Effect of donor *HSD17B13* genotype on patient survival after liver transplant: a retrospective cohort study

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Summary

Background Several genetic variants are associated with chronic liver disease. The role of these variants in outcomes after liver transplantation (LT) is uncertain. The aim of this study was to determine if donor genotype at risk-associated variants in *PNPLA3* (rs738409 C>G, p.I148M) and *HSD17B13* (rs72613567 T>TA; rs80182459, p.A192Lfs*8) influences post-LT survival.

Methods In this retrospective cohort study, data on 2346 adults who underwent first-time LT between January 1, 1999 and June 30, 2020 and who had donor DNA samples available at five large Transplant Immunology Laboratories in Texas, USA, were obtained from the United Network for Organ Sharing (UNOS). Duplicates, patients with insufficient donor DNA for genotyping, those who were <18 years of age at the time of transplant, had had a previous transplant or had missing genotype data were excluded. The primary outcomes were patient and graft survival after LT. The association between donor genotype and post-LT survival was examined using Kaplan–Meier method and multivariable-adjusted Cox proportional hazards models.

Findings Median age of LT recipients was 57 [interquartile range (IQR), 50–62] years; 837 (35.7%) were women; 1362 (58.1%) White, 713 (30.4%) Hispanic, 182 (7.8%) Black/African-American. Median follow-up time was 3.95 years. Post-LT survival was not affected by donor *PNPLA3* genotype but was significantly reduced among recipients of livers with two *HSD17B13* loss-of-function (LoF) variants compared to those receiving livers with no *HSD17B13* LoF alleles (unadjusted one-year survival: 82.6% vs 93.9%, $P < 0.0001$; five-year survival: 73.1% vs 82.9%, $P = 0.0017$; adjusted hazard ratio [HR], 2.25; 95% CI, 1.61–3.15 after adjustment for recipient age, sex, and self-reported ethnicity). Excess mortality was restricted to those receiving steroid induction immunosuppression (crude 90-day post-LT mortality, 9.3% [95% CI, 1.9%–16.1%] vs 1.9% [95% CI, 0.9%–2.9%] in recipients of livers with two vs no *HSD17B13* LoF alleles, $P = 0.0012$; age, sex, and ethnicity-adjusted HR, 2.85; 95% CI, 1.72–4.71, $P < 0.0001$). No reduction was seen among patients who did not receive steroid induction (90-day mortality 3.1% [95% CI, 0%–7.3%] vs 2% [95% CI, 0.9%–3.1%], $P = 0.65$; adjusted HR, 1.17; 95% CI, 0.66–2.08, $P = 0.60$).

Interpretation Donor *HSD17B13* genotype adversely affects post-LT survival in patients receiving steroid induction. Additional studies are required to confirm this association.

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Research in context

Evidence before this study

We searched the PubMed database using the search terms "liver transplantation", "donor genotype", "recipient genotype", and "PNPLA3" or "HSD17B13", to identify studies published before December 31, 2022, that reported on the impact of liver-disease associated variants in PNPLA3 and HSD17B13 on outcomes after liver transplantation (LT). We also reviewed the references cited in the identified papers. Variants in PNPLA3 and HSD17B13 are well described genetic risk factors that influence chronic liver disease progression. A few previous studies have examined the impact of these variants in the donor or the recipient on disease recurrence after LT, although the findings have been inconclusive. Whether the presence of these variants in the donor livers influences long-term survival of liver transplant recipients is not known.

Added value of this study

This cohort study is the first, to our knowledge, to investigate the association of donor PNPLA3 and HSD17B13 genotype with patient survival following LT. We found that recipients of livers from donors homozygous for loss-of-function (LoF) mutations in *HSD17B13* had significantly increased mortality compared to other recipients. Excess mortality was restricted to patients treated with high-dose corticosteroids; in this group, recipients of two *HSD17B13* LoF alleles had >4-fold higher 90-days post-LT mortality compared to recipients of no *HSD17B13* LoF alleles.

Implications of all the available evidence

Presence of HSD17B13 LoF alleles in donors reduces post-LT survival, particularly in patients treated with high-dose steroids.

Introduction

End-Stage Liver Disease (ESLD) is a leading cause of death in the USA.[1] The only treatment for ESLD is replacement of the organ by liver transplantation (LT). The etiology of liver diseases resulting in transplantation has changed over the past decade. Until recently, the most common indication for LT was hepatitis C infection,[2] but the advent of direct-acting antiviral therapies[3] has made nonalcoholic fatty liver disease (NAFLD) and alcohol-related liver disease (ALD) the leading indications for LT.[4]

One-year survival after LT has improved dramatically over the last three decades owing to development of better patient selection strategies, more effective immunosuppressive agents, advances in surgical techniques, and perioperative care.[5] No such improvements have been seen in long-term post-LT survival,[6–8] in part due to long-term sequelae of immunosuppression. The identification of risk factors associated with post-LT survival will be required to improve long-term patient outcomes.

Several genetic variants influence susceptibility to chronic liver disease of different etiologies. The most prevalent and impactful of these is a missense variant in *PNPLA3* (p.I148M, rs738409),[9] that is associated with a 1.5 to 4-fold increase in risk of hepatic steatosis,[9–11] steatohepatitis,[12,13] fibrosis,[12–14] cirrhosis,[12,14–21] hepatocellular carcinoma (HCC),[20,22,23] and mortality,[24–26] in patients with NAFLD, ALD, hepatitis C and mixed disease etiologies.[27–30]

More recently, two common putative loss-of-function (LoF) variants in *HSD17B13* (a splice-site variant rs72613567 T>TA, and a frameshift variant rs80182459, p.A192Lfs*8) were shown to be associated with a ~50% reduction in risk of progressive liver disease,[31,32] including fibrosis, cirrhosis, and HCC among patients with ALD, NAFLD,[20,33,34] and hepatitis C.[33,35]

Several studies have investigated whether recipient or donor genotype at *PNPLA3* or *HSD17B13* loci was associated with acute rejection, incident diabetes, or recurrent liver disease after LT.[36–46] However, most studies included small, single-centre cohorts and reported conflicting findings. No previous study has examined the effect of donor genotype on post-LT survival. Here, we tested the hypothesis that donor genotypes at *PNPLA3* and *HSD17B13* loci impact post-transplant survival in a large, multi-ethnic cohort of adult first-time LT recipients (n = 2346) using data from the United Network for Organ Sharing (UNOS). Although other genetic variants also alter liver disease progression,[47–49] they are either less common (e.g., TM6SF2 p.E167K[47] or *CIDEB*[49]) or have more variable effect sizes (e.g., MARC1 p.A165T).[48] Accordingly, here we focused on the two most common and impactful modifiers of liver disease risk to test the importance of donor genotype in long-term outcomes after LT.

