Supplementary Material

***APOL1* Genotype and Renal Function in African American Live Donors**

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METHODS:

Study Design and Oversight

We conducted an observational cohort study of donors who donated a kidney between 1993 and 2010 at two transplant centers in Detroit, MI. All donors provided written informed consent. The study protocols were approved by the institutional review board at both recruitment sites and genetic testing site i.e. National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (NIH). The study adhered to the *Declaration of Istanbul*.

Study Subjects

Live kidney donors. We identified 249 African American live kidney donors who were free from hypertension and diabetes prior to donation. Race was self-reported. Five of the 249 donors died during follow-up and were excluded. Of the remaining 244 donors, 136 (56%) consented to participate (Figure S1). Pre-donation serum creatinine values and age at time of donation were used to calculate baseline GFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[1]

At follow-up, blood pressure, height and weight were measured. Blood sample was collected to measure serum creatinine via enzymatic assay and a sample was sent to NIH for *APOL1* genotyping. A urine sample was collected to measure albumin and creatinine to calculate urine albumin to creatinine ratio (uACR). Five donors unable to come for a study visit, mailed a sample of their saliva for genotyping and relevant study information was abstracted from their medical records. All serum creatinine values spanning the period from 12-months after donation (to allow for renal compensation to occur) until last follow up were abstracted, and eGFR was calculated via the CKD-EPI formula using serum creatinine value and age at time of follow- up. Accuracy of CKD-EPI study equation is lower at GFR < 60 mL/min/1.73m2. We used abbreviated Modification of diet in renal disease (MDRD) study equation to estimate post-donation eGFR[2].

Donors were queried about ever receiving chronic dialysis or being transplanted, and when this occurred follow-up time was truncated at time of ESRD onset. Their last serum creatinine value prior to starting dialysis was used to compute eGFR. United Network of Organ Sharing (UNOS) was also contacted by each participating center to determine if any of their donors who had donated during study period (i.e. eligible for study) were on transplant wait list or received a kidney transplant. First-degree donor-recipient relationship was defined as a biological parent or child, or full sibling, as reported by the donor. If the donor was unrelated to the recipient, the medical chart was reviewed to see if they had a family history of ESRD in a first-degree relative.

Non-donor control subjects. A comparable group of African Americans were selected from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study.[3] We used techniques of restriction and matching to select a healthy comparison group of non-donors from the CARDIA cohort, described in detail in our previous paper.[4] After reviewing baseline records of 2,637 participants, 958 (36%) of CARDIA participants fulfilled the restriction criteria to be considered suitable for live kidney donation. The second step consisted of finding non-donors who matched donors with regards to baseline age, gender, systolic blood pressure, family history of ESRD in first-degree relative, APOL1 genotype, and duration of follow up. A total of 45 non-donors from 1985 CARDIA exam cohort were initially matched to 45 live kidney donors. The above process was repeated with 1995, 2000, and 2005 exam cohort to find suitable controls. At the end, 115 non-donors were successfully matched to 115 donors (Figure S2). Of the 21 donors excluded, 14 could not be matched for follow-up time. A total of 26 (23%) controls were used more than once but from different exam year and therefore for any given CARDIA exam year there were no duplicate controls.

Serum creatinine values from subsequent CARDIA exam years (after enrollment exam years) were used to calculate eGFR at various time points in follow-up via the CKD-EPI equation. Serum creatinine was measured by enzymatic assay. Information on need for chronic dialysis or transplant among CARDIA participants was also ascertained from their most recent follow-up interviews. Family history of ESRD in first-degree relative was also collected at last visit.[5]

APOL1 Genotyping. Three single nucleotide polymorphism in APOL1 were typed using Taqman assays. The G1 risk allele was defined by missense mutations at rs73885319 and rs60919145, and the G2 allele was the 6-bp deletion at rs71785313. APOL1 high-risk genotype was characterized as presence of ≥ 2 risk alleles (G1/G1, G1/G2 or G2/G2). APOL1 low-risk genotype was defined as 1 or no risk alleles.

