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Deceased donor multidrug resistance protein 1 and caveolin 1 gene variants may influence allograft survival in kidney transplantation

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Abstract

Variants in donor multidrug resistance protein 1 (ABCB1) and caveolin 1 (CAV1) genes are associated with renal allograft failure after transplantation in Europeans. Here we assessed transplantation outcomes of kidneys from 368 African American (AA) and 314 European American (EA) deceased donors based on 38 single nucleotide polymorphisms (SNPs) spanning ABCB1 and 16 SNPs spanning CAV1, including previously associated index and haplotype-tagging SNPs. Tests for association with time to allograft failure were performed for the 1,233 resultant kidney transplantations, adjusting for recipient age, sex, ethnicity, cold ischemia time, PRA, HLA match, expanded-criteria donation, and APOL1- nephropathy variants in AA donors. Interaction analyses between APOL1 with ABCB1 and CAV1 were performed. In a meta-analysis of all transplantations, ABCB1 index SNP rs1045642 was associated with time to allograft failure and other ABCB1 SNPs were nominally associated, but not CAV1 SNPs. ABCB1 SNP rs1045642 showed consistent effects with the 558 transplantations from EA donors, but not with the 675 transplantations from AA donors. ABCB1 SNP rs956825 and CAV1 SNP rs6466583 interacted with APOL1 in transplants from AA donors. Thus, the T allele at ABCB1 rs1045642 is associated with shorter renal allograft survival for kidneys from American donors. Interactions between ABCB1 and CAV1 with APOL1 may influence allograft failure for transplanted kidneys from AA donors.

Keywords
African American; allograft failure; ABCB1; APOL1; CAV1; kidney transplantation

Introduction

Genetic variations in organ donors and recipients have the potential to impact outcomes after transplantation. In Europeans, variations in the donor multidrug resistance protein 1 (ABCB1) and caveolin 1 (CAV1) genes are associated with kidney allograft survival. In a similar fashion, the G1 and G2 coding variants in the powerful apolipoprotein L1 gene (APOL1) have dramatic effects on time to renal allograft failure after transplantation from African American (AA) deceased donors, and variants in SHROOM3 predispose to renal allograft fibrosis. In contrast, variation in APOL1 in recipients of kidney transplants does not impact outcomes. APOL1 G1 and G2 nephropathy-risk variants are virtually limited to populations with recent African ancestry. These variants produce ethnic-specific risk, as they are nearly absent in individuals with European, Hispanic, and Asian ancestry.

Based on the potential for ethnic-specific differences in risk allele frequencies, it is important to validate the effects of kidney-donor gene variants possibly impacting allograft survival in members of different racial/ethnic groups. Assessment of variation along the full length of implicated genes is also required due to ancestry-specific haplotype block structures and to further refine the position of potential functional variants. Testing a single, previously associated, index genetic variant may be insufficient for full interrogation of effects of that gene on transplant outcomes in other ethnic groups. The present report...
assessed effects of variation in the *ABCB1* and *CAV1* genes of deceased European American (EA) and AA kidney donors on transplant outcomes. Haplotype-tagging single nucleotide polymorphisms (htSNPs) spanning these genes were evaluated and genetic association analyses for time to renal allograft failure were performed for the resultant transplantations. Adjustment was done for the impact of *APOL1* risk variants and interactions between *ABCB1* and *CAV1* htSNPS with *APOL1* were tested.

**Results**

The genetic association analyses for 675 kidney transplantations from AA donors were based on the results of two kidneys from the same donor separately engrafted in 102 Alabama and 205 North Carolina transplantations and one kidney engrafted from 17 Alabama and 44 North Carolina donors. Eight kidney transplantations were performed prior to 2001, 86 from 2001 to 2006, 397 from 2006 to 2010, and 184 after 2010. The median (first quartile, third quartile) follow-up duration after engraftment was 34.3 months (13.8, 57.9 months). Table 1 lists demographic characteristics of transplant recipients (57.8% of whom were African Americans) and of AA deceased organ donors. Median donor and recipient ages were 37.0 and 50.0 years, respectively; 59.2% of donors and 58.4% of recipients were male. Median terminal serum creatinine concentration was 1.1 mg/dl, peak panel reactive antibody (PRA) titer 5%, cold ischemia time (CIT) 22.0 hours, and number of HLA mismatches 5. Peak PRA titers exceeded 20% in 31.7%, 34.1%, and 31.9% of the recipients of Alabama AA, North Carolina AA, and North Carolina EA kidneys, respectively (p=0.71); induction immunosuppression was administered to 92.3%, 89.7%, and 92.7% of recipients of Alabama AA, North Carolina AA, and North Carolina EA kidneys, respectively (p=0.29).

The genetic association analyses for 558 kidney transplantations from EA donors were based on the results of two kidneys from the same donor separately engrafted in 244 North Carolina transplantations and one kidney engrafted from 70 North Carolina donors; 270 transplantations were performed from 2006 to 2010 and 288 after 2010. The median (first quartile, third quartile) follow-up duration after engraftment was 23.7 months (12.1, 33.9 months). Table 2 lists demographic characteristics of transplant recipients (42.3% of whom were African Americans) and of EA deceased organ donors. Median donor and recipient ages were 44.0 and 55.0 years, respectively; 60.5% of donors and 55.2% of recipients were male. Median terminal serum creatinine concentration was 0.9 mg/dl, peak PRA 6%, CIT 23.0 hours, and number of HLA mismatches 4. Immunosuppression varied by center, but generally included antibody induction with a calcineurin inhibitor (CNI) and an antiproliferative agent, with or without corticosteroids.

In the meta-analysis of 1,233 transplantations from all deceased AA and EA kidney donors, no SNPs in *CAV1*, including the previously associated index SNP rs4730751, met statistical significance for association with time to renal allograft failure in the fully-adjusted model that accounted for donor *APOL1* risk status in the AA subset and recipient, age, sex and ancestry (AA vs. non-AA), HLA match, CIT, PRA level (0% vs. >0%) and expanded-criteria donor (ECD) vs. standard-criteria donor (SCD) kidneys in AA and EA donors, Table 3. In contrast, the previously associated *ABCB1* index SNP rs1045642 identified in
European studies (hazard ratio [HR] 1.32, p=0.04, additive model) and five other ABCB1 htSNPs displayed nominal evidence of association; the “T” allele in rs1045642 denoted risk for early allograft failure, opposing findings in the European report. The tested allele and the tested allele frequency for the EA subset in Table 3 corresponded to the minor allele in the AA subset. Only ABCB1 and CAV1 SNPs common to AA and EA donors, meeting quality control metrics and with sufficient counts with two copies of the minor allele were analyzed in the meta-analysis. Thus, not all genotyped SNPs were included or shown in Table 3.

