced in CKD.[9-11] Angiotensin II and other inflammatory mediators are important mediators of reduced K_f and represent novel therapeutic targets to acutely and chronically improve GFR.[9-11] K_f is currently not targeted by any broadly active therapies. ■

UNMET NEEDS IN THE CURRENT STANDARD OF CARE (SOC) FOR CKD DUE TO ALPORT SYNDROME AND OTHER INSULTS

Key Points: Current SOC for CKD Insufficient to Prevent Disease Progression

Despite treatment with current SOC RAAS inhibitors, a natural history study of patients with Alport syndrome confirm progressive loss of kidney function[12]

While these interventions may have consistent effects on BP and proteinuria, effects on clinically important kidney outcomes, such as the progressive decline of eGFR and ESKD, are more modest[13-16]

SOC to reduce CKD progression includes:

- BP control (AASK, MDRD)[13,14]
- RAAS inhibitors that suppress angiotensin II signaling and lower proteinuria (RENAAL, IDNT)[15,16]
- SGLT2i-mediated decrease in intraglomerular pressure (CREDENCE) with other possible mechanisms presently being studied[17-21]

Incidence of ESKD remains unacceptably high, supporting the need for additional interventions earlier in the course of the disease targeting complementary pathways to reduce progression of CKD[22]

Unfortunately, there are no currently approved therapies for Alport syndrome, and these patients typically receive conservative management including annual monitoring of kidney function, dietary and lifestyle management, BP control, and prescription of RAAS inhibitors including ACEi or ARBs.[23-26] Overactivity of intrarenal RAAS is involved in the progression of CKD, and RAAS inhibitors have been shown to protect kidney function by decreasing BP and proteinuria.[27] However, while these interventions may have consistent effects on BP and proteinuria, effects on clinically important kidney outcomes, such as the progressive decline of eGFR, are more modest and do not alter the subsequent progressive decline in kidney function.[13-16]

The RAAS inhibitors losartan and irbesartan were demonstrated to suppress angiotensin II signaling and lower proteinuria in the Reduction of Endpoints in NIDDM (noninsulin-dependent diabetes mellitus) with the Angiotensin II Antagonist Losartan (RENAAL) trial and the Irbesartan Diabetic Nephropathy Trial (IDNT), respectively.[15,16]

In the RENAAL trial, patients with type 2 diabetes and nephropathy who received losartan experienced an average reduction in the level of proteinuria (the urinary albumin-to-creatinine ratio) of 35%, whereas in patients who received a traditional BP-lowering regimen (ie, the placebo group), the urinary albumin-to-creatinine ratio tended to increase ($P<0.001$ for the overall treatment effect).[15] Although patients who received losartan had a significantly greater acute fall in eGFR during the first 3 months compared with patients in the placebo group, a significantly slower long-term mean decline of eGFR was observed thereafter.[8] According to the intention-to-treat analysis, 327 patients in the losartan group (43.5%), as compared with 359 in the placebo group (47.1%) reached the primary composite end point of a doubling of the serum creatinine concentration, ESKD, or death, corresponding to a 16% reduction in the risk of the primary composite end point ($P=0.02$).[15]

The SGLT2i canagliflozin was recently demonstrated to cause a decrease in the risk of kidney failure in patients with type 2 diabetes and albuminuric CKD who were receiving RAAS inhibitors for at least 4 weeks before randomization in the Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial.[17]

In the CREDENCE trial, the level of proteinuria (urinary albumin-to-creatinine ratio) was an average of 31% lower during follow up in patients receiving canagliflozin than in those on placebo.[17] Although patients who received canagliflozin had a significantly greater acute fall in eGFR during the first 3 weeks compared with patients in the placebo group, a significantly slower long-term mean decline of eGFR was observed thereafter.[17] The event rate of the primary composite outcome of ESKD, doubling of the serum creatinine level, or kidney or cardiovascular (CV) death was significantly lower in the canagliflozin group (43.2 per 1000 patient-years) than in the placebo group (61.2 per 1000 patient-years), which resulted in a 30% lower relative risk (hazard ratio [HR], 0.70; 95% confidence interval [CI], 0.59 to 0.82; *P*=0.00001).[17]

