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Genetic variation in *Caveolin-1* correlates with long-term pancreas transplant function

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Abbreviations: Type 1 Diabetics (T1D), Hazard ratio (HR), Caveolin-1 (*Cav1*), single nucleotide polymorphism (SNP), simultaneous pancreas kidney (SPK), isolated pancreas (IP) transplant, pancreas after kidney transplant (PAK), pancreas transplants alone (PTA), minor allele frequency (MAF), Hardy Weinberg Equilibrium (HWE), donor after brainstem death (DBD), donor after circulatory death (DCD), 95% confidence limits (95% CI), bronchiolitis obliterans syndrome (BOS), insulin receptor (IR) & untranslated region (UTR).

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ABSTRACT (196 words):

Pancreas transplantation is a successful treatment for a selected group of people with type 1 diabetes. Continued insulin production can decrease over time and identifying predictors of long-term graft function is key to improving survival. The aim of this study was to screen subjects for variation in the Caveolin-1 gene (*Cav1*), previously shown to correlate with long-term kidney transplant function. We genotyped 435 pancreas transplant donors and 431 recipients who had undergone pancreas transplantation at the Oxford Transplant Centre, UK, for all common variants in *Cav1*. Death-censored cumulative events were analysed using Kaplan-Meier and Cox regression. Unlike kidney transplantation, the rs4730751 variant in our pancreas donors or transplant recipients did not correlate with long-term graft function (P=0.331-0.905). Presence of rs3801995 TT genotype (P=0.009) and rs9920 CC/CT genotype (P=0.010) in our donors did however correlate with reduced long-term graft survival. Multivariate Cox regression (adjusted for donor and recipient transplant factors) confirmed the association of rs3801995 (P=0.009, HR=1.83;[95% CI=1.16-2.89]) and rs9920 (P=0.037, HR=1.63;[95% CI=1.03-2.73]) with long-term graft function. This is the first study to provide evidence that donor *Cav1* genotype correlates with long-term pancreas graft function. Screening *Cav1* in other datasets is required to confirm these pilot results.

BRIEF COMMUNICATION (3000 words maximum- 3000 words)

INTRODUCTION

Whole organ pancreas transplantation has the potential to provide life-long independence from exogenous insulin injections for people with type 1 diabetes (T1D) with poor glycaemic control and/or severe secondary complications (1, 2). Whilst early complications are common after transplantation, patient survival is >95%-98% at one year (3). Patients with a functioning pancreas transplant achieve insulin independence, improved hypoglycaemic awareness and there is increasing evidence of stabilisation or improvement in secondary diabetic complications (2). Although graft survival can exceed 20 years, graft function decreases over time (4). Although one year post transplant 85% of patients have a functioning pancreas graft (defined as insulin production), this falls to around 68% five years post-transplant (5). Loss of pancreas graft function, and subsequent return to exogenous insulin, is strongly associated with transplant patient mortality (6). Hyperglycaemia is a late feature of graft loss, by which time graft failure is usually irreversible. Currently it is not possible to predict when a graft is likely to fail.

Whilst recent progress has been made in identifying clinical features associated with long-term pancreas transplant function, including development of de novo HLA alloantibodies (Hazard Ratio (HR)=4.66) (7) and an abnormal glucose tolerance test within two weeks after transplantation (HR=1.66) (8), only limited work has been undertaken looking at potential genetic associations with long-term pancreas transplant function. Work in the related, but more established, field of renal transplantation, has identified several potential genetic factors that correlate with long-term graft function (9-12) including, most convincingly, variation in the Caveolin-1 gene (*Cav1*), which encodes invaginations within the plasma membrane involved

in signal transduction and tissue fibrosis (13). Presence of the *Cav1* rs4730751 single nucleotide polymorphism (SNP) AA genotype in kidney donors was associated with increased risk of allograft failure, HR=1.56-1.97, in two independent UK cohorts (13). Importantly, the effect size of this variant is comparable with that of female donor gender and donor hypertension, both recognised as relevant clinical indicators of transplant success. Whilst some small datasets have looked at potential genetic predictors of long-term pancreas graft function, including *MICA/MICB*, *HLA-DRA* and *vitamin D* (14-16), currently no genetic factors have been identified that correlate with long-term pancreas function. Identifying genetic factors associated with long-term pancreas function might provide an opportunity to intervene early to preserve beta cell function and prolong the benefits of a functioning graft.

The aim of this study, therefore, was to screen all common variation within *Cav1* in 435 pancreas transplant donors and 431 recipients who underwent transplantation at the Oxford Transplant Centre, UK, to determine if *Cav1* variation is associated with long-term pancreas graft function.

MATERIALS AND METHODS

Patients

This was a single centre, retrospective study. Anonymized DNA and matched clinical data, including both donor and recipient transplant related variables (Table 1), were obtained retrospectively from pancreas transplant donors and recipients at the Oxford Transplant Centre, UK, between 2002 and 2012. This was limited to those in which surplus DNA was available within our tissue typing laboratory (originally obtained from whole blood to undertake tissue matching and stored for further testing as clinically indicated). The patient cohort consisted of DNA from 435 transplants; 315 (72.4%) simultaneous pancreas kidney (SPK), 120 isolated pancreas (IP) transplants, including 68 (15.6%) pancreas after kidney transplant (PAK), 38 (8.7%) pancreas transplants alone (PTA) and 14 second transplants (3.2%) (Table 1). The study was approved by the United Kingdom National Research Ethics Committee (REC reference 12/SC/0655; Protocol dated 22/10/12 Version 1.0).

As previously described (7), all transplants were performed with systemic venous drainage. Enteric ductal drainage was used in all except three recipients, who received IP transplants with bladder drainage due to a change in center protocol. All recipients received alemtuzumab induction immunosuppression (Campath 30 mg, days 1 and 2, Genzyme Corporation, Boston, MA), with maintenance immunosuppression therapy consisting of tacrolimus, initially at 0.5 mg/kg twice a day (bd), titrated to maintain trough levels between 8 ng/mL and 10 ng/mL throughout the follow-up, and mycophenolate mofetil, at 750 mg bd, with dose adjustments as clinically indicated (7). Trough serum tacrolimus levels and absolute neutrophil counts were monitored regularly on an outpatient basis. Mycophenolate levels were not monitored. No steroids were used in maintenance immunotherapy.

Tag SNP Selection and Genotyping

Genotyping data for the 36.39kb *Cav1* region (Phase 2, NCBI Build 36, Caucasian (CEU) population) was downloaded from the International Haplotype Mapping Project website (http://www.hapmap.org). Our analysis of Hapmap genotyping data identified 53 SNPs with minor allele frequencies (MAF) of ≥5% in the CEU population within this region. Tagger pairwise function within Hapmap was used to assign tag SNPs. Twelve tag SNPs: rs4730751, rs959173, rs3801995, rs12672038, rs9886215, rs11773845, rs926198, rs3779512, rs9920, rs1049337, rs3807986 and rs4730748, were chosen to capture the majority of the common variation within this gene and the surrounding area with a minimum r^2 of 0.80 (for information on SNPs captured see Supplementary Table 1). All tag SNP genotypes represent the nucleotide variants present at that SNP associated with a given trait (see Supplementary Table 1 for further details on location and nucleotides present at each SNP). We had >0.80 power to detect an OR≥1.51 for MAF=0.10. Fluorescence-based genotyping assays for all SNPs were purchased from Life Technologies, Paisley, UK and genotyped on an ABI7900HT using Taqman® genotyping technologies (Life Technologies, Paisley, UK). All variants were subject to Hardy Weinberg Equilibrium (HWE) analysis to ensure accurate genotyping (P<0.05 was considered significant deviation from HWE).

Outcome Measures and Statistical Analysis

Anonymized demographic data and donor and recipient transplant related variables were extracted for all samples (Table 1). Quantitative parametric data were compared between groups using Student's t-test or the Mann–Whitney U test in the case of nonparametric distribution. Cross-tabulated data were analysed by the chi-squared test. Our primary outcome measure was defined as graft failure. Return to exogenous insulin was used as a surrogate marker of graft failure (pancreas graft biopsies were not performed as part of the clinical

protocol). A secondary outcome measure in our SPK patients was kidney graft failure, defined as return to kidney dialysis. Death censored cumulative events were analysed using Kaplan-Meier methods, with the log-rank test used for intergroup comparison. Time-to-event analyses were performed using a Cox proportional hazards model. Gene variation and other relevant clinical/demographic characteristics were initially examined by a series of univariate analyses, followed by multiple regression analysis incorporating any variables showing evidence of univariate association ($P \leq 0.20$). Statistical packages IBM SPSS Statistics 20 (IBM United Kingdom Limited, Portsmouth, UK) and MINITAB 16 (MINITAB LTD, Coventry, United Kingdom) was used for all data analysis.

