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Association of Genetic Variants With Rapid Fibrosis: Progression After Liver Transplantation for Hepatitis C

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Abstract

Background—Recurrence of hepatitis C, the main indication for liver transplantation in the United States, leads to rapid fibrosis progression and worse outcomes compared to other indications. While clinical variables play a role, they are insufficient to explain all inter-patient variability in posttransplant fibrosis progression. Genetic factors associated with hepatitis C virus (HCV) outcomes have been identified, but limited studies have been conducted in the context of HCV-related liver transplantation. Therefore, the purpose of this study was to examine candidate genes related to the immune response and rate of fibrosis in subjects undergoing liver transplantation for HCV.

Methods—One hundred twelve recipients with detailed posttransplant fibrosis and clinical information were genotyped using 25 single nucleotide variants (SNVs), including five SNVs within the IL28B gene region. Associations between SNVs and rapid fibrosis progression were performed controlling for pertinent clinical variables and haplotype analyses for the IL28B gene were completed.

Results—Significant multivariable associations were found for rs8099917 (IL28B), rs1991401 (DDX5), rs4969168 (SOC3), and rs7976497 (MLEC). The minor allele was protective against rapid fibrosis progression for the IL28B SNV (G allele), MLEC SNV (T allele), and DDX5 SNV (G allele). For the SOC3 SNV, the minor allele (A) increased the risk for rapid fibrosis

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progression. Additionally, two recipient haplotype structures for IL28B were significantly associated with rapid fibrosis progression.

Conclusions—These findings indicate that recipient genetic factors play a role in posttransplant HCV-related fibrosis progression. Molecular studies of these pathways may elucidate the pathogenesis of posttransplant fibrosis progression and provide risk prediction markers.

Keywords

Hepatitis C virus; Liver transplantation; Candidate gene study

Hepatitis C continues to present difficult clinical challenges. Treatment of active infection is complex, expensive, and often unsuccessful, making hepatitis C virus (HCV) the most common indication for liver transplantation (LT) (1). The clinical course after transplantation is also significantly worse for patients with HCV compared to all other indications for liver transplant (LT) (2, 3). Patients who are viremic at the time of LT universally suffer re-infection of the graft and may develop brisk progression of fibrosis in the transplanted organ (1, 4). For unclear reasons, however, there is profound inter-individual variability in the rate of histologic liver disease with HCV recurrence, suggesting that genetic factors condition susceptibility (3, 5). While certain pre-LT factors such as donor age, severity of illness pre-LT, as well as recipient and donor race have been associated with an increased risk of disease progression and worse post-LT outcomes (5–15), these factors account for only a small proportion of variability in disease progression and graft failure rates post-LT. Improved understanding of the genetic or other recipient factors that drive rapid progression of fibrosis in these patients could help predict individuals at high risk and potentially improve therapy.

Genetic factors have recently been shown to influence the response to treatment of HCV infection and the likelihood of progression to cirrhosis in non-HCV liver disease. A single nucleotide variant (SNV) falling in the intergenic region near IL28B is strongly associated with spontaneous clearance of HCV and response to treatment (16, 17). Concurrently, seven other loci collectively allow estimation of a cirrhosis risk score and are associated with more rapid progression to cirrhosis (18). Other gene regions have also been implicated in fibrosis progression, including the DEAD box protein 5 (DDX5) (19, 20), PNPLA3 (21–23), and suppressor of cytokine signaling-3 (SOC-3) (24). However, only limited studies have focused on contribution of host genetics to fibrosis progression in HCV-related LT. Studies focusing on the IL28B region and HCV-related LT have produced conflicting results (25–29). These studies have used different IL28B SNVs, often focusing only one SNV, and have used different phenotypes, which makes comparison of results difficult. No study has conducted an IL28B haplotype analysis with post-LT fibrosis progression. Limited other studies have focused on other gene regions. Evidence from a single study links recipients with higher cirrhosis risk scores to progression post-LT (30). On the other hand, a separate locus strongly predictive of cirrhosis—PNPLA3—had no significant association with progression after HCV-related LT (31).

