Online Data Supplement

Title: Variation in *PTX3* is Associated With Primary Graft Dysfunction After Lung Transplantation

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PGD Grading: Chest radiographs were independently assessed by two trained physicians with adjudication of conflicts(1). The classification kappa for PGD was 0.95. Chest radiographs were examined immediately after transplant (T0) and at 24, 48, and 72 hours post-transplant with scores reported at each time point (2-7).

Genotyping Method: The following SNPs were chosen for genotyping because they identify known coding variants: rs35948036 (synonomous), rs3816527 (nonsynonomous), rs35415718 (nonsynonomous), and rs4478039 (nonsynonomous). Genomic DNA was extracted from buffy coat using the Qiagen Qiamp 96 blood kit (Qiagen, Valencia, CA). Negative controls were included in 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. Genotyping of *PTX3* tagging SNPs was performed using two separate platforms:

Platform 1: We used a custom 50K single nucleotide polymorphism (SNP) genotyping array, called the HumanCVD BeadChip™ (manufactured by Illumina, Inc.® (San Diego,CA)) designed to assay SNPs in candidate genes and pathways affecting cardiovascular, pulmonary, inflammatory, and metabolic phenotypes (8). This array was designed in collaboration with the Institute for Translational Medicine and Therapeutics (ITMAT) at the University of Pennsylvania, the Broad Institute, and the National Heart Lung and Blood Institute (NHLBI)-supported Candidate-gene Association Resource (CARe) Consortium. 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 import to cardiac, pulmonary, and metabolic phenotypes (8). Quality control thresholds for each SNP to be included in the analysis

included genotyping call ≥ 95%; test of Hardy-Weinberg equilibrium (HWE) by chi-square testing on the whole population yielding a p value ≥ 10-6; and MAF ≥ 0.01 overall. The following SNPs were genotyped using this platform: rs9289983, rs1456099, rs2120243, rs2305619, rs3816527, rs3845978.

Platform 2: The following SNPs were genotyped using Taqman® (Applied Biosystems, Foster City, CA) rs35415718, rs35948036, rs55757068, rs4478039. The call rate for all SNPs genotyped using Taqman® was >95%.

Measurement of PTX3 Concentration: Blood samples from all study subjects were collected prior to transplant and at 6 and 24 hours after allograft reperfusion. Samples were centrifuged within 30 minutes and then stored at -80°C prior to subsequent analysis. The intra-assay coefficient of variation for the ELISA (Alexis Biochemicals, Switzerland) was 5.7% (9).

Statistical Analysis: Three of the 10 SNPs genotyped had a MAF<5% in the transplant population and were not included in the analysis. Haplotypes were inferred using standard expectation maximization algorithms in Haploview (10) and the confidence interval (CI) criteria (11). Each haplotype was tested for association with PGD, adjusting for key clinical covariates and population stratification. Significance was determined using the χ^2 test.

Power Calculation: We had 654 lung transplant recipients for the candidate gene association study. Given a PGD incidence of approximately 29% in this population (7, 9) we calculated that we had 80% power to detect variants with MAF ≥ 0.05 with a minimum relative risk (RR) of 1.6, at an alpha level of 0.007. All power calculations

were performed using Power Calculator for Genetic Studies and Power for Genetic Association Analyses (12, 13).

Results: The results of the SNP-association study among patients with a pre-operative diagnosis of IPF are presented in Supplementary Table E1.

rs number	Minor Allele	Risk Allele	MAF affected	MAF unaffected	OR (95% CI)	p	Location
rs9289983	G	A	0.40	0.50	0.7 (0.5, 1.0)	0.06	5’ upstream
rs1456099	A	T	0.42	0.52	0.7 (0.5, 1.0)	0.047	5’ upstream
rs2120243	A	A	0.47	0.39	1.5 (1.0, 2.2)	0.04	5' upstream
rs2305619	T	T	0.53	0.45	1.4 (1.0, 2.1)	0.06	Intron
rs3816527	G	G	0.42	0.38	1.3 (0.9, 1.9)	0.2	Coding
rs55757068	C	C	0.09	0.06	1.1 (0.5, 2.2)	0.8	Intron
rs3845978	C	C	0.09	0.06	1.2 (0.6, 2.6)	0.6	Intron

Supplemental Table E1.

