



Published in final edited form as:

J Thorac Cardiovasc Surg. 2015 February ; 149(2): 596–602.e3. doi:10.1016/j.jtcvs.2014.09.077.

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

Edward Cantu, MD^{1,*}, Rupal J. Shah, MD^{2,3,*}, Wei Lin, PhD³, Zhongyin J. Daye, PhD, MS³, Joshua M. Diamond, MD, MSCE^{2,3}, Yoshikazu Suzuki, MD, MS¹, John H. Ellis, BA¹, Catherine F. Borders, BA¹, Gerald A. Andah, BA¹, Ben Beduhn, BA¹, Nuala J. Meyer, MD, MS^{2,3}, Melanie Ruschefski, BA^{2,3}, Richard Aplenc, MD, MS^{3,4}, Rui Feng, PhD^{3,*}, and Jason D. Christie, MD, MS^{2,3,*} for the Lung Transplant Outcomes Group Investigators

¹Cardiovascular Surgery Division, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

²Pulmonary, Allergy, and Critical Care Division, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

³Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

⁴Children's Hospital of Philadelphia, Philadelphia, PA

Abstract

Objective—Oxidant stress pathway activation during ischemia reperfusion injury may contribute to the development of primary graft dysfunction (PGD) after lung transplantation. We hypothesized 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

© 2014 by The American Association for Thoracic Surgery. All rights reserved.

Corresponding Author: Edward Cantu III, MD, Division of Cardiovascular Surgery, University of Pennsylvania Perelman School of Medicine, 3400 Spruce St., 6 Silverstein Pavilion, Philadelphia, PA 19104, Telephone: 215-615-6582, Fax: 215-349-5798, edward.cantu@uphs.upenn.edu.

*These authors have contributed equally to this work

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

EC and RJS participated in study design and coordination and drafted the manuscript. WL and ZJD performed the statistical analysis. JMD, YS, JHE, CFB, GAA and NJM helped to draft the manuscript. JDC, RA, and RF conceived of the study, and participated in its design and coordination and helped to draft the manuscript. MR performed laboratory analyses and aided in study design. All authors read and approved the final manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

PGD association using a sequencing kernel association test. Analyses were adjusted for clinical confounding variables and population stratification.

Results—392 donors and 1038 recipients met genetic quality control standards. 30% of subjects developed grade 3 PGD within 72 hours. Donor NADPH Oxidase 3 (*NOX3*) was associated with PGD ($p=0.01$) with 5 individual significant loci (p -values between 0.006 and 0.03). In recipients, variation in glutathione peroxidase (*GPXI*) and NRF-2 (*NFE2L2*) was significantly associated with PGD ($p=0.01$ for both). The *GPXI* association included 3 individual loci (p -values between 0.006 and 0.049) and the *NFE2L2* association included 2 loci ($p=0.03$ and 0.05). Significant epistatic effects influencing PGD susceptibility were evident between three different donor blocks of *NOX3* and recipient *NFE2L2* ($p=0.026$, $p=0.017$ and $p=0.031$).

Conclusions—Our study prioritizes *GPXI*, *NOX3*, and *NFE2L2* genes for future research in PGD pathogenesis, and highlights a donor-recipient interaction of *NOX3* and *NFE2L2* that increases PGD risk.

Keywords

Primary Graft Dysfunction; Lung Transplantation; Oxidant Stress; Genes

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 post-transplant period.² PGD affects 10–30% of all subjects 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, 4, 5}

Although the pathophysiology of PGD is incompletely understood, ischemia reperfusion injury leading to activation of the oxidant stress pathway is thought to be a major contributor to its development.⁶ In support of this notion, Williams, et al identified antioxidant system dysfunction prior to lung transplantation that persisted for up to a year after transplantation.⁷ Furthermore, the antioxidant N-acetylcysteine was protective against graft dysfunction in a small randomized trial.⁸ Additionally, 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, thought due to evolutionary pressures from infectious and environmental stimuli. Prior studies have implicated genetic variation in oxidant stress genes altering acute lung injury (ALI) risk.^{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 clinical trials.gov # NCT00457847.