Given the clinical burden of HCV and the poor outcomes after transplantation, there is an urgent need to better understand the genetic factors that influence the risk of fibrosis

progression post-LT. Identification of predictive genotypes can be clinically helpful by serving as a marker of disease progression and can also help identify genes that may be important in the pathogenesis of disease progression for HCV as well as other LT indications. Accordingly, we conducted a candidate gene study for post-LT early HCV fibrosis progression and examined numerous recipient SNVs of the IL28B gene region, allowing for both a genotype and haplotype analysis, as well as included SNVs for the cirrhosis risk score genes and other fibrosis-related candidate genes.

RESULTS

Study Demographics

One hundred twelve recipients met study entry criteria (see Figure S1, **SDC**, <http://links.lww.com/TP/A920>). Rapid fibrosis progressors were more often black compared to slow progressors (Table 1; 50.0% vs. 22.4%; $P=0.01$). A trend was apparent for rapid fibrosis progressors to have older donors (40.6 ± 16.9 years vs. 35.4 ± 14.7 ; $P=0.1$); no other significant group differences were noted, including no difference in the percent of subjects treated for an episode of acute cellular rejection nor in average cold ischemia time.

Multivariable Associations of SNVs With Rapid Fibrosis Progression

Multivariable logistic regression models were run for each candidate SNV, controlling for recipient gender, donor age, as well as recipient and donor race. For each analysis, the minor allele was considered the “risk” allele with additive, dominant, and recessive models run for each SNV as described in the Methods. Table 2 provides the odds ratios (OR) for each SNV and model, as well as the minor allele frequency. Four SNVs had significant results in these multivariable analyses: rs8099917 (IL28B gene), rs7976497 (MLEC gene), rs1991401 (DDX5 gene), and rs4969168 (SOC3 gene). For the IL28B SNV rs8099917, the minor allele, G, was protective against rapid fibrosis progression, with an additive model controlled OR of 0.48 (0.24–0.97; $P=0.042$). No other IL28B gene SNV reached or neared significance. The MLEC rs7976497 minor allele, T, exhibited a protective effect, with an additive model OR of 0.40 (0.21–0.76; $P=0.005$). The DDX5 SNV, rs1991401, also displayed a protective effect for the minor allele, G, with an additive model OR of 0.48 (0.23–0.97; $P=0.04$). Finally, the SOC3 gene minor allele for rs4969168, A, increased the risk for rapid fibrosis progression, with an additive model OR of 2.09 (1.12–3.89; $P=0.02$). No other SNV, including all the cirrhosis risk score SNVs, had a significant association with rapid fibrosis progression.

A separate analysis was conducted including only the non-Hispanic white subjects, and the significant associations remained for rs8099917 (IL28B gene) and rs1991401 (DDX5 gene). The associations for the MLEC and SOC3 genes approached significance, with the associations following the same directional trend compared to the full cohort analyses. Table S1 (see **SDC**, <http://links.lww.com/TP/A920>) displays the multivariable ORs for the non-Hispanic white subpopulation for all SNVs.

IL28B Haplotype Analyses

Because of inclusion of multiple IL28B SNVs, a haplotype analysis was conducted for this gene region. The two haplotype blocks formed by the IL28B SNVs are displayed in Figure 1. The first block consisted of rs12972991 and rs12980275 while the second block was made up of rs11881222, rs12979860, and rs8099917. The haplotypes frequencies and results of the covariate-adjusted haplotype associations are presented in Table 3. Two three-allele haplotypes of rs11881222, rs12979860, and rs8099917 yielded two significant results for rapid fibrosis progression. The G-T-G haplotype of was protective against rapid fibrosis progression (OR: 0.42 [0.21–0.84]; $P=0.15$), while the A-T-T haplotype was associated with an increased risk of rapid fibrosis progression (OR: 3.81 [1.53–9.50]; $P=0.004$).