Intensive treatment to reduce BP levels to below the goals recommended by guidelines does not consistently further reduce the rate of eGFR decline or decrease the chance of developing ESKD.[13,14] A subgroup analysis of the Modification of Diet in Renal Disease (MDRD) study in patients with CKD suggested that strict BP control slows the decline in eGFR only in those patients with proteinuria.[14] In secondary analyses comparing the effects of BP control in black and white MDRD study participants, a greater beneficial effect of a low mean arterial pressure goal on GFR decline (*P*=0.02 for both races) was demonstrated in patients with higher baseline urine protein excretion.[14] The results of the African American Study of Kidney Disease and Hypertension (AASK) trial further demostrated that BP reduction to levels below current guidelines for CV risk reduction was ineffective as a strategy to prevent progression of hypertensive nephrosclerosis in patients with hypertensive kidney disease.[13] In this study, the mean GFR slope from baseline through 4 years did not differ significantly between the lower BP group (-2.21 [0.17] mL/min/1.73 m^2/y) and the usual BP group (-1.95 [0.17] mL/min/1.73 m^2/y; *P*=0.24). Additionally, the lower BP goal did not significantly reduce the rate of the clinical composite outcome of reduction in GFR by 50% or more from baseline, ESKD, or death (risk reduction for lower BP group=2%; 95% CI, -22% to 21%; *P*=0.85).[13] Interestingly, baseline proteinuria was a strong predictor of GFR decline in this study.[13]

The Effect of Strict Blood Pressure Control and ACE Inhibition on the Progression of CRF (chronic renal failure) in Pediatric Patients (ESCAPE) Trial assessed the long-term renoprotective effect of intensified BP control among children who were receiving a fixed high dose of an ACEi. A total of 385 children, ages 3 to 18 years, with CKD received ramipril at a dose of 6 mg per square meter of body surface area per day. Patients were randomly assigned to intensified or conventional BP control, achieved by the addition of antihypertensive therapy that does not target RAAS with a follow-up of 5 years. The primary end point was the time to a decline of 50% in the GFR or progression to ESKD. A total of 29.9% of the patients in the group that received intensified BP control reached the primary end point, as compared with 41.7% in the group that received conventional BP control (HR, 0.65; 95% CI, 0.44 to 0.94; *P*=0.02). The study demonstrated that intensified BP control, with target 24-hour BP levels in the low range of normal, provides substantial benefit with respect to kidney function among children with CKD.[28]

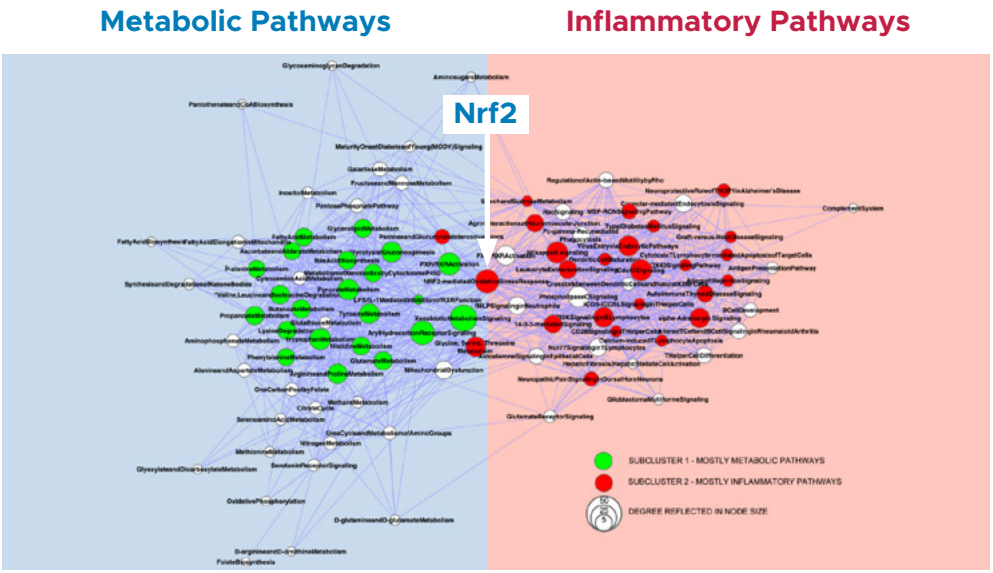
The natural rate of progression of CKD in 165 patients with Alport syndrome was recently evaluated in the observational, global, multicenter, ATHENA study.[12] The mean slope decline in measured GFR and eGFR at 24 weeks of follow-up was approximately -4 mL/min/y compared to baseline.[12,29] Progressive loss of kidney function was observed in this study despite more than 80% of the patients with Alport syndrome receiving either an ACEi or an ARB.[12] These findings, and those from RAAS inhibitor and SGLT2i trials in patients with type 2 diabetes and nephropathy, show that RAAS/SGLT2i inhibition is not sufficient to prevent progression of eGFR loss and ESKD.[12,15,16] With the incidence of ESKD in the US remaining unacceptably high, additional pathways and processes involved in the progression of CKD need to be characterized and hopefully targeted by novel therapies.[22] To that end, targeting chronic inflammation is broadly recognized as an approach that could prevent CKD progression.[30,31]