Methods

Study design and population

In this retrospective cohort study, prospectively collected data were obtained from UNOS. The study was conducted with approval from the University of Texas Southwestern Institutional Review Board (UTSW IRB) and in accordance with institutional regulations. Since the study involved retrospective analysis of previously collected deidentified data, the requirement to obtain informed consent was waived. We have followed the STROBE reporting guidelines for cohort studies.

The study included adult, first-time LT recipients who had donor DNA sample available at five large Transplant Immunology Laboratories (HLA Labs) in Texas. Donor DNA samples were retrospectively collected from HLA labs at UTSW, UT San Antonio, Baylor University Medical Center Dallas, Baylor University Houston, and UT Health Science Center at Houston. UNOS database was then used to link donor data to follow up and outcomes of LT recipients. HLA Labs are required to store samples for at least 10 years. Most patients (98%) for whom we had donor DNA available received LT between 2010 and 2020, with a small proportion receiving LT prior to 2010. Most of the patients in the linked dataset received LT at transplant centres affiliated with the HLA labs, from which we obtained samples, with a smaller proportion of patients receiving LT at other transplant centres, if their donor had been evaluated for another (non-liver) organ transplant at the specified HLA labs. Additional details regarding the study population are provided in Supplementary Material.

In total, 4167 donor DNA samples were obtained from 5 HLA Labs. After excluding duplicate samples (n = 159), samples with no DNA (n = 343) or no consent (n = 11), samples with insufficient DNA for genotyping (n = 469), the remaining donor samples were matched to 3317 LT recipients in UNOS. Records were excluded if the liver was not used for transplant (n = 418); recipient was <18 years of age (n = 432); recipient had had a previous transplant (n = 118); or genotype data were missing (n = 3). The final dataset included 2346 adult patients (≥18 years of age) who received first-time LT in one of 89 US transplant centres between January 1, 1999 and June 30, 2020 (Fig. 1). The distribution of LTs by centre and year is shown in Supplementary Table S1.

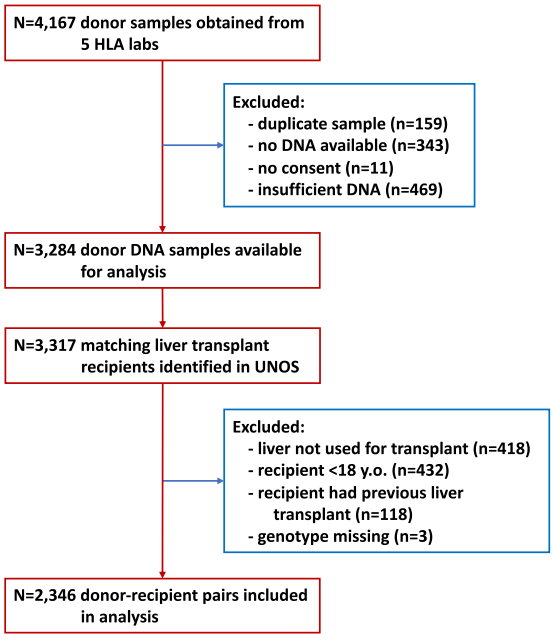

Fig. 1: **Study flow diagram**.

Recipient and donor characteristics

Demographic and clinical characteristics of donors and recipients were obtained from UNOS. Race/ethnicity was self-reported and classified as non-Hispanic White, non-Hispanic Black, Hispanic, or Other (Asian, American Indian/Alaska Native, Native Hawaiian/other Pacific Islander, Multiracial). Indication for LT and liver disease etiology were defined based on diagnostic codes provided in UNOS (Supplementary Table S2). MELD scores were available for patients receiving LT after January, 2002.

Immunosuppressive treatments

Data on induction immunosuppression therapy given at the time of surgery were obtained from UNOS. A total of 1212 (52%) patients received high-dose steroids; of those, 579 (24.7%) also received Simulect (basiliximab) or another agent. The remaining patients received induction immunosuppression with other agents (n = 149, 6.4%) or no induction immunosuppression (n = 874, 37.3%), and n = 111 (4.7%) had missing data. Missing data on steroids were imputed using multiple imputation (see Supplementary Methods).

Outcomes

The primary outcomes were patient and graft survival after LT. Patient mortality was defined as death from any cause. Graft failure was defined as recipient death or graft failure requiring re-transplantation. Follow-up time was defined as time from LT to last encounter with the transplant centre, graft failure, or death. Cause of death was classified using the primary and contributory cause of death codes in UNOS (Supplementary Table S3).

Genotyping

Donor DNA samples were genotyped for *PNPLA3* rs738409, *HSD17B13* rs72613567 and rs80182459 by Real-Time PCR as described.[9,31] Genotype frequencies were in Hardy–Weinberg equilibrium (using exact test in the R package 'HardyWeinberg').

Kidney and heart transplant data

We matched donor genotypes to data on kidney and heart transplants using UNOS. The inclusion/exclusion criteria were similar to those used for the primary cohort. The kidney transplant dataset included 4039 adult patients (≥18 years of age), who underwent first-time kidney transplant between 1996 and 2020. The heart transplant dataset included 1296 adult patients (≥18 years of age), who underwent heart transplant between 1996 and 2020.

Sensitivity analysis

For the primary analysis, patient data from all transplant centres were pooled. To investigate whether results varied between centres, we performed an analysis stratified by centre. Centers, for which we had available data for >100 transplants (7 centres), were analysed individually, whereas those with data on ≤100 transplants were combined into groups: 51–100 transplants (2 centres, a total of 115 patients); 11–50 transplants (9 centres, 198 patients); ≤10 transplants (71 centres, 246 patients). Centre-specific estimates were pooled using random-effects meta-analysis. To assess the effect of each individual centre on the combined estimate, we performed a leave-one-out sensitivity analysis. In addition, to account for possible heterogeneity in outcomes, we performed a mixed-effects analysis of the pooled cohort with robust standard errors to account for patient clustering by centre, and an analysis restricted to the 7 largest centres, with an adjustment for centre.