Frequency of Rapid Fibrosis Progression for Genotypes of Significant SNVs

For each SNV that reached significance in multivariable logistic regression models, the frequencies of rapid fibrosis progressors are displayed for each minor/minor, minor/major, and major/major allele combination in Figure 2. For rs8099917 (IL28B), rs7976497 (MLEC), and rs1991401 (DDX5), the major allele was associated with higher proportions of rapid fibrosis progression, whereas for rs4969158 (SOC-3), the minor allele was associated with higher proportions of rapid fibrosis progression.

DISCUSSION

HCV is the main indication for LT in the United States; unfortunately, recurrence is universal and histological progression can be severe, leading to increased rates of graft loss and mortality. Thus, it is valuable to identify predictors of disease progression and understand factors that alter such progression. While recent efforts have identified critical genetic markers for other HCV outcomes, there exists a paucity of studies examining the association of genes with post-LT HCV fibrosis progression. Therefore, the objective of this study was to examine the association of recipient candidate genes SNVs with rapid fibrosis progression post-LT.

This candidate gene study provided the opportunity to examine the role of not only IL28B SNVs but other candidate gene SNVs associated with the development of HCV-related liver fibrosis after LT. To our knowledge, this is the first study to examine multiple IL28B SNVs simultaneously in a post-LT HCV fibrosis progression study. This allowed a comparison of IL28B SNVs results in a single study, as well as the completion of haplotype analyses. Additionally, this is the first study to examine a more comprehensive list of candidate genes that could be relevant for post LT HCV related fibrosis progression. Such analyses allowed the identification of new potential pathogenic genes in the setting of HCV-related LT. Additional strengths include the rigorous post-LT histologic progression criteria and exclusion of patients treated for HCV recurrence or multiple courses of acute rejection, or both, to limit confounding variables.

In multivariable analyses, four SNVs of different genes were significantly associated with rapid fibrosis progression after HCV-related LT. For the IL28B gene, the minor allele, G, of SNV rs8099917 was protective against rapid fibrosis progression both in multivariable

logistic regression of the whole study cohort as well as in a sub-analysis including only white recipients. These results are in contrast to other studies. Eurich et al. found no association with rs8099917 post-LT fibrosis progression, yet observed higher inflammation and higher ALT levels with the G allele (27). For rs1297860, several studies have now found the T allele to be associated with more rapid fibrosis progression (25, 28, 29). However, Lange et al. found no association with post-LT viremia or aminotransferase levels and this recipient genotype (26). The discrepant results among the various studies may be secondary to differences within the cohorts with respect to donor or recipient variables, variable outcome measurements, and the small sample sizes of all studies. Our study had a higher percentage of black Americans, which can impact SNV findings because of allelic variation. We also excluded subjects who underwent treatment for HCV, which was not consistent with other studies. Further, we excluded recipients with greater than one episode of ACR to limit confounding, and thus had a lower frequency of ACR treatment in our population compared to other studies. To better clarify the association of IL28B gene polymorphisms and HCV post-LT fibrosis progression, larger populations with accurately quantitated fibrosis progression post-LT will need to be utilized. It is known that the IL28B gene encodes the type III interferon (IFN)- λ 3, which is induced by viral infections and exhibits anti-viral activity (32, 33), making it plausible for mutational changes within this region to alter these functions.

This is the first study to examine IL28B haplotype associations. Two haplotype structures of the IL28B region were highly associated with rapid fibrosis progression—one conferring protection against progression, with the other associated with a greater risk of fibrosis in the first 2 years. While these findings are of interest, the limited sample size requires the need for additional studies to substantiate these haplotype results.