Association analyses for time to allograft failure limited to recipients of AA donor kidneys are shown in Supplementary Table S2. Results are presented in fully-adjusted models as above, with additional adjustment for donor APOL1 risk (recessive). Three of the 38 ABCB1 SNPs were nominally associated with time to allograft failure (additive models): rs10808071 (hazard ratio [HR] 0.68, p=0.045), rs10264990 (HR 1.53, p=0.019), and rs17327624 (HR 1.50, p=0.02). The ABCB1 SNP rs1045642 was not associated with allograft failure. Supplementary Table S2 shows that only one of 16 CAV1 htSNPs was nominally associated with time to allograft failure: rs4730748 (HR 1.97, p=0.03 recessive model). Additional adjustments for recipient diabetic kidney disease and donor age did not alter association results in the meta-analysis or the analyses within each race group (data not shown).

Association results for time to allograft failure in recipients of EA donor kidneys are presented in Supplementary Table S3. Three of 32 ABCB1 SNPs were nominally associated with time to kidney allograft failure (additive models): index SNP rs1045642 (HR 1.66, p=0.04), rs6949448 (HR 1.52, p=0.04), rs2235046 (HR 1.47, p=0.05); rs1045642 supported the reported association in European kidney donors with the same direction of effect. Among 14 CAV1 htSNPs, rs3807992 (HR 1.54, p=0.03, additive model) and rs9920 (HR 1.92, p=0.01, additive model) were nominally associated with time to allograft failure.

We performed immunostaining of ABCB1 and CAV1 proteins in non-diseased human kidney cryosections to confirm their presence in renal cells and determine whether APOL1 interaction analyses might be clinically relevant. APOL1 protein localization in kidney tissue has been reported, with high levels (and cellular uptake) in podocytes; lower APOL1 protein levels are seen in renal tubular cells and glomerular endothelial cells and APOL1 protein and mRNA are absent in mesangial cells. In the current analyses, robust ABCB1 fluorescence was observed in mesangial cells and smooth muscle cells of renal arterioles; ABCB1 was also present in endothelial cells in glomeruli and medium-sized renal arterioles. Although ABCB1 fluorescence was observed in renal tubule cells, it was considerably less intense than the staining in mesangial cells (Supplementary Figures S1-S5). CAV1 was present in mesangial cells, smooth muscle cells in renal arterioles, and endothelial cells of glomeruli and medium-sized renal arterioles (Supplementary Figures S6-S9). CAV1 was not enriched in proximal tubule cells or podocytes (Supplementary Figures S10-S11).

Fluorescence of low intensity for ABCB1 and higher intensity for APOL1 proteins in renal tubule cells, coupled with CAV1 and APOL1 proteins in glomerular endothelial cells and arteriolar cells, supported performance of gene-gene interaction analyses on renal transplantation outcomes. Low levels of ABCB1 expression in tubules may amplify the toxicity of APOL1 G1/G2 nephropathy-risk variants upon CNI treatment after kidney transplantation.
Table 4 displays results of testing for genetic interaction between \textit{ABCB1} and \textit{CAV1} SNPs with the powerful \textit{APOL1} G1 and G2 nephropathy-risk variants in transplantation of allografts from AA donors. The recessive model was used to define \textit{APOL1}-mediated risk.\textsuperscript{13} Significant interaction effects with \textit{APOL1} were observed for \textit{ABCB1} htSNP rs956825 (p=0.001; dominant model) and \textit{CAV1} htSNP rs6466583 (p=0.004; recessive model), revealing potentially important gene-gene interactions on time to renal allograft survival (Figures 1 and 2).

In silico prediction softwares (SIFT/Polyphen) are not available for potential effects of non-coding SNPs. RegulomeDB, a tool that queries multiple data resources and annotates SNPs with respect to known and predicted regulatory elements, including DNAase hypersensitivity, transcription factor binding sites and promoter regions, in intergenic regions, was explored. Among the six SNPs associated in the meta-analysis (Table 3), the following scores returned: rs1045642 (synonymous), rs10808071 (3a), rs6949448 (7), rs1202168 (5), rs1202179 (2b), and rs2188526 (5), where lower scores indicate increased support from multiple datasets. The scores returned in this analysis were not exceedingly strong (e.g., rs1202179 was assigned a score of 2b, which can be interpreted as support from 4 of 9 resources). This is not surprising as htSNPs variants were chosen to capture variation, as opposed to functional implications.

\section*{Discussion}

This is the first report evaluating common genetic variation in \textit{CAV1} and \textit{ABCB1} in American deceased organ donors for impact on time to allograft failure after kidney transplantation. \textit{ABCB1} index SNP rs1045642 was selected since it was putatively functional and associated with renal allograft survival in a European report; 37 additional \textit{ABCB1} htSNPs were selected to comprehensively assess common variation. In American deceased kidney donors, rs1045642 revealed an effect on time to allograft failure in the same direction as reports of genetic risk for CNI-toxicity. However, the direction of these associations opposed that reported in Europeans for time to renal allograft failure. Variation in \textit{CAV1} did not significantly impact transplant outcomes from the meta-analysis of AA and EA donors.\textsuperscript{2,4} In addition, SNPs in \textit{ABCB1} and in \textit{CAV1} appeared to interact with donor \textit{APOL1} nephropathy-risk variants to impact time to allograft failure in kidneys transplanted from deceased AA donors. These analyses comprise the first \textit{APOL1}-second gene interaction analyses performed in kidney transplantation.

