Oxidant stress regulatory genetic variation in recipients and donors contributes to risk of primary graft dysfunction after lung transplantation

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Objective: Oxidant stress pathway activation during ischemia reperfusion injury may contribute to the development of primary graft dysfunction (PGD) after lung transplantation. We hypothesized that oxidant stress gene variation in recipients and donors is associated with PGD.

Methods: Donors and recipients from the Lung Transplant Outcomes Group (LTOG) cohort were genotyped using the Illumina IBC chip filtered for oxidant stress pathway genes. Single nucleotide polymorphisms (SNPs) grouped into SNP sets based on haplotype blocks within 49 oxidant stress genes selected from gene ontology pathways and literature review were tested for PGD association using a sequencing kernel association test. Analyses were adjusted for clinical confounding variables and population stratification.

Results: Three hundred ninety-two donors and 1038 recipients met genetic quality control standards. Thirty percent of patients developed grade 3 PGD within 72 hours. Donor NADPH oxidase 3 (*NOX3*) was associated with PGD ($P = .01$) with 5 individual significant loci (P values between .006 and .03). In recipients, variation in glutathione peroxidase (*GPX1*) and NRF-2 (*NFE2L2*) was significantly associated with PGD ($P = .01$ for both). The *GPX1* association included 3 individual loci (P values between .006 and .049) and the *NFE2L2* association included 2 loci ($P = .03$ and .05). Significant epistatic effects influencing PGD susceptibility were evident between 3 different donor blocks of *NOX3* and recipient *NFE2L2* ($P = .026$, $P = .017$, and $P = .031$).

Conclusions: Our study has prioritized *GPX1*, *NOX3*, and *NFE2L2* genes for future research in PGD pathogenesis, and highlights a donor-recipient interaction of *NOX3* and *NFE2L2* that increases the risk of PGD. (J Thorac Cardiovasc Surg 2015;149:596-602)

See related commentary on pages 602-3.

Supplemental material is available online.

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Funding sources: National Institutes of Health Heart, Lung and Blood Institute grants HL087115, HL081619, HL096845, HL115354, HL114626, HL116656, and HL090021; and Robert Wood Johnson Foundation grant RWJ11642. Rui Feng has received grant support from the National Institutes of Health General Medical Sciences (RO1GM088566).

Disclosures: Nuala J. Meyer has received lecturing fees from the American Thoracic Society and the American College of Chest Physicians. All other authors have nothing to disclose with regard to commercial support.

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Received for publication April 9, 2014; revisions received Aug 19, 2014; accepted for publication Sept 23, 2014; available ahead of print Oct 17, 2014.

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0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2014.09.077>

Primary graft dysfunction (PGD) is a form of acute lung injury that develops within 72 hours of lung transplantation. It is defined by the presence of hypoxemia and radiographic infiltrates in the allograft,¹ and is the major cause of death in the early posttransplant period.² PGD affects 10% to 30% of all patients receiving lung transplantation,³ and is associated with an increased risk of bronchiolitis obliterans syndrome, prolonged hospitalization, and increased short- and long-term mortality.^{1,3-5}

Although the pathophysiology of PGD is incompletely understood, ischemia reperfusion injury leading to activation of the oxidant stress pathway is believed to be a major contributor to its development.⁶ In support of this notion, Williams, and colleagues⁷ identified antioxidant system dysfunction before lung transplantation that persisted for up to a year after transplantation. Furthermore, the

Abbreviations and Acronyms

ALI	= acute lung injury
COPD	= chronic obstructive pulmonary disease
GPX1	= glutathione peroxidase-1 gene
GSTM2	= glutathione S-transferase mu 2 gene
HWE	= Hardy-Weinberg equilibrium
LD	= linkage disequilibrium
LTOG	= Lung Transplant Outcomes Group
MAF	= minor allele frequencies
MDS	= multidimensional scaling
NFE2L2	= nuclear factor (erythroid-derived 2)-like 2 gene
NOS1AP	= nitric oxide synthase 1 adaptor protein gene
NOS3	= nitric oxide synthase 3 gene
NOX3	= NADPH oxidase 3 gene
PGD	= primary graft dysfunction
PON1	= paraoxonase 1 gene
ROS	= reactive oxygen species
SKAT	= sequencing kernel association test
SNP	= single nucleotide polymorphism

antioxidant *N*-acetylcysteine was protective against graft dysfunction in a small randomized trial.⁸ In addition, targeted delivery of catalase, an antioxidant enzyme, reduced ischemia reperfusion injury in an experimental model of lung transplantation.⁹ These and other studies provide evidence that oxidative stress is an important mediator of lung allograft dysfunction after transplantation.

