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.

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 colleagues[7] identified antioxidant system dysfunction before lung transplantation that persisted for up to a year after transplantation. Furthermore, the

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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 Qiamp 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 markers[23] 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 method[24] 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