JCI The Journal of Clinical Investigation

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J Clin Invest. 2021;131(22):e154676. https://doi.org/10.1172/JCI154676.

Commentary

APOL1 G1 and G2 variants are established risk factors for nondiabetic kidney disease. The presence of two APOL1 risk variants in donor kidneys negatively impacts kidney allograft survival. Because of evolutionary pressure, the APOL1 risk variants have become common in people from Africa and in those with recent African ancestry. APOL1 risk variant proteins are expressed in kidney cells and can cause toxicity to these cells. In this issue of the *JCI*, Zhang, Sun, and colleagues show that recipient APOL1 risk variants negatively affect kidney allograft survival and T cell–mediated rejection rates, independent of donor APOL1 genotype or recipient ancestry. The authors provide evidence that APOL1 risk variants play an immunomodulatory role in T cells and NK cells in the setting of kidney transplantation. These findings have important clinical implications that require further investigation.

APOL1 risk variants in kidney transplantation: a modulation of immune cell function

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APOL1 G1 and G2 variants are established risk factors for nondiabetic kidney disease. The presence of two APOL1 risk variants in donor kidneys negatively impacts kidney allograft survival. Because of evolutionary pressure, the APOL1 risk variants have become common in people from Africa and in those with recent African ancestry. APOL1 risk variant proteins are expressed in kidney cells and can cause toxicity to these cells. In this issue of the *JCI*, Zhang, Sun, and colleagues show that recipient APOL1 risk variants negatively affect kidney allograft survival and T cell–mediated rejection rates, independent of donor APOL1 genotype or recipient ancestry. The authors provide evidence that APOL1 risk variants play an immunomodulatory role in T cells and NK cells in the setting of kidney transplantation. These findings have important clinical implications that require further investigation.

APOL1 and kidney risk

Two genome sequence variants in the APOL1 gene on chromosome 22q strongly associate with an increased risk of nondiabetic kidney diseases such as focal segmental glomerulosclerosis, hypertension-related nephropathy, HIV-associated nephropathy, sickle cell nephropathy, and lupus-associated nephropathy (1–5). These risk variants are known as G1 and G2 and are nearly always found linked in the *trans* phase when both are present. G1 results in a recombinant APOL1 protein with two point mutations (S342G and I384M), and G2 results in a recombinant protein with two amino acid deletion mutations (N388_Y389del). These variants are found only in populations with recent African ancestry and at high frequency in areas where *Trypanosoma brucei rhodesiense* and *T.b. gambiense* infections are common. Homozygosity or compound heterozygosity (G1/G1, G1/G2, or G2/G2) for these variants increases the risk of nephropathy. The presence of two risk variants conferred seventeen-fold higher odds (95% CI, 11–26) for focal segmental glomerulosclerosis and twenty-nine-fold higher odds (95% CI, 13–68) for HIV-associated nephropathy (2). Thirteen percent of African Americans (AAs) possess two risk variants (considered a high-risk genotype), and 87% have APOL1 low-risk genotypes (~39% G0G1/G0G2; ~48% G0G0; ref. 1). Although the strength of association with kidney disease is high, the majority of AAs with two risk variants do not develop chronic kidney disease, suggesting that other factors are required for disease to occur (6, 7).

APOL1 function and the kidney

APOL1 is a lethal trypanolytic factor, and this mechanism has been well studied. *T.b. rhodesiense* and *T.b. gambiense* have developed resistance to wild-type APOL1, resulting in increased acute infections in East and West Africa, respectfully (8, 9). The G1 variant reduces the risk of *T.b. gambiense* infection, and the G2 variant reduces the risk of *T.b. rhodesiense* infection (1, 10). The G1 and G2 variants are presumed to have reached high frequencies in West Africa as a result of positive selection (1). There have been many studies investigating the action of APOL1 in mammalian cells and in the kidney (11–14). APOL1 mRNA and protein have been found in human podocytes, glomerular endothelial cells, and renal tubular cells (15, 16). Expression of the G1 or G2 APOL1 variants in mouse podocytes produced proteinuria, podocyte effacement, glomerulosclerosis, and interstitial fibrosis, all features found in patients with APOL1-associated focal segmental glomerulosclerosis (17). Disease severity in this model correlated with variant protein expression levels. In most cases, two risk variants are required for toxicity, and it is thought that a gene dosage effect increases risk variant APOL1 protein expression (18–20). A certain threshold level of plasma membrane expression of risk variant APOL1 protein causes podocyte toxicity (21). Interestingly, it is also possible that the immune system plays a role in APOL1-associated nephropathy. Individuals with JC polyoma viruria and APOL1 risk variants appear to be less likely to develop nephropathy (22). Presumably, clearance of JC polyoma virus indicates a heightened immune response that drives APOL1 risk variant expression in these patients relative to expression in patients with persisting JC viruria.