METHODS

Study Population and Data Collected

Subjects were selected from the Lung Transplant Outcomes Group (LTOG), which is a multi-center, prospective cohort study of lung transplant recipients that has been previously described.^{13–17} University of Pennsylvania institutional review board (Federal-wide Assurance # 00004028) approval and informed written consent was obtained prior to recruitment of subjects. Consecutive subjects 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 two blinded readers as previously described.^{20, 21} We used any Grade 3 PGD occurring within the first 72 hours following lung transplantation as our primary PGD definition (noted as “PGD” henceforth).²¹ Potential confounding variables including both recipients’ and donors’ characteristics were tested for their association with PGD. Recipient age, predisposing diagnosis, and use of cardiopulmonary bypass were significant at the 0.05 level and thus were included in the genetic models.¹⁸

Collection and Processing of Biological Samples

Whole blood collected prior to 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 Qiamap 96 blood kit (Qiagen, Valencia, CA). Negative controls were included with all DNA extraction runs. Extracted DNA from PGD and non-PGD subjects were plated together on each 96-well microplate, and lab 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 50K 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, CA).²² The array was designed to evaluate all non-synonymous coding SNPs with minor allele frequencies (MAF) > 0.01 , as well as provide coverage for a number of loci with MAF > 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 $\geq 95\%$; chi-square testing of Hardy-Weinberg equilibrium (HWE) on the whole population yielding a p value $\leq 10^{-6}$; and MAF ≥ 0.01 overall. We sought to apply a computational methodology (SNPset analysis) which 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 PubMed search conducted 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 online supplement for list of genes, eTable 1).

Following 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 adjustment for population stratification.

SNPset 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 three 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 SNPset analyses are presented in the supplemental material. We first tested the associations between PGD and each recipient's SNPset and then between PGD and each donor's SNPset, 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 SNPsets using SKAT.²⁷ For each interaction test of donor SNP set 1 and recipient SNP set 2, all pair-wise cross-products of SNPs within set 1 and SNPs within set 2 were included in the function h of the model (Equation 1 in Supplement). 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 nonlinear effect of gene-set interactions. Due to the multiple comparison adjustment inherent in SNPset analysis, a p-value of 0.05 was considered significant for all SNPset comparisons.

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. 30% (95% confidence interval: 27.5, 33.0) of subjects (314 out of 1038) developed grade 3 PGD within 72 hours. Baseline demographics for study subjects are summarized in Table 1. Recipient race, pre-transplant diagnosis and cardiopulmonary bypass use differed by PGD. These differences have previously been described as associated with PGD.³

Table 2 presents the results of the main effect SNPset associations. Four recipient genes were associated with elevated PGD risk including, glutathione peroxidase (*GPX1*), an enzyme important in detoxification of hydrogen peroxide, ($p=0.01$); and nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*), encoding the NRF-2 transcription factor that up-regulates expression of several phase I and phase II reactive oxygen species (ROS) detoxifying proteins (block 1 $p=0.024$ and block 2 $p=0.005$). There was also borderline significance for nitric oxide synthase 3 (*NOS3*), responsible for production of nitric oxide ($p=0.028$); and glutathione S-transferase mu 2 enzyme (*GSTM2*) which regulates glutathione

production ($p=0.048$). 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 two SNPs in tight linkage disequilibrium (LD), rs3811699 ($p=0.006$) and rs1800668 ($p=0.012$), located in the 5' promoter region and first exon, which have previously been described to have functional consequences in transcription,²⁸ and rs9818758 ($p=0.046$) located in the intergenic region near *GPX1*, 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 ($p=0.029$) and rs1806649 ($p=0.049$). 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 (eFigure 1).¹⁰

In donors, *NOX3* ($p=0.01$), nitric oxide synthase 1 adaptor protein (*NOS1AP*, $p=0.048$) and paraoxonase-1 (*PONI*, $p=0.03$) were associated with the development of PGD. Within *NOX3*, rs3749930, a coding non-synonymous SNP had the strongest association with PGD ($p=0.006$). 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. Additionally, several intronic SNPs within *NOX3* associated with increased PGD risk.