The second SNV associated with post-LT fibrosis progression was the rs4969168 SNV of the SOC-3 gene. The SOC-3 gene is a member of the suppressor of cytokine signaling genes, which are inhibitors of cytokine signaling by direct binding to the activated JAK receptor of the JAK-STAT pathway, an important pathway for interferon-stimulated gene production (34). Recent studies have illustrated that SOC-3 hepatic expression impacts HCV replication (35), interferon-gamma responses (36), HCV treatment response (37), and hepatic fibrosis (24). Intriguingly, one study found an association with hepatic SOC-3 expression levels and black race (37). The present study is the first to find an association of a SOC-3 gene SNV with HCV post-LT fibrosis, with the minor allele, A, being associated with an increased risk of rapid fibrosis progression.

DDX5 is an ATP-dependent RNA helicase and transcription modulator; recent studies have illustrated associations of SNVs for this gene with advanced fibrosis in patients with HCV (20) as well as increased transcription of fibrogenic genes in hepatic stellate cells (19). There are no published studies that have examined the association of this gene with post-LT fibrosis progression. We found a significant association with polymorphisms of the rs1991401 SNV and rapid fibrosis progression, with the G allele providing a protective affect for the phenotype. Hepatic stellate cells play a fundamental role in the fibrogenesis process and hepatic cell activation has been shown to be a good marker of subsequent

fibrosis progression post-LT. Further work identifying the role the DDX5 plays in the fibrogenesis process post-LT may help to identify markers for rapid fibrosis progression.

Finally, this study identified an association between polymorphisms of the rs7976497 SNP of the malectin gene (MLEC) and rapid fibrosis progression after HCV-related LT; the minor allele, T, was protective against rapid fibrosis progression. The MLEC encodes a carbohydrate-binding protein of the endoplasmic reticulum and is thought to play a role in protein N-glycosylation (38). The role this gene plays in HCV recurrence and fibrogenesis is yet to be defined.

Limiting this analysis was the small sample size and the lack of donor genetic results. While examining donor genetic factors is also critical, the uncertain interaction between the recipient and donor graft makes the statistical genetic analyses difficult, especially with small sample sizes. Additionally, the main objective of this study was to look at the primary effect of recipient genetic markers. Several additional variables impact posttransplant fibrosis. In this study, we accounted for significant confounders identified in the cohort rather than incorporating all variables known to be associated with fibrosis into the final models. Further, we were unable to assess for donor steatosis or posttransplant viral load levels. Finally, this study was not designed nor powered to develop a predictive model incorporating the significant variants. Future studies validating such findings and with larger sample size could consider the development of a predictive model.

In summary, in this candidate gene study of patients with HCV we identified four SNVs of different genes (IL28B, SOC-3, DDX5, and MLEC) that were associated with fibrosis progression post-LT. Further studies with larger samples sizes will be needed to validate these markers and deep sequencing of the pertinent regions will be necessary to identifying causal variants. Mechanistic studies of these genes and associated pathways could help define the pathogenesis of HCV-related post-LT fibrosis progression and RNA-based designs could be employed to examine the association of gene expression with posttransplant fibrosis progression.

MATERIALS AND METHODS

Study Population and Design

Study subjects were drawn from a retrospective cohort of patients undergoing LT for HCV between 1999 and 2008. For inclusion, subjects had to meet at least one of the following post-LT biopsy criteria: (a) at least two protocol biopsies within the first 2 years post-LT; (b) only one biopsy, but with stage three or greater fibrosis; or (c) only one biopsy at month 24 with stage 0 or 1 fibrosis. Patients were excluded if they were co-infected with hepatitis B virus or human immunodeficiency virus, were treated with antivirals for HCV recurrence within the first 2 years, had more than one treatment course for acute cellular rejection (ACR), died within the first 30 days after LT, or did not have an available pretransplant liver biopsy to allow for DNA extraction. ACR was defined as receipt of treatment for ACR; standard practice for the treatment of ACR was high dose corticosteroids for 1 to 3 days. Of the 182 HCV-related liver transplants during the study period, 112 adult patients (72 white and 40 black) met study criteria and were included for analyses (see Figure S1, **SDC**, [http://](#)