The oxidant stress pathway is rich in genetic variation, believed to be to the result of evolutionary pressures from infectious and environmental stimuli. Previous studies have implicated genetic variation in oxidant stress genes altering the risk of acute lung injury (ALI).¹⁰⁻¹² In this study, we sought to identify donor and recipient variation in oxidative stress genes associated with PGD using a large multicenter cohort study and a candidate gene platform. This study has previously been registered as clinicaltrials.gov NCT00457847.

METHODS**Study Population and Data Collected**

Participants were selected from the Lung Transplant Outcomes Group (LTOG), which is a multicenter, prospective cohort study of lung transplant recipients that has been previously described.¹³⁻¹⁷ The University of Pennsylvania institutional review board (Federal-wide Assurance no. 00004028) approval and informed written consent was obtained before recruitment. Consecutive individuals transplanted between January 1, 2002, and December 31, 2009, from 10 LTOG centers were enrolled. Clinical data were collected prospectively as described elsewhere.^{18,19} PGD grade was determined using the consensus definition of the International Society of Heart and Lung Transplantation using 2 blinded readers as previously described.^{20,21} We used any grade 3 PGD occurring

within the first 72 hours after lung transplantation as our primary definition of PGD (noted as PGD henceforth).²¹ Potential confounding variables including both recipient and donor characteristics were tested for their association with PGD. Recipient age, predisposing diagnosis, and use of cardiopulmonary bypass were significant at the .05 level and thus were included in the genetic models.¹⁸

Collection and Processing of Biological Samples

Whole blood collected before organ procurement (donor) or organ implantation (recipient) was centrifuged, and buffy coat fractions were aliquoted and stored at -80°C until DNA extraction using the Qiagen Qiaamp 96 blood kit (Qiagen, Valencia, CA). Negative controls were included with all DNA extraction runs. Extracted DNA from PGD and non-PGD individuals were plated together on each 96-well microplate, and laboratory personnel were unaware of the PGD status of each sample at the time of laboratory analysis.

Genotyping and Quality Control

We used the HumanCVD BeadChip (IBC chip), a custom 50,000 single nucleotide polymorphism (SNP) genotyping array designed to assay SNPs in candidate genes and pathways affecting cardiovascular, pulmonary, inflammatory, and metabolic phenotypes (Illumina, Inc, San Diego, Calif).²² The array was designed to evaluate all nonsynonymous coding SNPs with minor allele frequencies (MAF) greater than 0.01, as well as provide coverage for several loci with MAF greater than 0.02 of potential importance to cardiac, pulmonary, and metabolic phenotypes.²² Quality control thresholds for each SNP to be included in the analysis included genotyping call greater than or equal to 95%; χ^2 testing of Hardy-Weinberg equilibrium (HWE) on the whole population yielding a *P* value of 10^{-6} or greater; and MAF greater than or equal to 0.01 overall. We sought to apply a computational methodology (SNP set analysis) that incorporated oxidant stress as a biologically plausible mediator of PGD. To accomplish this, we filtered the IBC BeadChip for 49 oxidant stress genes identified through a PubMed search conducted in May 2011 using the search terms “oxidant stress,” “polymorphism,” “genetic” limited to human and manually curated search results. Identified genes were further expanded using gene ontology pathways (see the Online Data Supplement for the list of genes, Table E1).

After genotyping, outliers were detected and removed using a genome-wide similarity metric.²³ Population stratification was determined using multidimensional scaling (MDS) analysis using all markers²³ and the resulting components were used in adjustments for population stratification.