Supplemental Figure E1 represents a box plot of PTX3 plasma concentration measured 24 hours post-transplantation stratified by genotype at the rs2120243 locus in 47 European ancestry patients with IPF as a pre-operative diagnosis. Supplemental Figure E2 represents a box plot of pre-transplant PTX3 concentration stratified by genotype at the rs2305619 locus. The p-value reported for both figures is from the nonparametric test for trend. The horizontal line indicates the median concentration and the upper and lower limits of the boxes indicate the interquartile range.

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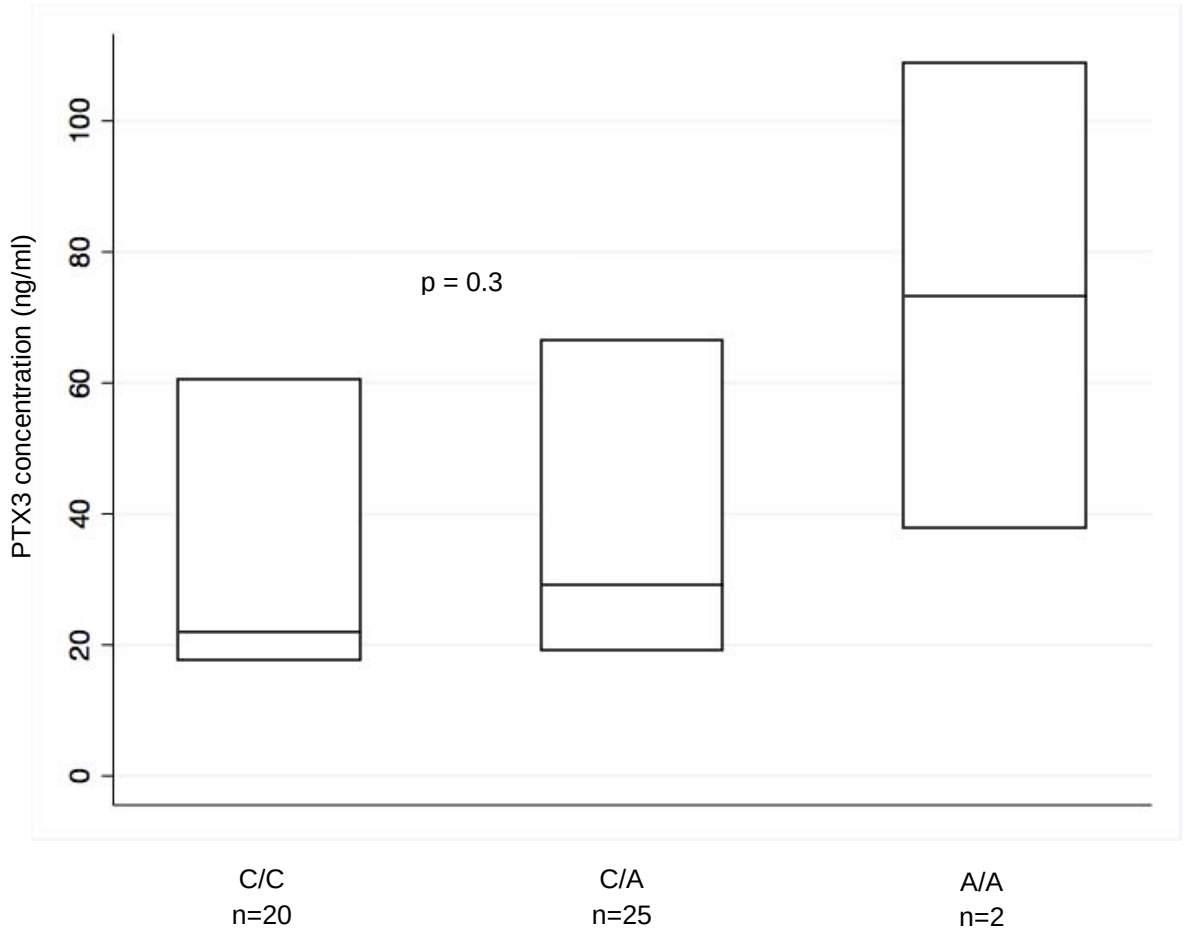
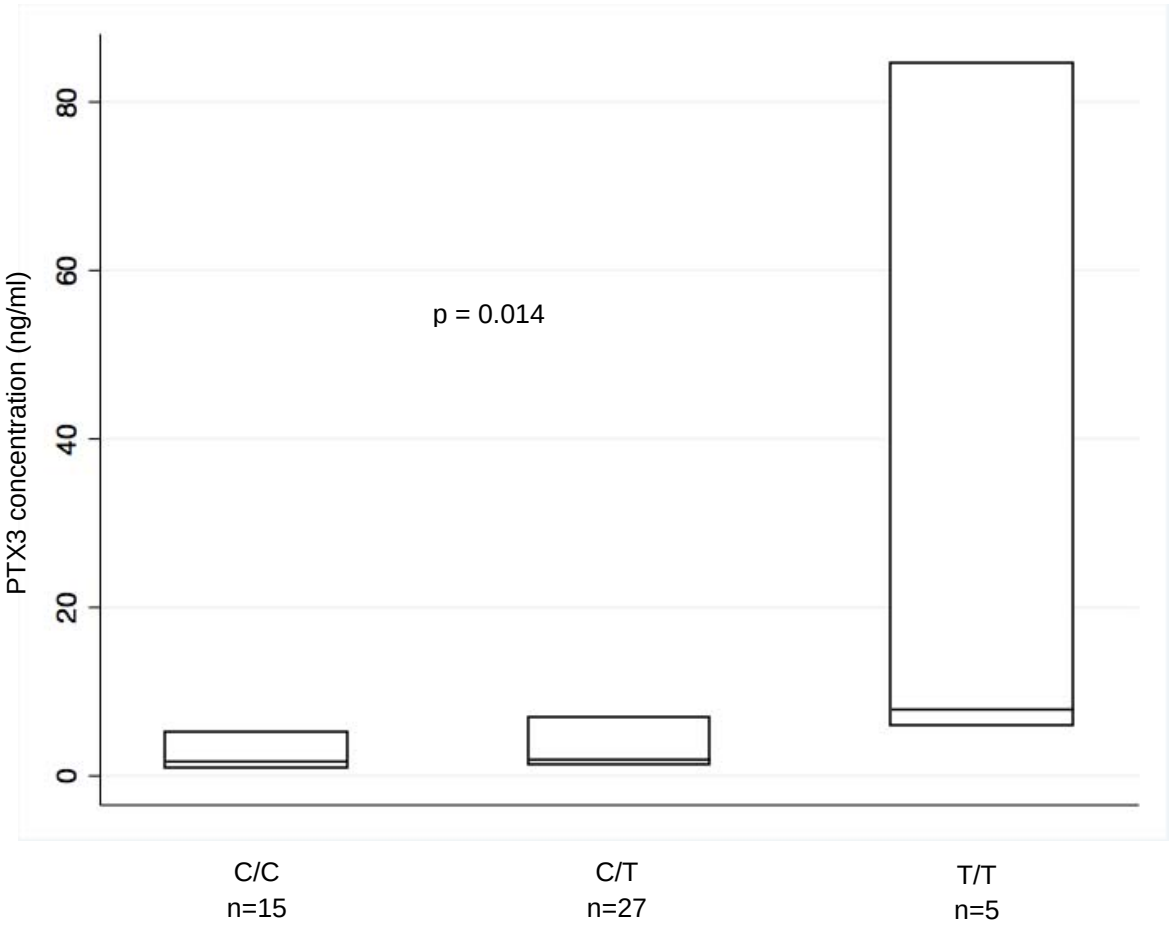


Supplemental Figure E1.



Supplemental Figure E2.