RESULTS

Demographics

Four hundred and thirty five pancreas transplants were included in this study (Table 1). Three hundred and fifteen (72.4%) received an SPK transplant, and 120 (27.6%) received an Isolated Pancreas (IP) transplant. The PTA, PAK and second pancreas transplant groups had equivalent graft outcomes. The SPK group had comparatively superior graft outcomes (as previously reported (8)). Two hundred and ninety one (92.7%) SPK recipients received pancreases from donors after brainstem death (DBD) compared to 80 (66.7%) of IP recipients. This difference was statistically significant ($P=6.00x10^{-12}$) and was principally due to UK organ allocation procedures (previously described (7)). A difference in donor BMI between SPK (Mean BMI=24.25) and IP (Mean BMI=23.28) groups was also detected, which could be due to differences in allocation scoring for SPK vs IP. The groups were otherwise comparable for donor and recipient characteristics, and cold ischemia time (P=0.807-0.090) (Table 1).

Cav1 Genotyping in Donors

In the pancreas transplant donors all 12 tag SNPs screened were in HWE (P=0.96-0.06) (Supplementary Table 2). Within our transplant recipient cohort there were 84 events and 351 censored events, with median follow up of 42 months. Kaplan-Meier analysis of death-censored pancreas allograft function, revealed presence of the *Cav1* rs4730751 AA genotype variant, previously shown to correlate with decreased allograft function in kidney transplants (13), was not associated with decreased long-term pancreas graft survival (P=0.331) (Figure 1). Presence of the *Cav1* rs3801995 TT genotype however did correlate with reduced median graft survival (78 months) compared to donors with CT (98 months) or CC (111 months) genotypes (P=0.009) (Figure 1). Presence of the *Cav1* rs9920 CC and CT genotype (genotypes

were grouped together as only one donor had the CC genotype) also correlated with reduced median graft survival (93 months) compared to the TT genotype (112 months) (P=0.010). None of the other nine *Cav1* tag SNPs screened in the donors showed any association with long-term graft function (P=0.792-0.100).

Cav1 Genotyping in Recipients

In the pancreas transplant recipients, rs9920 (P=0.02) and rs3807986 (P=0.03) were out of HWE, suggesting the results should be viewed with caution. All other *Cav1* tag SNPs screened in the recipients were in HWE (0.96-0.10) (Supplementary Table 2). None of the twelve *Cav1* tag SNPs screened in the recipient was shown to influence long-term pancreas function (P=0.986-0.066) (see Figure 1 for rs4730751, rs3801995 and rs9920 Kaplan-Meier plots).

Multivariate Analysis of Donor Cav1 r3801995 and rs9920 SNPs

Gene variation and other clinical/demographic features of the cohort were analysed by Cox regression. Initial univariate analysis confirmed association of donor rs3801995 TT genotype (P=0.004, HR=2.74 [95% confidence intervals (CI)=1.38-5.44]) and rs9920 CC/CT genotype (P=0.037, HR=1.83 [95% CI=1.14-2.93]) with reduced long-term graft function. Additionally, univariate analysis identified operation type (Second operation $P=1.04x10^{-4}$, PAK P=0.037 and PTA P=0.009 compared against SPK), donor type (0.097), donor age (P=0.149) and recipient gender (0.177) as showing some univariate association (Table 2). Multivariate analysis of the rs3801995 SNP and clinical features, further supported a role for rs3801995 TT genotype in reduced long-term graft function (P=0.009, HR=1.83 [95% CI=1.16-2.89]), along with second operation ($P=2.71x10^{-5}$, HR=5.05 [95% CI=2.37-10.76]) and PTA operation (P=0.032, HR=1.80 [95% CI=1.06-3.42] compared to SPK. Multivariate analysis of the rs9920 SNP

supported association of the CC/CT genotype (P=0.037, HR=1.63 [95% CI=1.03-2.73]), along with second operation (P=0.005, HR=2.25 [1.28-3.97]), with reduced long-term graft function.

As multivariate analysis identified second transplant as being associated with reduced long-term pancreas function compared to SPK, we wanted to ensure presence of a second transplant was not driving rs3801995 TT or rs9920 CC/CT genotype associations. We repeated our Kaplan-Meier analysis removing all 14 second transplants and both rs3801995 TT genotype (Log Rank P=0.004) and rs9920 CC/CT genotype (Log Rank P=0.010) remained associated with reduced long-term pancreas function, with rs4730751 still showing no association with long-term pancreas transplant function (P=0.493) (Supplementary Figure 1). Multivariate analysis further confirmed a role for rs3801995 TT genotype (P=0.011, HR=2.52 [95% CI=1.23-5.17] and rs9920 CC/CT genotype (P=0.043, HR=1.67 [95% CI=1.02-2.80] in reduced long-term pancreas function (Supplementary Table 3), suggesting that over-representation of second transplants within these genotypes was not driving this effect. Interestingly, rs11773845 AA genotype in our single transplant only donor cohort showed a borderline association with reduced long-term graft function (P=0.053), however this effect was not present after multivariate analysis (P=0.153) (data not shown). No other *Cav1* tag SNP in the single transplant only donors showed any association with long-term graft function (P=0.866-0.086). None of the 12 *Cav1* tag SNPs screened in the recipients undergoing a single transplant were associated with graft survival (P=0.997-0.057, data not shown).

Role of Cav1 on long-term kidney function in our SPK transplants

As a result of the link between *Cav1* variation and long-term kidney transplant function (13), we also investigated *Cav1* in our SPKs for a role in long-term kidney function. When looking at kidney outcomes, there were 25 events and 269 censored events, with a median follow up of

45 months. When investigating *Cav1* SNP variation on kidney transplant outcome none of the 12 tag SNPs screened showed any association with long-term kidney transplant outcome (P=0.123-0.938), including rs4730751 previously associated with long-term kidney function (13). In the SPK recipients, none of the 12 tag SNPs showed any association with kidney outcome (P=0.996-0.128). The rs959173 SNP showed weak association with long-term kidney transplant survival (P=0.049), being driven by three recipients with CC genotypes. When combining rs959173 CC and CT genotypes compared against TT genotypes no association with long-term transplant function was detected (P=0.819) suggesting the original association was probably due to small numbers of CC genotypes. No other *Cav1* tag SNP in the recipients showed any association with long-term kidney function (P=0.987-0.436).

DISCUSSION

Screening all common variation within *Cav1* in our Oxford pancreas transplant donors revealed presence of rs3801995 TT genotype and rs9920 CC/CT genotypes correlated with reduced long-term graft function. This is the first time a potential role for *Cav1* variation in long-term pancreas transplant outcome has been reported. Interestingly however, we did not detect a role for rs4730751 genotype, previously reported to be associated with long-term kidney transplant function (13), in reduced long-term pancreas transplant or long-term kidney transplant function in our SPK patients.

Further support for *Cav1* in long-term transplant outcomes comes from 503 lung transplant recipients where *Cav1* rs3807989 GG genotype (tagged by rs11773845 in our cohort) was associated with greater mortality (P=0.04) (17). In an independent lung transplant cohort, screening four *Cav1* variants (rs3807989, rs3807994 (tagged by rs3801995 in our cohort), rs10256914 (tagged by rs4730751 in our cohort) and rs12154695) in 20 patients with bronchiolitis obliterans syndrome (BOS), a characteristic of chronic allograft rejection after lung transplant, compared to 80 lung transplant recipients without BOS, revealed that 35% of BOS positive recipients had the rs3807989 TT genotype compared to 8% of BOS negative recipients (OR=6.13 [95% CI=1.85-20.41]) (18). Genotyping of 11 of our 12 *Cav1* tag SNPs (rs4730748 was not screened) in large T1D collections (>7500 cases and >9045 controls) showed no association with T1D (19, 20), further suggesting that *Cav1* variation plays a direct role in transplant survival.

Caveolin-1 is an essential component of caveolae, small invaginations within the plasma membrane which act as specialized lipid rafts involved in cell signal transduction and protein

interaction (21-24). Caveolin-1 plays a key role in numerous tissue remodelling and fibrotic pathways, including regulating TGF-β1 signalling/expression, controlling cell matrix remodelling, fibroblast adhesion/migration and in cell stretching, proliferation and apoptosis (22, 25). Decreased *Cav1* expression has also been reported in affected tissues from several human fibrotic diseases including idiopathic pulmonary fibrosis, scleroderma and systemic sclerosis, with re-introduction of *Cav1* function shown to rescue these phenotypes (25). Upon histological assessment of kidney graft failure, recipients who received donor organs with the rs4730751 AA genotype, had a predominant increase in interstitial fibrosis compared to donors with non-AA genotypes (13). Only *Cav1* variation in the donors, and not the recipients, was shown to play a role in long-term pancreas graft function, further suggesting that this effect is intrinsic to the donor organ rather than the recipient. Whilst our clinical protocol does not allow for routine biopsy and histological grading of pancreas graft failure, making it difficult to assess the degree of fibrosis, increased fibrosis due to *Cav1* variation could be affecting long-term pancreas function.

