Genetic Inhibition of *APOL1* Pore Forming Function Prevents APOL1 Kidney Disease

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INTRODUCTION

•*APOL1* high risk variants are associated with CKD progression to ESRD for all forms of nondiabetic kidney disease as well as with acute kidney injury.

•*APOL1* high risk variants affect kidney transplant outcomes. Allografts from *APOL1* high-risk donors have short allograft survival and donors with *APOL1* high risk genotype are at risk of kidney disease..

•However not all patients with a high-risk genotype will develop kidney disease. Gene-gene interactions and gene environment interactions are considered as second hits.

We hypothesize that in humans the presence of *APOL1* p.N264K could modify the risk of CKD, ESKD and other renal phenotypes observed in patients with an *APOL1* high risk genotype

METHODS

•**Design:** cross-sectional analyses in 121,492 African ancestry participants from the MVP. Replication: Vanderbilt BioVU (n=14,386) and in the NIH All of US (n=15,320).

•**Exposures** *APOL1* high-risk group (2RVs) with and without *APOL1* p.N264K. Comparison to *APOL1* low-risk group (1 or 0 risk variants) with and without p.N264K were also performed, and an additive interaction was tested.

•**Main Outcomes** The co-primary outcomes were CKD and ESKD among non-diabetic patients.

•**Analytical approach:** Sequential logistic regression models.

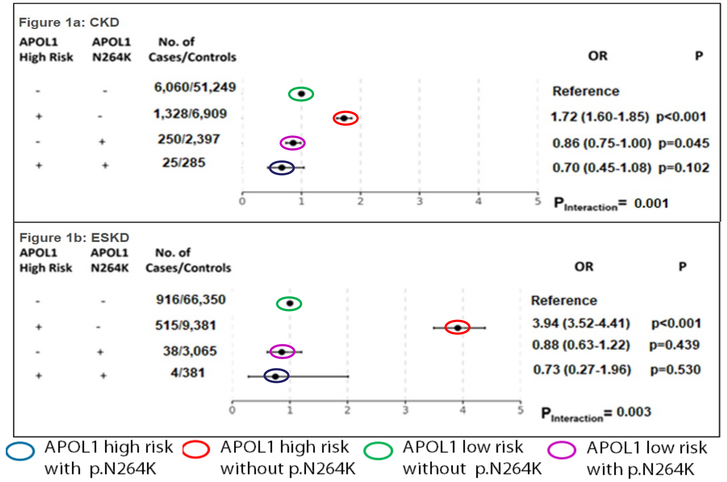
•**Functional genomics studies** were completed by overexpressing APOL1 G1/G2 with and without p.N264K in human podocytes and HEK cells.

RESULTS

Table 1. Clinical characteristics of the study cohort.