Statistical analysis

Statistical analyses were performed using R statistical software version 3.6.3. Baseline characteristics were expressed as median (interquartile range [IQR]) or as number (%). Categorical variables were compared using Fisher's exact test. Continuous variables were compared using ANOVA and linear regression models. An inverse-normal transformation was applied to variables with non-normal distributions. Survival probabilities were estimated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazards models were used to estimate the effect of genotypes on post-LT outcomes after adjustment for covariates. We considered three progressively adjusted models: Model 1 adjusted for recipient characteristics (age, sex, and ethnicity); Model 2 adjusted in addition for donor characteristics (ethnicity, sex, and age); and Model 3, including Model 2 covariates plus recipient BMI, diabetes mellitus status, and MELD score. Genotypes were coded as 0 for reference allele homozygotes, 1 for heterozygotes, and 2 for alternate allele homozygotes. Since the two *HSD17B13* variants are mutually exclusive (i.e., never occur on the same chromosome) and both are predicted to result in a loss of function,[31,32] we pooled the two HSD17B13 variants for the primary analysis and coded the *HSD17B13* genotype as 0, 1, and 2 for donors with no, one, or two LoF variants, as previously described.[50] The assumption of proportional hazards was checked using Schoenfeld residuals. Interactions between genotype and other factors were tested by including multiplicative interaction terms in the model. Centre-specific estimates were pooled using random-effects meta-analysis in the R package 'meta'. Heterogeneity was assessed using Cochran's Q and I^2 statistics. Multiple imputation was performed using the R package 'mice', with 5 multiple imputations performed and the results averaged across the imputed datasets (see Supplementary Methods for more details). All tests were two-sided and P Values < 0.05 were considered statistically significant. In genetic analysis, significance level was set at 0.025 (0.05/2) to correct for the number of genotypes tested. For interaction analysis, the significance level was set to 0.1. Additional sensitivity analyses were performed after excluding patients with missing data on steroids, adjusted or stratified by liver disease etiology, and time of transplant (1999–2013, 2014–2016, and 2017–2020), and separately for two HSD17B13 variants, as described in Supplementary Methods.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. J.C.C., H.H.H., and M.P.M. had full access to the dataset and had final responsibility for the decision to submit for publication.

Results

Baseline characteristics of recipients and donors are listed in Table 1. Median age of LT recipients was 57 years [IQR, 50–62 years], and 837 (35.7%) were women. Non-Hispanic Whites comprised the majority of recipients (n = 1362; 58.1%); 30.4% (n = 713) were Hispanic, 7.8% (n = 182) non-Hispanic Black, and 3.8% (n = 89) other ethnicities. The most common indications for LT were alcohol-related hepatitis and cirrhosis (23.4%), hepatitis C (20.2%), HCC (15.2%), and fatty liver or NASH (10.6%).

Just over half of the donors (51.4%) were White, 29.5% were Hispanic, 16.5% were Black, and 2.6% were of other races/ethnicities. A total of 38% (n = 889) of donors were women. Ancestry-specific frequencies of genetic variants in LT donors were similar to those reported previously (Supplementary Table S4).[9,31,32]

Over a median follow-up time of 3.95 [IQR, 3.88–3.99] years, 381 deaths and 48 re-transplantations occurred; of these, 87 (22.8%) deaths occurred within 90 days of surgery. The overall patient and graft survival rates were 92.4% and 90.9% at one year, 81.9% and 80.2% at five years, and 63.8% and 61.3% at 10 years, respectively.

Characteristic	Total	Number of *HSD17B13* LoF alleles			*P* Value
		0 alleles	1 allele	2 alleles	
	(N = 2346)	(n = 1380)	(n = 823)	(n = 143)	
Recipients:					
Age at transplant, median (IQR), years	57 (50–62)	57 (50–63)	57 (50.5–62)	54 (47–61)	0.078
Female sex, number (%)	837 (35.7)	478 (34.6)	302 (36.7)	57 (39.9)	0.34
Male sex, number (%)	1509 (64.3)	902 (65.4)	521 (63.3)	86 (60.1)	0.34
Race/ethnicity, number, (%)					
White	1362 (58.1)	801 (58)	476 (57.8)	85 (59.4)	0.94
Black	182 (7.8)	97 (7)	72 (8.7)	13 (9.1)	0.27
Hispanic	713 (30.4)	437 (31.7)	234 (28.4)	42 (29.4)	0.27
Other	89 (3.8)	45 (3.3)	41 (5)	3 (2.1)	0.080
Body mass index, median (IQR), kg/m^2	28.2 (24.7–32.5)	28.3 (24.7–32.7)	28.2 (24.7–32.2)	28.2 (24.5–31.7)	0.64
Diabetes, number (%) (n = 2313)					
No	1669 (72.2)	981 (72.3)	578 (70.9)	110 (78)	0.22
Type I	17 (0.7)	12 (0.9)	5 (0.6)	0 (0)	0.66
Type II	617 (26.7)	360 (26.5)	226 (27.7)	31 (22)	0.37
Unknown	10 (0.4)	4 (0.3)	6 (0.7)	0 (0)	0.28
Liver diagnosis at listing, number (%)					
Alcohol related cirrhosis and hepatitis	550 (23.4)	327 (23.7)	191 (23.2)	32 (22.4)	0.94
HCV hepatitis/cirrhosis	473 (20.2)	280 (20.3)	160 (19.4)	33 (23.1)	0.58
HBV hepatitis/cirrhosis	32 (1.4)	13 (0.9)	16 (1.9)	3 (2.1)	0.072
Alcohol related cirrhosis with hepatitis C	104 (4.4)	59 (4.3)	37 (4.5)	8 (5.6)	0.71
Fatty liver (NASH)	248 (10.6)	149 (10.8)	85 (10.3)	14 (9.8)	0.93
Cryptogenic cirrhosis	154 (6.6)	89 (6.4)	56 (6.8)	9 (6.3)	0.96
Acute Hepatic Necrosis	83 (3.5)	42 (3)	33 (4)	8 (5.6)	0.17
Primary biliary cirrhosis	125 (5.3)	73 (5.3)	46 (5.6)	6 (4.2)	0.85
Autoimmune	54 (2.3)	29 (2.1)	24 (2.9)	1 (0.7)	0.22
Hepatoma - hepatocellular carcinoma	356 (15.2)	225 (16.3)	111 (13.5)	20 (14)	0.19
Other or N/A	167 (7.1)	94 (6.8)	64 (7.8)	9 (6.3)	0.67
Transplant type, number, (%)					
Whole	2312 (98.6)	1358 (98.4)	813 (98.8)	141 (98.6)	0.78
Split	34 (1.4)	22 (1.6)	10 (1.2)	2 (1.4)	0.78
Urgent Transplant, number (%)	65 (2.8)	38 (2.8)	21 (2.6)	6 (4.2)	0.49
Multiorgan Transplant, number (%)	299 (12.7)	166 (12)	113 (13.7)	20 (14)	0.45
Recipient life support, number (%)	149 (6.4)	94 (6.8)	47 (5.7)	8 (5.6)	0.58
MELD score, median (IQR)[a] (n = 2342)	25 (15–34)	25 (14–34)	24 (15–33)	27 (17–35)	0.49
Serum Creatinine, median (IQR), mg/dL	1.2 (0.8–1.9)	1.2 (0.8–1.9)	1.2 (0.8–2.1)	1.2 (0.9–1.9)	0.11
Bilirubin, median (IQR), mg/dL	4.6 (1.7–13.7)	4.5 (1.7–14.2)	4.4 (1.7–12.6)	5.6 (2.2–17)	0.56
Serum sodium, median (IQR), mg/dL (n = 2318)	137 (134–140)	137 (134–140)	137 (133–140)	138 (135–140)	0.52
Ascites, number (%)					
Absent	532 (22.7)	325 (23.6)	182 (22.1)	25 (17.6)	0.24
Slight	1089 (46.5)	643 (46.7)	374 (45.4)	72 (50.7)	0.50
Moderate	720 (30.7)	409 (29.7)	267 (32.4)	44 (31)	0.40
Unknown/NA	1 (0)	0 (0)	0 (0)	1 (0.7)	0.061
Induction treatment, number (%)					
None	874 (37.3)	523 (37.9)	296 (36)	55 (38.5)	0.63
Steroids	1212 (51.7)	719 (52.1)	426 (51.8)	67 (46.9)	0.49
Steroids alone	633 (27)	362 (26.2)	238 (28.9)	33 (23.1)	0.22
Steroids + Other	579 (24.7)	357 (25.9)	188 (22.8)	34 (23.8)	0.27
Other	149 (6.4)	90 (6.5)	48 (5.8)	11 (7.7)	0.63
Unknown/Missing data	111 (4.7)	48 (3.5)	53 (6.4)	10 (7)	**0.0024**
Follow-up time (years), median (95% CI)	3.95 (3.88–3.99)	3.94 (3.82–3.99)	3.95 (3.79–4.00)	4.33 (3.61–4.97)	0.48