Sensitivity analyses of individual donor and recipient SNPs with respect to cardiopulmonary bypass and IPF demonstrated only small effects on point estimates. Only one of the 10 variants (rs231948) demonstrated a change in the directionality of the risk (eTable 3). Taken together, 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 one 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 2 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 development of PGD ($p=0.026$, $p=0.017$ and $p=0.031$, respectively).

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 post-transplant PGD risk, using SNPset 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 employ these techniques in a lung transplant cohort, and our results identify that *GPX1*, *NFE2L2*, *NOS3*, *GSTM2*, *NOX3*, *NOS1AP* and *PONI* 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 up-regulation 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 acute lung injury following 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 SNP's within *NFE2L2* described in our current study (rs6726395 and rs1806649) are 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; eFigure 2b). 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 two genes for further research into their function of increased PGD risk.

GPX1 is an intracellular anti-oxidant 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, three *GPX1* SNPs (rs1800668, rs9818758, rs3811699) were significantly associated with PGD. Two of the three SNPs (rs1800668, rs3811699) are in tight LD ($D' = 1$, $R^2 = 0.88$, eFigure 2) 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 three SNP (rs1800668, rs9818758, rs3811699) minor alleles, indicating that either lower GPX1 expression is protective, or that the putative functional inference from prior 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 prior 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 in 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. Additionally, SNPset methods as we have employed them

assume that SNPs within known genes associate with a complex trait, an approach that ignores potential trait-associated SNPs which are not located within genes.³⁰ Third, although we did not perform a simple adjustment for multiple comparisons in our post-analysis p-values (such as a Bonferroni adjustment), SNPset methods do not require post-analysis multiple comparison adjustment, as the method accounts for the number of comparisons.²⁵ Additionally, our methods did not employ multiple adjustments for donor-specific factors given the limited information available. Finally, 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 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 multi-institutional cohort studies will possess enough statistical power to clarify 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 importance of oxidant stress detoxifiers in lung recipients and ROS generators in lung donors. Effects of their interaction suggests a complex mechanism of injury of which oxidant stress is a component and therefore should stimulate further inquiry of *GPX1*, *NFE2L2*, *NOX3*, *PON1* and *GSTM2* participation in PGD pathogenesis. Additionally, if validated, these gene variants may allow improved risk stratification of transplant recipients for therapies targeted at oxidant balance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: HL087115, HL081619, HL096845, HL115354, HL114626, HL116656, RWJ11642 and HL090021

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

Abbreviations

PGD	primary graft dysfunction
ALI	acute lung injury
LTOG	Lung Transplant Outcomes Group
SNP	single nucleotide polymorphism
MAF	minor allele frequencies
HWE	Hardy- Weinberg equilibrium
MDS	multidimensional scaling
SKAT	sequencing kernel association test

IPF	idiopathic pulmonary fibrosis
COPD	chronic obstructive pulmonary disease
NOX3	NADPH oxidase-3 gene
GPX1	glutathione peroxidase-1 gene
NFE2L2	nuclear factor (erythroid-derived 2)-like 2 gene
GSTM2	glutathione S-transferase mu 2 gene
ROS	reactive oxygen species
NOS3	nitric oxide synthase 3 gene
LD	linkage disequilibrium
NOS1AP	nitric oxide synthase 1 adaptor protein gene
PONI	paraoxonase-1 gene