Interestingly, *Cav1* knockout mice not only show impaired fibrosis but also exhibit lipid and metabolic disorders including insulin resistance (21, 26). Caveolin-1 is an essential scaffolding protein required for correct folding of the insulin receptor (IR), and without *Cav1* the IR is not correctly formed, leading to insulin resistance (21, 24). Humans with severe insulin resistance have been found to have mutations within the IR caveolin binding motif (27-30), suggesting *Cav1* could be contributing to reduced long-term graft function through other mechanisms in addition to fibrosis.

It is interesting that we did not detect evidence for association of rs4730751, previously associated with kidney transplant outcomes, with either pancreas or kidney outcome in our

cohort. There are several potential reasons for this, i) differences in patient selection or time for follow up for renal transplant compared to those selected for SPK or IP transplant, ii) a functioning pancreas graft is known to extend kidney transplant function and may negate effects of rs4730751 in our cohort, iii) the rs4730751 SNP could have a smaller effect on long-term pancreas transplant function which we are not currently powered to detect or iv) whilst *Cav1* SNP frequencies in our Oxford Pancreas and Birmingham Kidney transplant cohorts were similar (13) (data not shown), subtle geographical differences in *Cav1* SNP allele frequency between these populations could be affecting their role in transplant outcome.

There are limitations to this study that should be considered when interpreting the results. First, whilst the majority of our pancreas donors and recipients were Caucasian (>95%), we do not routinely store donor or recipient ethnicity within our centre as part of our clinical protocol. Reassuringly, all *Cav1* tag SNPs in our donors were in HWE, which would be unlikely if ethnic differences were contributing to the variation seen in *Cav1* tag SNP genotype frequencies. As *Cav1* SNP frequencies vary between different ethnic groups, and it is worth noting that effects found in our mainly white Caucasian dataset need to be replicated in other ethnic groups before inferences can be made on the role of *Cav1* in non-Caucasian populations. Second, we have only been able to study a single data set, and as such data should be viewed as preliminary and awaiting replication. Before the clinical utility of this variant can be established further screening of *Cav1* and fine-mapping the surrounding region in a second, and ideally larger independent pancreas transplant cohorts, will be required to confirm the association and that the primary effect, if present, is the result of polymorphism within *Cav1* itself. Once independent replication of these associations has been achieved, functional studies will enable us to determine how *Cav1* variation in the donor is affecting gene expression and function leading to reduced long-term graft function, whilst being mindful of how features such as donor

age and donor type (DBD vs DCD) could be impacting upon *Cav1* expression. This new found knowledge can then be translated clinically into the identification of novel drugs, or repurposing of currently available drugs, to extend long-term graft function in patients carrying risk *Cav1* genotypes.

In summary, this study is the first to screen *Cav1* in pancreas transplant donors and recipients and provide preliminary evidence for a role of *Cav1* variation in donors in long-term pancreas transplant function. Replication of these *Cav1* data in additional pancreas transplant cohorts is now required to confirm these potentially important findings.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

FIGURE LEGENDS

Figure 1: Kaplan-Meier Plots for role *Cav*1 rs4730751, rs3801995 and rs9920 SNP Variation in Oxford Transplant Centre Donors and Recipients on Long-term Pancreas Graft function A: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Donors, B: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Recipients, C: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Donors, D: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Recipients, E: Kaplan-Meier plot for variation of *Cav1* rs9902 in Pancreas Transplant Donors & F: Kaplan-Meier plot for variation of *Cav1* rs9902 in Pancreas Transplant Recipients

Table 1: Characteristics of 435 Pancreas Transplant Donors and Recipients from the Oxford Transplant Centre

Data are expressed as numbers (%) unless otherwise stated. For age and BMI the mean is given with the standard deviation in brackets. Cold Ischemia Time (CIT), Body Mass Index (BMI)

Table 2: Univariate and Multivariate Analysis of *Cav1* rs3801995 and rs9920 Donor Genotype on Long-term Pancreas Transplant Function

Bold represents any association seen after univariate analysis ($P<0.20$) or multivariate analysis ($P<0.05$). Simultaneous Pancreas Kidney (SPK, Pancreas After kidney (PAK), Pancreas Transplant Alone (PTA), Second Pancreas Transplant (2ND), Hazard Ratio (HR), Human Leukocyte Antigen (HLA), Body Mass Index (BMI), Donor After Brainstem Death (DBD) & Donor After Circulatory Death (DCD)

DESCRIPTION OF SUPPORTING MATERIAL

Supplementary Figure S1: Kaplan-Meier Plots for role of *Cav1* rs4730751, rs3801995 and rs9920 SNP variation in Oxford Transplant Centre Donors and Recipients on Long-term Pancreas Graft function without those who received a second transplant A: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Donors, B: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Donors, C: Kaplan-Meier plot for variation of *Cav1* rs9920 in Pancreas Transplant Donors

Supplementary Table S1: *Cav1* tag SNPs screened in our Oxford Transplant Cohort and other common SNPs that they capture

Supplementary Table S2: *Cav1* Tag SNP genotype frequencies and Hardy Weinberg Equilibrium in our Oxford Pancreas Transplant donors and recipients

Hardy Weinberg Equilibrium (HWE), Single Nucleotide Polymorphism (SNP)

Supplementary Table S3: Univariate and Multivariate Analysis of *Cav1* rs3801995 and rs9920 Donor Genotype on Long-term Pancreas Transplant Function with all those with a Second Transplant Removed

Bold represents any association seen after univariate analysis (P<0.20) or multivariate analysis (P<0.05). Simultaneous Pancreas Kidney (SPK, Pancreas After kidney (PAK), Pancreas Transplant Alone (PTA), Hazard Ratio (HR), Human Leukocyte Antigen (HLA), Body Mass Index (BMI), Donor After Brainstem Death (DBD) & Donor After Circulatory Death (DCD)