\textit{ABCB1} is important to evaluate in American deceased kidney donors, particularly African Americans, based on the results of association studies in Europeans and given the role of the ABCB1 protein in transporting CNIs from cells and preventing intracellular accumulation with potential for tubulointerstitial kidney disease.\textsuperscript{14,15} The present report thoroughly interrogated common variation in \textit{CAV1} and \textit{ABCB1} via a haplotype-tagging approach. The previously identified and putatively functional \textit{ABCB1} index variant rs1045642 showed association with time to renal allograft failure in kidneys donated by EAs (Supplementary Table S3) and in the meta-analysis of transplantations from AA and EA donors (Table 3), but in the opposite direction of the European report (no association was seen in analyses limited to transplantations from AA donors; Supplementary Table S2). Although the “T”
allele in rs1045642 (C3435T) denoted risk for early allograft failure in our report, versus longer allograft survival in the European report.\textsuperscript{4} The TT genotype is reported to reduce p-gp renal expression.\textsuperscript{16} This effect should enhance risk for CNI-toxicity. Consistent with our findings, reports in independent French, Belgian and German studies reveal that the rs1045642 T allele in donor kidneys was associated with risk of CNI-nephropathy/renal allograft injury.\textsuperscript{2,17,18} It is possible that these controversial results for \textit{ABCB1} SNP rs1045642 in these reports, compared to the European study,\textsuperscript{4} reflect unique environmental exposures between centers or kidney donors.\textsuperscript{19}

As \textit{ABCB1} protein transports nephrotoxic CNIs out of cells, cells lacking (or expressing lower amounts) of this protein, an effect potentially related to allelic variation, could be more vulnerable to CNI nephrotoxicity. Localization studies suggest that CNIs may accumulate in renal tubule cells, likely due to the lower level of this efflux-pump membrane protein in these cells.\textsuperscript{2,15,20,21} The immunofluorescence staining for \textit{ABCB1} protein was sparse in human renal tubule cells (Supplementary Figure S5) relative to that in mesangial and glomerular endothelial cells (Supplementary Figures S1 and S3). This finding supported potential gene-gene interactions between kidney donor \textit{ABCB1} and \textit{APOL1} on transplant outcomes related to interstitial fibrosis. Marked renal tubule injury and interstitial damage are observed in native kidney \textit{APOL1}-associated nephropathy,\textsuperscript{22} and failed transplanted kidneys from donors with two \textit{APOL1} nephropathy-risk variants have similar findings.\textsuperscript{6}

Therefore, down-regulation of \textit{ABCB1}, coupled with \textit{APOL1} nephropathy-risk variants (G1 or G2) has the potential to enhance interstitial injury in transplanted kidneys. In interaction analyses with \textit{APOL1}, a significant effect with \textit{ABCB1} hSNP rs956825 (\textit{p}=0.001, dominant model) was observed for kidneys from AA donors (Table 4 and Figure 1). \textit{ABCB1} mRNA levels tended to be lower in transformed lymphoblastoid cell lines from Yoruba in Ibadan Nigerians (YRI) with the rs956825 minor allele A (Stouffer \textit{p}=0.06, additive model; HapMap-Sanger gene expression database; http://www.hapmap.org and http://ftp.sanger.ac.uk/pub/genevar). We are creating primary renal tubule cell lines from African American kidneys and will perform expression quantitative trait loci (eQTL) studies to assess effects of rs956825 on renal \textit{ABCB1} gene expression when sufficient samples are available.

This study failed to replicate the previously reported \textit{CAV1} variant rs4730751 association with renal allograft survival for AA and EA donors.\textsuperscript{3} An effect of this \textit{CAV1} variation for European donor kidneys was previously observed in two cohorts, with weaker evidence in a third.\textsuperscript{3} Potential mechanisms for studying kidney allograft failure due to variation in \textit{CAV1} includes differential entry of nephropathic BK polyoma virus from the urothelium into the kidney through caveolar pathways (with subsequent allograft failure due to BK nephropathy)\textsuperscript{23} and/or effects on transforming growth factor \textit{β} (TGF\textit{β}) signaling.\textsuperscript{24} As with \textit{ABCB1}, \textit{CAV1} hSNP rs6466583 significantly interacted with \textit{APOL1} for allograft survival in kidneys from AA donors (\textit{p}=0.004, recessive model; Table 4, Figure 2).

The present report has the limitation of a relatively short post-transplant follow-up period for assessing allograft failures, particularly for kidneys from EA donors. However, we note that \textit{APOL1} nephropathy-risk variants were associated with an increased risk for allograft failure early after transplantation, within two to three years.\textsuperscript{6,7} The short post-transplant follow-up
is likely to affect the statistical power as the survival analyses are powered by the number of
‘events’ (allograft failures) instead of the overall sample size. Re-analysis of these datasets
in the future, after more events have been accumulated, may reveal effects that were missed
in the current analysis. In addition, we were unable to link \textit{ABCB1} gene variants with CNI
toxicity as a cause of allograft failure because SRTR does not contain this variable.
Recurrent disease was listed as the etiology of allograft failure in 9.0\% of DDKTs from AA
donors and 4.3\% of EA donors.

The putatively functional \textit{ABCB1} variant rs1045642 independently associated with time to
renal allograft failure after transplantation from all deceased American donors and from EA
donors alone. Common \textit{CAV1} variants did not associate with transplant outcomes for
kidneys from American donors. Variation in the powerful \textit{APOL1} nephropathy-risk gene,
known to play an important role in determining outcomes after transplantation from AA
deceased kidney donors, was taken into account in the current analyses.\cite{6,7} With
identification of \textit{ABCB1} effects, pharmacogenomic analyses based on variations in \textit{ABCB1}
should be considered. These types of studies could improve renal allograft outcomes in CNI-
treated patients; serum levels of the drug may not accurately reflect the potential for CNI-
mediated renal toxicity after transplantation. The current results also suggest that variants in
\textit{CAV1} and \textit{ABCB1} may interact with \textit{APOL1} and influence renal allograft failure. This is
worthy of additional study to assess genetic risk for renal allograft failure in donors of
multiple racial/ethnic groups.