Groups by APOL1 status. The donors with 0 and 1 APOL1 renal risk alleles were grouped together as low-risk genotype and those with 2 risk alleles are referred to as high-risk genotype. The non-donors were also grouped similarly based on number of APOL1 renal risk alleles.

Outcomes

The primary outcome for all study subjects was average annual change in eGFR from one year after cohort entry. We also examined the following secondary outcomes: 1) urinary albumin excretion estimated via spot uACR and microalbuminuria defined by a random uACR >30 mg/g (SI units: >3.0 mg/mmol); and 2) hypertension defined by a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or the use of antihypertensive medications. These outcomes were compared between donors with and without APOL1 high-risk genotype, and also between donor and matched non-donor controls.

Statistical analysis

Data are presented as mean (standard deviation) for normally distributed data and median [inter-quartile range] for non-parametric data. We assessed differences in baseline characteristics between groups using independent sample t-tests, Mann-Whitney U test, Fisher exact tests before matching; paired t-tests, Wilcox signed-rank test, McNemar's test or Mantel–Haenszel test, as appropriate after matching. We used the 'greedy matching' macro[6] to match non-donors to donor at 1:1 ratio by the following: age (5-year interval), gender, duration of follow up (5-year interval), family history of ESRD in a first-degree relative, systolic blood pressure (15 mmHg interval) and APOL1 genotype (low- vs. high-risk). The number of available eligible non-donors determined the donor sample size. The paired t-tests or Wilcox signed-rank test were used, as appropriate, to compare blood pressure, eGFR and uACR at follow-up. The mixed-effect model with repeated measure was used to compare the rate of decline in renal function. We tested for interaction in the matched donor non-donor outcomes to see if the effects were modified by the presence or absence of APOL1 risk. All tests of statistical significance were two-tailed tests, and we interpreted $\alpha < 0.05$ as statistically significant. We used SAS version 9.4 (SAS Institute. Cary, NC) to perform the analyses.

Supplementary Table S1: Comparison of baseline characteristics of live kidney donors and non-donors matched by *APOL1* risk status and other characteristics.

	Donors (N=115)	Non-donors (N=115)	p-value***
Age, years	35±8	34±8	<0.001
Women	64%	64%	*
Weight, kg	82±18	82±17	0.921
Body mass index, kg/m^2	29±5	29±5	0.828
Systolic blood pressure, mmHg	119±9	116±9	<0.001
Diastolic blood pressure, mmHg	73±8	73±9	0.620
Serum creatinine, mg/dL	0.89±0.17	0.86±0.15	0.067
CKD-EPI eGFR, mL/min/1.73m^2	108±19	113±13	0.040
Medical insurance, Yes	64%	87%	<0.001
Education			0.017
0-8th grade	2%	0%	
9-11th grade	13%	35%	
High school	36%	40%	
Some college	38%	37%	
Bachelors	8%	17%	
Postgraduate	3%	3%	
Individual Income			0.016
<$12,000	14%	6%	
$12,000 to $25,000	22%	12%	
> $25,000	64%	82%	
Employed, full- or part-time, yes	87%	90%	0.217
First-degree relative with ESRD, yes	77%	77%	*
Family history of hypertension, yes	73%	74%	0.368
APOL1 status			*
0 risk allele	47%	39%	

1 risk allele	41%	49%	
2 risk alleles	12%	12%	
Duration of follow-up, years	11.6[9.1-12.8]	9.9[9.3-14.6]	0.012

Non-donors were matched to donors with regards to baseline age (within five years), sex, baseline systolic blood pressure (within five mm Hg), family history of ESRD in first-degree relative, *APOL1* genotype, and duration of follow up (within five years).

Data are presented as mean±standard deviation or median [25th-75th percentile].

To convert serum creatinine from mg/dl to µmol/l, multiply by 88.

* p value not generated because these variables were hard matched.