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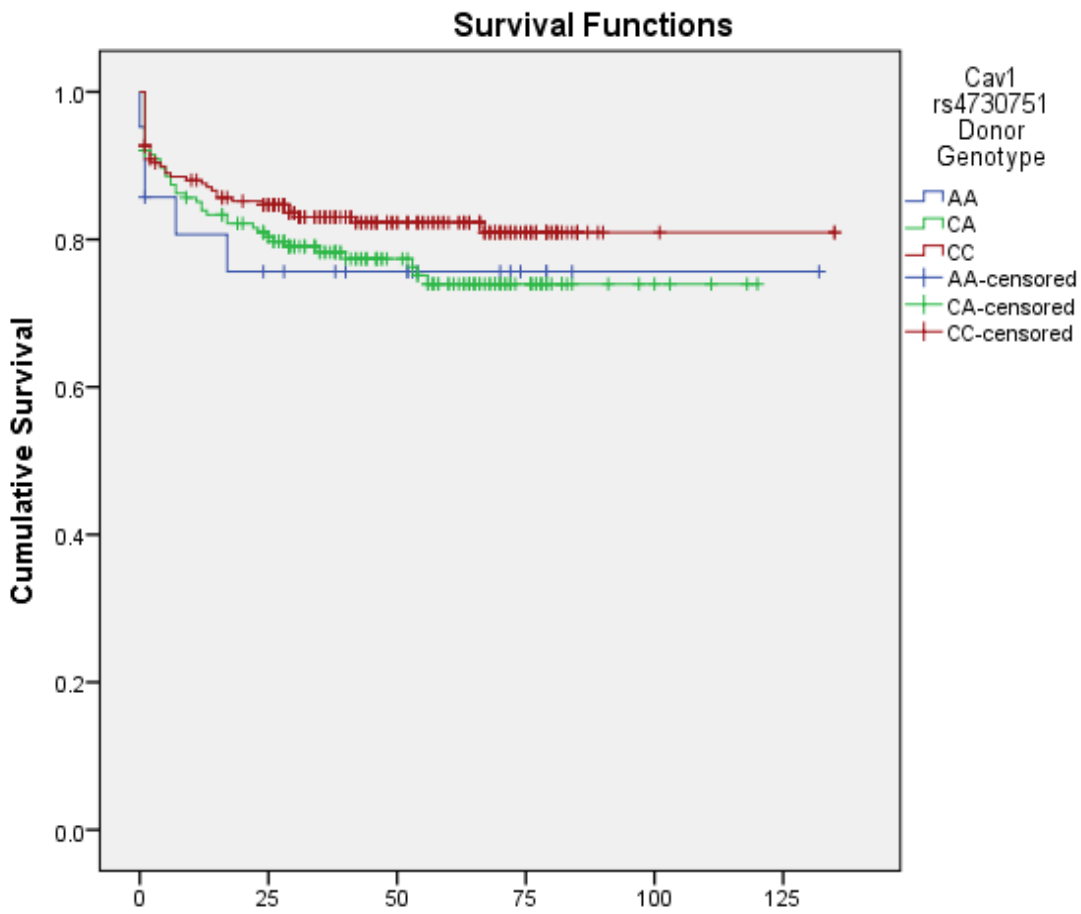
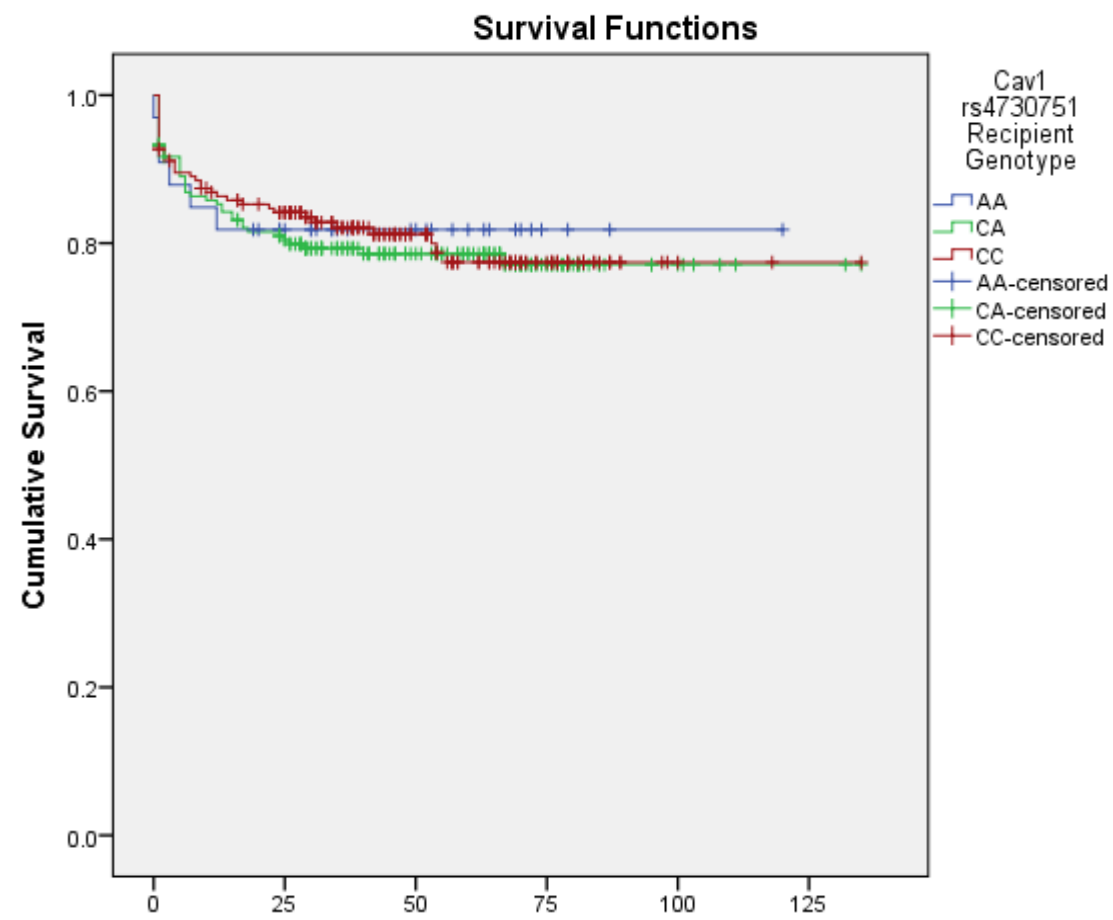
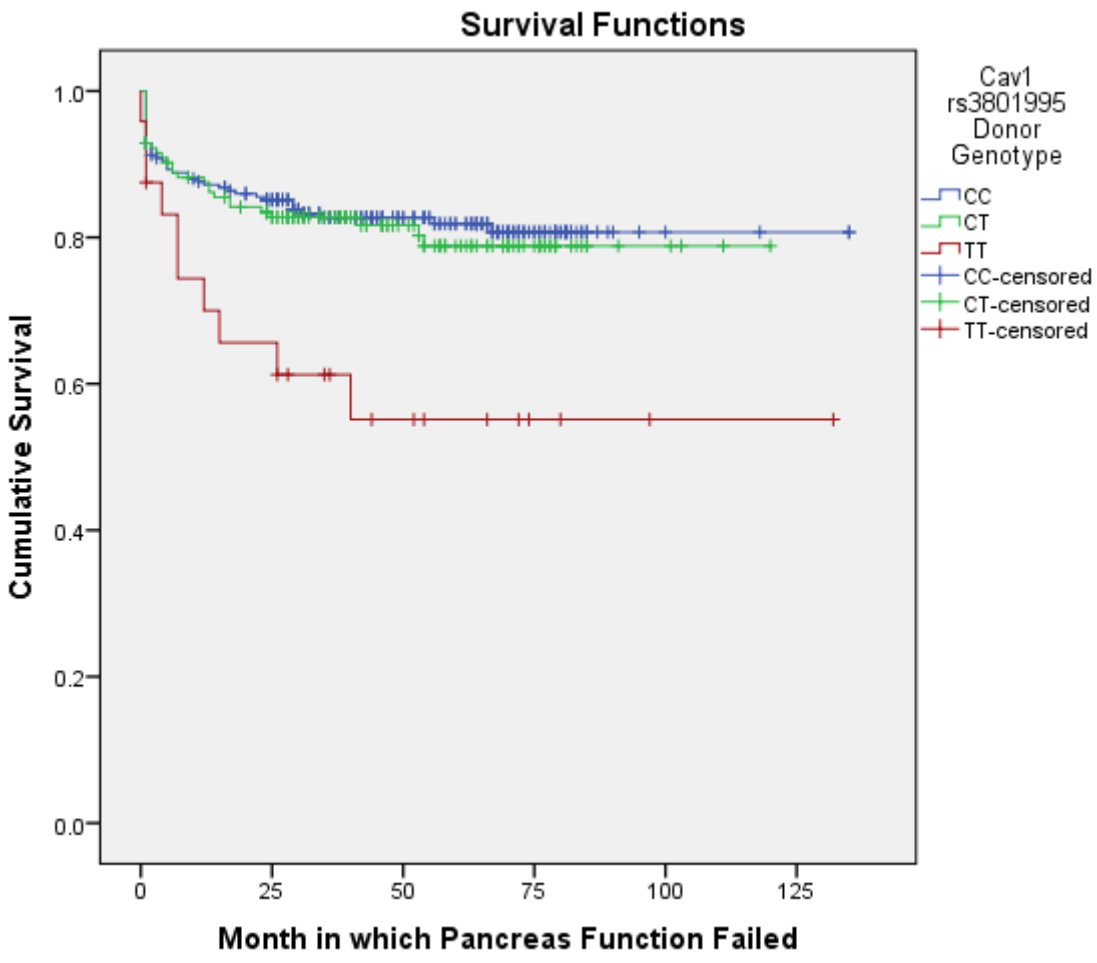