INFLAMMATION AS DETERMINANT OF PROGRESSIVE CKD IN ALPORT SYNDROME

Inflammation and Metabolic Dysfunction Promote GFR Loss in Patients With CKD

Determining common molecular pathways underlying the etiology of progressive CKD due to a wide variety of initial insults has long been a goal of scientists and has recently been facilitated through the use of a genome-wide association study in combination with transcriptomic analysis.[32] A comprehensive network of pathways in patients with progressive CKD has been constructed using data from genome-wide association studies and molecular analyses of kidney biopsies obtained from 157 patients with CKDs including thin basement membrane nephropathy, focal segmental glomerulosclerosis, membranous glomerulonephritis (MGN), minimal change disease (MCD), diabetic nephropathy (DN), hypertensive nephropathy (HTN), IgA nephritis (IgAN), and lupus nephritis (LN).[32] Graphic presentation of the network of pathways reveals that the majority of the pathways aggregate in either an inflammation- or a metabolism-related cluster, corresponding to 2 major hallmarks of CKD (**Figure 3**).[32] Nrf2, the transcriptional regulator of cellular response to injury, was identified as the link between these 2 clusters, suggesting that it is a key factor underlying progressive CKD.[32]

Figure 3. Pathways That Correlate With GFR in Patients With CKD[32]



Nodes display significantly (P<0.05) enriched pathways derived from 2 or more lists of coregulated candidate genes, and node size reflects the number of connections to other pathways (degree).

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Inflammatory Pathway Activation in CKD Is Caused by Resident Kidney Cells Adapting a Proinflammatory State

Key Points: Chronic Inflammation is Evidenced by Resident Kidney Cells Producing Proinflammatory Cytokines and Chemokines

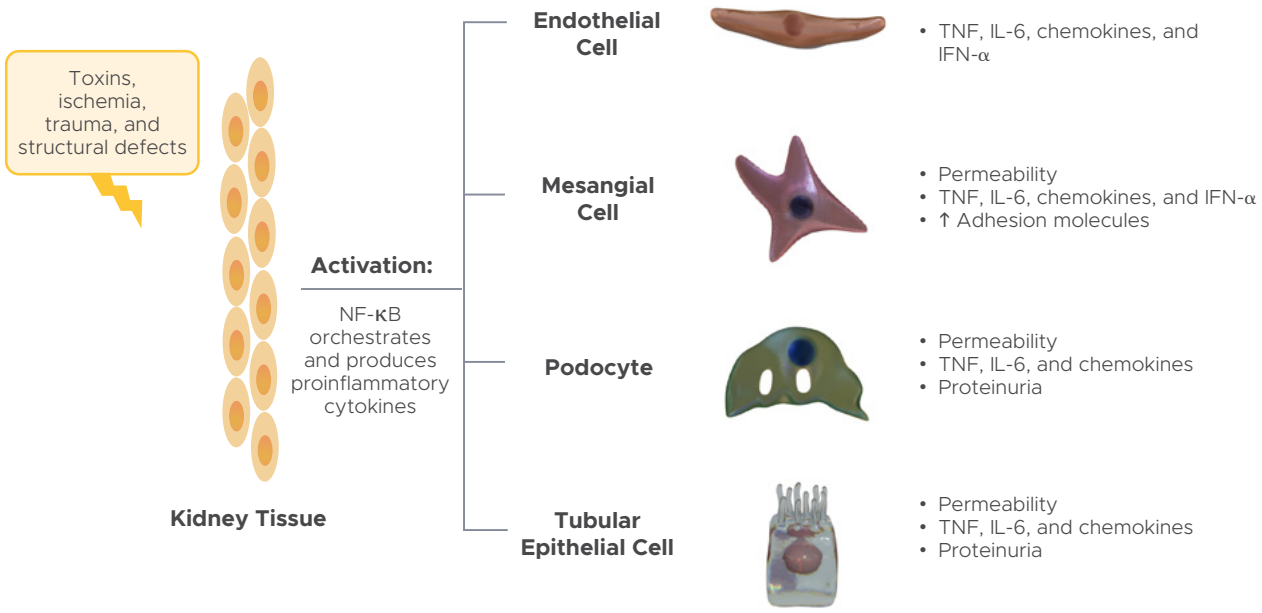
Acute inflammation is marked by infiltrating white blood cells[33]
- Neutrophils, macrophages, dendritic cells, and T and B lymphocytes