SNP Set Analysis

SNPs were grouped into sets based on haplotype blocks within 49 genes identified to be in the oxidant stress pathway. The haplotype blocks were initially determined using the confidence interval method²⁴ implemented in Haploview, and small blocks were modified to include at least 3 common SNPs.

We used a logistic kernel machine method, sequencing kernel association test (SKAT), to test the joint effect of SNP sets on PGD. The method has previously been shown to have improved power to detect association by reducing the total number of tests being performed, jointly testing the multiple SNPs surrounding causal variants, and incorporating nonlinear and epistatic effects.²⁵ Detailed methods of SNP set analyses are presented in Appendix E1. We first tested the associations between PGD and each recipient's SNP set and then between PGD and each donor's SNP set, adjusting for recipient age, recipient diagnosis, use of cardiopulmonary bypass, and population stratification.²⁶

After donor and recipient genes with significant association were identified, we used the quadratic terms to test the interactions between significant matched donor and recipient SNP sets using SKAT.²⁷ For

FIGURE 1. Enrollment design. LTOG, Lung Transplant Outcomes Group; PGD, primary graft dysfunction.

each interaction test of donor SNP set 1 and recipient SNP set 2, all pairwise cross products of SNPs within set 1 and SNPs within set 2 were included in the function h of the model (Equation E1). We fitted a multivariate logistic model with coded SNPs as an approximation to the nonparametric function and the top SNPs that were most associated with PGD were chosen as covariates to be adjusted in the model for testing the nonlinear effect of gene-set interactions.

RESULTS

There were 1038 lung transplant recipients and 392 lung donors who met genetic quality control standards (Figure 1) with 338 representing a donor-recipient pair. Thirty percent (95% confidence interval, 27.5, 33.0) of patients (314 of 1038) developed grade 3 PGD within 72 hours. Baseline demographics for the participants in the study are summarized in Table 1. Recipient race, pretransplant diagnosis, and use of cardiopulmonary bypass differed by PGD. These differences have previously been described as associated with PGD.³

Table 2 presents the results of the main effect SNP set associations. Four recipient genes were associated with increased risk of PGD: glutathione peroxidase (*GPX1*), an enzyme important in detoxification of hydrogen peroxide ($P = .01$); nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*), encoding the NRF-2 transcription factor that upregulates expression of several phase I and phase II reactive oxygen species (ROS) detoxifying proteins (block 1, $P = .024$; block 2, $P = .005$); there was also borderline

significance for nitric oxide synthase 3 (*NOS3*), responsible for production of nitric oxide ($P = .028$); and glutathione *S*-transferase mu 2 enzyme (*GSTM2*), which regulates glutathione production ($P = .048$). Individual recipient SNPs that differed most between PGD and non-PGD included 3 SNPs within *GPX1* and 2 within *NFE2L2*. Within *GPX1*, PGD associations included 2 SNPs in tight linkage disequilibrium (LD), rs3811699 ($P = .006$) and rs1800668 ($P = .012$), located in the 5' promoter region and first exon, which have previously been described to have functional consequences in transcription,²⁸ and rs9818758 ($P = .046$) located in the intergenic region near *GPX1*, which has been associated with inflammatory bowel disease.²⁹ Within *NFE2L2*, there were 2 SNPs located in the first intron that were significantly associated with PGD; rs6726395 ($P = .029$) and rs1806649 ($P = .049$). These SNPs were in moderate LD ($R^2 = 0.35$; $D' = 0.93$) with SNPs previously reported to have function and ALI association by our group (Figure E1).¹⁰

In donors, *NOX3* ($P = .01$), nitric oxide synthase 1 adaptor protein (*NOS1AP*, $P = .048$), and paraoxonase 1 (*PON1*, $P = .03$) were associated with the development of PGD. Within *NOX3*, rs3749930, a coding nonsynonymous SNP had the strongest association with PGD ($P = .006$). The SNP marks a G/T nucleotide conversion resulting in a threonine to lysine substitution at position 171 in a transmembrane portion of the NOX-3 protein. The variant T allele resulting in a lysine was associated with a decreased risk of PGD compared with the ancestral G allele. In addition, several intronic SNPs within *NOX3* were associated with increased risk of PGD.