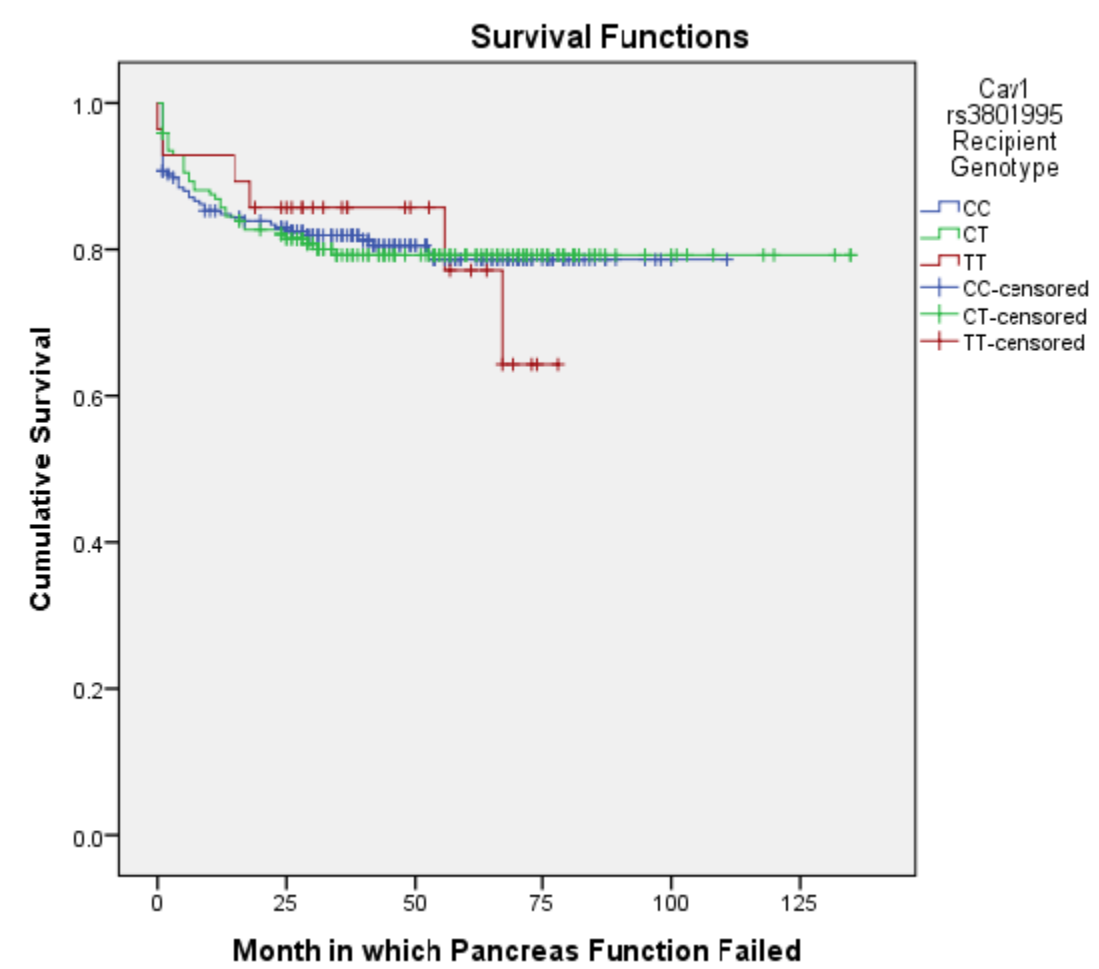
A: Log Rank X^2=2.21, P=0.331



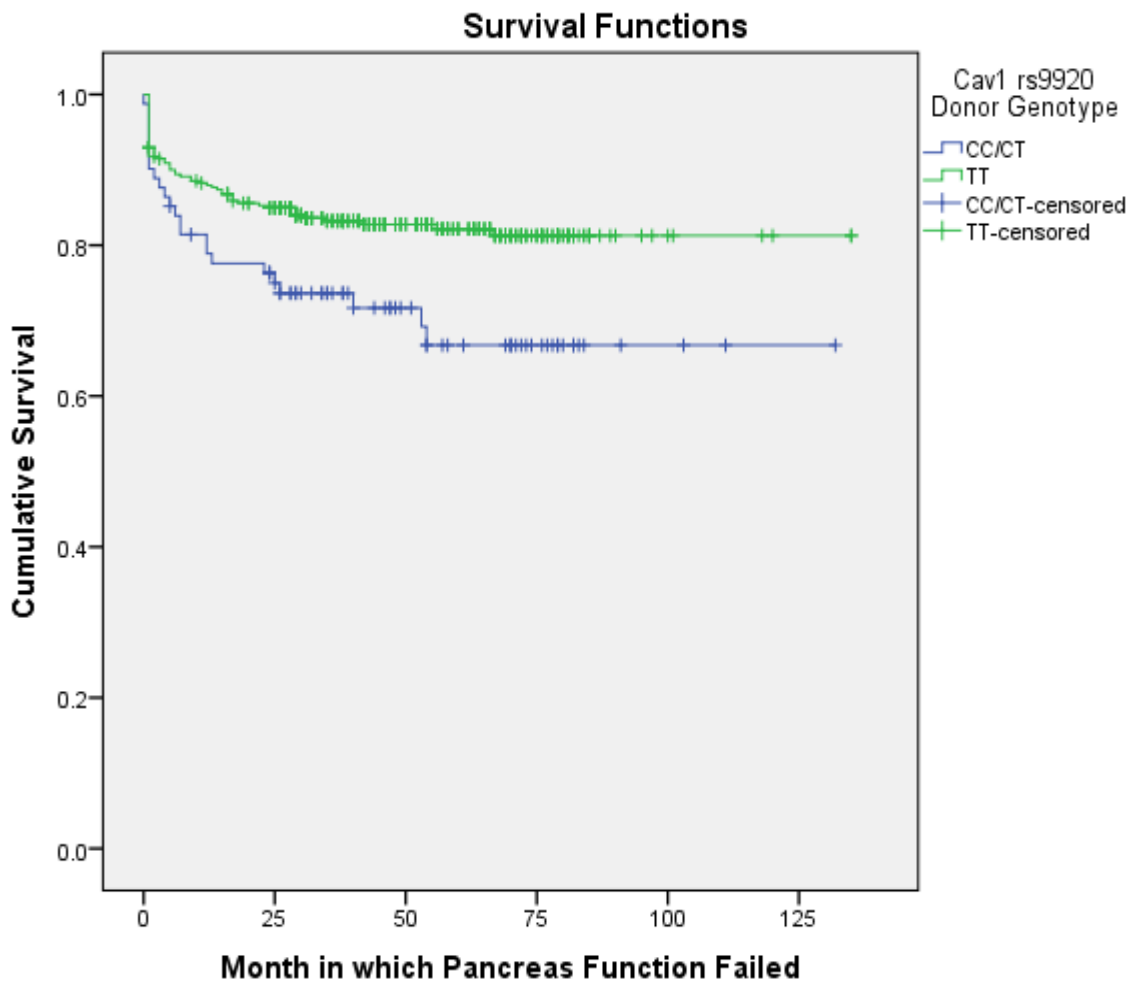
B: Log Rank X^2=0.20, P=0.905



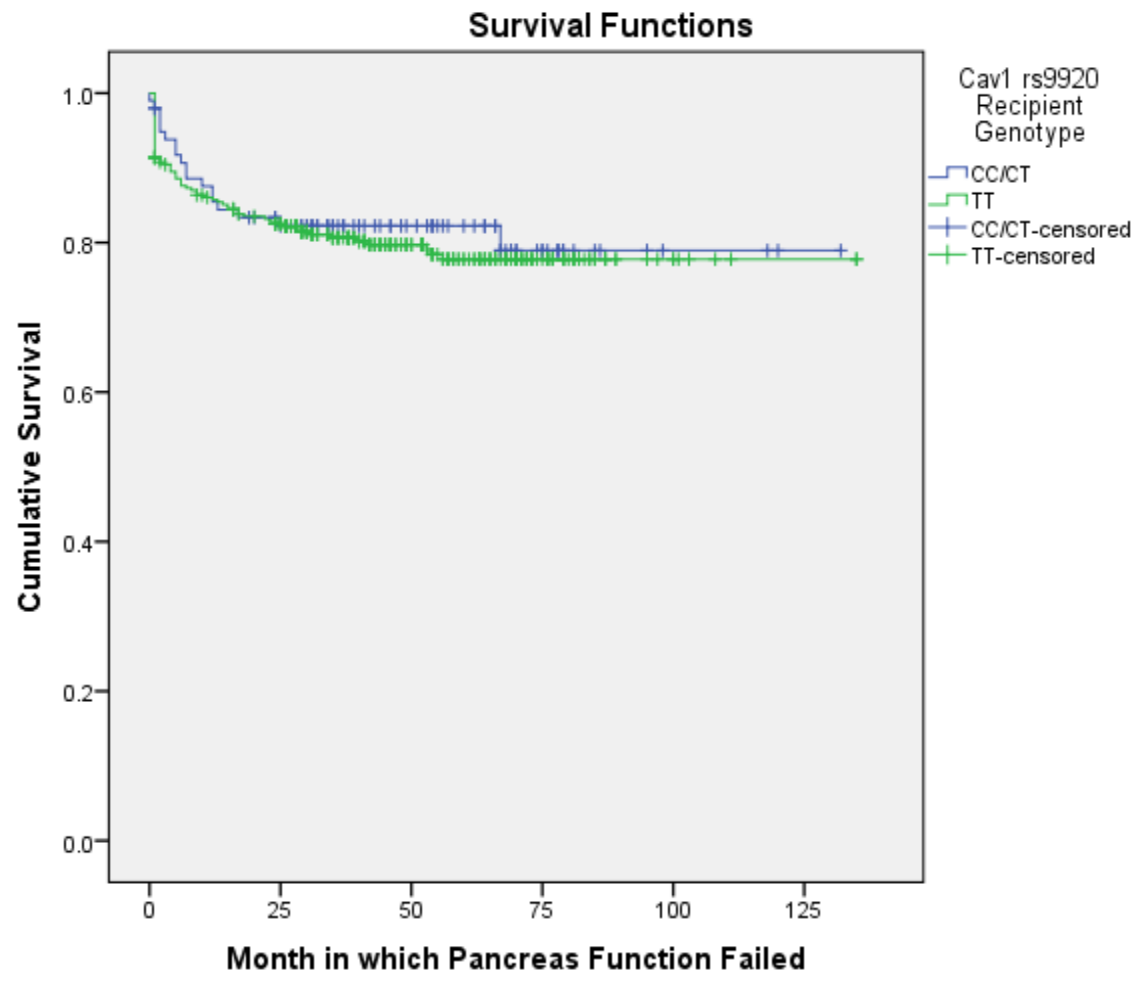
C: Log Rank X^2=9.38, P=0.009



D: Log Rank X^2=0.03, P=0.99



E: Log Rank X^2=6.62, P=0.010



F: Log Rank X^2=0.17, P=0.68

Figure 1: Kaplan-Meier Plots for role of *Cav*1 rs4730751, rs3801995 and rs9920 SNP Variation in Oxford Transplant Centre Donors and Recipients on Long-Term Pancreas Graft function A: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Donors, B: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Recipients, C: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Donors, D: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Recipients, E: Kaplan-Meier plot for variation of *Cav1* rs9902 in Pancreas Transplant Donors & F: Kaplan-Meier plot for variation of *Cav1* rs9902 in Pancreas Transplant Recipients

Table 1: Characteristics of 435 Pancreas Transplant Donors and Recipients from the Oxford Transplant Centre

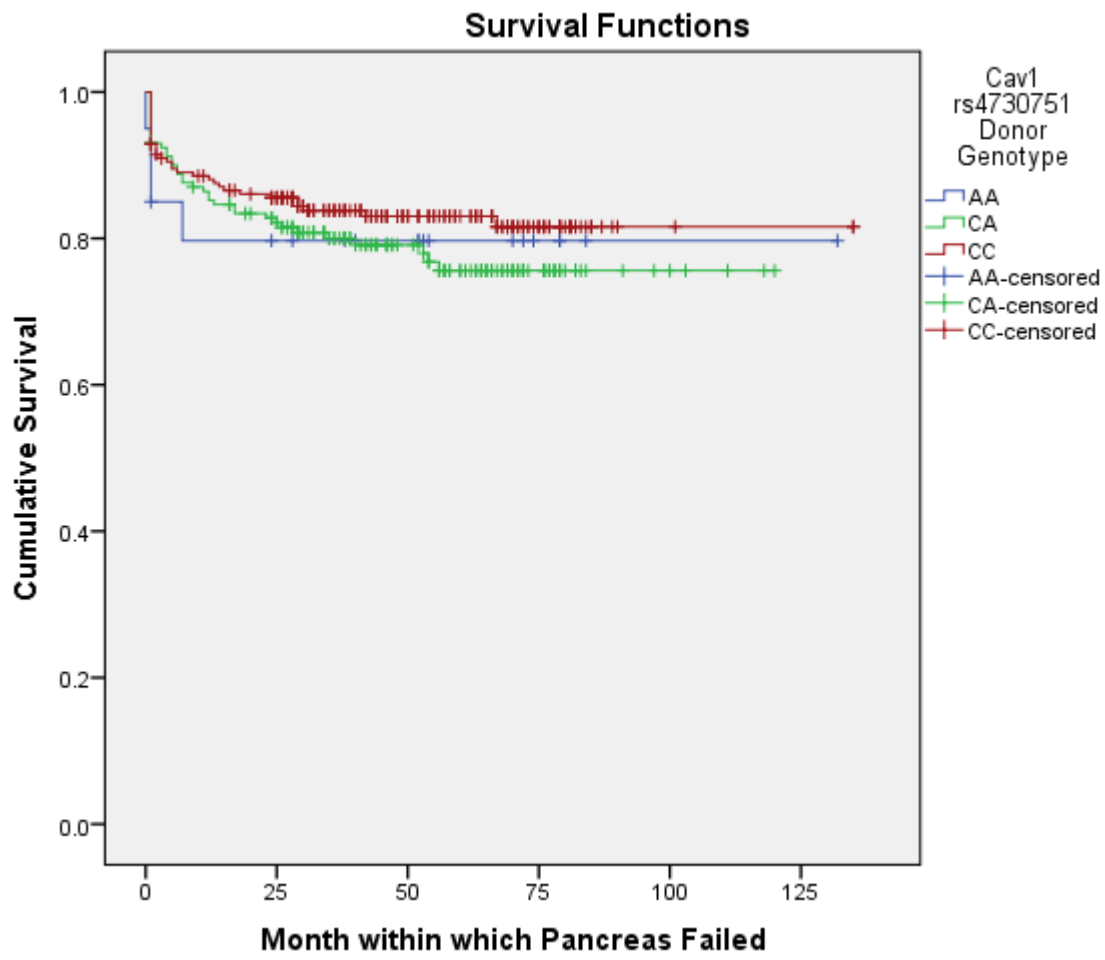
Cohort Characteristics of Pancreas Transplant Donors and Recipients		Total Cohort		SPK		IP		P
Type of Operation	**Simultaneous Pancreas Kidney (SPK)**	315	(72.4)	315	(72.4)			