Chronic inflammation, however, is evidenced by activation of resident kidney cells adapting a proinflammatory response[11,34-38]
- Endothelial cells, mesangial cells, podocytes, and tubular epithelial cells

Activated resident kidney cells produce proinflammatory cytokines and chemokines responsible for both an acute effect on kidney function and for perpetuating chronic inflammation leading to kidney fibrosis[11,35,39]

Acute inflammation is marked by the infiltration of white blood cells, including neutrophils, macrophages, dendritic cells, and T and B lymphocytes, to initiate an essential immune response to address the underlying insult before tissue repair can take place.[33,40] The activated stress response pathways of damaged kidney epithelial cells lead to the secretion of cytokines and vasoactive factors.[40] In the presence of persistent cellular insult however, the acute inflammatory response evolves into chronic inflammation, which plays a central role in the progression of Alport syndrome and other CKDs.[41] Chronic inflammation is evidenced by activation of resident kidney cells adapting a proinflammatory response (**Figure 4**).[11,34-38]

Figure 4. Activation of Resident Kidney Cells Produces Proinflammatory Cytokines and Chemokines[42]



IFN-α, interferon-alpha; IL-6, interleukin 6; TNF, tumor necrosis factor.
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These resident kidney cells include endothelial cells, mesangial cells, podocytes, and tubular epithelial cells.[11,34-36,38] The endothelial cells of the glomerulus are uniquely adapted for selective filtration and permeability.[11] Mesangial cells play a role in glomerular contraction, modulating K_f and filtration surface area, which help regulate GFR.[43] Oxidative stress and inflammation provoke dynamic and chronic actions leading to GFR decline.[43] Dynamic action includes the reactive oxygen species (ROS)-induced mesangial cell contraction and glomerular endothelial dysfunction due to decreased nitric oxide bioavailability, causing reduction of K_f.[43] Podocyte foot contraction and effacement is an adaptive response to oxidative stress and inflammation in order to prevent podocyte loss.[44] As a consequence, the quality of glomerular filtration is reduced due to denudation of the glomerular basement membrane (GBM).[44]

GFR decline as a result of chronic action involves an increase in inflammatory cytokines and extracellular matrix deposition, which causes structural changes, such as tubulointerstitial fibrosis and mesangial expansion.[43] Activated mesangial cells produce chemokines and cytokines, which act on mesangial cells themselves and on other resident glomerular cells or leukocytes. If mesangial cell activation is ongoing, extracellular matrix (ECM) accumulation in the interstitial space leads to interstitial fibrosis, followed by glomerulosclerosis.[11]

In the classic 5/6 nephrectomy hyperfiltration model of CKD, NF-κB is activated and transforming growth factor beta (TGF-β) is upregulated.[45] NF-κB is a regulator of proinflammatory genes that orchestrates and produces hundreds of inflammatory cytokines and mediators.[41,46,47]

Activation of TGF-β causes progressive development of fibrosis.[48] Podocytes express TGF-β following the onset of proteinuria,[49] which promotes transformation of epithelial and mesangial cells into fibroblasts and myofibroblasts.[48] TGF-β also causes podocytes to produce ECM proteins that accumulate in the tubulointerstitium.[49] Tubular epithelial cells and myofibroblasts are all capable of synthesizing TGF-β at different stages during the development of kidney fibrotic lesions.[35] Activated resident kidney cells produce proinflammatory cytokines and chemokines responsible for perpetuating chronic inflammation leading to kidney fibrosis.[11,35,39]