[links.livingscience.com/TP/A920](https://www.livingscience.com/TP/A920)). Race was self-reported and was consistent with what was reported both at the University and submitted to the United Network for Organ Sharing nationwide database. All subjects were infected with genotype 1 virus (genotype 1: sub-type not identifiable [2.2%]; 1a: [77.3%]; 1b: [20.5%]). HCV subtype did not vary among slow progressors or rapid fibrosis progressors. All donors were HCV antibody negative. Patients were treated with a standard maintenance immunosuppression regimen consisting of a calcineurin inhibitor and mycophenolate mofetil, and received steroid tapering dosing per protocol. The study was approved by the University of Illinois at Chicago's Institutional Review Board, and all liver transplant events were at this institution. The lead author and two others (J.E.L., N.M., S.C.) were on faculty at this institution at the time the study was initiated.

Histologic Assessment

A single blinded pathologist staged all biopsies using the Knodell fibrosis scale (F0–F4) (39). The primary outcome was fibrosis stage during the first 2 years post-LT. Rapid fibrosis progression was defined as F2 by 12 months post-LT or F3 or F4 anytime in the first 2 years; the remaining subjects were categorized as slow fibrosis progressors.

Clinical Data

Clinical data were extracted from an existing database and included the following information: donor and recipient demographics, comorbidities, surgical complications, dates of treatment for HCV recurrence and rejection dates, standard pre-LT laboratory data, pre- and post-LT viremia and aminotransferase levels, and survival or follow-up information.

DNA Extraction and Single Nucleotide Polymorphism Genotyping

DNA was extracted from pre-LT liver biopsies or explants that were stored as fresh frozen paraffin-embedded (FFPE) blocks. Three 6-Hm-thick slices of each FFPE block were taken and placed in Eppendorf tubes for DNA extraction using the QIAamp DNA FFPE Tissue Kit (Qiagen, USA). The kit tissue procedure allows for paraffin removal, sample lysis, heat treatment to reverse formalin crosslinking, DNA binding, sample purification, and DNA concentration. Genotyping was performed using mass spectrometry on the MassARRAY Analyzer 4 system (Sequenom, Inc, USA).

Candidate Single Nucleotide Variants (SNVs)

SNVs were selected from previous studies that showed associations of particular genes with liver fibrosis or other HCV outcomes, or both. In particular, the SNVs previously identified from the cirrhosis risk score genome-wide study were included (18). Additional SNVs previously published to be associated with liver fibrosis were also analyzed (19, 21, 23, 40). Finally, five SNVs of the IL28B gene, which is associated with HCV treatment response and spontaneous HCV clearance, were also included (16, 17).

Analytic Procedures

SNVs were excluded from analyses if there were greater than 10% of missing genotypes or significant deviation from Hardy-Weinberg equilibrium ($P < 0.001$). The primary outcome

was rapid fibrosis progression, as described above. To test for associations between rapid fibrosis progression status and each single SNV, we fitted logistic regression models in which each SNV was presented as a predictor variable whose values were equal to the number of copies of the minor allele (0, 1, 2) for additive mode, presence of at least one copy of the minor allele (0, 1) for dominant mode, or presence of two copies of the minor allele (0, 1) for recessive mode. In each fitted model, recipient sex, donor age, as well as recipient and donor race were included as covariates. Analyses were done both as combined and stratified by self-reported race.