Sensitivity analyses of individual donor and recipient SNPs with respect to cardiopulmonary bypass and idiopathic pulmonary fibrosis demonstrated only small effects on point estimates. Only 1 of the 10 variants (rs231948) demonstrated a change in the directionality of the risk (Table 3 and Table E3). These results suggest the effects of the individual SNPs are important and not the result of known unbalanced clinical risk factors.

Individual SNP interactions between the 338 matched donor-recipient pairs are presented in Figure 2. Within recipient *NFE2L2*, blocks 1 and 2 (5 kb and 34 kb in length) are separated by 10 kb with 1 SNP in the intervening region not in LD with either block. Donor *NOX3* was divided into 5 blocks. Block 1 was the largest of the blocks (21 kb) containing 14 SNPs with a 4-kb intervening region with 7 SNPs upstream of block 2. Table E2 displays the significant interactions that were detected between donor and recipient genes. As displayed in Figure 2, donor *NOX3* block 3 and block 5 were demonstrated to have significant epistatic effects with recipient *NFE2L2* blocks 1 and 1 and 2 in their association with the development of PGD ($P = .026$, $P = .017$, and $P = .031$, respectively).

TABLE 1. Donor and recipient characteristics*

	No PGD (n = 724)	PGD (n = 314)	P value
Donor age, y ± SD	34.48 ± 14.31	34.64 ± 13.97	.872
Female donor, n (%)	277 (38)	136 (43)	.128
Donor race, n (%)			.965
White	457 (63)	205 (65)	
African American	145 (20)	59 (19)	
Hispanic	85 (12)	34 (11)	
Other	30 (4)	13 (4)	
Recipient age, y ± SD	54.02 ± 12.91	53.09 ± 12.57	.277
Female recipient, n (%)	322 (44)	143 (46)	.786
Recipient race, n (%)			.001
White	632 (87)	245 (78)	
African American	49 (7)	46 (15)	
Hispanic	24 (3)	14 (4)	
Other	18 (2)	9 (3)	
Diagnosis, n (%)			<.001
COPD	109 (15)	26 (8)	
IPF	282 (39)	177 (56)	
Other	333 (46)	111 (35)	
Bilateral treatment, n (%)	73 (10.1)	29 (9.2)	.758
CPB use, n (%)	221 (31)	174 (55)	<.001

PGD, Primary graft dysfunction; SD, standard deviation; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; CPB, cardiopulmonary bypass. *Comparison of individual cohorts with and without donor/recipient pairing is available in the supplemental materials.

CONCLUSIONS

PGD is a complex trait that has been associated with both inherent donor and recipient clinical factors.³ In this study, we have defined both the main effects and the interactions in key oxidant stress genes from lung transplant donors and recipients that increase the risk of PGD after transplant, using SNP set analysis. Like most complex traits, PGD most likely involves many SNPs with modest effects; therefore this technique is particularly well suited.³⁰ This study is the first to use these techniques in a lung transplant cohort, and our results identify that *GPX1*, *NFE2L2*, *NOS3*, *GSTM2*, *NOX3*, *NOS1AP*, and *PON1* should be prioritized for future research efforts. Most importantly, the demonstration of donor-recipient interaction between putatively functional SNPs in genes encoding *NOX3* and *NRF2* (*NFE2L2*) should focus future research efforts on these mediators.

NFE2L2 encodes NRF-2, a zinc finger transcription factor responsible for the upregulation of several key reactive oxygen detoxification enzymes.³¹ A hypofunctioning promoter variant in *NFE2L2* (rs6721961) has previously been shown by our group to have an association with increased risk for ALI after major trauma.¹⁰ This variant and others previously described by our group are not contained within the IBC chip used in our current study. However, the individual SNPs within *NFE2L2* described in our current study (rs6726395 and rs1806649) are