Inflammatory Mediators in CKD

Chronic inflammation in CKD is a multifactorial process involving many proinflammatory mediators, including initiating factors (cytokines), reinforcing factors, and signaling and transcription factors (**Figure 5**). These inflammatory mediators promote remodeling, fibrosis, and acute and chronic loss of function in CKD.[41]

Chronic inflammation is a complex process involving various inflammatory mediators that[4,48]:

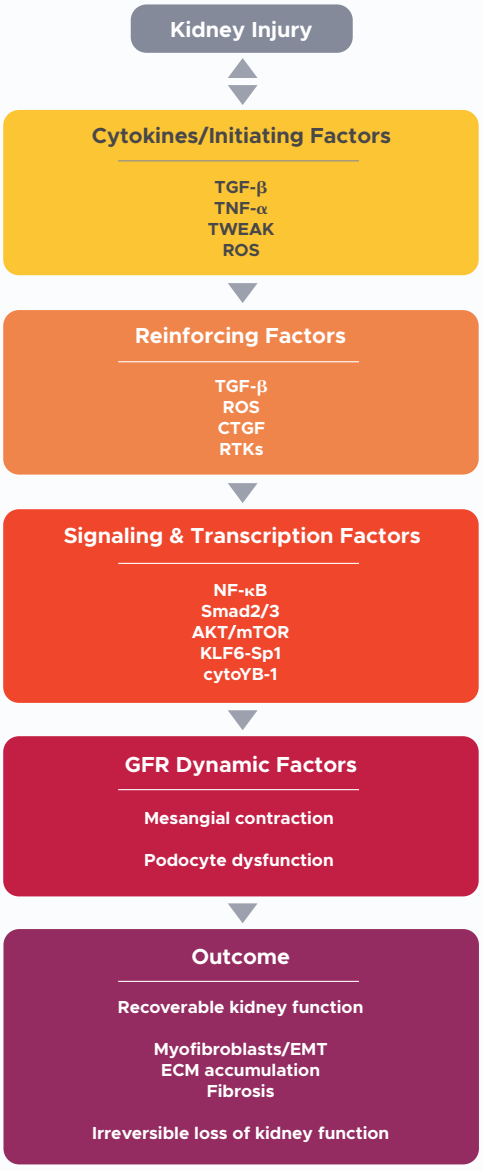
Promote remodeling and fibrosis

- NF-κB is a regulator of proinflammatory genes that orchestrates and produces hundreds of inflammatory cytokines and mediators[41,46,47]
- TNF-α and other cytokines activate neighboring kidney cells and recruit macrophages to damaged tissue, which contribute to progressive glomerulosclerosis[4,48]
- TGF-β promotes transformation of epithelial and mesangial cells into fibroblasts and myofibroblasts resulting in fibrosis[4,48-52]

Reduce kidney function acutely and chronically

- TGF-β1 and connective tissue growth factor (CTGF) contribute to altered glomerular structural and functional properties of the Alport GBM with irregular thickening, splitting, and increased permeability[4,51,52]
- TGF-β1 and platelet-derived growth factor (PDGF) are important mediators of interstitial fibrosis with TGF-β1 upregulation occurring in nearly every form of CKD; interstitial ECM accumulation contributes to functional loss[53]
- Structural changes contribute to progressive loss of GFR[52,54]

Figure 5. Mediators in CKD[41]



EMT, epithelial-mesenchymal transition.
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Nrf2 Is a Key Regulator of the Anti-Inflammatory Pathway in CKD

- Nrf2 is a transcription factor that regulates the expression of hundreds of genes involved in the antioxidant response, metabolism and lipid regulation, and mitochondrial function[5,55,56]
- A cytosolic inhibitor, kelch-like ECH-associated protein 1 (Keap1), retains Nrf2 in the cytoplasm until activated by disease triggers[5,55]

Nrf2-Keap1 system plays a key role in the resolution phase of inflammation by opposing inflammatory and oxidative damage[43,57-59]