We also explored possible associations with various joint allelic configurations of IL28B SNVs through haplotype association analysis. The software Haploview (41) was used to compute estimates of linkage disequilibrium (LD) for each pair of SNVs by the standard D-prime method (42) and to determine the haplotype blocks—regions with no evidence of historical recombination events, but significant level of LD. The haplotype blocks were defined by the four-gamete test (43, 44) as implemented in Haploview. Analysis included all haplotypes with frequencies of at least 10% within the two constructed blocks. All the haplotypes were individually tested for association with rapid fibrosis progression status by using logistic regression models as described above for tests of genotype association. Sex, donor age, recipient, and donor race were adjusted for in each haplotype-phenotype analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1. Plot of linkage disequilibrium between single nucleotide variations (SNVs) in IL28B gene. The degree of linkage disequilibrium expressed as R^2 is shown in each linkage box.

FIGURE 2.

Frequency of rapid fibrosis progression for each genotype of significant SNVs is displayed for each allelic combination: minor/minor, minor/major, and major/major.

TABLE 1

Demographics of slow progressors and non-progressors versus rapid fibrosis progressors

Variable	Slow progressors N=58	Rapid fibrosis progressors N=54	P
% Female	31.0%	35.2%	NS
Recipient age ^a	52.5±9.2	52.7±7.1	NS
% Recipient AA	22.4%	50.0%	0.01
Donor age ^a	35.4±14.7	40.6±16.9	0.1
% Donor female	36.2%	36.0%	NS
% Donor AA	31.0%	24.1%	NS
% HCC	27.6%	22.2%	NS
Pre-LT MELD ^a	27.1±6.9	26.0±8.7	NS
Pre-LT log viral load ^a	5.2±1.3	5.1±1.2	NS
Cold ischemia time, hr ^a	7.1±1.0	5.8±0.5	NS
One treatment of acute cellular rejection (ACR) during first 2 years ^b	13.8%	20.4%	NS

^a Mean±standard deviation.^b Of note, exclusion criteria—specific subjects could not have greater than one treatment for ACR.

AA, African American; HCC, hepatocellular carcinoma; LT, liver transplant; MELD, model for end-stage liver disease.