*** denotes McNemar's, paired t-test

Supplementary Table S2: Comparison of pre-donation characteristics between donors that did and did not participate in study

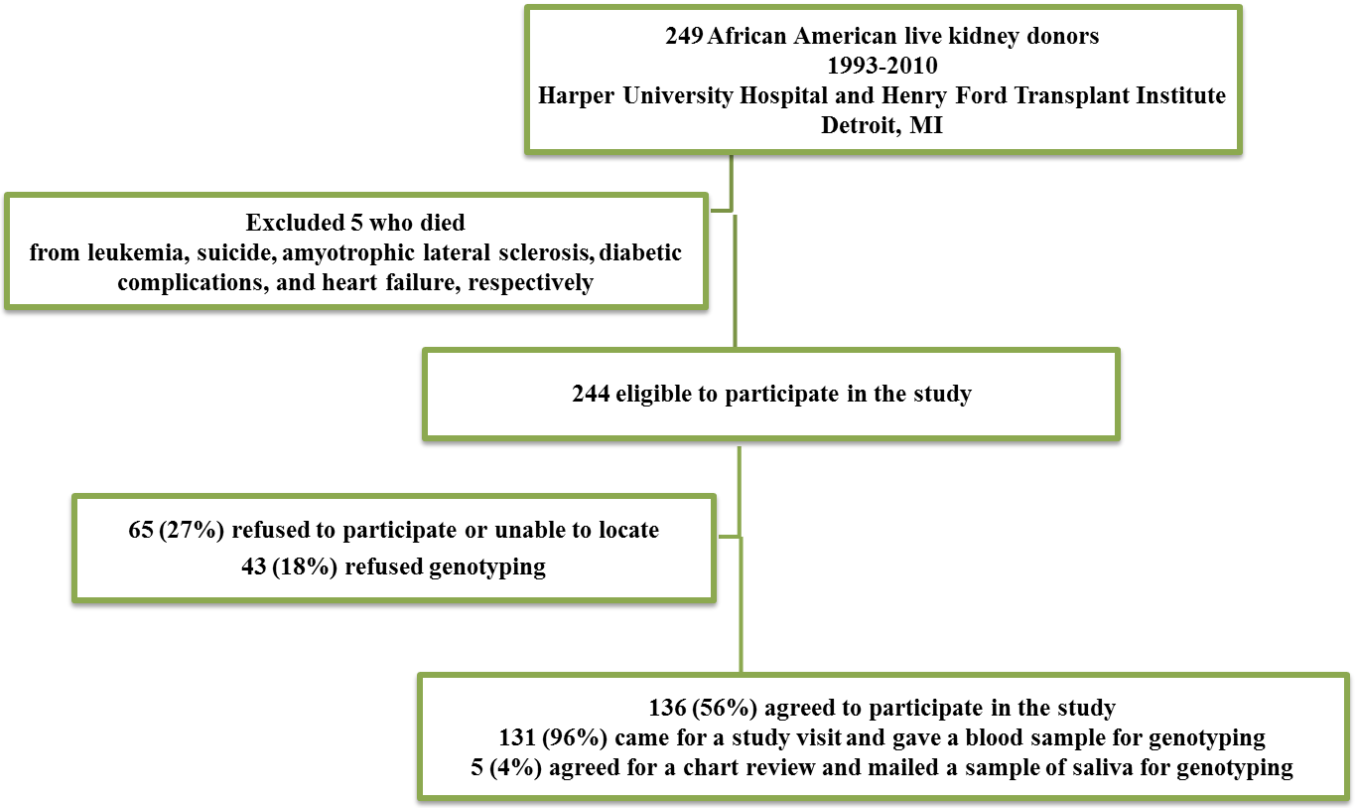
	Non-participant Donors (N=108)	Participant Donors (N=136)	p-value
Age, years	36±9	37±9	0.38
Women	61%	64%	0.64
Weight, kg	84±18	83±17	0.51
Body-mass Index, kg/m^2	30±6	29±6	0.50
Systolic blood pressure, mm Hg	119±11	120±10	0.17
Diastolic blood pressure, mm Hg	74±8	73±8	0.35
Serum Creatinine, mg/dL	0.89±0.17	0.90±0.18	0.55
CKD-EPI eGFR, mL/min/1.73 m^2	110±19	107±20	0.15
Family history of hypertension, Yes	76%	72%	0.48
Family history of ESRD, Yes	76%	78%	0.71
Relationship to the recipient			0.37
Parent	20%	9%	
Sibling	31%	38%	
Child	20%	29%	
Spouse	8%	9%	
Others	21%	15%	

Data are presented as mean±standard deviation or median [25th-75th percentile].