(Table 1 continues on next page)

Characteristic	Total	Number of *HSD17B13* LoF alleles			*P* Value
		0 alleles	1 allele	2 alleles	
	(N = 2346)	(n = 1380)	(n = 823)	(n = 143)	
(Continued from previous page)					
Donors:					
Age, median (IQR), years	35 (23–48)	35 (23–49)	34 (22–47)	36 (24–51)	0.84
Pediatric donor (age <18 years), number (%)	192 (8.2)	115 (8.3)	67 (8.1)	10 (7)	0.91
Female sex, number (%)	889 (37.9)	525 (38)	316 (38.4)	48 (33.6)	0.54
Male sex, number (%)	1457 (62.1)	855 (62)	507 (61.6)	95 (66.4)	0.54
Race/ethnicity, number, (%)					
White	1205 (51.4)	620 (44.9)	489 (59.4)	96 (67.1)	**<0.0001**
Black	388 (16.5)	213 (15.4)	148 (18)	27 (18.9)	0.21
Hispanic	691 (29.5)	521 (37.8)	157 (19.1)	13 (9.1)	**<0.0001**
Other	62 (2.6)	26 (1.9)	29 (3.5)	7 (4.9)	**0.013**
Body mass index, median (IQR), kg/m^2	26.5 (23.1–30.8)	26.6 (23.1–31.3)	26.2 (22.9–29.8)	26.6 (23.7–31.2)	0.43
Cold ischemia time, median (IQR), hours	5.8 (4.4–7.4)	5.7 (4.3–7.3)	5.8 (4.5–7.4)	6.1 (4.7–7.7)	0.086
Non-heart beating donor, number (%)	70 (3)	42 (3)	25 (3)	3 (2.1)	0.92
Deceased donor heavy alcohol use, number (%)					
N	2003 (85.4)	1185 (85.9)	703 (85.4)	115 (80.4)	0.22
Y	286 (12.2)	160 (11.6)	103 (12.5)	23 (16.1)	0.27
Unknown or missing	57 (2.4)	35 (2.5)	17 (2.1)	5 (3.5)	0.48
BAR score, median (IQR)	10 (5–14)	10 (4–14)	9 (5–14)	11 (6–14)	0.84
DRI score, median (IQR) (n = 2333)	1.4 (1.2–1.7)	1.4 (1.2–1.7)	1.4 (1.2–1.6)	1.3 (1.1–1.6)	**0.0002**
DRI group, number (%) (n = 2333)					
1	108 (4.6)	48 (3.5)	53 (6.5)	7 (4.9)	**0.0055**
2	1339 (57.4)	790 (57.5)	465 (57.1)	84 (58.7)	0.93
3	710 (30.4)	417 (30.3)	245 (30.1)	48 (33.6)	0.69
4	176 (7.5)	120 (8.7)	52 (6.4)	4 (2.8)	**0.0092**
Marginality score, number (%)					
0	1611 (68.7)	948 (68.7)	564 (68.5)	99 (69.2)	0.99
1	681 (29)	402 (29.1)	237 (28.8)	42 (29.4)	0.98
2	52 (2.2)	28 (2)	22 (2.7)	2 (1.4)	0.59
3	2 (0.1)	2 (0.1)	0 (0)	0 (0)	0.59
Modified marginality score, median (IQR)	2 (1–2)	2 (1–2)	1 (1–2)	2 (1–3)	0.56
Terminal AST, median (IQR), U/L	46 (27–88)	46 (26–87)	46 (27–90)	41 (25–77.5)	0.72
Terminal ALT, median (IQR), U/L	35 (21–70)	35 (21–67)	35 (22–75)	35 (20.5–64)	0.86
Deceased Donor Steroids (within 24 h of procurement), number (%) (DDR Form)	1061 (45.2)	626 (45.4)	371 (45.1)	64 (44.8)	0.99
Deceased Donor Steroids, number (%) (DonorNet Form)	1917 (81.7)	1113 (80.7)	683 (83)	121 (84.6)	0.27
Steatosis (fat >5%), number (%)	343 (14.6)	195 (14.1)	123 (14.9)	25 (17.5)	0.51
Macrosteatosis (fat >30%), number (%)	37 (1.6)	23 (1.7)	13 (1.6)	1 (0.7)	0.89
Elevated AST (>499), number (%)	42 (1.8)	26 (1.9)	14 (1.7)	2 (1.4)	0.97
Donor HBV Core Antibody, number (%)	64 (2.7)	40 (2.9)	22 (2.7)	2 (1.4)	0.68
Deceased Donor Antibody to Hep-C Virus, number (%)	54 (2.3)	38 (2.8)	12 (1.5)	4 (2.8)	0.12
Deceased Donor Cause of Death, number (%)					
Anoxia	604 (25.7)	360 (26.1)	214 (26)	30 (21)	0.41
Cerebrovascular/Stroke	688 (29.3)	409 (29.6)	232 (28.2)	47 (32.9)	0.48
Head Trauma	952 (40.6)	550 (39.9)	343 (41.7)	59 (41.3)	0.70
CNS Tumor	15 (0.6)	10 (0.7)	4 (0.5)	1 (0.7)	0.69
Other	87 (3.7)	51 (3.7)	30 (3.6)	6 (4.2)	0.90
Allocation Type, number (%)					
Local	1721 (73.4)	1006 (72.9)	614 (74.6)	101 (70.6)	0.50
Regional	573 (24.4)	345 (25)	188 (22.8)	40 (28)	0.30
Foreign	52 (2.2)	29 (2.1)	21 (2.6)	2 (1.4)	0.70
Allocation out of area, number (%)	625 (26.6)	374 (27.1)	209 (25.4)	42 (29.4)	0.50

Bolded values indicate $P < 0.05$. [a]MELD (Model for End-Stage Liver Disease) scores were available for patients receiving LT after January, 2002.