ARE, antioxidant response element; Cys, sensor cysteine residues; Ub, ubiquitin.
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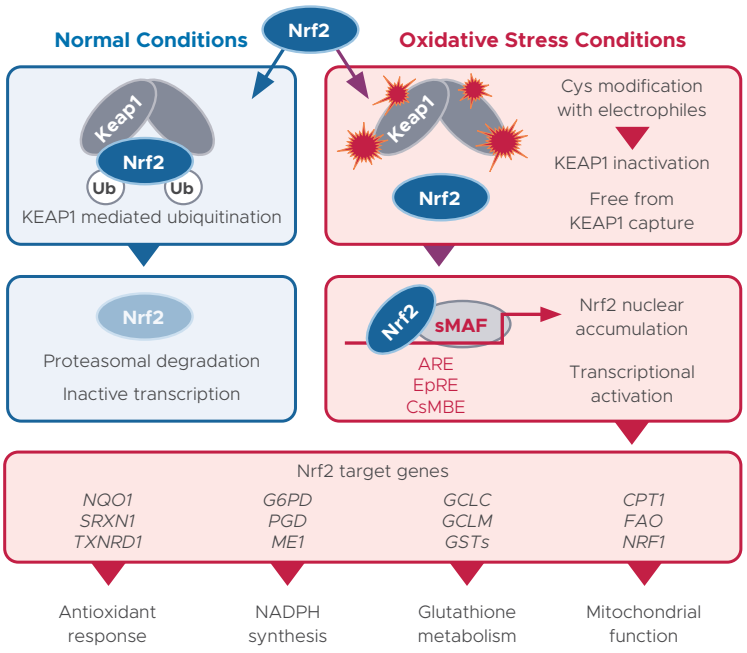
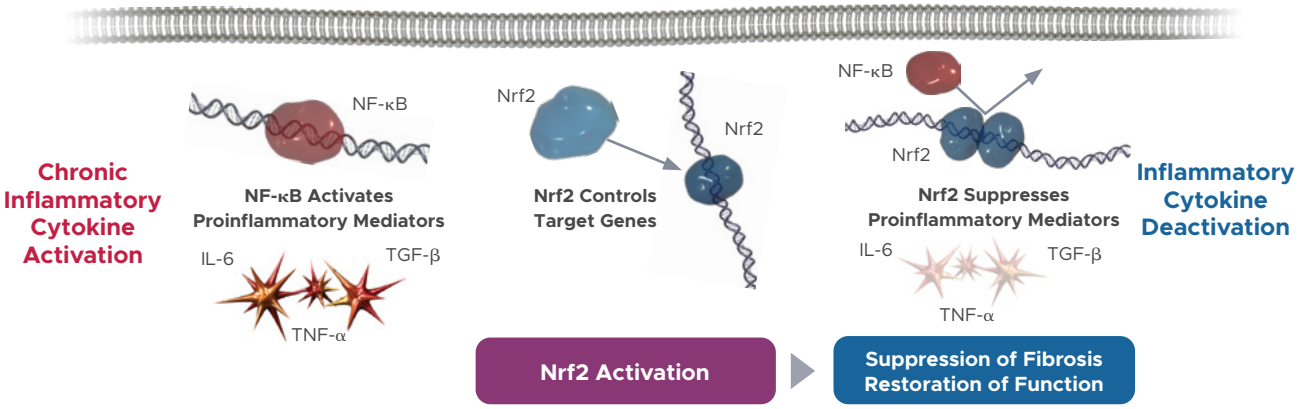
Molecular Mechanism of Nrf2[59]



Resolution of the natural immune response to kidney injury is orchestrated by Nrf2, but CKD is associated with impaired activation of the Nrf2-Keap1 system.[5,60] Keap1 is a cytosolic inhibitor that retains Nrf2 in the cytoplasm until activated by disease triggers.[9,55,61] Nrf2 accumulates in the nucleus and helps resolve inflammation through direct and indirect mechanisms.[56] Nrf2 directly induces transcription of anti-inflammation genes and suppresses proinflammatory gene transcription.[56] By inhibiting inflammation, Nrf2 is associated with suppressing the proinflammatory transcription factor, NF-κB, and subsequent proinflammatory cytokine production (**Figure 6**).[58] Nrf2 also indirectly counteracts inflammation involving ROS/reactive nitrogen species (RNS) and inhibits migration and infiltration of immune cells.[56]

Figure 6. Nrf2 Activation Can Suppress Fibrosis and Restore Kidney Function[47,58,62,63]



Nrf2 directly controls the expression of proinflammatory mediators and facilitates the resolution of inflammation leading to suppression of fibrosis and restoration of function.

