A major population disparity in CKD has long been documented in United States registry data. Therefore, in areas where sleeping sickness is endemic, mutant APOL1 homozygotes possess an advantage provided by even a single parental G1 or G2 allele against the pathogenicity of Trypanosoma brucei rhodesiense. Among whites age 40–49, the rate (per million population adjusted for gender) rose 61 percent between 2000 and 2011, to 28.1. Approximately 30% of African Americans carry either the G1 or G2 APOL1 allele and 13 % carry the dreaded combination G1/G2.

Approximately 8.2% of African Americans face an 8.2% lifetime risk for ESRD, and are 3.5-fold more likely to require RRT, with a disproportionate number on dialysis compared with transplantation as a result of poorer transplantation rates and outcomes and the development of progressive adult nephropathy.

The discovery of two common variants located on Chromosome 22 that encode the APOL1 protein provided an explanation for much of the increase in the rates of ESKD in patients of African ancestry in the USA, which had been a previously mysterious aspect of health disparities research. The rate for African Americans of the same age fell 5.8 percent to 304 per million in 2000 and 2011, to 28.1. The importance role of genetic factors was suggested by the epidemiologic observations of Freedman et al., who observed that many patients of African ancestry with ESKD had close relatives who had also been treated with dialysis.

Two major coding variants associated with renal disease have been identified: the G1 allele, consisting of two amino acid substitutions, and the G2 allele, defined by a 2-nucleotide deletion at the C terminus.

The high frequency of these RVSs (renal risk variants) in African-Americans and the Yoruba (Nigeria) and their absence in Europeans and Asians suggest that positive selection increased the frequency of these APOL1 RVSs in Africa.

Evolution of the primate trypanolytic factor APOL1

- This landmark study examined the trypanolytic activity of APOL1 risk variants in vivo.
- G2-expressing mice exhibited a prolonged and increased survival compared with G1-expressing mice.
- The rate for African Americans of the same age fell 5.8 percent to 304 per million population — nearly 11 times greater than that of their white counterparts.
- Among whites age 40–49, the rate (per million population adjusted for gender) rose 61 percent between 2000 and 2011, to 28.1.
EPIDEMIOLOGIC STUDIES OF APOL1 NEPHROPATHY
The OR for kidney disease in individuals with two APOL1 risk alleles (G1/G1, G1/G2 and G2/G2) among different studies

Population-based studies (Dallas Heart Study)

- This cohort study (1825 African Americans and 1042 European Americans) confirmed the APOL1 risk association and reported that non-diabetic African Americans with two APOL1 risk alleles exhibited a 3-fold increase in the risk of microalbuminuria and a 4-fold increase in CKD (eGFR < 60 mL/min per 1.73 m²) (OR 3.9).
- These associations were relatively more modest (i.e., the ORs were lower) than those observed in the original case-control studies, likely because the patients exhibited less severe disease in the population studies.


Longitudinal studies reveal progression factors in individuals with APOL1-associated CKD

- This population study of patients with a 25-year follow-up in the Atherosclerosis Risk in Communities Study (ARIC study) reported that individuals with two-risk alleles of APOL1 demonstrated a 1.49-fold increased risk for the development of CKD compared with those with one or zero-risk alleles.
- Among those who had CKD, those with two-risk alleles exhibited a 2.2-fold increased risk of progression to ESKD compared with participants with zero or one-risk allele over a median follow-up period of 19.7 years.
- These more modest associations are similar to the results obtained in the Dallas Heart Study but have the advantage of >25 years of follow-up data.


The African American Study of Kidney Disease and Hypertension (AASK) and the Chronic Renal Insufficiency Cohort (CRIC) study

- Both longitudinal CKD cohorts, have confirmed the strong association of APOL1 risk variants with progressive kidney disease.
- In the AASK study, 58% of the patients with two-risk variants exhibited a doubling of serum creatinine or ESKD, irrespective of the medication that was administered or the level of blood pressure control that was achieved.


The CRIC study

- Among the 2955 patients for whom adequate genotyping data were available, 48% were African Americans, and 45.5% had diabetes.
- African Americans with two APOL1 risk alleles exhibited a more rapid decline in eGFR and higher composite renal outcomes than African Americans with zero or one-risk allele or European American patients, regardless of diabetes status.

Systemic lupus erythematosus

- Larsen et al. described the association between two APOL1 risk variants and collapsing glomerulopathy.
- It is unclear whether the finding of collapsing glomerulopathy in cases of lupus represents a distinct lupus nephritis variant or a coincidental association between these two diseases in a patient population prone to developing both diseases.
End-Stage Renal Disease in African Americans With Lupus Nephritis Is Associated With APOL1

- Freedman et al. have also recently demonstrated the strong impact of the presence of two APOL1 risk variants on the development of ESKD in patients with lupus and a more rapid progression of kidney disease with a shortened time to the start of dialysis.
- The difference of 2 years in the median time from diagnosis of ESRD to development of ESKD represents an important 32% shorter median time from ESRD onset to ESKD development in patients with, versus those without, the risk GLU/L2 genotype.