APOL1 and kidney transplant outcomes

Given our current understanding of APOL1 risk variants, it would be reasonable to assume that APOL1 risk variants impact kidney transplant outcomes through a donor mechanism. Reeves-Daniel et al. studied 136 kidney transplants from 106 AA donors (23). Their multivariate model accounting for the donor's African ancestry, expanded donation criteria, as well as the recipient's age and sex, HLA mismatch, cold isch-

▶ **Related Article: https://doi.org/10.1172/JCI146643**

Conflict of interest: The author has declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2021;131(22):e154676. https://doi.org/10.1172/JCI154676.

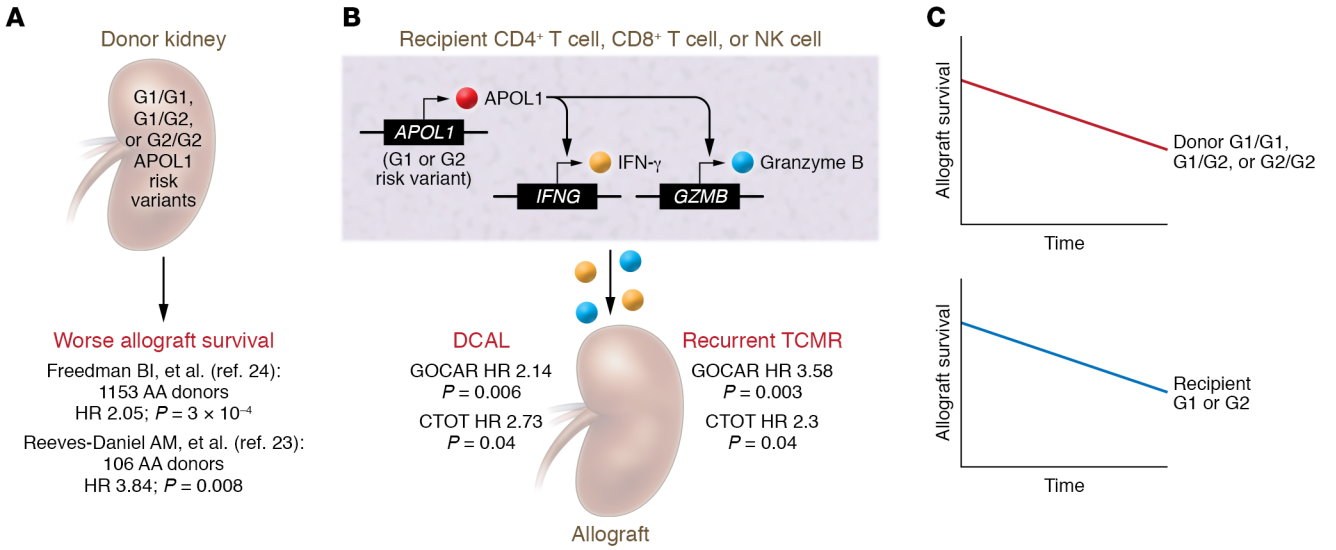

Figure 1. APOL1 risk variants influence kidney transplantation outcomes via intrinsic and extrinsic mechanisms. (**A**) Previous studies showed worse allograft survival when donors had two APOL1 risk factors (23, 24). Within the kidney, APOL1 risk variants act intrinsically, causing podocyte toxicity in a dose-dependent manner. (**B**) Zhang, Sun, and colleagues showed that when the recipient had APOL1 G1 or G2, there was an association with death-censored allograft loss and recurrent T cell–mediated rejection. APOL1 risk variants act through an extrinsic, immune-mediated pathway, damaging the kidney through activation of T and NK cells (28). DCAL, death-censored allograft loss; TCMR, T cell–mediated rejection. (**C**) APOL1 risk variants negatively influence allograft survival with time when kidney transplant recipients carry the risk variants or receive a transplant from an APOL1 donor.

emia time, and panel-reactive antibodies revealed that graft survival was significantly shorter in donor kidneys with two APOL1 risk variants (HR 3.84; P = 0.008; Figure 1). In this study, the recipient's race was not included in the fully adjusted model. Subsequent larger studies reported similar findings. Freedman et al. genotyped 478 kidney transplants from AA donors (24). They reported a significant negative effect on time to allograft failure for donor kidneys with two APOL1 risk variants (HR 2.00; P = 0.03). This multivariate analysis was adjusted for HLA mismatches, cold ischemia time, the donor's age, the recipient's age and sex, and the recipient's race. Freedman had previously studied 675 kidney transplants from AA donors and reported that shorter allograft survival was associated with two donor APOL1 risk variants (HR 2.26; P = 0.001; ref. 25). A combined analysis of these 675 plus 478 kidney transplants from AA donors shows a similar result (HR 2.05; $P = 3 \times 10^{-4}$; Figure 1 and ref. 24).