\textbf{Methods}

\textbf{DNA samples}

Aliquots of stored DNA from deceased AA and EA kidney donors at Wake Forest School of
Medicine (WFSM) and deceased AA donors from University of Alabama at Birmingham
School of Medicine (UAB) were sent to the Center for Genomics and Personalized Medicine
Research at WFSM for \textit{ABCB1} and \textit{CAV1} genotyping (and \textit{APOL1} G1 and G2 variant
genotyping in AA donors).\cite{7} The UAB Institutional Review Board (IRB) permitted
participation because materials came from deceased individuals and WFSM received IRB
approval for genotyping donor DNA samples and linking results to outcomes from kidney
transplantation based on United Network of Organ Sharing (UNOS) identification numbers
in the Scientific Registry of Transplant Recipients (SRTR). For AA donors, analyses were
conducted for 675 deceased donor kidney transplantations (DDKTs) of 221 organs
recovered by the Alabama Organ Center and 454 organs recovered and/or transplanted in
North Carolina. For EA donors, analyses included 558 DDKTs with organs procured and/or
transplanted in North Carolina. Outcomes were evaluated in the SRTR for DDKTs
performed throughout the United States. This study used data from the SRTR. The SRTR
data system includes data on all donor, wait-listed candidates, and transplant recipients in
the US, submitted by the members of the Organ Procurement and Transplantation Network
(OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of
Health and Human Services provides oversight to the activities of the OPTN and SRTR
contractors.
Genotyping
To identify htSNPs, the genomic intervals (± 10kb) containing \textit{ABCB1} and \textit{CAV1} were extracted from the HapMap Genome Browser (Release #28) from representative African YRI (Yoruba in Ibadan, Nigeria) and European-derived CEU (Utah residents with ancestry from northern and western Europe) populations. Haplotype tagging was performed in Haploviev to capture SNPs with a minor allele frequency (MAF) greater than 5% at an $r^2$ threshold of 0.80, first in Yoruba (YRI) and then supplemented with additional SNPs from CEU to tag any differential linkage disequilibrium (LD) block structure. Successfully genotyped htSNPs had $r^2$ values >0.80 for \textit{ABCB1} and \textit{CAV1} in EAs and AAs and effectively captured common variation across both genes. Two \textit{APOL1} G1 nephropathy-risk SNPs (rs73885319; rs60910145) and an insertion/deletion for the G2 risk allele (rs71785313) were genotyped. Genotyping was performed using the Sequenom MassArray system (Sequenom, Inc.; San Diego, CA) in the WFSM Center for Genomics and Personalized Medicine Research. PCR primers were designed in MassARRAY Assay Design 3.1 (Sequenom, Inc.) and genotypes were analyzed using MassARRAY Typer (Sequenom, Inc.; San Diego, CA). Call rates were >90%. In EA and AA samples, respectively, 7 and 4 blind duplicates were genotyped with 99.6% and 100% concordance rates.

Statistical Analysis
The outcome of interest was time to allograft failure, determined by the interval between the date of kidney transplantation and the date of allograft loss (return to dialysis, nephrectomy, or repeat transplantation). The date of final observation was censored in the event of death with a functioning allograft or at the most recent follow-up (before November 30, 2013) in recipients with functioning allografts. Cox proportional hazard models were then fitted. The sandwich estimator was used to obtain a robust estimation of covariance matrix associated with the parameter estimates to account for the correlation between allograft failure rate and time to failure of kidneys donated by a single individual to two recipients. This approach has been consistent and robust to several misspecifications of the Cox model. The fully adjusted model accounted for donor \textit{APOL1} risk status in the AA subset and recipient age, sex, and ancestry (AA vs non-AA) HLA match, CIT, PRA level (0% vs. >0%) and ECD vs. SCD kidney in AA and EA donors. A meta-analysis of association between SNPs and outcomes after transplantation of kidneys from AA and EA donors was performed. This analysis was conducted only for SNPs that passed our quality filters, for which the COXPH routinely coded in the R package survival (Therneau T (2014). A Package for Survival Analysis in S. R package version 2.37-7, \url{http://CRAN.R-project.org/package=survival}) reached convergence, yielded valid results, and was common to both ethnic groups. The weighted average of the parameter estimates for the AA and EA subsets and the associated variance were computed after ensuring that results were computed with respect to the same allele, the minor allele in the AA subset. Weights were computed as the inverse of the variance of each parameter in each subset and normalized to ensure that they added up to 1. P-values for the meta-analysis were computed based on the cumulative distribution function of the normal distribution whereby observed Z-values were computed as the ratio of estimated meta-analysis parameter and its standard error. Metaanalysis hazard
ratios were computed as the exponential of the meta-analysis parameter. Genetic association analyses were also performed separately for recipients of AA deceased-donor kidneys and for recipients of EA deceased-donor kidneys.

Gene-level testing accounting for total variation in \textit{ABCB1} and \textit{CAV1} was performed. For each, the quantity $Z^T \Sigma Z$ was computed where $Z$ represents the vector of Z-values observed for each htSNP in the fully adjusted model under the additive mode of inheritance and $\Sigma$ is the matrix of $r^2$ values observed between htSNPs located within each gene. This sum is expected to follow a chi-square distribution with $k$ degrees of freedom, where $k$ is the rank of LD matrix $\Sigma$. This approach is similar to the one proposed by Liu et al.,\cite{27} except that the observed LD matrix was used. P-values for the gene-level test were calculated using the large-sample-theory chi-square distribution and permutation tests.

Interaction analyses with \textit{APOL1} nephropathy risk variants were performed only for recipients of AA donor kidneys, as EA donors essentially lack these risk variants. This analysis included the centered cross-product term of each SNP by the \textit{APOL1} nephropathy-risk variant in the model that already contained the main effects.

**\textit{ABCB1} and \textit{CAV1} protein localization in human kidney by immunofluorescence**

Immunofluorescence (IF) localization of ABCB1 and CAV1 proteins was performed in non-diseased kidney cryosections from EAs and AAs using established protocols (see Supplementary Methods).\cite{28} APOL1 protein localizations in the kidney have been reported.\cite{12} Primary antibodies and antibody dilutions are listed in Supplementary Table S1.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**