Table 1: **Donor and recipients characteristics stratified by donor *HSD17B13* genotype.**

Survival after liver transplant according to donor genotype

We hypothesized that presence of PNPLA3(148-M) in donors would be associated with decreased post-LT survival. Baseline characteristics of LT recipients did not differ significantly according to donor *PNPLA3* genotype (Supplementary Table S5). Contrary to expectation, donor PNPLA3-I148M genotype was not associated with post-LT patient ($P = 0.96$; Fig. 2A; Table 2) or graft survival ($P = 0.67$; Supplementary Figure S1A; Supplementary Table S6).

Next, we tested whether recipients of livers with *HSD17B13* LoF variants would have improved post-LT survival. Unexpectedly, patients receiving livers with two *HSD17B13* LoF alleles had *decreased* survival compared to recipients of livers homozygous for the reference allele ($P < 0.0001$; Fig. 2A; unadjusted 1-year post-LT survival: 82.6% [95% CI, 76.5%–89.2%] vs 93.9% [95% CI, 92.6%–95.2%], $P < 0.0001$; 5-year post-LT survival, 73.1% [95% CI, 65.3%–81.8%] vs 82.9% [95% CI, 80.4%–85.3%], $P = 0.0017$, Supplementary Table S7). Survival did not differ between those receiving livers with one vs no *HSD17B13* alleles ($P = 0.11$; Fig. 2A right; 5-year survival, 81.9% [95% CI, 78.8%–85.2%] vs 82.9% [95% CI, 80.4%–85.3%], Supplementary Table S7). Baseline characteristics of LT recipients did not differ according to donor *HSD17B13* genotype (Table 1). In multivariable-adjusted Cox regression models, patients receiving livers with two *HSD17B13* LoF alleles had more than two-fold higher hazard of death (HR, 2.25; 95% CI, 1.61–3.15; $P < 0.0001$, Table 2) compared to recipients of livers with no *HSD17B13* LoF alleles. No difference was observed between patients receiving livers with one *HSD17B13* vs no LoF variants (HR, 1.19; 95% CI, 0.96–1.48; $P = 0.11$, Table 2). Similar results were observed after adjustment for donor characteristics (ethnicity, age, and sex) and in fully adjusted models (Table 2), as well as for graft survival (Supplementary Figure S1B and Supplementary Table S6).

The adverse effects of *HSD17B13* LoF alleles became apparent early after LT. Cumulative 90-day post-LT mortality was >4.5-fold higher in recipients of livers with two vs no *HSD17B13* LoF alleles (12.2% [95% CI, 6.6%–17.5%] vs 2.6% [95% CI, 1.8%–3.5%]; $P < 0.0001$; Supplementary Table S7). Similar results were obtained for graft survival (Supplementary Figure S1B and Supplementary Table S7).

Survival after kidney or heart transplantation not related to donor genotype

To determine if the effect of *HSD17B13* genotype was specific to LT, we examined the association of donor *HSD17B13* genotype with survival after kidney or heart transplantation. Characteristics of kidney and heart donors and recipients are presented in Supplementary Tables S8 and S9. No association was observed between donor *HSD17B13* genotype and patient or graft survival (Fig. 2B–C, Supplementary Figure S1C and D) for either of these procedures.

Association stratified by transplant centre

In a sensitivity analysis stratified by centre, all centre-specific HRs for recipients of 2 versus 0 *HSD17B13* alleles were greater than 1 (random-effects meta-analysis pooled HR, 2.82; 95% CI, 1.58–5.02), although some heterogeneity between centres was observed ($I^2 = 53\%$, $P = 0.02$, Fig. 3). Centre 6 appeared to be an outlier in this analysis; therefore, we repeated the analysis, excluding centre 6. The pooled HR was moderately attenuated but remained highly statistically significant in this analysis, or in sensitivity analysis excluding other centres one at a time (Supplementary Figure S2). Similar results were observed for graft survival (Supplementary Figure S3).

Donor *HSD17B13* genotype alters survival in patients receiving steroid induction

Increased 90-day mortality in recipients of two *HSD17B13* LoF alleles suggested that donor *HSD17B13* genotype modulates susceptibility to factors acting early in the post-transplant period. To determine if available individual-level or centre-level factors might explain these outcomes, we performed post-hoc interaction analysis. The effect of donor *HSD17B13* genotype on post-LT survival did not differ between males and females or between recipient or donor ethnicities (*P*-interaction>0.05, Supplementary Table S10). We did not observe common causes of death among recipients of 2 HSD17B13 alleles (Supplementary Table S11).

We then tested if the association between donor *HSD17B13* genotype and patient or graft survival was modified by induction immunosuppression therapy, in particular steroids, since prior data suggested that HSD17B13 may be involved in steroid metabolism.[31,51] The frequency of immunosuppressive regimens did not differ between donor *HSD17B13* genotypes (Table 1).

We observed an interaction between homozygous *HSD17B13* genotype and steroid induction on patient survival ($P = 0.035$), although the result was not statistically significant after correcting for the number of interactions tested (Bonferroni corrected *P* Value = 0.14). Recipients of livers with two vs no *HSD17B13* LoF alleles experienced nearly a 3-fold increase in mortality hazard if they received steroid induction (Fig. 4A, $P = 0.0001$; HR, 2.85; 95% CI, 1.72–4.71, $P < 0.0001$, Supplementary Table S10; 90-day post-LT mortality, 9.3% [95% CI, 1.9%–16.1%] vs 1.9% [95% CI, 0.9%–2.9%] in recipients of livers with two vs no *HSD17B13* LoF alleles, $P = 0.0012$; Supplementary Table S13), but no difference was observed among those who did not receive steroids ($P > 0.05$, Fig. 4B; HR, 1.17; 95% CI, 0.66–2.08, Supplementary Table S10; 90-day mortality 3.1% [95% CI, 0%–7.3%] vs 2% [95% CI, 0.9%–3.1%],