TABLE 2. Genes associated with primary graft dysfunction*

Cohort	Gene	P value	No. of SNPs
Recipients (n = 1038)	<i>GPX1</i> block 1	.014	8
	<i>NFE2L2</i> block 1	.024	3
	<i>NFE2L2</i> block 2	.005	13
	<i>NOS3</i> block 1	.028	8
Donors (n = 392)	<i>GSTM2</i> block 1	.048	6
	<i>NOX3</i> block 2	.01	3
	<i>NOX3</i> block 3	.05	12
	<i>NOS1AP</i> block 4	.048	11
	<i>PON1</i> block 4	.035	13
Interaction (n = 338)	Donor <i>NOX3</i> block 3 with recipient <i>NFE2L2</i> block 1	.026	
	Donor <i>NOX3</i> block 5 with recipient <i>NFE2L2</i> block 1	.017	
	Donor <i>NOX3</i> block 5 with recipient <i>NFE2L2</i> block 2	.031	

SNP, Single nucleotide polymorphism. *Statistical tests of significance generated using the quadratic kernel function.

significantly associated with PGD in lung transplant recipients and are in moderate LD with the previously described promoter SNP not in the IBC chip (rs6721961, $D' = 1$; $R^2 = 0.118$ and $D' = 1$; $R^2 = 0.052$, respectively; Figure E1, B). Variation in this gene in lung transplant recipients may increase susceptibility to PGD by reducing transcription of ROS detoxifying enzymes, and a resultant dampened oxidant stress response.

Interactions between recipient *NFE2L2* and donor *NOX3* were also associated with PGD risk. However, the functional consequences of the Thr171Lys substitution (rs3749930) in *NOX3* is unknown and the function of the *NOX3* intronic variants (rs231956, rs13207865, rs23948, and rs1546894) have thus far not been described. Because any hypothesized mechanism behind the interaction of recipient *NFE2L2* and donor *NOX3* on PGD susceptibility is speculative at this time, fine mapping of variants and/or cellular functional studies aimed at clarifying this finding are logical next research steps. Our study is useful to prioritize these 2 genes for further research into their function in increased risk of PGD.

GPX1 is an intracellular antioxidant enzyme that reduces harmful hydrogen peroxide to water.³² Genetic polymorphisms in *GPX1* have been demonstrated to affect transcription and alter response to vascular oxidant stress and risk of cardiovascular disease in humans.²⁸ In our study, 3 *GPX1* SNPs (rs1800668, rs9818758, rs3811699) were significantly associated with PGD. Two of the 3 SNPs (rs1800668, rs3811699) are in tight LD ($D' = 1$, $R^2 = 0.88$, Figure E1) and have previously been shown to enhance oxidant injury in cell culture through decreased expression of *GPX1* in the setting of oxidant stress.²⁸ Our results demonstrate protective effects associated with these 3 SNP (rs1800668, rs9818758, rs3811699) minor alleles, indicating that either lower *GPX1* expression is protective

TABLE 3. Individual single nuclear polymorphisms associated with primary graft dysfunction

Cohort	SNP	MA	MAF_A	OR	95% CI	P value	Gene	Location
Recipient	rs9818758	A	0.13	0.74	0.54, 0.99	.048	GPX1	Intergenic
	rs1800668	T	0.25	0.74	0.58, 0.94	.012	GPX1	UTR
	rs3811699	G	0.26	0.72	0.57, 0.91	.006	GPX1	5' upstream
	rs6726395	T	0.43	0.79	0.64, 0.98	.029	NFE2L2	Intron
	rs1806649	A	0.18	0.76	0.58, 0.99	.049	NFE2L2	Intron
Donor	rs3749930	T	0.06	0.38	0.20, 0.76	.006	NOX3	Coding nonsynonymous
	rs231956	C	0.53	1.49	1.05, 2.12	.024	NOX3	Intron
	rs13207865	T	0.29	1.57	1.06, 2.33	.024	NOX3	Intron
	rs231948	T	0.54	0.57	0.3, 1.0	.037	NOX3	Intron
	rs1546894	A	0.02	0.31	0.11, 0.89	.029	NOX3	Intron

SNP, Single nucleotide polymorphism; MA, minor allele; MAF_A, minor allele frequency of those affected; OR, odds ratio; CI, confidence interval; A, adenine; T, thymine; UTR, untranslated region; G, guanine; C, cytosine.

or that the putative functional inference from previous studies does not apply to pulmonary ischemia reperfusion injury. Therefore, future study is warranted in suitable models that apply to PGD.