References

- Christie JD, Carby M, Bag R, Corris P, Hertz M, Weill D, et al. Report of the isHLT working group on primary lung graft dysfunction part ii: Definition. A consensus statement of the international society for heart and lung transplantation. *J Heart Lung Transplant*. 2005; 24:1454–9. [PubMed: 16210116]
- Christie JD, Edwards LB, Kucheryavaya AY, Aurora P, Dobbels F, Kirk R, et al. The registry of the international society for heart and lung transplantation: Twenty-seventh official adult lung and heart-lung transplant report--2010. *J Heart Lung Transplant*. 2010; 29:1104–18. [PubMed: 20870165]
- Suzuki Y, Cantu E, Christie JD. Primary graft dysfunction. *Semin Respir Crit Care Med*. 2013; 34:305–19. [PubMed: 23821506]
- Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med*. 2007; 175:507–13. [PubMed: 17158279]
- Zhang YX, Fan H, Shi Y, Xu ST, Yuan YF, Zheng RH, et al. Prevention of lung ischemia-reperfusion injury by short hairpin rna-mediated caspase-3 gene silencing. *J Thorac Cardiovasc Surg*. 2010; 139:758–64. [PubMed: 19969310]
- de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion-induced lung injury. *Am J Respir Crit Care Med*. 2003; 167:490–511. [PubMed: 12588712]
- Williams A, Riise GC, Anderson BA, Kjellstrom C, Schersten H, Kelly FJ. Compromised antioxidant status and persistent oxidative stress in lung transplant recipients. *Free Radic Res*. 1999; 30:383–93. [PubMed: 10342331]
- Rega FR, Wuyts WA, Vanaudenaerde BM, Jannis NC, Neyrinck AP, Verleden GM, et al. Nebulized n-acetyl cysteine protects the pulmonary graft inside the non-heartbeating donor. *J Heart Lung Transplant*. 2005; 24:1369–77. [PubMed: 16143259]
- Kozower BD, Christofidou-Solomidou M, Sweitzer TD, Muro S, Buerk DG, Solomides CC, et al. Immunotargeting of catalase to the pulmonary endothelium alleviates oxidative stress and reduces acute lung transplantation injury. *Nat Biotechnol*. 2003; 21:392–8. [PubMed: 12652312]
- Marzec JM, Christie JD, Reddy SP, Jedlicka AE, Vuong H, Lanken PN, et al. Functional polymorphisms in the transcription factor nrf2 in humans increase the risk of acute lung injury. *FASEB J*. 2007; 21:2237–46. [PubMed: 17384144]