1. Gautreaux MD, Freedman BI. Genotypic variation and outcomes in kidney transplantation: donor and recipient effects. Kidney Int. 2013; 84:431–433. [PubMed: 23989355]
2. Hauser IA, Schaeffeler E, Gauer S, et al. \textit{ABCB1} genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. J Am Soc Nephrol. 2005; 16:1501–1511. [PubMed: 15772250]
3. Moore J, McKnight AJ, Simmonds MJ, et al. Association of caveolin-1 gene polymorphism with kidney transplant fibrosis and allograft failure. JAMA. 2010; 303:1282–1287. [PubMed: 20371787]
4. Moore J, McKnight AJ, Dohler B, et al. Donor ABCB1 variant associates with increased risk for kidney allograft failure. J Am Soc Nephrol. 2012; 23:1891–1899. [PubMed: 23064017]
5. DeMeyer M, Haufroid V, Elens L, et al. Donor age and ABCB1 1199G>A genetic polymorphism are independent factors affecting long-term renal function after kidney transplantation. J Surg Res. 2012; 178:988–995. [PubMed: 22839481]
6. Reeves-Daniel AM, Depalma JA, Bleyer AJ, et al. The APOL1 Gene and Allograft Survival after Kidney Transplantation. Am J Transplant. 2011; 11:1025–1030. [PubMed: 21486385]
7. Freedman BI, Julian BA, Pastan SO, Israni AK, Kirk AD, Gaston RS, Stratta RJ, Mohan S, Adams PL, Palanisamy A, Reeves-Daniel AM, Divers J. Apolipoprotein L1 gene variants in deceased organ donors are associated with renal allograft failure. Journal of the American Society of Nephrology. 2014 Abstract.
8. Menon MC, Chuang PY, Li Z, et al. Intronic locus determines SHROOM3 expression and potentiates renal allograft fibrosis. J Clin Invest. 2014
9. Lee BT, Kumar V, Williams TA, et al. The APOL1 Genotype of African American Kidney Transplant Recipients Does Not Impact 5-Year Allograft Survival. Am J Transplant. 2012; 12:1924–1928. [PubMed: 22487534]
10. Freedman BI, Divers J, Palmer ND. Population Ancestry and Genetic Risk for Diabetes and Kidney, Cardiovascular, and Bone Disease: Modifiable Environmental Factors May Produce the Cures. Am J Kidney Dis. 2013
11. Keaton JM, Cooke Bailey JN, Palmer ND, et al. A comparison of type 2 diabetes risk allele load between African Americans and European Americans. Hum Genet. 2014
12. Ma L, Shelness GS, Snipes JA, et al. Localization of APOL1 Protein and mRNA in the Human Kidney: Nondiseased Tissue, Primary Cells, and Immortalized Cell Lines. J Am Soc Nephrol. 2014
13. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010; 329:841–845. [PubMed: 20647424]
14. Fakata KL, Elmquist WF, Swanson SA, et al. Cyclosporin A has low potency as a calcineurin inhibitor in cells expressing high levels of P-glycoprotein. Life Sci. 1998; 62:2441–2448. [PubMed: 9651111]
15. Joy MS, Nickeleit V, Hogan SL, et al. Calcineurin inhibitor-induced nephrotoxicity and renal expression of P-glycoprotein. Pharmacotherapy. 2005; 25:779–789. [PubMed: 15927895]
16. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A “silent” polymorphism in the MDR1 gene changes substrate specificity. Science. 2007; 315:525–528. [PubMed: 17185560]
17. Naesens M, Lerut E, de JH, et al. Donor age and renal P-glycoprotein expression associate with chronic histological damage in renal allografts. J Am Soc Nephrol. 2009; 20:2468–2480. [PubMed: 19762492]
18. Woillard JB, Rerolle JP, Picard N, et al. Donor P-gp polymorphisms strongly influence renal function and graft loss in a cohort of renal transplant recipients on cyclosporine therapy in a long-term follow-up. Clin Pharmacol Ther. 2010; 88:95–100. [PubMed: 20505666]
19. Rosenquist JN, Lehrer SF, O’Malley AJ, et al. Cohort of birth modifies the association between FTO genotype and BMI. Proc Natl Acad Sci U S A. 2015; 112:354–359. [PubMed: 25548176]
20. Joy MS, Hogan SL, Thompson BD, et al. Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. Nephrol Dial Transplant. 2007; 22:1963–1968. [PubMed: 17395652]
21. Cattaneo D, Ruggenenti P, Balldelli S, et al. ABCB1 genotypes predict cyclosporine-related adverse events and kidney allograft outcome. J Am Soc Nephrol. 2009; 20:1404–1415. [PubMed: 19470683]
22. Larsen CP, Beggs ML, Saeed M, et al. Histopathologic findings associated with APOL1 risk variants in chronic kidney disease. Mod Pathol. 2014
23. Gilbert JM, Benjamin TL. Early steps of polyomavirus entry into cells. J Virol. 2000; 74:8582–8588. [PubMed: 10954560]
24. Razani B, Zhang XL, Bitzer M, et al. Caveolin-1 regulates transforming growth factor (TGF)-beta/SMAD signaling through an interaction with the TGF-beta type I receptor. J Biol Chem. 2001; 276:6727–6738. [PubMed: 11102446]

25. Cox DR. Regression models and life tables. J Royal Stat Soc. 1972; 34B:187–197.

26. Lin DY, Wei LJ. The robust inference for the Cox Proportional Hazards Model. J Am Stat Assoc. 1989; 84:1074–1078.

27. Liu JZ, McRae AF, Nyholt DR, et al. A versatile gene-based test for genome-wide association studies. Am J Hum Genet. 2010; 87:139–145. [PubMed: 20598278]

28. Willingham MC. Fluorescence labeling of surface antigens of attached or suspended tissue-culture cells. Methods Mol Biol. 1999; 115:113–119. [PubMed: 10098173]
Figure 1.
Kaplan-Meier plots with time to allograft failure based on the significant interaction (p=0.001, dominant model) between *APOL1* risk variants (recessive; *APOL1*=2 signifies risk) and *ABCB1* haplotype-tagging single nucleotide polymorphism rs956825 (rs956835=1/2 signifies risk).
Figure 2.
Kaplan-Meier plots with time to allograft failure based on the significant interaction (p=0.004, recessive model) between $APOL1$ risk variants (recessive; $APOL1=2$ signifies risk) and $CAV1$ haplotype-tagging single nucleotide polymorphism rs6466583 (rs6466583=2 signifies risk).
Table 1
Demographic data for 368 African American kidney donors and 675 resultant transplantations

| Donor characteristics (N=368) |  |  |  |  |  |
|---|---|---|---|---|---|
| **Categorical variables** | N | % |  |  |  |
| Donor gender (% male) | 218 | 59.2% |  |  |  |
| Standard criteria donor (%) | 296 | 80.4% |  |  |  |
| **Continuous variables** | N | 1st Quartile | Median | Mean | Standard Deviation | 3rd Quartile |
| Donor age (years) | 368 | 20.0 | 37.0 | 35.3 | 17.5 | 50.0 |
| Donor terminal serum creatinine (mg/dl) | 300 | 0.85 | 1.1 | 1.2 | 0.7 | 1.5 |