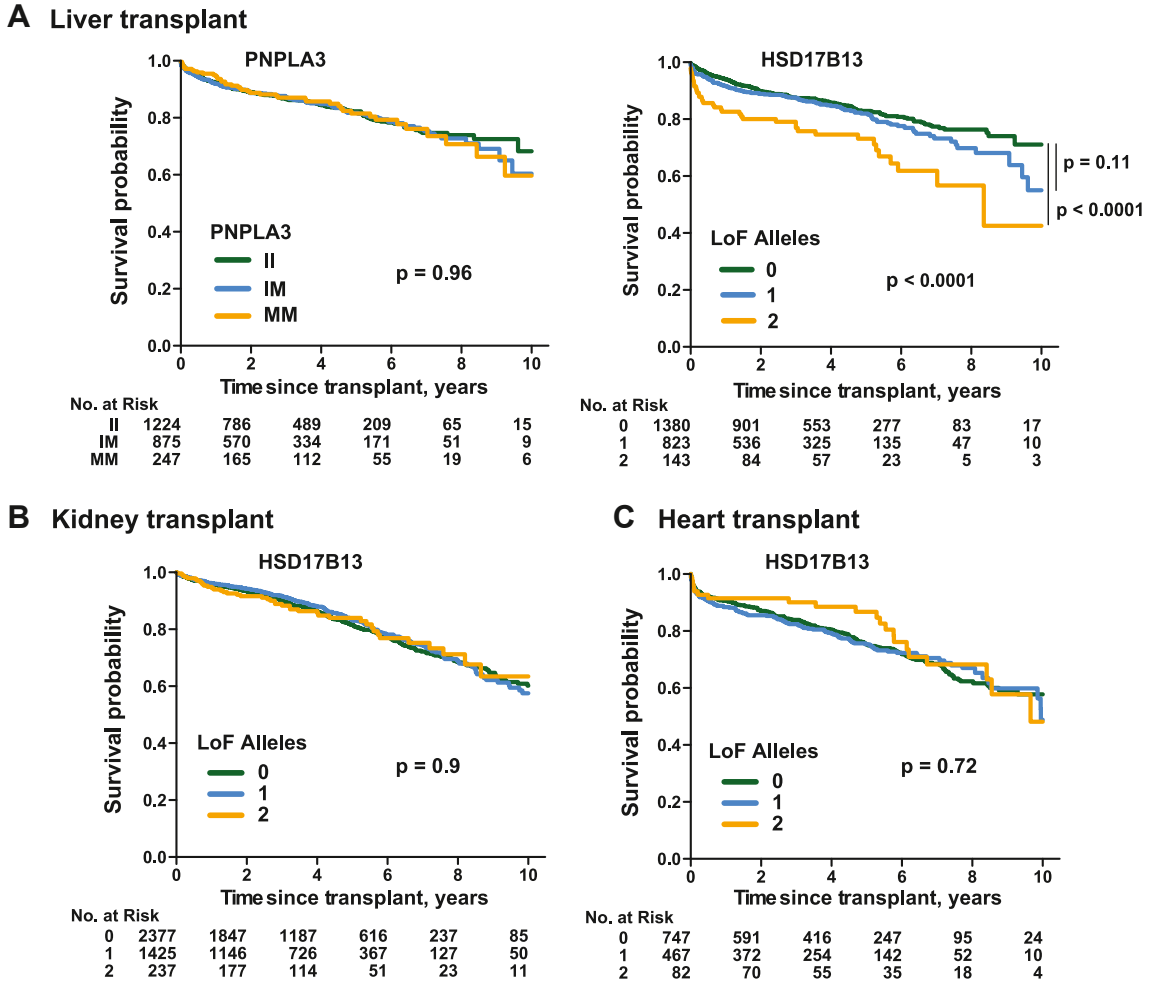

***Fig. 2:* Kaplan–Meier estimates of overall patient survival according to donor genotype.** (A) Survival after liver transplant by donor *PNPLA3* (left) and *HSD17B13* (right) genotype. (B) Survival after kidnedy transplant by donor *HSD17B13* genotype. (C) Survival after heart transplant by donor *HSD17B13* genotype. *P* Values calculated by log-rank test.

$P = 0.65$, Supplementary Table S13). No common causes of death were observed among recipients of 2 HSD17B13 LoF alleles, regardless of steroid induction treatment (Supplementary Table S11).

The frequency of steroid induction use varied widely among transplant centres (Fig. 3). In meta-regression, the proportion of patients receiving steroid induction accounted for a substantial proportion of variability in outcomes (residual heterogeneity $I^2 = 27\%$, $P = 0.21$).

In sensitivity analysis, we found identical results when we used multiple imputation to fill in missing data on steroid induction, imputed steroid use based on review of centre practices, or performed complete-case analysis (Supplementary Table S14). Furthermore, we observed a similar association of donor *HSD17B13* genotype with post-LT survival among patients with missing steroid data (Supplementary Figure S4B). Exploratory analyses revealed no difference in the association of donor *HSD17B13* genotype between patients receiving steroids only (n = 633) or steroids in combination with other IS regimens (n = 579) ($P = 0.84$) (data not shown). In contrast, no association of donor *HSD17B13* genotype with survival among kidney or heart transplant recipients was observed regardless of steroid induction status (Supplementary Figure S5).

Sensitivity analyses

In sensitivity analysis stratified by underlying liver disease etiology, the association between donor HSD17B13 genotype and patient survival was only significant among patients with non-viral disease, and was not significant in those with Hep-C or Hep-B infection (Supplementary Table S15), although disease etiology was not associated with survival in the full cohort

Model	Genotype	N	N events	Genotypic HR (95% CI)	*P* Value
PNPLA3 I148M					
Model 1	II	1224	195	1 [Reference]	–
	IM	875	143	1.00 (0.81–1.24)	0.99
	MM	247	43	0.99 (0.71–1.37)	0.93
Model 2	II	1224	195	1 [Reference]	–
	IM	875	143	0.97 (0.77–1.21)	0.76
	MM	247	43	0.91 (0.64–1.30)	0.61
Model 3	II	1201	192	1 [Reference]	–
	IM	864	141	0.95 (0.76–1.20)	0.69
	MM	244	43	0.94 (0.66–1.34)	0.73
HSD17B13 LoF alleles					
Model 1	0	1380	201	1 [Reference]	–
	1	823	138	1.19 (0.96–1.48)	0.11
	2	143	42	2.25 (1.61–3.15)	<0.0001
Model 2	0	1380	201	1 [Reference]	–
	1	823	138	1.25 (1.00–1.56)	0.051
	2	143	42	2.42 (1.71–3.42)	<0.0001
Model 3	0	1355	199	1 [Reference]	–
	1	814	136	1.22 (0.97–1.52)	0.088
	2	140	41	2.50 (1.76–3.54)	<0.0001

Estimates obtained from Cox Proportional Hazards models. Model 1: adjusted for recipient age, sex, and self-reported ethnicity. Model 2: adjusted for Model 1 covariates + donor characteristics (ethnicity, sex, and age). Model 3: adjusted for Model 2 covariates + recipient BMI, diabetes mellitus status, and MELD score.