Increased Burden of Cardiovascular Disease in Carriers of APOL1 Genetic Variants

- Individuals with two APOL1 risk alleles exhibit a 2-fold increased risk of cardiovascular disease events compared with individuals with no risk alleles, irrespective of the presence of clinical kidney disease.
- These studies suggest the endothelium is a target for APOL1-mediated injury, with accumulation of subendothelial proteins or the formation of intima as a consequence of injury or in concert to mediate injury.
- The endothelium regulates the activation and inhibition of coagulation and fibrinolysis. In healthy individuals, the endothelium serves as an anticoagulant, unless injury exposes subendothelial tissue factor, which triggers activation of the coagulation pathway.

Diabetes mellitus

- The original case-control studies, as well as the Dallas Heart Study, demonstrated a lack of association between kidney disease in diabetic patients and the APOL1 risk variants.
- However, the ARIC study reported an association of two risk variants of APOL1 in diabetic patients with CKD.
- In their publication, Parsa et al. analyzed APOL1 loci in the CRIC study and reported a strong association between kidney disease in diabetic patients and its progression.
- APOL1 is involved in autophagy, a lysosomal degradation process that removes damaged organelles and protein aggregates to maintain intracellular homeostasis in times of stress.
- The autophagy pathway is essential for podocyte integrity and is inherently dysregulated (suppressed) in cases of diabetic kidney disease.
- Speculations are that the effects of APOL1 protein on autophagy may be limited in the setting of DKD.
- Is there a possible association of APOL1 risk alleles with nephropathy in diabetic subjects that may not represent diabetic nephropathy per se, but rather APOL1-associated nephropathies (e.g., focal segmental glomerulosclerosis)?

Circulating APOL1 versus kidney-expressed APOL1

- APOL1, a 43 kDa protein that belongs to the apolipoprotein family, is the only member of this family that produces a secreted protein, which is bound to circulating HDL particles.
- APOL1 is expressed in various organs, including the kidney.
- APOL1 is involved in the autophagy pathway.
- One of the fundamental questions with regard to APOL1-associated kidney disease is whether the risk lies in the circulating APOL1 or the APOL1 that is expressed in the kidney.

Kidney transplantation

- Reeves-Daniel et al. described the relationship between the APOL1 risk allele status of the donor kidney and transplant outcomes and suggested that the risk for kidney allograft outcomes is associated with the two risk allele status of the donor kidney.
- Kidneys from deceased African-American donors that harboured two APOL1 risk variants failed more rapidly after kidney transplantation than kidneys from donors with zero or one risk variant.

There are some concerns to this study because the ‘deceased’ in this case was kidney transplant ‘dead’ not deceased donor kidney transplant. The study also included a rather small number of deceased donor kidney transplant cases, which may have introduced selection bias.

Renewed attention has been directed to cause experts in the field to suggest that all deceased African American kidney donors should be screened for APOL1.
Kidney Transplantation

- Lee et al. reported that the APOL1 two risk allele status of the kidney transplant recipient does not impact the kidney transplant outcome.
- Taken together, these two studies suggest that the APOL1 risk allele association is mediated by the gene product (isoform that is endogenously expressed within the kidney, not the circulating APOL1).

Second hits

- Only a subset of individuals who carry two APOL1 risk alleles develops kidney disease.
- The lifetime risk for kidney disease in individuals with HIV infection, in the absence of antiretroviral therapy, is estimated to peak very early: 5% to 10% at age 40, whereas the lifetime risk for FSGS is 4%.
- A fundamental question remains: what are the contributing factors that either trigger progressive CKD or, conversely, protect individuals with two APOL1 risk alleles from CKD.

Nephrotropic viral infection

- A leading candidate for a second hit that can explain the genetic epidemiologic observations is human nephrotropic viral infection.
- The prototype for a viral second hit is HIV.
- The lifetime risk of kidney disease in patients with two APOL1 risk alleles has been shown to rise from < 5 to > 50% in the presence of untreated or undertreated HIV infection.
- Successful anti-retroviral therapy, which reduces the yearly decline in the eGFR, is thought to be one of the most important pieces of evidence indicating that HIV represents an important, pathogenic factor.
- Fine et al. described the utility of APOL1 risk variants for the prediction of histology in non-HIVAN forms of HIV kidney disease, which are increasingly more common and comprised >70% of a recent series of patients.
- Patients with two risk variants were more likely to exhibit focal segmental sclerosis (76%) and hypertensive kidney disease (10%).