There are several limitations of this study. First, although several of our implicated variants have suggested function in regulating transcription from previous studies, it is not possible to establish functional causation with our study design. However, our results serve to prioritize these specific oxidant stress genes for future study. Second, gaps in our knowledge on oxidant stress gene inducers, regulators, and modulators may have led us to exclude genes with important function in regulating oxidative stress.³⁰ We designed a strategy that was as inclusive as possible, relying both on existing gene ontology pathways

as well a comprehensive literature search. In addition, SNP set methods as we have used them assume that SNPs within known genes associate with a complex trait, an approach that ignores potential trait-associated SNPs that are not located within genes.³⁰ Third, although we did not perform a simple adjustment for multiple comparisons in our postanalysis *P* values (such as a Bonferroni adjustment), SNP set methods do not require postanalysis multiple comparison adjustment, as the method accounts for the number of comparisons.²⁵ In addition, our methods did not use multiple adjustments for donor-specific factors given the limited information available. Our findings have not been replicated in an independent dataset. Unfortunately, there is no existing cohort to validate our multicenter findings available at this time. Work is

FIGURE 2. SNP set block interaction. Individual SNPs are represented by a rectangle. Blocks are delineated by dotted lines. The highest intensities are evident in donor *NOX3* block 3 with recipient *NFE2L2* block 1 and in donor *NOX3* block 3 with recipient *NFE2L2* blocks 1 and 2. SNP, Single nucleotide polymorphism.

underway by our group and others to standardize prospective sample collection in order to develop cohorts from which validation of identified genetic variants and pathways important in PGD can be tested. Given the many possible confounding factors inherent in lung transplantation and the modest effects of potential factors, only large multiinstitutional cohort studies will have enough statistical power to clarify the effects of these identified factors.

In summary, we have demonstrated an association between genes regulating oxidative stress and PGD. The effects of these associations suggest the importance of oxidant stress detoxifiers in lung recipients and ROS generators in lung donors. The effects of their interaction suggest a complex mechanism of injury of which oxidant stress is a component and therefore should stimulate further inquiry into the participation of *GPX1*, *NFE2L2*, *NOX3*, *PON1*, and *GSTM2* in the pathogenesis of PGD. In addition, if validated, these gene variants may allow improved risk stratification of transplant recipients for therapies targeted at oxidant balance.

We would like to acknowledge E.J. Demissie for coordinating all data collection efforts for the LTOG study investigators.

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EDITORIAL COMMENTARY

One step closer to the elimination of primary graft dysfunction

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See related article on pages 596-602.

Primary graft dysfunction (PGD) is a significant cause of morbidity and mortality after lung transplants. As supported by the findings in the study published by Cantu and colleagues¹ in this issue of the *Journal*, as many as 30% of patients will have development of grade 3 PGD, defined by a Pao₂ to inspired oxygen fraction ratio less than 200 along with diffuse allograft infiltrates on chest radiography. Implicated in PGD is ischemia-reperfusion injury, which our laboratory has studied and published on extensively, as well as activation of the oxidant stress pathway. Although studies have shown that genetic variations alter the risk of acute lung injury, there are few data on human subjects describing the genes responsible for PGD after lung transplants. This article examines genetic variations associated with PGD in both donors and recipients of transplanted lungs.

FIGURE 1. Lungs from a porcine donor without a beating heart undergoing rehabilitation with ex vivo lung perfusion.

Cantu and colleagues¹ completed a large, multicenter prospective cohort study of lung transplant donors and recipients selected from the Lung Transplant Outcomes Group. DNA samples from 1038 lung transplant recipients and 392 lung donors were analyzed for the presence of 49 oxidant stress genes with single-nucleotide polymorphism set analysis. The data demonstrated that *GPX1*, *NFE2L2*, *NOS3*, *GSTM2*, *NOX3*, *NOS1AP*, and *PON1* were associated with grade 3 PGD and

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Disclosures: Authors have nothing to disclose with regard to commercial support. Received for publication Sept 30, 2014; accepted for publication Oct 1, 2014; available ahead of print Nov 7, 2014.