| Recipient characteristics (N=675) |  |  |  |  |  |
|---|---|---|---|---|---|
| **Categorical variables** | N | % |  |  |  |
| Recipient gender (% male) | 394 | 58.4% |  |  |  |
| Recipient ethnicity (% African American) | 390 | 57.8% |  |  |  |
| Allograft failure (%) | 117 | 17.3% |  |  |  |
| **Continuous variables** | N | 1st Quartile | Median | Mean | Standard Deviation | 3rd Quartile |
| Peak PRA (%) | 674 | 0.0 | 5.0 | 23.8 | 32.8 | 38.0 |
| Recipient age (years) | 675 | 38.0 | 50.0 | 47.8 | 15.6 | 60.0 |
| Recipient body mass index (kg/m²) | 621 | 23.6 | 26.9 | 27.6 | 5.7 | 31.4 |
| Cold ischemia time (hours) | 641 | 16.1 | 22.0 | 23.5 | 11.2 | 28.3 |
| HLA mismatch (#) | 675 | 4.0 | 5.0 | 4.3 | 1.4 | 5.0 |
| Duration of transplant follow-up (months) | 675 | 13.8 | 34.3 | 39.5 | 30.1 | 57.9 |
Table 2
Demographic data for 314 European American kidney donors and 558 resultant transplantations

| Donor characteristics (N=314) |   |   |   |   |   |
|-------------------------------|---|---|---|---|---|
| **Categorical variables**     |   |   |   |   |   |
| Donor gender (% male)         | 190 | 60.5% |
| Standard criteria donor (%)   | 238 | 75.8% |
| **Continuous variables**      |   |   |   |   |   |
| Donor age (years)             | 314 | 25.0 | 44.0 | 40.7 | 18.2 | 55.0 |
| Donor terminal serum creatinine (mg/dl) | 314 | 0.7 | 0.9 | 1.2 | 1.4 | 1.4 |

| Recipient characteristics (N=558) |   |   |   |   |   |
|-----------------------------------|---|---|---|---|---|
| **Categorical variables**         |   |   |   |   |   |
| Recipient gender (% male)         | 308 | 55.2% |
| Recipient ethnicity (% African American) | 236 | 42.3% |
| Allograft failure (%)             | 47 | 8.4% |
| **Continuous variables**         |   |   |   |   |   |
| Peak PRA (%)                      | 555 | 0.0 | 6.0 | 23.4 | 32.9 | 40.0 |
| Recipient age (years)             | 558 | 6.0 | 55.0 | 52.6 | 15.1 | 64.0 |
| Recipient body mass index (kg/m²) | 549 | 23.7 | 27.5 | 28.1 | 6.1 | 32.1 |
| Cold ischemia time (hours)        | 543 | 16.3 | 23.0 | 26.3 | 14.0 | 34.0 |
| HLA mismatch (#)                  | 557 | 3.0 | 4.0 | 3.7 | 1.7 | 5.0 |
| Duration of transplant follow-up (months) | 558 | 12.1 | 23.7 | 22.5 | 10.2 | 33.9 |
### Table 3

Meta-analysis: *ABCB1* and *CAV1* single SNP associations with time to allograft failure in 1,233 recipients of African American and European American donor kidneys