Table 2: **Association of Donor PNPLA3 and HSD17B13 Genotype with Patient survival.**

(P = 0.53, Supplementary Table S16), and no significant interaction between disease etiology and donor *HSD17B13* genotype was detected. We also observed a consistent association between donor *HSD17B13* genotype and patient survival in the analysis stratified by time period (Supplementary Table S17), stratified by donor ethnicity (Supplementary Table S10), and accounting for clustering of patients by centre (Supplementary Table S16). Finally, we observed a similar association for donor *HSD17B13* rs72613567 genotype alone (Supplementary Figure S6). No association was observed between donor *HSD17B13* rs80182459 genotype and patient survival. However, because this variant is specific to individuals of African ancestry, and only a small fraction of donors were of African ancestry (n = 388 or 16.5%), our study was not powered to detect this effect.

Discussion

The major finding of this study was that two common genetic variants associated with progressive liver disease have qualitatively different effects on post-LT survival.

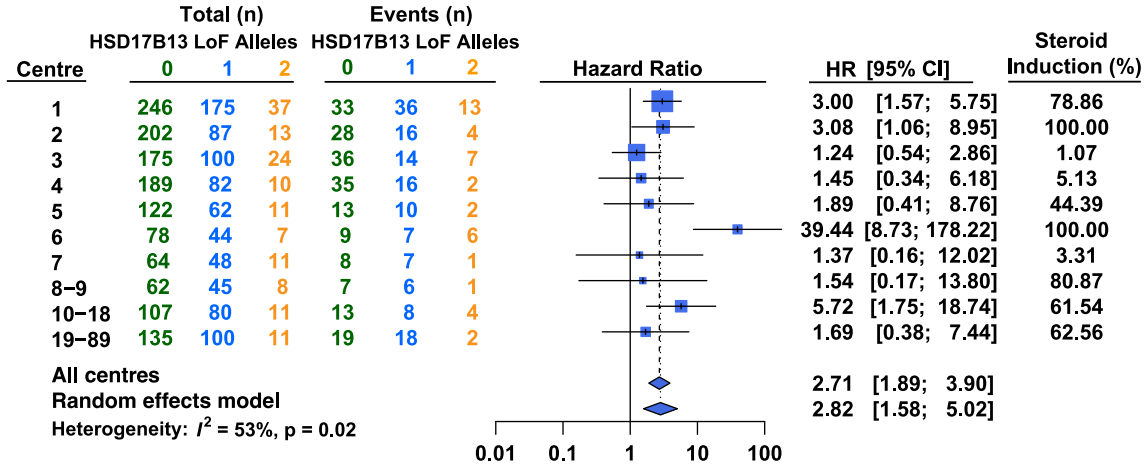

Fig. 3: **Association of donor HSD17B13 genotype with patient survival stratified by transplant centre.** Pooled estimate calculated using random-effects meta-analysis model.

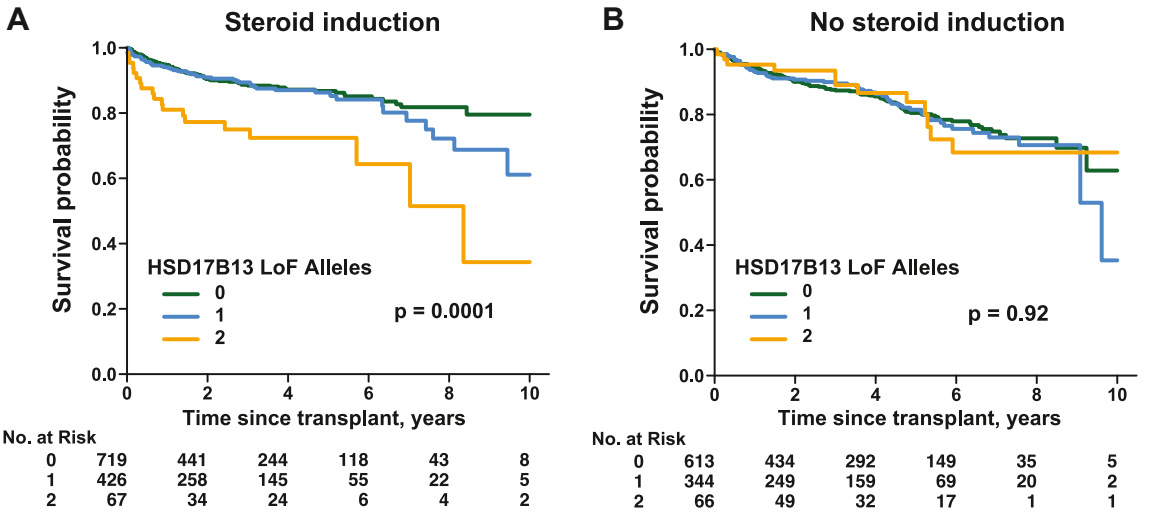

Fig. 4: **Kaplan–Meier estimates of patient survival according to donor HSD17B13 genotype and steroid induction**. (A) Patients receiving steroid induction. (B) Patients receiving no steroid induction.

PNPLA3-148-M allele, which doubles the risk of liver disease among unselected individuals,[11] had no detectable effect on survival after LT. In contrast, LoF variants in *HSD17B13* that confer protection from progressive liver disease[31,32] were associated with decreased post-LT survival. Individuals receiving livers with two *HSD17B13* LoF alleles had >4.5-fold higher 90-day post-LT mortality compared to recipients of livers with no *HSD17B13* LoF alleles. Decreased survival was only observed in individuals receiving steroid induction; no effect of donor *HSD17B13* genotype was seen among patients not treated with high-dose corticosteroids. Taken together, these data indicate that factors contributing to post-LT outcomes differ from those that impact liver disease incidence and progression.

Although patient survival was numerically lower among recipients of livers with one vs no *HSD17B13* LoF alleles, the difference was not significant. Therefore, the effect of donor *HSD17B13* genotype on post-LT survival was more consistent with a recessive model. This differs from previous studies, which found an additive effect of *HSD17B13* on liver disease progression.[20,31] The lack of effect of heterozygous donor *HSD17B13* genotype on post-LT survival may be related to the relatively smaller size of our study compared to previous studies, which looked at association with disease risk, not post-LT survival.