It is important to note that only one study, by Lee et al., specifically addresses the question of recipient APOL1 risk variants and kidney transplant outcomes (26). Lee and colleagues performed a retrospective study of 119 AA kidney transplant recipients originally enrolled in a study of β3 integrin variants and acute rejection (27). Approximately half of these recipients carried two APOL1 risk variants. When controlling for age and diabetes mellitus, they found no statistically significant difference in allograft survival for recipients with two APOL1 risk variants compared with the low, zero (0-risk), or single (1-risk) APOL1 risk variant groups (HR 0.96, 95% CI 0.61–1.49, P = 0.840). Unadjusted allograft survival and allograft survival censoring for patient death (death-censored allograft survival) showed no difference between the 0-risk, 1-risk, or 2-risk variant groups.

Recipient APOL1 risk variants and kidney transplantation

In this issue of the *JCI*, Zhang, Sun, and colleagues examined whether recipient APOL1 G1 or G2 risk variants impacted kidney transplant outcomes independently of donor APOL1 risk variants (28). Using data from the Genomics of Chronic Allograft Rejection (GOCAR) study (29), the authors found that the number of recipient APOL1 G1/G2 risk variants (R-nAPOL1) was associated with an increased risk of death-censored allograft loss, independent of the recipient's ancestry and the donor's APOL1 genotype (HR = 2.14; P = 0.006). This association was also found in a subgroup of AA and Hispanic recipients (HR = 2.36; P = 0.003). R-nAPOL1 was also associated with recurrent T cell–mediated rejection (HR = 3.58; P = 0.003). These findings were validated using data from the Clinical Trials in Organ Transplantation-01/17 (CTOT) study (30). The GOCAR discovery data set included 385 donor-recipient pairs, and the CTOT validation data set included 122 pairs. In both cohorts, genome-wide genotyping was performed (excluding the MHC region) to estimate the proportion of African ancestry (pAFR). Using this approach, the researchers applied a quantitative metric to adjust for ancestry in their analysis of R-nAPOL1 and transplantation outcomes. The findings by Zhang, Sun, and co-authors are notable, because they are the first to show that recipient APOL1 risk variants associate with a risk of allograft loss when censoring for patient death (death-censored allograft loss). This association is contrary to the findings of the study by Lee et al., in which paired APOL1 genotyping of donors was not performed (26). In fact, most studies of APOL1-associated outcomes in transplantation did not perform complete paired donor-recipient APOL1 genotyping (23–25). Another finding by Zhang, Sun, and colleagues was the additive effect of each risk allele on allograft survival, as shown by Kaplan-Meier survival curves stratified by R-nAPOL1 (28).

As described above, evidence to date suggests that APOL1 risk variants act through toxic effects in kidney cells. Zhang, Sun, and co-authors provide some mechanistic evidence for the modulation of immune cell function by APOL1 risk variants. The authors used four strategies to investigate immune cell function. First, they used the DICE (Database of Immune Cell Expression, quantitative trait loci [eQTLs], and epigenomics) database of immune cell gene expression in healthy individuals (31). From this database, they examined cell-specific gene expression in 22 AA individuals, five of whom carried at least one APOL1 variant. In individuals with a risk variant, they found an immune activation signature in $CD4^+$ T cells and cytotoxic $CD56^{dim}$ NK cells. Second, single-cell RNA-Seq revealed upregulation of similar immune activation pathways in $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in two individuals with at least one APOL1 risk allele compared with two AA individuals with the G0/G0 genotype. Third, differential expression of immune activation pathways associated with one or two APOL1 risk alleles were found by bulk RNA-Seq of peripheral blood from 60 pre-transplant patients. Finally, Zhang, Sun, and colleagues showed that APOL1 mRNA and protein are expressed in peripheral blood cells (28).

Summary and future directions

Zhang, Sun, and co-authors report an association between recipient APOL1 risk variants and kidney allograft failure, contrary to previous findings and assumptions. The authors also found an association between APOL1 risk variants and T cell–mediated rejection, which may have a greater impact in clinical practice (28). For example, patients with APOL1 risk variants may require more frequent surveillance and more intense immunosuppression. The ongoing APPOLLO study aims to genotype 2600 AA kidney donors (32). If many of the study recipients are genotyped for APOL1 risk variants, this large study will help to clarify the relevance of recipient APOL1 risk variants in the setting of kidney transplantation. From a mechanistic perspective, Zhang, Sun, and colleagues report data showing that APOL1 risk variants may alter immune cell function, in particular T cell and NK cell function (28). However, the specific mechanisms driving these changes remain to be elucidated. Regardless, this study by Zhang, Sun, and co-authors (28) suggests that APOL1 risk variants influence kidney transplantation outcomes by mechanisms both intrinsic and extrinsic to the kidney (Figure 1). This study highlights the complexity of the relationship between APOL1 and kidney disease. It is important to note that the mechanism driving recipient-related APOL1 pathology was not thoroughly defined in this study. Furthermore, these findings must be replicated by future studies.

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