| Gene - SNP | Position | African American | European American | Additive Model | Dominant Model | Recessive Model |
|------------|----------|------------------|-------------------|---------------|---------------|----------------|
|            |          | MA | MAF | TA | TAF | HR | Direction | p-value | HR | Direction | p-value | HR | Direction | p-value |
| **ABCB1**  |          |    |     |    |     |    |          |        |    |          |        |    |          |        |
| rs17209837 | 87495506 | C | 0.279 | C | 0.155 | 0.92 | -- | 0.73 | 0.92 | ++ | 0.66 | 1.08 | + | 0.84 |
| rs1055302  | 87503600 | A | 0.303 | A | 0.143 | 0.92 | -- | 0.75 | 0.92 | ++ | 0.64 | 1.05 | + | 0.88 |
| rs17064    | 87504154 | T | 0.143 | T | 0.069 | 0.86 | -- | 0.53 | 0.86 | -- | 0.48 | 0.98 | + | 0.98 |
| rs1045642  | 87509329 | T | 0.198 | T | 0.516 | 1.32 | ++ | 0.04 | 1.32 | ++ | 0.12 | 1.79 | ++ | 0.03 |
| rs10808071 | 87511492 | G | 0.216 | G | 0.207 | 0.65 | -- | 0.01 | 0.65 | -- | 0.02 | 0.43 | -- | 0.15 |
| rs6949448  | 87512498 | T | 0.226 | T | 0.423 | 1.22 | ++ | 0.04 | 1.22 | ++ | 0.26 | 1.77 | ++ | 0.01 |
| rs7787082  | 87527735 | G | 0.476 | G | 0.829 | 1.00 | ++ | 0.43 | 1.00 | + | 0.98 | 1.38 | ++ | 0.14 |
| rs10234411 | 87535576 | T | 0.241 | T | 0.465 | 1.20 | ++ | 0.08 | 1.20 | ++ | 0.31 | 1.59 | ++ | 0.04 |
| rs2032588  | 87550127 | T | 0.204 | T | 0.061 | 0.84 | -- | 0.52 | 0.84 | -- | 0.37 | 1.30 | + | 0.50 |
| rs2235023  | 87561136 | A | 0.267 | A | 0.067 | 0.96 | -- | 0.96 | 0.96 | ++ | 0.82 | 1.14 | + | 0.64 |
| rs956825   | 87562959 | A | 0.177 | A | 0.310 | 0.98 | -- | 0.42 | 0.98 | + | 0.92 | 0.63 | -- | 0.22 |
| rs120168   | 87566646 | T | 0.266 | T | 0.435 | 1.43 | ++ | 0.01 | 1.43 | ++ | 0.04 | 1.59 | ++ | 0.04 |
| rs950978   | 87571151 | T | 0.122 | T | 0.293 | 1.10 | + | 0.77 | 1.10 | + | 0.61 | 0.43 | -- | 0.21 |
| rs10264990 | 87573299 | C | 0.200 | C | 0.341 | 1.28 | + | 0.43 | 1.28 | + | 0.15 | 0.93 | + | 0.88 |
| rs1202179  | 87574963 | G | 0.423 | G | 0.361 | 0.73 | -- | 0.05 | 0.73 | -- | 0.06 | 0.74 | -- | 0.21 |
| rs1202184  | 87584585 | A | 0.143 | A | 0.498 | 1.34 | ++ | 0.06 | 1.34 | ++ | 0.14 | 1.42 | ++ | 0.21 |
| rs1202182  | 87585988 | C | 0.316 | C | 0.361 | 0.84 | -- | 0.11 | 0.84 | -- | 0.30 | 0.62 | -- | 0.09 |
| rs17327624 | 87587501 | T | 0.180 | T | 0.202 | 1.30 | -- | 0.12 | 1.30 | -- | 0.14 | 1.15 | + | 0.74 |
| rs2188526  | 87591246 | A | 0.154 | A | 0.466 | 1.46 | ++ | 0.05 | 1.46 | ++ | 0.04 | 1.15 | + | 0.63 |
| rs3789243  | 87591570 | C | 0.441 | C | 0.489 | 1.27 | ++ | 0.28 | 1.27 | ++ | 0.24 | 1.13 | + | 0.56 |
| **CAV1**   |          |    |     |    |     |    |          |        |    |          |        |    |          |        |
| rs4730748  | 116527541| G | 0.205 | G | 0.188 | 0.71 | -- | 0.32 | 0.71 | -- | 0.07 | 1.62 | + | 0.08 |
| rs3807986  | 116537771| A | 0.444 | A | 0.747 | 1.08 | ++ | 0.81 | 1.08 | + | 0.64 | 0.98 | + | 0.93 |
| rs4730751  | 116540796| A | 0.199 | A | 0.280 | 0.99 | ++ | 0.81 | 0.99 | ++ | 0.96 | 1.24 | ++ | 0.46 |
| Gene - SNP | Position | African American | European American | Additive Model | Dominant Model | Recessive Model |
|------------|----------|-----------------|-------------------|---------------|---------------|----------------|
|            |          | MA   | MAF   | TA    | TAF  | HR   | Direction | p-value | HR   | Direction | p-value | HR   | Direction | p-value |
| rs10270569 | 116542728 | T    | 0.224 | T     | 0.275 | 1.01 | ++        | 0.85    | 1.01 | +         | 0.94    | 1.11 | ++         | 0.73    |
| rs11773845 | 116551247 | A    | 0.349 | A     | 0.555 | 0.88 | --        | 0.23    | 0.88 | --        | 0.50    | 0.76 | --         | 0.24    |
| rs729949   | 116554851 | A    | 0.336 | A     | 0.281 | 1.12 | --        | 0.42    | 1.12 | +         | 0.51    | 1.07 | ++         | 0.79    |
| rs3807992  | 116557191 | A    | 0.367 | A     | 0.277 | 1.10 | -+        | 0.54    | 1.10 | +         | 0.57    | 0.96 | -+         | 0.86    |
| rs8713     | 11659743  | C    | 0.282 | C     | 0.189 | 1.07 | -+        | 0.88    | 1.07 | +         | 0.69    | 0.87 | -+         | 0.68    |
| rs2052106  | 116566611 | G    | 0.386 | G     | 0.667 | 0.98 | --        | 0.63    | 0.98 | +         | 0.90    | 0.85 | -+         | 0.52    |

MA – minor allele; MAF – minor allele frequency; TA – tested allele; TAF – tested allele frequency; HR – Hazard Ratio. *ABCB1* and *CAV1* hSNPs common to African American and European American donors, meeting quality control metrics, and with sufficient counts with two copies of the minor allele are included.
Table 4
Interaction analysis of *APOL1* nephropathy-risk variants (recessive) with *ABCB1* and *CAV1* on time to allograft failure in recipients of African American donor kidneys