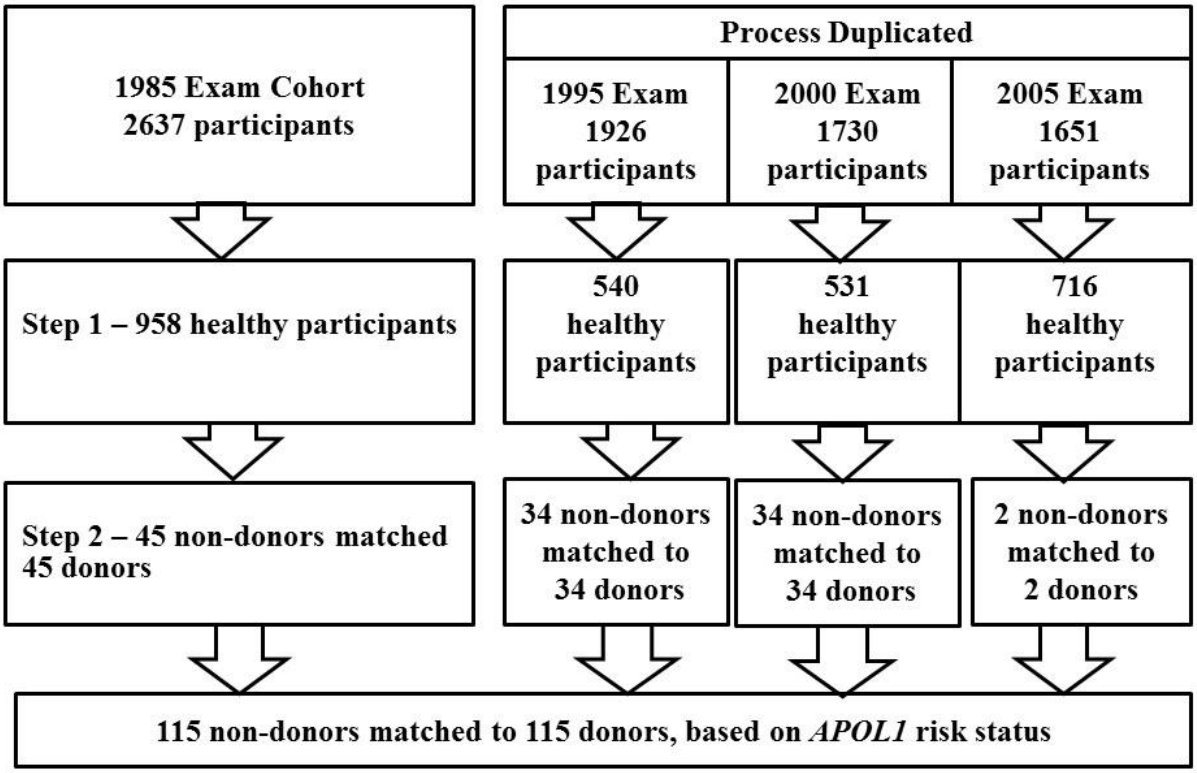
To convert serum creatinine from mg/dl to µmol/l, multiply by 88.4.

Family history of hypertension and ESRD was defined as first degree relative with these conditions.

Supplementary Figure S1: Flow-chart for Donor Selection



Supplementary Figure S2: Flow chart of Selection of healthy non-donors from CARDIA matched for APOL1 renal risk genotype



REFERENCES

1. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12.

2. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-70.

3. Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol 1988;41:1105-16.

4. Doshi MD, Goggins MO, Li L, Garg AX. Medical outcomes in African American live kidney donors: a matched cohort study. Am J Transplant 2013;13:111-8.

5. CARDIA Exam Components-All Years. 2010. (Accessed at http://www.cardia.dopm.uab.edu/exam-materials2/exam-components.)

6. . (Accessed at http://www.cardia.dopm.uab.edu/study-information)