	Isolated Pancreas (IP)	120				120		
	Pancreas After Kidney (PAK)	68	(15.6)			68	(15.6)	
	Pancreas Transplant Alone (PTA)	38	(8.7)			38	(8.7)	
	Second Pancreas Transplant	14	(3.2)			14	(3.2)	
Donor Type	**Donor after brainstem death (DBD)**	371	(85.5)	291	(92.7)	80	(66.7)	6.00×10^{-12}
	Donor after circulatory death (DCD)	63	(14.5)	23	(7.3)	40	(33.3)	
	CIT (SD)	688.31	(172.8)	683.1	(178.0)	702.1	(158.3)	0.158
HLA mismatches	**0-4 mismatches**	297	(70.4)	215	(70.7)	82	(69.5)	0.804
	5-6 mismatches	125	(29.6)	89	(29.3)	36	(30.5)	
Donor	**Age, Mean (SD), year**	36.80	(13.49)	37.48	(13.84)	35.03	(12.34)	0.090
Donor	**BMI, Mean (SD)**	23.98	(4.20)	24.25	(4.53)	23.28	(3.06)	0.031
Donor Gender	**Female**	213	(49.1)	160	(51.0)	53	(44.2)	0.206
	Male	221	(51.0)	154	(49.0)	67	(55.8)	
Recipient	**Age, Mean (SD), year**	43.3	(8.05)	43.6	(8.01)	42.70	(8.14)	0.298
Recipient	**BMI, Mean (SD)**	25.32	(3.83)	25.39	(3.90)	25.15	(3.68)	0.807
Recipient Gender	**Female**	179	(41.2)	121	(38.5)	58	48.3	0.064
	Male	255	(58.8)	193	(61.5)	62	51.7	

Data are expressed as numbers (%) unless otherwise stated. For age and BMI the mean is given with the standard deviation in brackets. Cold Ischemia Time (CIT), Body Mass Index (BMI), Standard Deviation (SD)