| Gene   | SNP       | Fully-adjusted model |                |                |                |                |                |                |
|--------|-----------|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|        |           | Additive model       | Dominant model | Recessive model |                |                |                |                |
|        |           | HR       | SE  | p-value | HR       | SE  | p-value | HR       | SE  | p-value |
| *ABCB1*| rs17209837| 1.84    | 0.51| 0.23    | 1.63    | 0.52| 0.35    | 7.85    | 1.06| 0.05    |
|        | rs6946119 | 0.56    | 0.83| 0.49    | 0.54    | 0.87| 0.48    | NA      | 0.00| NA      |
|        | rs1055302 | 1.57    | 0.48| 0.35    | 1.43    | 0.53| 0.49    | 4.20    | 1.11| 0.20    |
|        | rs17064   | 2.65    | 0.53| 0.06    | 2.20    | 0.54| 0.14    | NA      | 0.67| NA      |
|        | rs2235047 | 1.24    | 0.44| 0.63    | 1.34    | 0.55| 0.59    | NA      | 0.88| NA      |
|        | rs1045642 | 2.46    | 0.50| 0.07    | 2.81    | 0.52| 0.05    | NA      | 0.00| NA      |
|        | rs10808071| 0.27    | 0.60| 0.03    | 0.26    | 0.62| 0.03    | 0.00    | 1.27| NA      |
|        | rs17149699| 2.42    | 0.45| 0.05    | 2.36    | 0.53| 0.11    | 6.95    | 1.20| 0.11    |
|        | rs6949448 | 0.98    | 0.40| 0.96    | 0.90    | 0.54| 0.84    | 1.44    | 0.53| 0.50    |
|        | rs4148749 | 4.15    | 0.66| 0.03    | 4.19    | 0.69| 0.04    | NA      | 0.00| NA      |
|        | rs7779562 | 2.25    | 0.43| 0.06    | 2.32    | 0.57| 0.14    | 4.14    | 0.71| 0.05    |
|        | rs2373589 | 1.07    | 0.36| 0.86    | 1.56    | 0.56| 0.43    | 0.36    | 1.06| 0.34    |
|        | rs4148740 | NA      | NA  | NA      | NA      | 0.00| NA      | NA      | 0.00| NA      |
|        | rs7787082 | 0.79    | 0.48| 0.61    | 0.67    | 0.55| 0.47    | 0.81    | 0.76| 0.79    |
|        | rs10274587| 0.57    | 0.55| 0.31    | 0.53    | 0.62| 0.31    | 0.00    | 1.27| NA      |
|        | rs10234411| 0.92    | 0.41| 0.83    | 0.82    | 0.53| 0.71    | 1.43    | 0.50| 0.47    |
|        | rs2235041 | 1.05    | 0.59| 0.94    | 0.75    | 0.81| 0.73    | NA      | 1.30| NA      |
|        | rs3789246 | 1.66    | 0.53| 0.34    | 1.51    | 0.56| 0.46    | NA      | 0.00| NA      |
|        | rs12720066| NA      | NA  | NA      | NA      | 0.00| NA      | NA      | 0.00| NA      |
|        | rs2032588 | 0.75    | 0.50| 0.57    | 0.68    | 0.56| 0.49    | 1.18    | 0.97| 0.86    |
|        | rs2235023 | 0.55    | 0.45| 0.18    | 0.45    | 0.53| 0.14    | 0.69    | 0.96| 0.70    |
|        | rs956825  | 4.55    | 0.47| 0.00    | 5.00    | 0.56| 0.00    | 9.43    | 1.13| 0.05    |
|        | rs1202168 | 1.28    | 0.36| 0.50    | 1.47    | 0.52| 0.46    | 1.10    | 0.59| 0.88    |
|        | rs6950978 | 2.47    | 0.59| 0.13    | 2.39    | 0.60| 0.15    | NA      | 0.00| NA      |
| Gene   | SNP       | Fully-adjusted model | Additive model | Dominant model | Recessive model |
|--------|-----------|-----------------------|----------------|----------------|----------------|
|        |           | HR       | SE | p-value | HR   | SE | p-value | HR   | SE | p-value |
|        | rs10264990 | 0.85   | 0.55 | 0.77 | 0.80 | 0.57 | 0.69 | NA | 0.00 | NA |
|        | rs1202179 | 0.62 | 0.32 | 0.14 | 0.66 | 0.54 | 0.44 | 0.29 | 0.68 | 0.07 |
|        | rs13226726 | 1.52 | 0.69 | 0.55 | 2.31 | 1.39 | 0.55 | 2.31 | 1.39 | 0.55 |
|        | rs4728705 | 1.46 | 0.83 | 0.65 | 1.37 | 0.83 | 0.70 | NA | 0.00 | NA |
|        | rs1202184 | 1.30 | 0.52 | 0.61 | 1.39 | 0.53 | 0.54 | NA | 0.00 | NA |
|        | rs1211152 | 0.47 | 0.36 | 0.35 | 0.55 | 0.35 | 0.55 | NA | 0.00 | NA |
|        | rs1202182 | 0.47 | 0.36 | 0.04 | 0.35 | 0.55 | 0.06 | 0.38 | 0.71 | 0.17 |
|        | rs1202182 | 1.14 | 0.43 | 0.76 | 1.04 | 0.51 | 0.94 | 2.33 | 1.03 | 0.41 |
|        | rs2188526 | 1.27 | 0.35 | 0.49 | 1.65 | 0.51 | 0.33 | 1.52 | 1.04 | 0.68 |
|        | rs3789243 | 1.23 | 0.35 | 0.56 | 1.23 | 0.55 | 0.71 | 1.48 | 0.64 | 0.54 |
|        | rs2214104 | 0.29 | 1.22 | 0.31 | 0.31 | 1.23 | 0.34 | NA | 0.00 | NA |
|        | rs2157928 | 1.46 | 0.59 | 0.52 | 1.17 | 0.57 | 0.79 | NA | 0.00 | NA |
|        | rs1015415 | 1.36 | 0.70 | 0.66 | 1.44 | 0.70 | 0.61 | NA | 0.00 | NA |
|        | rs6972098 | 1.02 | 0.40 | 0.96 | 1.23 | 0.52 | 0.70 | 0.45 | 1.16 | 0.49 |
| CAV1   | rs975028  | 2.45 | 0.46 | 0.05 | 2.69 | 0.53 | 0.06 | 0.57 | 1.28 | 0.66 |
|        | rs4730748 | 0.42 | 0.66 | 0.18 | 0.52 | 0.68 | 0.33 | NA | 0.00 | NA |
|        | rs6466583 | 2.37 | 0.36 | 0.02 | 2.22 | 0.71 | 0.27 | 4.49 | 0.53 | 0.004 |
|        | rs3807986 | 0.55 | 0.43 | 0.16 | 0.35 | 0.54 | 0.06 | 0.67 | 0.91 | 0.67 |
|        | rs4730751 | 0.80 | 0.65 | 0.73 | 0.67 | 0.69 | 0.56 | 2.84 | 1.48 | 0.48 |
|        | rs10270569 | 0.76 | 0.60 | 0.64 | 0.60 | 0.62 | 0.41 | 3.58 | 1.48 | 0.39 |
|        | rs3779514 | 2.09 | 0.32 | 0.02 | 3.75 | 0.54 | 0.01 | 0.82 | 0.51 | 0.69 |
|        | rs1173845 | 0.71 | 0.43 | 0.42 | 0.54 | 0.57 | 0.27 | 0.86 | 0.86 | 0.86 |
|        | rs729949  | 0.57 | 0.40 | 0.16 | 0.49 | 0.52 | 0.17 | 0.52 | 0.83 | 0.43 |
|        | rs3807992 | 0.68 | 0.37 | 0.29 | 0.66 | 0.52 | 0.42 | 0.47 | 0.78 | 0.34 |
|        | rs8713   | 0.79 | 0.48 | 0.63 | 0.67 | 0.55 | 0.47 | 1.47 | 1.22 | 0.75 |
|        | rs9920   | 4.37 | 1.08 | 0.17 | 2.56 | 1.50 | 0.53 | NA | 0.00 | NA |
|        | rs17138812 | 0.57 | 0.63 | 0.37 | 0.63 | 0.87 | 0.60 | NA | 1.00 | NA |
| Gene     | SNP      | Fully-adjusted model |               |               |               |               |
|----------|----------|-----------------------|---------------|---------------|---------------|---------------|
|          |          | Additive model        | Dominant model| Recessive model|
|          |          | HR  | SE  | p-value | HR  | SE  | p-value | HR  | SE  | p-value |
| rs11974088 | 0.61    | 0.70 | 0.48 |         | 0.65 | 0.70 | 0.54   | NA  | 2.00 | NA      |
| rs2052106  | 0.72    | 0.43 | 0.45 |         | 0.54 | 0.56 | 0.27   | 0.95 | 0.84 | 0.95    |

HR – hazard ratio; SE – standard error; NA – not applicable due to low minor allele frequency counts