The reason for increased mortality in recipients of livers with HSD17B13 deficiency treated with steroid induction is unknown. One possibility is that HSD17B13 plays a role in response to surgical trauma associated with LT. The lack of association between *HSD17B13* genotype and mortality following kidney or heart transplantation is not consistent with this notion. The observation that the effect of *HSD17B13* variants on survival was only apparent in individuals who underwent steroid induction suggests that HSD17B13 (17β Hydroxysteroid Dehydrogenase-Type-13) may have a role related to the metabolism of corticosteroids. Other members of the HSD17B family catalyse interconversion of 17β-hydroxyl steroids and 17-ketoandrogens and estrogens. HSD17B13 can also catalyse similar reactions *in vitro*,[31,51] though it is unclear if any of these substrates have relevance *in vivo*. A recent study found that *HSD17B13* rs72613567 variant was associated with lower serum levels of total testosterone, 17-OH- progesterone, and androstenedione in a cohort of Polish women with polycystic ovary syndrome (PCOS).[52] The high prevalence of genetic deficiency of HSD17B13 in the general population and lack of apparent adverse health sequelae suggest the enzyme is not required for steroid homeostasis under usual conditions. Steroid induction involves the administration of synthetic corticosteroids at supra-physiologic doses, rarely used outside the context of short-term immunosuppression. HSD17B13 may be required for the catabolism of these corticosteroids and/or one of their metabolites.

The specificity of the association of donor *HSD17B13* genotype on recipient survival in LT, and not in kidney or heart transplant, is consistent with the hypothesis that the impact of the *HSD17B13* variants is mediated by their effect on the enzymatic activity in the liver. It would be interesting to test whether a similar association is observed between recipient *HSD17B13* genotype and patient survival in kidney and heart transplant. We did not have data on recipient genotypes and could not test this hypothesis. However, this should be investigated in future studies.

Previous studies have examined the association of donor and recipient genotypes with post-LT outcomes. Several studies found that the presence of PNPLA3(148-M) in donor livers was associated with post-transplant steatosis[41–43] and fibrosis progression.[37] Yet, other studies found that recipient, not donor, PNPLA3(148-M) genotype was associated with post-LT steatosis.[39,40] In this study, no association was found between donor *PNPLA3* genotype and post-LT survival. These seemingly discrepant findings may reflect complex interactions among multiple risk factors. In the general population, the effect of PNPLA3(148-M) is greatly magnified by adiposity,[53] insulin resistance,[54] and alcohol use.[15,21] After LT, patients are encouraged to make healthy life-style changes, such as weight loss and alcohol cessation, to protect new liver from injury. It is possible that these changes in lifestyle are sufficient to offset the detrimental effect of PNPLA3(148-M). It is also possible that the effect of PNPLA3(148-M) on liver disease incidence does not occur within the timeframe of this study. People with PNPLA3(148-M) typically do not develop NAFLD until late middle age,[55] indicating an effect upon disease progression that takes decades to manifest. Median age at LT in our cohort was 57 years, and median follow-up was 3.95 years, perhaps not providing sufficient time to see the differences in incidence of ESLD or liver-related mortality. Alternatively, due to improvements in post-LT outcomes, most LT recipients die of causes unrelated to liver disease or graft function.[56] As such, it appears that donor *PNPLA3* genotype alone is unlikely to be a useful criterion for donor evaluation.

Data on the effect of *HSD17B13* on post-LT outcomes are sparse. One small study reported that *HSD17B13* rs6834314, which is in linkage disequilibrium with *HSD17B13* rs72613567, in the donor was associated with reduced risk of NAFLD recurrence after LT.[45] Intriguingly, two recent reports found that patients with advanced liver disease carrying *HSD17B13* rs72613567:TA allele had increased risk of liver-related mortality.[44,57] However, both studies examined the effect of constituent genotype in patients with advanced disease; neither has considered the effect of donor genotype on recipients of healthy livers from donors carrying *HSD17B13* LoF alleles. Future studies will be required to confirm these findings and elucidate the basis of the variable effects of *HSD17B13* on patient outcomes.

Current transplant practices rely heavily on the assessment of physical appearance, histological features, and immunologic compatibility of donor livers to determine graft suitability, perform donor-recipient matching, and predict post-LT outcomes. Donor genomic DNA is routinely isolated and used for high-resolution determination of human leukocyte antigens (HLA). Tests for *HSD17B13* genotypes performed concurrently with HLA screening hold the promise of identifying livers that may be suitable for LT but should not be subjected to steroid induction. These results support the potential value of expanded donor genetic testing to evaluate risk and personalize post-LT treatments.

The major limitation of this study is the lack of an independent replication cohort, which was not available at the time of the analysis. However, the consistency of the results across multiple transplant centres serves as internal validation of the data. Additionally, this is a retrospective study that was dependent on the accuracy of the available data. It is possible that steroid induction is a proxy for another, unmeasured variable. The interaction between donor genotype and steroid was discovered through post hoc analysis; thus, the results should be considered hypothesis generating and interpreted with caution. Nonetheless, the observed difference in the association of *HSD17B13* genotype with post-LT survival by high-dose corticosteroid treatment status suggests the presence of an in-vivo drug interaction with this enzyme in humans and provides a potential clue to understanding of the function of HSD17B13, although the mechanism behind such an interaction should be investigated in a comprehensive study. In addition, future studies will be required to investigate whether a similar gene–drug interaction exists in other contexts when patients receive high doses of steroids, such as recipients of other solid organ transplants, cancer patients, or patients with severe exacerbations of autoimmune diseases. Finally, we did not have recipient genotype data or genome-wide genotyping data on donors to control for genetic ancestry. Given the consistent replication of the results across several patient subgroups defined by donor and recipient ethnicity, and lack of attenuation of the association after adjustment for donor and recipient ethnicity, it is unlikely that the inclusion of these variables would alter the main conclusions of the current study. Nevertheless, the effects of these variables should be investigated in future studies.

In conclusion, this study constitutes the first observation of a negative health effect of donor genotype on survival of liver transplant recipients from donors carrying *HSD17B13* LoF alleles. If confirmed, the findings suggest that genetic screening of donors can guide and improve future care of LT patients.

Contributors

H.H.H., J.C.C., and M.P.M. conceived, designed, and directed the study. N.M.C., C.M.-H., J.G.S., P.J., M.A., C.S.H., P.A.V., C.L., and M.P.M. acquired the samples. J.K., N.M.C., D.S., and M.P.M. performed the analysis and created figures and tables. J.K., N.M.C., D.S., J.C.C., H.H.H., and M.P.M. wrote the manuscript. J.C.C., H.H.H., and M.P.M. had direct access to the data and verified the results reported in the manuscript. All authors contributed to data interpretation, provided critical revisions, and approved the final version of the manuscript.

Data sharing statement

Data analysed here can be requested from UNOS by authorized researchers with a signed data access agreement. Due to the sensitive nature of the data, individual level donor genotypes are not available for sharing.

Declaration of interests
All authors declare no competing interests.

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This study used data from the Organ Procurement and Transplantation Network (OPTN). The OPTN data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN contractor.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102350.

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