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APPENDIX E1. STATISTICAL METHODS FOR SNP SET ANALYSIS

For a given individual i , we use Y_i to denote the PGD, $x_{i1}, \dots, x_{im}$ to denote the adjusted covariates, and $z_{i1}, \dots, z_{ip}$ the p coded SNPs within a test set. The SKAT tests the hypothesis that $h(z_1, z_2, \dots, z_p) = 0$, in the semi-parametric model

$$\log \text{it}\{P(Y_i = 1)\} = \beta_0 + \beta_1 x_{i1} + \dots + \beta_m x_{im} + h(z_{i1}, z_{i2}, \dots, z_{ip}), \quad I = 1, \dots, n \quad (\text{Eq. 1})$$

where $\beta_0, \beta_1, \dots, \beta_m$ are unknown parameters, measuring the covariates effects, and $h(\cdot)$ is an unknown

function, summarizing the joint effect of SNPs $z_{i1}, \dots, z_{ip}$ on the PGD. $h(\cdot)$ can be written as $h(z_{i1}, z_{i2}, \dots, z_{ip}) = \sum_{j=1}^n \gamma_j K(z_i, z_j)$ where $K(\cdot, \cdot)$ is a user-specified positive semidefinite kernel function and coefficients $\gamma_1, \dots, \gamma_n$ are determined by the data.³¹ A score test statistic can be formulated as a function of the phenotype similarity and the kernel function $K(\cdot, \cdot)$, the genotype similarity of 2 individuals, and can be calculated under the null hypothesis without estimating the coefficients $\gamma_1, \dots, \gamma_n$. Although there are multiple choices of kernel functions, previous findings demonstrate that the IBS and quadratic kernels have much improved power over the linear kernel in the presence of epistasis.²²

FIGURE E1. Visualization of linkage disequilibrium. Individual SNPs within *GPX1* (A) and *NFE2L2* (B) were examined using Haploview software.³³ *GPX1* demonstrated more LD blocks than *NFE2L2* using the methodology of Gabriel and colleagues.²³ A block is created if 95% of informative (ie, noninconclusive) comparisons are strong LD. This method by default ignores markers with MAF <0.05.

TABLE E1. Oxidant stress pathway genes*

Genes	Proteins
<i>CAT</i>	Catalase
<i>CYBA</i>	Cytochrome <i>b</i> -245, α polypeptide chain
<i>CYBB</i>	Cytochrome <i>b</i> -245, β polypeptide chain
<i>DUSP1</i>	Dual specificity protein phosphatase 1
<i>EPHX2</i>	Epoxide hydrolase 2
<i>GPX1</i>	Glutathione peroxidase 1
<i>GPX2</i>	Glutathione peroxidase 2
<i>GPX3</i>	Glutathione peroxidase 3
<i>GPX4</i>	Glutathione peroxidase 4
<i>GPX5</i>	Glutathione peroxidase 5
<i>GPX6</i>	Glutathione peroxidase 6
<i>GPX7</i>	Glutathione peroxidase 7
<i>GSS</i>	Glutathione synthetase
<i>GSTA1</i>	Glutathione <i>S</i> -transferase α 1
<i>GSTM1</i>	Glutathione <i>S</i> -transferase μ 1
<i>GSTM2</i>	Glutathione <i>S</i> -transferase μ 2
<i>GSTM3</i>	Glutathione <i>S</i> -transferase μ 3
<i>GSTM5</i>	Glutathione <i>S</i> -transferase μ 5
<i>GSTO1</i>	Glutathione <i>S</i> -transferase ω 1
<i>GSTO2</i>	Glutathione <i>S</i> -transferase ω 2
<i>GSTP1</i>	Glutathione <i>S</i> -transferase π 1
<i>GSTT1</i>	Glutathione <i>S</i> -transferase θ 1
<i>HMOX1</i>	Heme oxygenase (decycling) 1
<i>MPO</i>	Myeloperoxidase
<i>MPV17</i>	Mitochondrial inner membrane protein
<i>MSRA</i>	Methionine sulfoxide reductase A
<i>NCF2</i>	Neutrophil cytosolic factor 2
<i>NCF4</i>	Neutrophil cytosolic factor 4
<i>NFE2L2</i>	Nuclear factor (erythroid-derived 2)-like 2
<i>NFKB1</i>	Nuclear factor of κ light polypeptide gene enhancer in B cells 1
<i>NOS1</i>	Nitric oxide synthase 1
<i>NOS1AP</i>	Nitric oxide synthase 1 adaptor protein
<i>NOS2A</i>	Nitric oxide synthase 2A, inducible
<i>NOS3</i>	Nitric oxide synthase 3
<i>NOX3</i>	Reduced nicotinamide adenine dinucleotide phosphate oxidase 3
<i>NOX4</i>	Reduced nicotinamide adenine dinucleotide phosphate oxidase 4
<i>NQO1</i>	Reduced nicotinamide adenine dinucleotide (phosphate) dehydrogenase (quinone) 1
<i>PIP3-E</i>	Phosphoinositide-binding protein 3-E
<i>PON1</i>	Paraoxonase 1
<i>PON2</i>	Paraoxonase 2
<i>PON3</i>	Paraoxonase 3
<i>SELS</i>	Selenoprotein S
<i>SFTPD</i>	Surfactant (pulmonary-associated) protein D
<i>SIRT2</i>	Nicotinamide adenine dinucleotide-dependent deacetylase sirtuin 2
<i>SOD1</i>	Superoxide dismutase 1
<i>SOD2</i>	Mitochondrial superoxide dismutase 2
<i>SOD3</i>	Extracellular superoxide dismutase 3
<i>TTN</i>	Titin
<i>TXNRD2</i>	Thioredoxin reductase 2