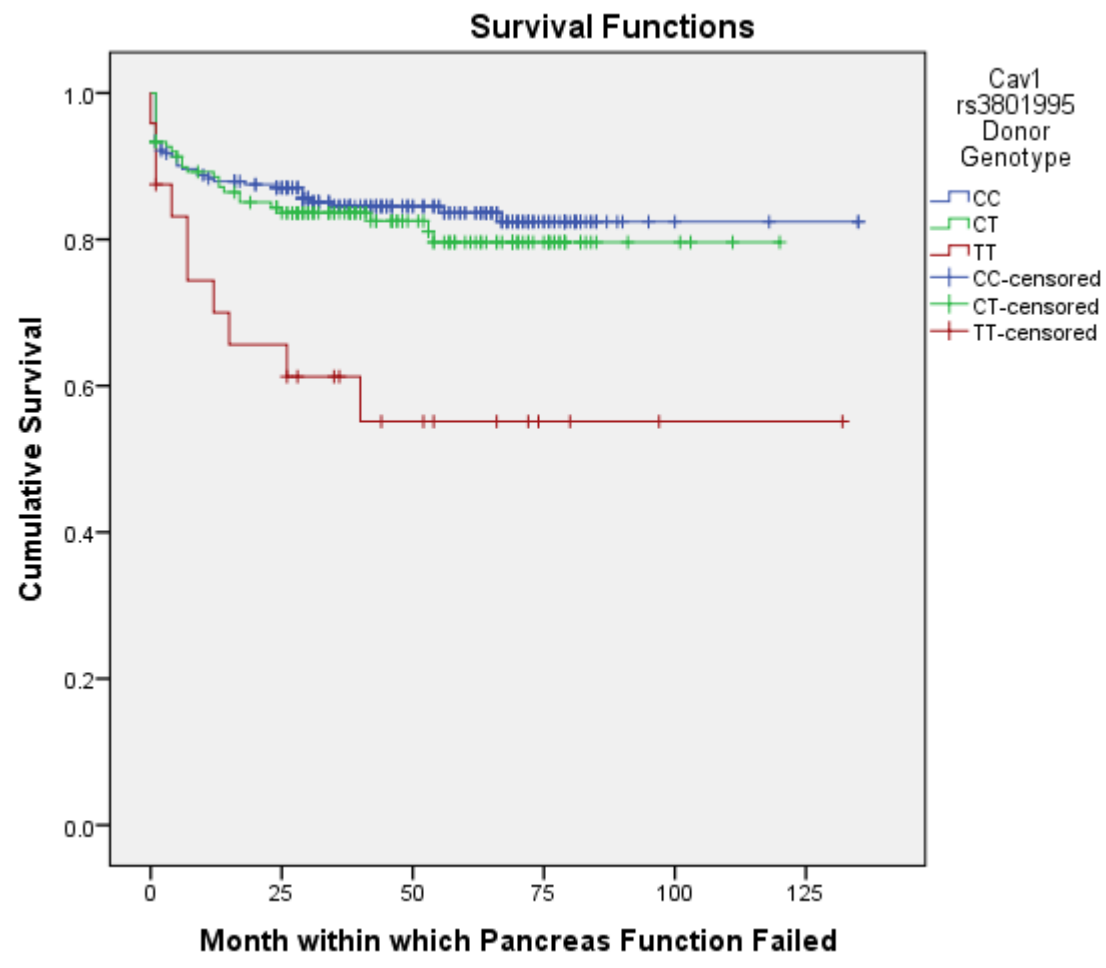
Table 2: Univariate and Multivariate Analysis of *Cav1* rs3801995 and rs9920 Donor Genotype on Long-Term Pancreas Transplant Function

		Univariate Analysis			Multivariate Analysis of *Cav1* rs3801995 donor genotype			Multivariate Analysis of *Cav1* rs9920 donor genotype		
		HR	**(95% CI)**	**P value**	**HR**	**(95% CI)**	**P value**	**HR**	**(95% CI)**	**P value**
***Cav1* rs3801995**	**Overall Effect**	**-**	**-**	**0.015**	**-**	**-**	**0.034**			
	CC genotype	1	Reference		1	Reference				
	CT genotype	1.10	(0.69-1.75)	0.702	0.75	(0.54-1.05)	0.094			
	TT genotype	**2.74**	**(1.38-5.44)**	**0.004**	**1.83**	**(1.16-2.89)**	**0.009**			
***Cav1* rs9902**	**TT genotype**	1	Reference					1	Reference	
	CC/CT genotype	**1.83**	**(1.14-2.93)**	**0.013**				**1.68**	**(1.03-2.73)**	**0.037**
Operation	**SPK**	**1**	**Reference**		**1**	**Reference**		**1**	**Reference**	
	2ND	**4.78**	**(2.26-10.15)**	**$1.08x10^{-4}$**	**5.05**	**(2.37-10.76)**	**$2.71x10^{-5}$**	**2.25**	**(1.28-3.97)**	**0.005**
	PAK	**2.01**	**(1.04-3.88)**	**0.037**	1.95	(0.95-4.02)	0.07	0.97	(0.58-1.62)	0.90
	PTA	**2.03**	**(1.19-3.47)**	**0.009**	**1.80**	**(1.06-3.42)**	**0.032**	0.97	(0.63-1.48)	0.88
Donor Type	**DCD**	**1.00**	**Reference**							
	DBD	**0.80**	**(0.61-1.04)**	**0.097**	0.90	(0.67-1.03)	0.48	0.89	(0.67-1.20)	0.45
Donor	**Age**	**1.01**	**(1.00-1.03)**	**0.149**	1.02	(1.00-1.03)	0.07	1.02	(1.00-1.03)	0.94
Donor	**BMI**	1.01	(0.96-1.05)	0.845						
Donor	**Gender**	1.21	(0.90-1.38)	0.315						
CIT		1.00	(1.00-1.00)	0.264						
Recipient	**Age**	0.99	(0.96-1.01)	0.310						
Recipient	**BMI**	1.01	(0.96-1.07)	0.694						
Recipient	**Male (reference)**	**0.86**	**(0.68-1.07)**	**0.177**	0.86	(0.68-1.09)	0.21	0.89	(0.70-1.12)	0.31
HLA mismatch	**5or6 (reference)**	0.94	(0.74-1.19)	0.60						

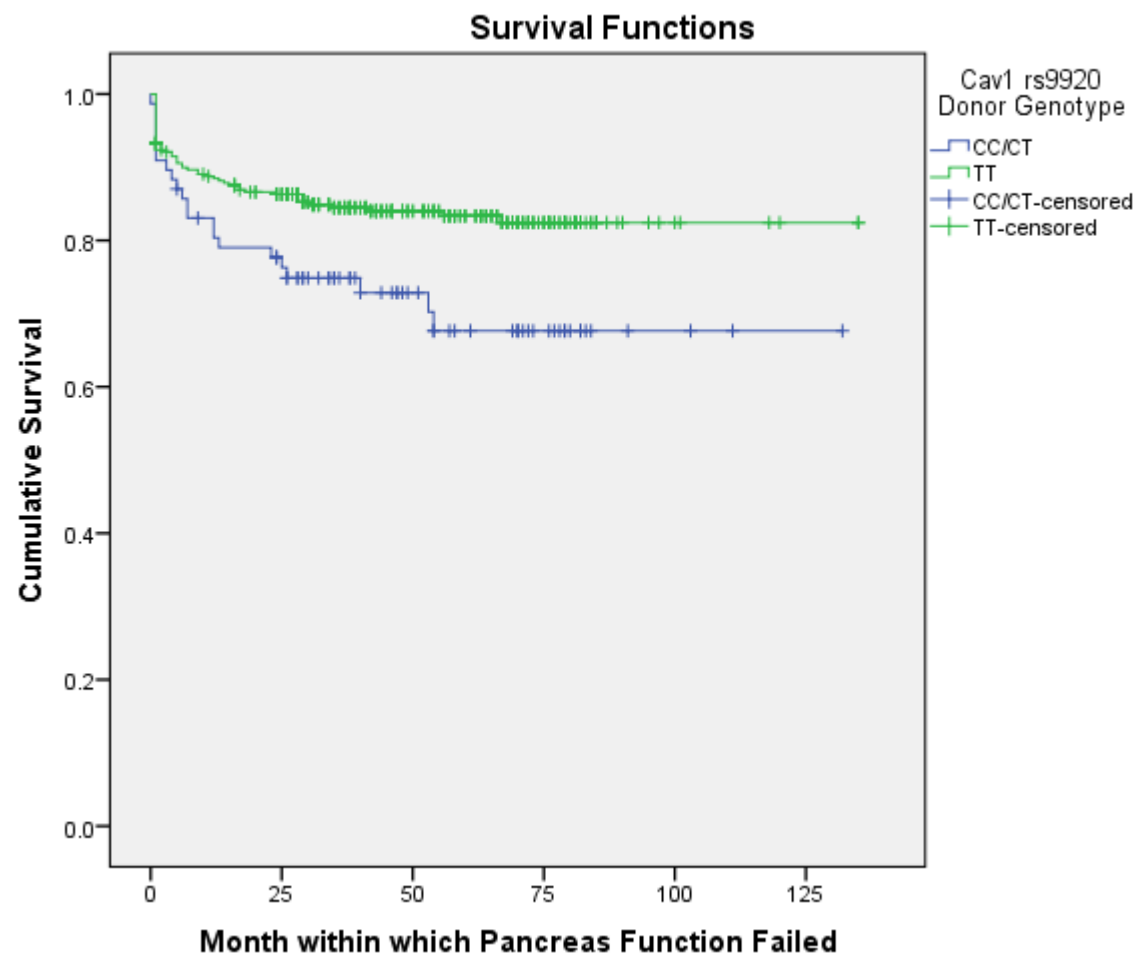
Bold represents any association seen after univariate analysis (P<0.20) or multivariate analysis (P<0.05). Simultaneous Pancreas Kidney (SPK, Pancreas After kidney (PAK), Pancreas Transplant Alone (PTA), Second Pancreas Transplant (2ND), Hazard Ratio (HR), Human Leukocyte Antigen (HLA), Body Mass Index (BMI), Donor After Brainstem Death (DBD) & Donor After Circulatory Death (DCD)