TABLE 2

Multivariable logistic regression analyses for each SNV: total cohort

Chr	SNP	Gene	Alleles (minor/major)	MAF (%)	Additive mode		Dominant mode		Recessive mode		
					OR	P	OR	P	OR	P	
Cirrhosis risk score SNVs											
3	rs17740066	STXBP5L	A/G	8.48	0.88 (0.32–2.46)	0.809	0.70 (0.23–2.17)	0.540	NA	NA	
15	rs2290351	AP3S2	A/G	33.03	0.88 (0.49–1.58)	0.679	0.91 (0.41–2.01)	0.812	0.73 (0.21–2.50)	0.614	
12	rs2878771	AQP2	C/C	20.19	1.20 (0.65–2.24)	0.563	1.23 (0.51–2.96)	0.645	1.51 (0.37–6.13)	0.568	
1	rs4290029	LOC	C/G	22.48	0.75 (0.39–1.47)	0.403	0.61 (0.26–1.44)	0.259	1.12 (0.22–5.82)	0.890	
11	rs886277	TRPM5	C/C	50.00	1.04 (0.59–1.82)	0.900	1.70 (0.67–4.31)	0.263	0.63 (0.24–1.64)	0.344	
9	rs4986791	TLR4	T/C	3.57	0.45 (0.09–2.33)	0.342	0.45 (0.09–2.33)	0.342	NA	NA	
8	rs62522600	AZIN1	A/A	0.93	1.76 (0.10–30.71)	0.697	1.76 (0.10–30.71)	0.697	NA	NA	
IL28B gene region SNVs											
19	rs12972991	IL28B	C/A	26.56	0.82 (0.43–1.55)	0.541	0.65 (0.27–1.61)	0.355	1.05 (0.28–4.02)	0.939	
19	rs12980275	IL28B	G/A	49.52	0.91 (0.50–1.64)	0.754	0.87 (0.34–2.24)	0.778	0.89 (0.34–2.37)	0.821	
19	rs11881222	IL28B	G/A	40.87	0.69 (0.37–1.28)	0.235	0.60 (0.25–1.42)	0.244	0.66 (0.20–2.16)	0.496	
19	rs12979860	IL28B	C/T	43.37	0.71 (0.38–1.33)	0.282	0.73 (0.28–1.89)	0.517	0.53 (0.17–1.60)	0.258	
19	rs8099917	IL28B	G/T	22.97	0.48 (0.24–0.97)	0.042	0.36 (0.15–0.86)	0.022	0.60 (0.12–3.03)	0.533	
Other fibrosis-related SNVs											
2	rs693	APOB	T/C	42.45	0.84 (0.48–1.45)	0.530	0.77 (0.32–1.83)	0.549	0.80 (0.30–2.12)	0.657	
4	rs3816873	MTTP	C/T	26.13	0.86 (0.46–1.59)	0.629	0.91 (0.41–2.03)	0.820	0.58 (0.13–2.59)	0.479	
5	rs12654264	HMGCR	T/A	30.00	0.68 (0.36–1.28)	0.236	0.63 (0.28–1.42)	0.262	0.56 (0.13–2.46)	0.444	
11	rs923346	LRP5	C/C	16.02	1.29 (0.62–2.69)	0.488	1.03 (0.41–2.60)	0.943	5.86 (0.59–57.78)	0.130	
11	rs2305508	CPT1A	G/A	29.09	0.67 (0.36–1.27)	0.219	0.56 (0.24–1.31)	0.181	0.70 (0.19–2.65)	0.602	
11	rs2229738	CPT1A	A/G	4.59	0.12 (0.01–1.01)	0.051	0.12 (0.01–1.01)	0.051	NA	NA	
11	rs2229113	IL10RA	A/A	29.25	1.45 (0.75–2.79)	0.270	1.37 (0.60–3.12)	0.455	2.47 (0.53–11.50)	0.249	
12	rs10774671	OAS1	G/G	39.90	1.42 (0.79–2.54)	0.238	2.08 (0.88–4.92)	0.096	1.05 (0.36–3.07)	0.926	
12	rs7976497	MLEC	T/C	37.85	0.40 (0.21–0.76)	0.005	0.42 (0.18–0.98)	0.045	0.13 (0.03–0.63)	0.011	
17	rs1991401	DDX5	G/A	27.73	0.48 (0.23–0.97)	0.042	0.28 (0.11–0.71)	0.007	0.98 (0.24–4.04)	0.983	
17	rs4969168	SOC3	A/G	29.63	2.09 (1.12–3.89)	0.020	3.26 (1.36–7.82)	0.008	1.92 (0.58–6.35)	0.284	
22	rs738409	PNPLA3	C/G	37.14	0.78 (0.44–1.38)	0.397	1.20 (0.52–2.77)	0.675	0.27 (0.08–0.92)	0.037	

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All candidate gene analyses controlled for recipient gender, donor age, recipient, and donor race.
MAF, minor allele frequency; SNV, single nucleotide variation; OR, odds ratio; NA, not applicable.

TABLE 3

Haplotype multivariable analyses for IL28B

SNVs combination	Haplotype	Frequency (%)	OR	<i>P</i>
rs12972991 rs12980275	C-G	27.00	0.75 (0.41–1.36)	0.339
rs12972991 rs12980275	A-G	24.20	1.75 (0.91–3.39)	0.096
rs12972991 rs12980275	A-A	48.90	0.83 (0.46–1.50)	0.540
rs11881222 rs12979860 rs8099917	G-T-G	23.70	0.42 (0.21–0.84)	0.015
rs11881222 rs12979860 rs8099917	A-C-T	42.70	0.79 (0.44–1.41)	0.425
rs11881222 rs12979860 rs8099917	G-T-T	16.10	1.83 (0.83–4.01)	0.132
rs11881222 rs12979860 rs8099917	A-T-T	16.30	3.81 (1.53–9.50)	0.004

SNV, single nucleotide variation; OR, odds ratio.