11. Arcaroli JJ, Hokanson JE, Abraham E, Geraci M, Murphy JR, Bowler RP, et al. Extracellular superoxide dismutase haplotypes are associated with acute lung injury and mortality. *Am J Respir Crit Care Med.* 2009; 179:105–12. [PubMed: 18948423]
12. Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. *Arch Biochem Biophys.* 2001; 389:84–93. [PubMed: 11370676]
13. Christie JD, Robinson N, Ware LB, Plotnick M, De Andrade J, Lama V, et al. Association of protein c and type 1 plasminogen activator inhibitor with primary graft dysfunction. *Am J Respir Crit Care Med.* 2007; 175:69–74. [PubMed: 17023732]
14. Christie JD, Shah CV, Kawut SM, Mangalmurti N, Lederer DJ, Sonett JR, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. *Am J Respir Crit Care Med.* 2009; 180:1010–5. [PubMed: 19661249]
15. Covarrubias M, Ware LB, Kawut SM, De Andrade J, Milstone A, Weinacker A, et al. Plasma intercellular adhesion molecule-1 and von willebrand factor in primary graft dysfunction after lung transplantation. *Am J Transplant.* 2007; 7:2573–8. [PubMed: 17908278]
16. Diamond JM, Meyer NJ, Feng R, Rushefski M, Lederer DJ, Kawut SM, et al. Variation in ptx3 is associated with primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med.* 2012; 186:546–52. [PubMed: 22822025]
17. Diamond JM, Akimova T, Kazi A, Shah RJ, Cantu E, Feng R, et al. Genetic variation in the prostaglandin e2 pathway is associated with primary graft dysfunction. *Am J Respir Crit Care Med.* 2014; 189:567–75. [PubMed: 24467603]
18. Christie JD, Kotloff RM, Pochettino A, Arcasoy SM, Rosengard BR, Landis JR, et al. Clinical risk factors for primary graft failure following lung transplantation. *Chest.* 2003; 124:1232–41. [PubMed: 14555551]
19. Diamond JM, Lee JC, Kawut SM, Shah RJ, Localio AR, Bellamy SL, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med.* 2013; 187:527–34. [PubMed: 23306540]
20. Christie JD, Van Raemdonck D, de Perrot M, Barr M, Keshavjee S, Arcasoy S, et al. Report of the isHLT working group on primary lung graft dysfunction part i: Introduction and methods. *J Heart Lung Transplant.* 2005; 24:1451–3. [PubMed: 16210115]
21. Christie JD, Bellamy S, Ware LB, Lederer D, Hadjiliadis D, Lee J, et al. Construct validity of the definition of primary graft dysfunction after lung transplantation. *J Heart Lung Transplant.* 2010; 29:1231–9. [PubMed: 20655249]
22. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k snp array for large-scale genomic association studies. *PLoS One.* 2008; 3:e3583. [PubMed: 18974833]
23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–75. [PubMed: 17701901]
24. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science.* 2002; 296:2225–9. [PubMed: 12029063]
25. Wu MC, Kraft P, Epstein MP, Taylor DM, Chanock SJ, Hunter DJ, et al. Powerful snp-set analysis for case-control genome-wide association studies. *Am J Hum Genet.* 86:929–42. [PubMed: 20560208]
26. Meyer NJ, Li M, Feng R, Bradfield J, Gallop R, Bellamy S, et al. Angpt2 genetic variant is associated with trauma-associated acute lung injury and altered plasma angiotensin-2 isoform ratio. *Am J Respir Crit Care Med.* 2011; 183:1344–53. [PubMed: 21257790]
27. Musani SK, Shriner D, Liu N, Feng R, Coffey CS, Yi N, et al. Detection of gene x gene interactions in genome-wide association studies of human population data. *Hum Hered.* 2007; 63:67–84. [PubMed: 17283436]
28. Hamanishi T, Furuta H, Kato H, Doi A, Tamai M, Shimomura H, et al. Functional variants in the glutathione peroxidase-1 (gpx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in japanese type 2 diabetic patients. *Diabetes.* 2004; 53:2455–60. [PubMed: 15331559]

29. Goyette P, Lefebvre C, Ng A, Brant SR, Cho JH, Duerr RH, et al. Gene-centric association mapping of chromosome 3p implicates mst1 in ibd pathogenesis. *Mucosal Immunol.* 2008; 1:131–8. [PubMed: 19079170]
30. Fridley BL, Biernacka JM. Gene set analysis of snp data: Benefits, challenges, and future directions. *Eur J Hum Genet.* 19:837–43. [PubMed: 21487444]
31. Cho HY, Reddy SP, Kleeberger SR. Nrf2 defends the lung from oxidative stress. *Antioxid Redox Signal.* 2006; 8:76–87. [PubMed: 16487040]
32. Lubos E, Mahoney CE, Leopold JA, Zhang YY, Loscalzo J, Handy DE. Glutathione peroxidase-1 modulates lipopolysaccharide-induced adhesion molecule expression in endothelial cells by altering cd14 expression. *Faseb J.* 24:2525–32. [PubMed: 20219985]
33. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of ld and haplotype maps. *Bioinformatics.* 2005; 21:263–5. [PubMed: 15297300]