*In addition to known classic oxidant stress genes, additional genes were identified through gene ontology and literature searches.

TABLE E2. Individual donor/recipient single nucleotide polymorphism interactions

SNP		Donor			Recipient			Statistics	
Donor	Recipient	Block	Gene	Function	Block	Gene	Function	OR_INT	P
rs163007	rs13005431	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	0.3025	.00019
rs163007	rs13001694	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	0.3093	.00029
rs151223	rs10183914	5	<i>NOX3</i>	Intron	1	<i>NFE2L2</i>	Intron	0.3292	.00099
rs163007	rs4243387	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	3.591	.00551
rs17086264	rs1806649	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	0.4149	.01738
rs10223752	rs13005431	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	3.106	.02495
rs11961314	rs13001694	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	2.949	.03266
rs11963380	rs4243387	5	<i>NOX3</i>	Intron	2	<i>NFE2L2</i>	Intron	0.3273	.04277
rs11963380	rs6726395	3	<i>NOX3</i>	Intron	1	<i>NFE2L2</i>	Intron	0.4406	.04875
rs11963380	rs2001350	3	<i>NOX3</i>	Intron	1	<i>NFE2L2</i>	Intron	0.113	.04897

OR_INT, Odds ratio for interaction.

TABLE E3. Individual single nucleotide polymorphisms associated with primary graft dysfunction

Cohort	SNP	All		No CPB		CPB		IPF	
		OR	P value	OR	P value	OR	P value	OR	P value
Recipient	rs9818758	0.74	.048	0.60	.011	0.91	.672	0.86	.475
	rs1800668	0.74	.012	0.62	.003	0.92	.608	0.86	.333
	rs3811699	0.72	.006	0.62	.003	0.92	.596	0.89	.465
	rs6726395	0.79	.029	0.84	.217	0.73	.034	0.85	.237
	rs1806649	0.76	.049	0.82	.242	0.62	.018	0.82	.265
Donor	rs3749930	0.38	.006	0.59	.230	0.44	.025	0.24	.004
	rs231956	1.49	.024	1.52	.077	1.32	.226	1.16	.530
	rs13207865	1.57	.024	2.23	.004	1.24	.424	1.63	.083
	rs231948	0.57	.037	1.76	.012	1.27	.280	1.46	.098
	rs1546894	0.31	.029	0.30	.117	0.35	.091	0.43	.187