A: Log Rank X^2=1.415, P=0.493



B: Log Rank X^2=11.28, P=0.004



C: Log Rank X^2=6.658, P=0.010

Supplementary Figure S1: Kaplan-Meier Plots for role of *Cav1* rs4730751, rs3801995 and rs9920 SNP variation in Oxford Transplant Centre Donors and Recipients on Long-Term Pancreas Graft function without those who received a second transplant A: Kaplan-Meier plot for variation of *Cav1* rs4730751 in Pancreas Transplant Donors, B: Kaplan-Meier plot for variation of *Cav1* rs3801995 in Pancreas Transplant Donors, C: Kaplan-Meier plot for variation of *Cav1* rs9920 in Pancreas Transplant Donors

Supplementary Table S1: *Cav1* tag SNPs screened in our Oxford Transplant Cohort and other common SNPs that they capture

Tag SNP Screened	Nucleotide variants present*	Location of SNP on chromosome 7	SNP Type	Variants Captured by tag SNP
rs959173	T-C	115969290	Intronic	rs959173, rs976739, rs1543293, rs6466586, rs6466584, rs6466583, rs3779514, rs1476833, rs6466585, rs2742125, rs1474510 & rs4727834
rs3801995	C-T	115977833	Intronic	rs3807990, rs729949, rs3757732, rs3757733, rs7804372, rs3801995, rs3807994, rs3807992, rs6466588 & rs3815412
rs12672038	G-A	115974342	Intronic	rs3807988, rs3807987, rs2052105, rs12672038, rs13233553, rs3807995, rs3801993, rs3801994, rs1049334 & rs12668226
rs9886215	A-G	115978887	Intronic	rs8713, rs9886219, rs2109516 ,rs9886215, rs6867, rs1049314 & rs6466587
rs11773845	C-A	115978537	Intronic	rs1997572, rs3807989 & rs11773845
rs4730751	C-A	115968086	Intronic	rs4730751, rs10270569 & rs10256914
rs926198	T-C	115954444	Intronic	rs926198 & rs917664
rs3779512	G-T	115958299	Intronic	rs9649394 & rs3779512
rs9920	T-C	115987328	3’ UTR	rs9920
rs1049337	C-T	115987823	3’ UTR	rs1049337
rs3807986	A-G	115965061	Intronic	rs3807986
rs4730748	A-G	115954831	Intronic	rs4730748

All Tag SNP locations are based on NCBI Build 36 Assembly, dbSNP126, *=Major-minor allele nucleotides present at each tag SNP.

Supplementary Table S2: *Cav1* Tag SNP genotype frequencies and Hardy Weinberg Equilibrium in our Oxford Pancreas Transplant donors and recipients

		Donors				Recipients			
***Cav1* SNP**	**Genotype**	**N**	**(%)**	**HWE X^2**	**HWE P**	**N**	**(%)**	**HWE X^2**	**HWE P**
rs959173	**TT**	295	(68.76)	3.12	0.08	304	(71.87)	1.56	0.21
	TC	115	(26.81)			113	(26.71)		
	CC	19	(4.43)			6	(1.42)		
rs3801995	**CC**	253	(58.70)	0.01	0.93	226	(53.30)	0.28	0.60
	CT	154	(35.73)			170	(40.09)		
	TT	24	(5.57)			28	(6.61)		
rs12672038	**GG**	370	(85.65)	0.85	0.36	369	(87.03)	0.60	0.44
	AG	61	(14.12)			52	(12.26)		
	AA	1	(0.23)			3	(0.71)		
rs9886215	**AA**	296	(69.16)	1.48	0.22	267	(63.12)	2.05	0.15
	AG	124	(28.67)			144	(34.04)		
	GG	8	(1.87)			12	(2.84)		
rs11773845	**CC**	149	(34.90)	0	0.96	141	(33.65)	0.33	0.57
	CA	206	(48.24)			209	(49.88)		
	AA	72	(16.86)			69	(16.47)		
rs4730751	**CC**	219	(52.64)	3.67	0.06	192	(45.93)	2.65	0.10
	CA	176	(42.31)			193	(46.17)		
	AA	21	(5.05)			33	(7.90)		
rs926198	**TT**	177	(40.88)	0.01	0.93	180	(42.55)	1.82	0.18
	CT	199	(45.96)			182	(43.03)		
	CC	57	(13.16)			61	(14.42)		
rs3779512	**GG**	134	(31.31)	0.20	0.66	117	(27.92)	1.14	0.28
	GT	215	(50.23)			219	(52.27)		
	TT	79	(18.46)			83	(19.81)		
rs9920	**TT**	353	(81.34)	2.60	0.11	325	(76.83)	5.09	0.02
	CT	80	(18.43)			97	(22.93)		
	CC	1	(0.23)			1	(0.24)		
rs1049337	**CC**	239	(55.71)	0.01	0.91	219	(52.02)	0	0.96
	CT	163	(38.00)			169	(40.14)		
	TT	27	(6.29)			33	(7.84)		
rs3807986	**AA**	243	(56.38)	0.15	0.70	253	(59.81)	4.76	0.03
	AG	164	(38.05)			158	(37.35)		
	GG	24	(5.57)			12	(2.84)		
rs4730748	**AA**	282	(65.28)	2.36	0.12	260	(61.46)	0	0.95
	AG	140	(32.41)			143	(33.81)		
	GG	10	(2.31)			20	(4.73)		

Hardy Weinberg Equilibrium (HWE), Single Nucleotide Polymorphism (SNP)

Supplementary Table S3: Univariate and Multivariate Analysis of *Cav1* rs3801995 and rs9920 Donor Genotype on Long-Term Pancreas Transplant Function with all those with a Second Transplant Removed

		Univariate Analysis			Multivariate Analysis of *Cav1* rs3801995 donor genotype			Multivariate Analysis of *Cav1* rs9920 donor genotype		
		HR	**(95% CI)**	**P value**	**HR**	**(95% CI)**	**P value**	**HR**	**(95% CI)**	**P value**
***Cav1* rs3801995**	**Overall Effect**	-	-	**0.007**	-	-	**0.034**			
	CC genotype	1	Reference		1	Reference				
	CT genotype	1.17	(0.71-1.91)	0.544	1.05	(0.64-1.73)	0.86			
	TT genotype	**3.06**	**(1.52-1.91)**	**0.002**	**2.52**	**(1.23-5.17)**	**0.011**			
***Cav1* rs9902**	**TT genotype**	1	Reference					1	Reference	
	CC/CT genotype	**1.85**	**(1.28-3.04)**	**0.015**				**1.67**	**(1.02-2.80)**	**0.043**
Operation	**SPK**	**1**	**Reference**		**1**	**Reference**		**1**	**Reference**	
	PAK	**2.00**	**(1.04-3.87)**	**0.039**	1.24	(0.78-1.97)	0.37	1.97	(0.97-3.99)	0.60
	PTA	**2.04**	**(1.19-3.48)**	**0.009**	**1.23**	**(0.83-1.82)**	**0.30**	**2.02**	**(1.14-3.58)**	**0.017**
Donor Type	**DCD**	**1.00**	**Reference**							
	DBD	**0.75**	**(0.57-0.98)**	**0.038**	0.86	(0.64-1.17)	0.33	0.74	(0.41-1.34)	0.33
Donor	**Age**	**1.01**	**(1.00-1.03)**	**0.188**	1.02	(1.00-1.04)	0.081	1.02	(1.00-1.03)	0.095
Donor	**BMI**	1.01	(0.96-1.05)	0.830						
Donor	**Gender**	1.16	(0.92-1.45)	0.210						
CIT		1.00	(1.00-1.00)	0.255						
Recipient	**Age**	0.99	(0.96-1.01)	0.281						
Recipient	**BMI**	1.02	(0.96-1.09)	0.520						
Recipient	**Male (reference)**	**0.85**	**(0.67-1.08)**	**0.177**	0.86	(0.67-1.09)	0.21	0.76	(0.46-1.24)	0.27
HLA mismatch	**5or6 (reference)**	0.90	(0.71-1.08)	0.403						

Bold represents any association seen after univariate analysis (P<0.20) or multivariate analysis (P<0.05). Simultaneous Pancreas Kidney (SPK), Pancreas After kidney (PAK), Pancreas Transplant Alone (PTA), Hazard Ratio (HR), Human Leukocyte Antigen (HLA), Body Mass Index (BMI), Donor After Brainstem Death (DBD) & Donor After Circulatory Death (DCD)