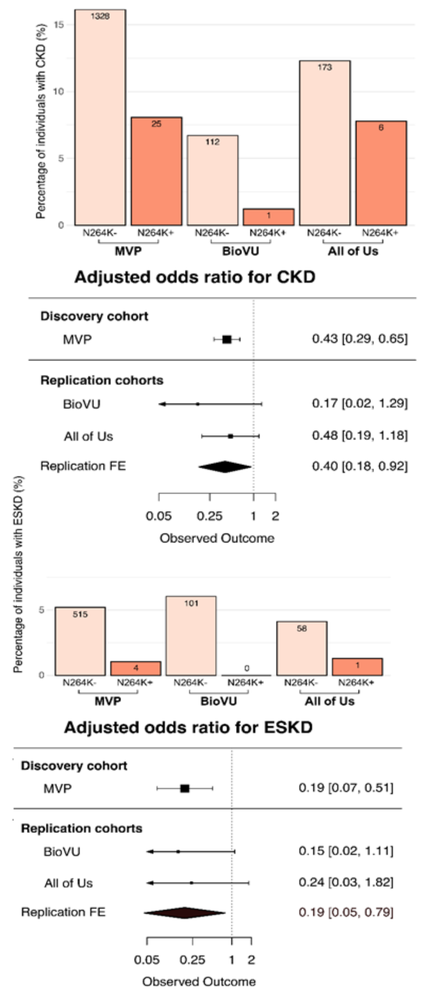
	Absence p.N264K alleles		Presence (≥1) p.N264K alleles	
	APOL1 Low Risk	***APOL1* High Risk**	*APOL1* Low Risk	***APOL1* High Risk**
Characteristics	N=101216	N=15022	N=4672	N=582
Age, median [IQR], years	59.0 [51.0;66.0]	59.0 [51.0;66.0]	59.0 [51.0;66.0]	60.0 [53.0;66.0]
Male, n, (%)	87266 (86.2%)	12922 (86.0%)	4059 (86.9%)	509 (87.5%)
eGFR, ml/min/1.73m2, median [IQR]	87.0 [71.4;103]	84.5 [69.0;101]	86.7 [71.6;102]	88.5 [73.1;103]
Diabetes, n, (%)	33950 (33.5%)	5126 (34.1%)	1569 (33.6%)	197 (33.8%)
Hypertension, n, (%)	67652 (66.8%)	10447 (69.5%)	3124 (66.9%)	403 (69.2%)
Systolic BP, mmHg, median [IQR]	130 [120;139]	130 [120;140]	130 [120;139]	131 [120;140]
Diastolic BP, mmHg, median [IQR]	79.0 [72.0;86.0]	79.0 [72.0;86.0]	79.0 [72.0;86.0]	79.0 [72.0;86.0]
RAAS inhibition	40480 (40.0%)	6201 (41.3%)	1877 (40.2%)	234 (40.2%)
Kidney Disease				
CKD, n, (%)	15179 (15)	**2896 (19.3%)**	685(3.6%)	**71(12.2%)**
ESKD, n, (%)	2973 (2.94%)	**1054 (7.02%)**	136 (2.91%)	**14 (2.41%)**
Outpatient dipstick proteinuria >= 2+, n, (%)	5340 (7.69%)	**1117 (10.8%)**	218 (6.81%)	**26 (6.27%)**
Nephrotic syndrome, n, (%)	684 (0.68%)	**191 (1.27%)**	31 (0.66%)	**4 (0.69%)**
FSGS, n, (%)	83 (0.08%)	**53 (0.35%)**	3 (0.06%)	**1 (0.17%)**

Figure 1 Association of *APOL1* high-risk group and *APOL1* p.N264K allele with non-diabetic CKD and non-diabetic ESKD among MVP participants.



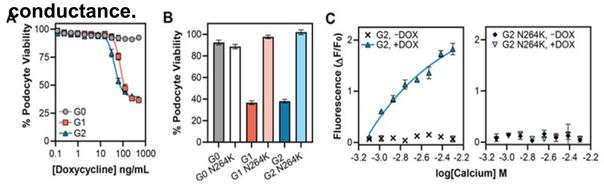
Logistic regression was used to evaluate the association of APOL1 high-risk and p.N264K allele genotype and ESKD. Odds ratios were adjusted for age, gender, 10 principal components of ancestry, body mass index, hypertension, and renin angiotensin aldosterone system blockade. .

Figure 2. Association of APOL1 high-risk Group with and without p.N264K allele with CKD & ESKD among MVP, BioVU and All of US participants.



RESULTS

Figure 3. APOL1 p.N264K blocks renal cytotoxicity by G1/G2 in podocytes and HEK cells through blocking ion channel conductance.



(A) APOL1 disease variants, G1 and G2, are toxic when overexpressed in human immortalized podocytes. APOL1 G0 overexpression does not affect podocyte cell viability. (B) The cytotoxicity of APOL1 G1 and G2 risk variants is attenuated by the N264K mutation (C) G2 APOL1 mediated calcium transit is blocked by the N264K mutation. Expression of APOL1 G2 or APOL1 G2 with p.N264K was induced with doxycycline and fluorescence was measured in the presence of increasing concentrations of calcium.

CONCLUSIONS

•In our study we have shown that in humans the presence of an N264K variant decreases the odds of CKD and ESKD in individuals with *APOL1* high risk mutations.

•In our functional genomics experiments *APOL1* p.N264K mutation impaired ion conductance and blocked the pore forming function of the *APOL1* high risk alleles.

•These observations provide the opportunity for personalized care in patients with *APOL1* high-risk genotypes.

•Pharmacologic inhibition that mimics this genetic mutation blocking the *APOL1* pore formation may treat AMKD.

•Large studies with longitudinal follow up like MVP may allowed further studies of gene*gene interactions, or gene environment interactions.

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