Participating centers and investigators in the Lung Transplant Outcomes Group

University of Pennsylvania (coordinating site): Jason Christie, M.D., M.S. (PI), Steven M. Kawut, M.D., M.S., Ejigayehu Demissie, M.S.N., Robert M. Kotloff, M.D., Vivek N. Ahya, M.D., James Lee, M.D., M.S., Denis Hadjiliadis, M.D., M.H.S., Melanie Rushefski, B.S., Richard Aplenc, M.D., Clifford Deutschman, M.D., M.S., Benjamin Kohl, M.D., Edward Cantu, M.D., Yoshikazu Suzuki, M.D., M.S., Joshua M. Diamond, M.D., M.S., Rupal J. Shah, M.D., and Laurel Kalman. Columbia University: David Lederer, M.D., M.S. (PI), Selim Arcasoy, M.D., Joshua Sonett, M.D., Jessie Wilt, M.D., Frank D’Ovidio, M.D., Lori Shah, M.D., Hilary Robbins, M.D., Matthew Bacchetta, M.D., Nilani Ravichandran, N.P., Genevieve Reilly, N.P., Jeffrey Okun, M.D., Debbie Rybak, B.A., Michael Koeckert, B.A., Robert Sorabella, B.A., Nisha Ann Philip, M.B.B.S., Nadine Al-Naamani, M.D., Matthew LaVelle, B.S., Megan Larkin, M.P.H., and Shefali Sanyal, B.S. Vanderbilt University: Lorraine Ware, M.D. (PI), Aaron Milstone, M.D. (PI), Jean Barnes, R.N., Stephanie Logan, R.N., Carla Ramsey, R.N., Thelma Walden, and Shaquita Claybrooks, R.N. Stanford University: Ann Weinacker, M.D. (PI), Susan Spencer Jacobs, M.S.N., Val Scott, M.S.N., and Tal Alfasi, M.S. University of Alabama, Birmingham: Keith Wille, M.D. (PI), and Necole Harris, R.N. Johns Hopkins University: Jonathan Orens, M.D. (PI), Ashish Shah, M.D., John McDyer, M.D., Christian Merlo, M.D., M.P.H., Matthew Pipeling, M.D., Reda Girgis, M.D., Karen Oakjones, R.N., and April Thurman. University of Michigan: Vibha Lama, M.D., M.S. (PI), Fernando Martinez, M.D., M.S., Emily Galopin, Douglas R. Armstrong R.N., M.S., and Mary Maliarik, B.S. Duke University: Scott M. Palmer, M.D., M.H.S. (PI), David Zaas, M.D., M.B.A., R. Duane Davis, M.D., Ashley Finlen-Copeland, M.S.W., Jessica Martissa, and William A. Davis. University of Chicago: Sangeeta Bhorade, M.D. (PI), and Mark Lockwood, R.N., M.S.N. University of Pittsburgh: Maria Crespo, M.D. (PI), Joseph Pilewski, M.D., Christian Bermudez, M.D., and Kathleen Hanze. Indiana University: David S. Wilkes, M.D., David Wilson Roe, M.D., Thomas Wozniak, M.D., Ronda L. McNamee, R.N., Kim A. Fox, R.N., Danyel F. Gooch, R.N., and Tonya Isaacs, R.N.