INFLAMMATION IS THE FINAL COMMON PATHWAY OF CKD PROGRESSION, IRRESPECTIVE OF DISEASE ETIOLOGY

Structural and Functional Change in the Kidney[31]

Continuous exposure of the kidney to harmful stimuli causes a persistent local inflammatory state.[40,41] This is due in part to an inadequate cellular Nrf2 response, which normally counters proinflammatory cytokine gene expression mediated by NF-κB, and a resultant failure to resolve inflammation.[56,64] The anti-inflammatory activity of Nrf2 involves key points of crosstalk with NF-κB signaling.[56] Both the NF-κB and Nrf2 transcription factors require and compete for the coactivator CBP/p300, which is an acetyltransferase enzyme that directly binds to these transcription factors and acetylates their respective DNA-binding capacities.[56] Persistent inflammation causes increased NF-κB activity, which depletes the amount of CBP/p300 that is available for Nrf2, hence reducing its capacity to regulate transcription of its target genes.[56] NF-κB is also able to bind and translocate Keap1 to the nucleus, which favors Nrf2 ubiquitination and degradation in this cellular compartment.[56,63,64]

Chronic inflammation is a multifactorial process, promoting disease progression and involving many proinflammatory mediators.[48] Combined, the proinflammatory transcription factors activated within, and the cytokines and chemokines produced by many cells within the kidney perpetuate the inflammatory environment, leading to structural and functional repercussions in kidney tissue.[31]

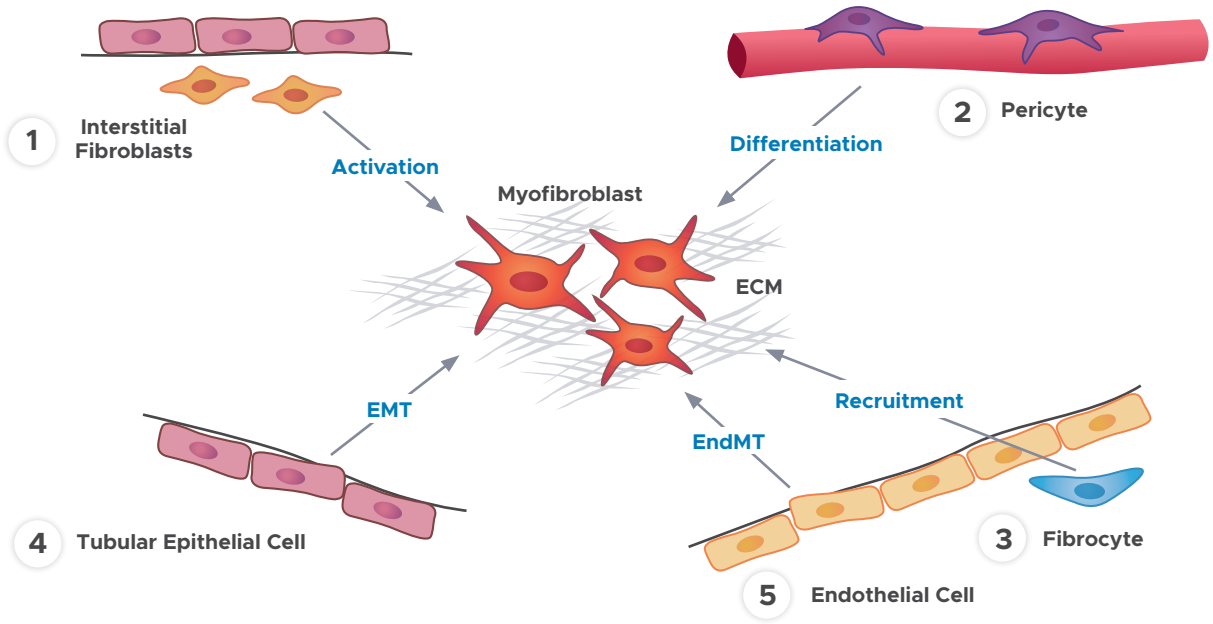
Fibrosis causes irreversible loss of kidney function:

- Tubules downstream of glomeruli that stop functioning become atrophic and fibrotic[66-68]
- Glomeruli upstream of tubules that become nonfunctional become atrophic and fibrotic[66-68]
- Interstitial fibrosis and tubular atrophy is a better predictor of long-term prognosis than severity of glomerular disease in almost all progressive forms of glomerular disease[66]
- Fibrosis arising from chronic inflammation disrupts normal kidney function, eventually leading to ESKD requiring dialysis or transplantation[4,31,48,69-71]
- Strategies are needed to target chronic inflammation before conversion to ESKD in Alport syndrome