MECHANISMS OF KIDNEY INJURY

- Another piece of evidence against the role of circulating APOL1 was demonstrated recently in in vivo model.
- The authors engineered APOL1 variants and expressed them in mice by hydrodynamic gene delivery (HGD).
- Increased hepatic necrosis was observed in mice that expressed the APOL1 risk variants, with a more pronounced effect observed with the G1 variant.
- The removal of the signal peptide did not reduce hepatic necrosis significantly, whereas the deletion of the helix at C terminus significantly decreased liver injury, suggesting that cellular toxicity was not related to the secretion of APOL1.

Association of APOL1 variants with mild kidney disease in the first-degree relatives of African American patients with non-diabetic end-stage renal disease

- A family study of first-degree relatives of African American patients with non-diabetic ESKD in the Natural History of APOL1–Associated Nephropathy Study reports that relatives of African Americans with non-diabetic ESKD are enhanced for APOL1 risk variants.
- After adjustment, two APOL1 risk variants only weakly predicted overt proteinuria and an eGFR < 60 mL/min per 1.73 m² [61].
- Other environmental 'second hits', most prominently viruses, are therefore necessary to explain the gaps between lifetime risks in individuals with the same genetic background.

Non-HIV Viral Infections as Second Hits

JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy

- Accounting for APOL1, results demonstrated reduced rates of albuminuria and kidney disease in African Americans with active JC urinary tract replication.
- JC polyomavirus is typically not nephrophytis-associated in kidney transplantation, and most individuals only develop urinary tract infections with a single polyomavirus strain (either BK or JC but not both).
- Infection with one virus seems to inhibit replication of the other, thereby protecting subjects with JC polymavirus from nephropathy.
- A report from Brazil also showed significantly higher rates of urine JC polyomavirus in healthy controls compared with subjects with ESKD.
- Brazilians have varying degrees of recent African ancestry, and these results are broadly consistent with the observation that African Americans without renal disease more often have JC virus than African Americans with CKD.
- Isolated JC virus is also associated with lower rates of acute renal allograft rejection after transplantation, further supporting protective effects of JC virus on the kidney.
Interactions between genes

- Freedman and colleagues have recently reported several interactions between certain genes and APOL1.
- The most prominent effect was observed in podocin (NPHS2), as well as other genes that include serologically defined colon cancer 8 (SDCCAG8) and a genomic locus near the bone morphogenetic protein 4 gene (BMP4).
- The effects of the interaction of these genes and the degree to which these genes increase or decrease the OR of the APOL1 risk allele have recently been quantified.

Pathways leading from genetic susceptibility to clinical kidney disease

- The simultaneous association between elevated procoagulant (factor VIIIc) and anticoagulant (protein C) levels is somewhat perplexing.
- The association with factor VIIIc, which is synthesized predominantly in endothelial cells and considered to be a marker of endothelial cell dysfunction and vascular damage, is consistent with its procoagulant role.
- The association with protein C is more difficult to explain and does not easily fit within the second-hit theory.
- The protein C pathway is activated when thrombin binds to thrombomodulin on the endothelial cell surface. The thrombin-thrombomodulin complex activates protein C far more efficiently when protein C is bound to the endothelial cell protein C receptor (EPCR).

Hemostatic Factors, APOL1, and ESRD Risk: Another Piece of the Puzzle?

- Tin et al. advance the understanding of APOL1-associated kidney disease, by demonstrating a potential pathogenic role for hemostatic factors.
- Using the approximately 20-year follow-up of the Atherosclerosis Risk in Communities (ARIC) study, the authors examine the molecular links between hemostatic factors, APOL1 risk variants, and ESRD.
- The authors identify incident ESRD by linking to the US Renal Data System.
- They divided the 13,337 participants into three groups by APOL1 categorization: European Americans, APOL1 low-risk African Americans (zero or one risk alleles), and APOL1 high-risk African Americans (two risk alleles).
- The core finding of the study was a significant interaction between the APOL1 high-risk genotype and factor VIIIc and protein C in the development of ESRD.

Crosstalk between the inflammatory and coagulation pathways, whereby each system augments the other

- When APC is released from EPCR, it has anticoagulant properties, yet when it is transiently complexed with EPCR and PAR1 (Protease Activated Receptor-1), APC initiates intracellular signaling that confers antiapoptotic and anti-inflammatory protection.
- It has been demonstrated in animal models and in human tissues that the conversion of protein C to APC in the inflammatory states is impaired, which may be associated with a decreased expression of thrombomodulin and EPCR in endothelial cells.
- A new mechanism of protein C resistance was recently described, whereby an endothelial-derived phospholipase can modify EPCR, which loses its ability to bind APC, thereby rendering EPCR unable to mediate APC’s cytoprotective activities.
- As the phospholipase expression can be enhanced by inflammation, this novel phenomenon of “cellular APC resistance” may explain the current finding of elevated protein C and increased ESRD risk.

Cellular APC resistance

- The components and reactions of the protein C pathway

(From: Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway, 2003 June)

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