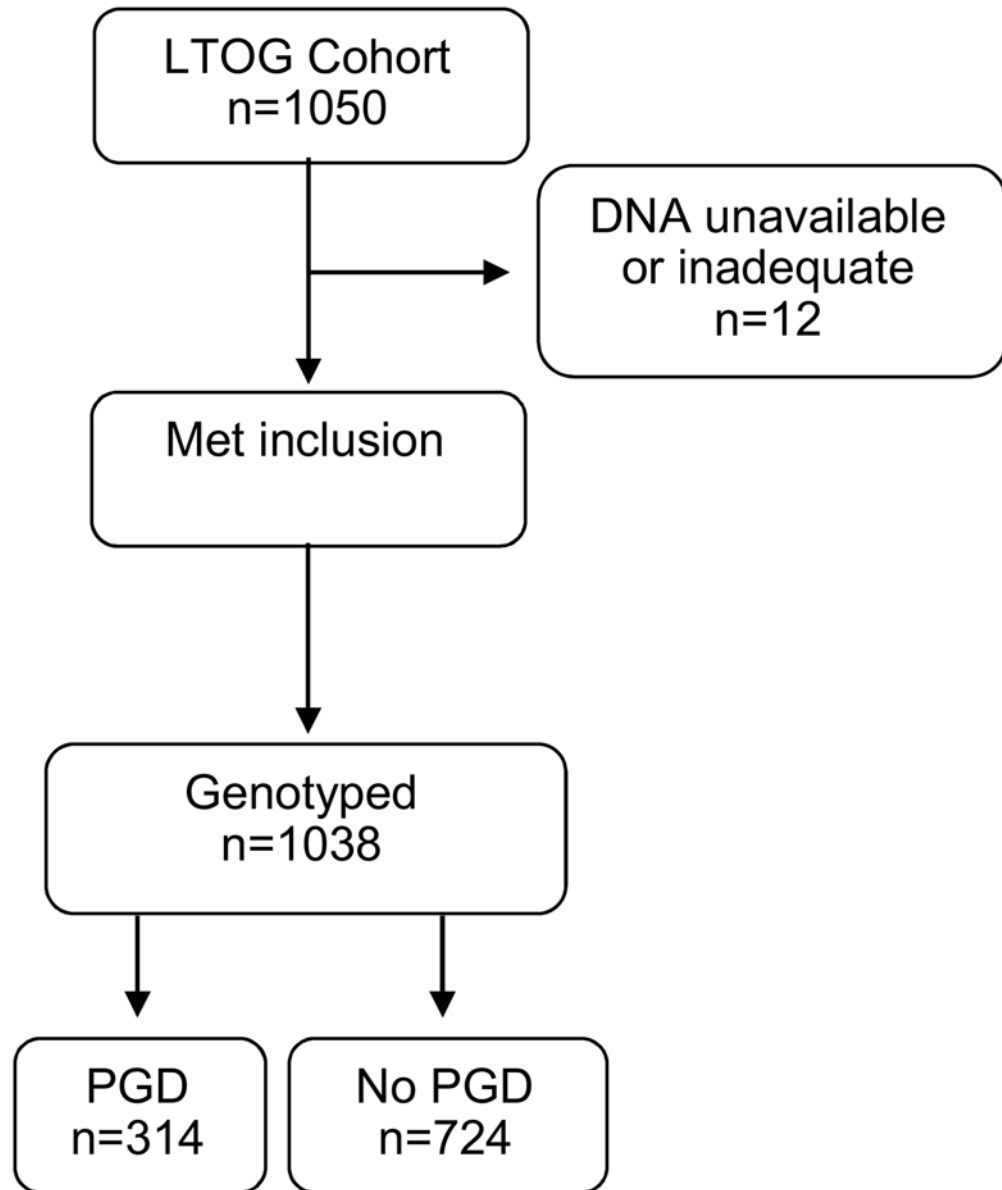
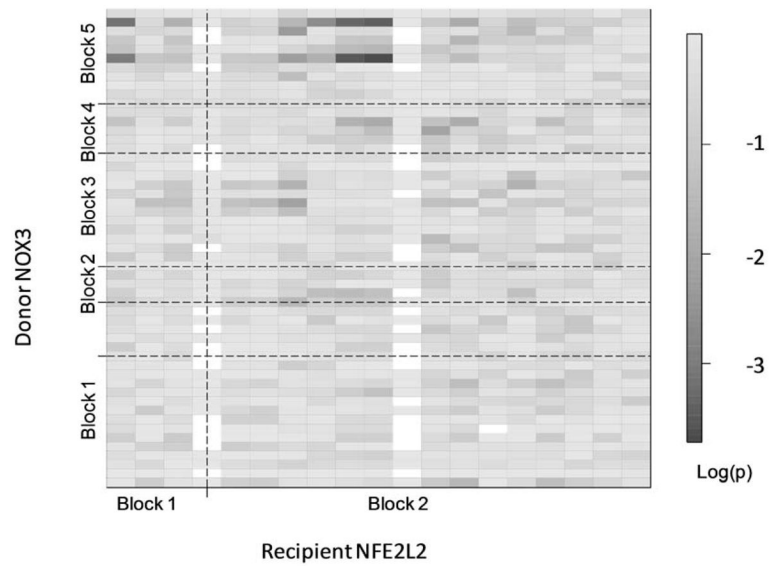


FIGURE 1.
Enrollment Design

**FIGURE 2.**

SNPset 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.