Inflammation Activates Various Cells and Processes Leading to Fibrosis

In Alport syndrome, the genetic mutations, which result in compromised composition of the GBM, renders the glomerulus susceptible to damage caused by biomechanical stress and triggers an inflammatory response leading to fibrosis.[30,31] Myofibroblasts play a key role in the development of fibrosis and a variety of molecular signals and processes contribute to their formation and activation. These include the activation of endothelial cells by vascular endothelial cell growth factor released from injured interstitial fibroblasts, kidney pericytes, fibrocytes, tubular epithelial cells, and endothelial cells, which produce PDGF and TGF-β (**Figure 7**).[72-74] Pericytes subsequently detach from endothelial cells and proliferate, spread, and migrate into the interstitium. Prolonged injury causes unstable vasculature, capillary loss, interstitial matrix expansion, and contraction of tissue architecture resulting in a phenotypic conversion to myofibroblasts.[72] Subsequently, myofibroblasts synthesize ECM components, leading to excessive collagen accumulation and fibrosis.[30,31] The consequent fibrosis results in irreversible loss of kidney function.[4,31,48,66-71]

Figure 7. Diverse Origins and Processes Involved in Myofibroblast Formation[73]



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SUMMARY

In summary, inflammation is a final common pathway of CKD progression regardless of disease etiology.[31] Strategies are needed to target Alport syndrome pathogenesis earlier before progression to ESKD. Current CKD standards of care to reduce CKD progression, including BP control and RAAS inhibitor use, are not sufficient to prevent progression of CKD.[13-16] Despite a large variety of initial insults, progression of CKD is a result of multiple distinct but related pathways reflecting abnormal cellular metabolism and chronic inflammation. Progressive CKD is associated with diminishing GFR, which is caused by the expression of multiple genes in interconnected pathways.[32] One of the most interconnected pathways is the Nrf2-mediated, oxidative stress response pathway, which is the central connection between these metabolic and inflammatory pathways.[32] Nrf2 is reduced in CKD and is an attractive target for novel therapies, being a key regulator of inflammation in CKD that is able to suppress inflammation, reduce fibrosis, and acutely and chronically restore kidney function.[5,32,55,61]

ACRONYMS

AASK, African American Study of Kidney Disease and Hypertension
ACEi, angiotensin-converting enzyme inhibitor
ARB, angiotensin receptor blockers
ARE, antioxidant response element
BP, blood pressure
CI, confidence interval
CKD, chronic kidney disease
COX-2, cyclo oxygenase 2
CREDENCE, Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation
CRF, chronic renal failure
CTGF, connective tissue growth factor
CV, cardiovascular
Cys, sensor cysteine residues
DN, diabetic nephropathy
ECM, extracellular matrix
eGFR, estimated glomerular filtration rate
EMT, epithelial-mesenchymal transition
ESCAPE, Effect of Strict Blood Pressure Control and ACE Inhibition on the Progression of CRF in Pediatric Patients
ESKD, end-stage kidney disease
GBM, glomerular basement membrane
GFR, glomerular filtration rate
HR, hazard ratio
HTN, hypertensive nephropathy
IDNT, Irbesartan Diabetic Nephropathy Trial
IFN-α, interferon-alpha
IgAN, IgA nephritis
IL-6, interleukin-6
Keap1, kelch-like ECH-associated protein 1
K_f, filtration coefficient
LN, lupus nephritis
MAP, mean arterial pressure
MCD, minimal change disease
MDRD, Modification of Diet in Renal Disease
MGN, membranous glomerulonephritis
NF-κB, nuclear factor kappa beta
NIDDM, noninsulin-dependent diabetes mellitus
NO, nitric oxide
Nrf2, nuclear factor erythroid 2–related factor 2
PDGF, platelet-derived growth factor
RAAS, renin-angiotensin-aldosterone system
RENAAL, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan
RNS, reactive nitrogen species
ROS, reactive oxygen species
SE, standard error
SGLT2i, sodium-glucose cotransporter-2 inhibitor
SOC, standard of care
TGF-β, transforming growth factor-beta
TNF, tumor necrosis factor
TNF-α, tumor necrosis factor-alpha
Ub, ubiquitin
US, United States

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