TABLE 1Donor and Recipient Characteristics^a

	No PGD (n=724)	PGD (n=314)	p-value
Donor Age	34.48±14.31	34.64±13.97	0.872
Female Donor	277 (38%)	136 (43%)	0.128
Donor Race			0.965
<i>Caucasian</i>	457 (63%)	205 (65%)	
<i>African American</i>	145 (20%)	59 (19%)	
<i>Hispanic</i>	85 (12%)	34 (11%)	
<i>Other</i>	30 (4%)	13 (4%)	
Recipient Age	54.02±12.91	53.09±12.57	0.277
Female Recipient	322 (44%)	143 (46%)	0.786
Recipient Race			0.001
<i>Caucasian</i>	632 (87%)	245(78%)	
<i>African American</i>	49 (7%)	46 (15%)	
<i>Hispanic</i>	24 (3%)	14 (4%)	
<i>Other</i>	18 (2%)	9 (3%)	
Diagnosis			<0.001
<i>COPD</i>	109 (15%)	26 (8%)	
<i>IPF</i>	282 (39%)	177 (56%)	
<i>Other</i>	333 (46%)	111 (35%)	
Bilateral Tx	73 (10.1%)	29 (9.2%)	0.758
CPB use	221 (31%)	174 (55%)	<0.001

^aComparison of individual cohorts with and without donor/recipient pairing is available in the supplemental materials. COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; IPF, idiopathic pulmonary fibrosis; PGD, primary graft dysfunction.

TABLE 2Genes associated with PGD^a

Cohort	Gene	P-value	No. SNPs
Recipients (n=1038)	<i>GPX1</i> block 1	0.014	8
	<i>NFE2L2</i> block 1	0.024	3
	<i>NFE2L2</i> block 2	0.005	13
	<i>NOS3</i> block 1	0.028	8
	<i>GSTM2</i> block 1	0.048	6
Donors (n=392)	<i>NOX3</i> block 2	0.01	3
	<i>NOX3</i> block 3	0.05	12
	<i>NOS1AP</i> block 4	0.048	11
	<i>PONI</i> block 4	0.035	13
Interaction (n=338)	Donor <i>NOX3</i> block 3 with recipient		
	<i>NFE2L2</i> block 1	0.026	
	Donor <i>NOX3</i> block 5 with recipient		
	<i>NFE2L2</i> block 1	0.017	
	Donor <i>NOX3</i> block 5 with recipient		
	<i>NFE2L2</i> block 2	0.031	

^a statistical tests of significance generated using the quadratic kernel function.

PGD, primary graft dysfunction; SNP, single nucleotide polymorphism.

TABLE 3

Individual SNPs associated with PGD

Cohort	SNP	MA	MAF_A	OR	95% CI	P-value	Gene	Location
Recipient	rs9818758	A	0.13	0.74	0.54, 0.99	0.048	<i>GPXI</i>	Intergenic
	rs1800668	T	0.25	0.74	0.58, 0.94	0.012	<i>GPXI</i>	UTR
	rs3811699	G	0.26	0.72	0.57, 0.91	0.006	<i>GPXI</i>	5' upstream
	rs6726395	T	0.43	0.79	0.64, 0.98	0.029	<i>NFE2L2</i>	Intron
	rs1806649	A	0.18	0.76	0.58, 0.99	0.049	<i>NFE2L2</i>	Intron
Donor	rs3749930	T	0.06	0.38	0.20, 0.76	0.006	<i>NOX3</i>	Coding non-synonymous
	rs231956	C	0.53	1.49	1.05, 2.12	0.024	<i>NOX3</i>	Intron
	rs13207865	T	0.29	1.57	1.06, 2.33	0.024	<i>NOX3</i>	Intron
	rs231948	T	0.54	0.57	0.3, 1.0	0.037	<i>NOX3</i>	Intron
	rs1546894	A	0.02	0.31	0.11, 0.89	0.029	<i>NOX3</i>	Intron

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