Received: 11 October 2018 | Revised: 1 March 2019 | Accepted: 1 March 2019

DOI: 10.1002/mgg3.689

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine Open Access WILEY

A risk assessment model of acute liver allograft rejection by genetic polymorphism of *CD276*

Xiaobo Yu[1,2,3,4,5] | **Bajin Wei**[2,3,4] | **Rong Su**[2,3,4] | **Jia Yao**[2,3,4] | **Xiaowen Feng**[1,2,3,4,5] | **Guoping Jiang**[1,2,3,4,5] | **Haiyang Xie**[1,2,3,4,5] | **Jian Wu**[1,2,3,4,5] | **Xiao Xu**[1,2,3,4,5] | **Min Zhang**[1,2,3,4,5] | **Shusen Zheng**[1,2,3,4,5] | **Lin Zhou**[1,2,3,4,5]

[1]Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

[2]NHFPC Key Laboratory of Combined Multi-organ Transplantation, Hangzhou, China

[3]Key Laboratory of the Diagnosis and Treatment of Organ Transplantation, CAMS, Hangzhou, China

[4]Key Laboratory of Organ Transplantation, Hangzhou, Zhejiang Province, China

[5]Collaborative Innovation Center for Diagnosis Treatment of Infectious Diseases, Hangzhou, China

Correspondence
Lin Zhou, 79 Qingchun Road, the First Affiliated Hospital of Zhejiang University, Hangzhou, Zhejiang 310003, China.
Email: zhoulin99@zju.edu.cn

Funding information
Natural Science Foundation of Zhejiang Province, Grant/Award Number: LY18H100003; Major program of National Natural Science Foundation of China, Grant/Award Number: 91542205; Zhejiang International Science and Technology Cooperation Project, Grant/Award Number: 2016C04003; Innovative Research Groups of National Natural Science Foundation of China, Grant/Award Number: 81721091; National S&T Major Project, Grant/Award Number: 2017ZX10203205 and 2018ZX10723204-006

Abstract

Background: Liver transplantation is an effective therapy for end-stage liver diseases and acute liver failure. After the operation, however, recipients may suffer grafts loss induced by alloimmune reaction, which is termed as acute allograft rejection. The interaction between costimulatory molecules, *CD276*, and its ligand, *TREML2*, promotes T cell-mediated immune response, as well as acute or chronic allograft rejection. Our research aimed at correlating genetic polymorphisms of *CD276/TREML2* with acute rejection, and evaluating its prognostic value of acute rejection after liver transplantation.

Methods: The study enrolled a total of 388 recipients. Among them, acute allograft rejection was observed in 54 cases. We performed single nucleotide polymorphism genotyping of *CD276*, including rs11072431, rs11574495, rs12593558, rs12594627, rs2127015, rs3816661 and rs7176654, and *TREML2*, including rs4714431, rs6915083, rs7754593, and rs9394767 from preoperative peripheral blood genome DNA.

Results: We found rs2127015 of *CD276*, rs6915083 and rs7754593 of *TREML2*, and HBV infection as well were associated with acute rejection. And, rs2127015 influences *CD276* expression. Moreover, we established a risk assessment model, composited by statistically proved risk factors.

Conclusion: By integrating both clinical and genetic variables, liver transplant recipients can be categorized into different risk groups, and might benefit from individualized therapies.

KEYWORDS
acute rejection, CD276, costimulatory molecule, liver transplantation, SNP

1 | INTRODUCTION

Liver transplantation is an effective therapy for end-stage liver diseases and liver failure. Since immunosuppressive agents have been introduced for clinical use, the incidence of allograft rejection decreases dramatically. However, acute rejection episode, normally resulted from inadequate immunosuppression, would still occur among 15%–45% recipients within months (Farges et al., 1996; Hubscher, 2009; Ingulli, 2010). And, it might lead to graft loss, increased risk of chronic organ dysfunction, and suboptimal long-term outcomes with decreased allograft half-life by 34% (Ingulli, 2010).

Allogeneic grafts induce fierce T cell-mediated immune responses in recipients, and the response is the main cause of rejection and graft dysfunction (Sanchez-Fueyo & Strom, 2011). T cells from both innate and adaptive immune system play central roles in regulating immune reactions during rejection episode (Clarkson & Sayegh, 2005; Ingulli, 2010; Rothstein & Sayegh, 2003). First, host T cells allorecognize donor-derived antigens, and are activated by costimulatory signals (Hubscher, 2009; Ingulli, 2010). During this step, antigens are recognized by the interaction between major histocompatibility complexes (MHC) on antigen-presenting cells (APC) and T-cell receptors (TCR) on T cells. Then the recognition stimulates T cells and alters the intracellular transcriptional profiles. Once activated, host T cells would undergo clonal expansion, differentiate into effector T cells, migrate into allograft, and accelerate the destruction of donor organ (Hubscher, 2009; Ingulli, 2010). It leads to mixed inflammatory cells infiltration, usually mononuclear, in portal tracts, and is the most common histology characteristic of acute rejection (Hubscher, 2009).

Recent studies indicated that costimulatory molecules might serve as important therapeutic targets for preventing allograft rejection. For instance, *B7* proteins, belonging to IgG superfamily, normally express on membrane of APCs. The best-studied *B7* proteins are *CD80* (OMIM #112203) and *CD86* (OMIM #601020). Depending on the counterparts engaged on T cells surface, they could provide either positive or negative costimulatory signal. T cells could be positively stimulated by APCs when *CD80* or *CD86* interacts with *CD28* (OMIM #186760), or negatively stimulated by *CTLA4* (OMIM #123890) (Clarkson & Sayegh, 2005). Commercial recombinant *CTLA4* protein has provided extended graft survival for renal recipients (Dell-Olio & Kelly, 2010; Post, Douglas, & Mulligan, 2005; Snanoudj, Zuber, & Legendre, 2010).

Initial works on *CD276* (OMIM #605715) suggested a positive costimulatory effect on T-cells activation. In conjunction with anti-CD3 monoclonal antibody, *CD276* positively stimulated T-cell proliferation, with enhanced *IFN*-γ production and *CD8* + cytotoxic activity. Although in a cardiac transplantation model, graft rejection developed rapidly in both *CD276*-/- mice and control mice equally, brief treatment of immunosuppressive regimens, to *CD276*-/- mice, led to prolonged survival time and decreased incidence of rejections (L. Wang et al., 2005). *CD276* also showed the effect of negative costimulation, such as inhibiting T-cell activation and effector cytokine production (Clarkson & Sayegh, 2005; Rothstein & Sayegh, 2003). *TREML2* (Triggering receptor expressed on myeloid cell-like transcript 2, OMIM #609715) has been identified as a ligand of *CD276* for positive costimulation (Hashiguchi et al., 2008; Kobori et al., 2010). Since *CD276* can positively activate T cells via *TREML2*, we speculated a participation of both molecules in graft intolerance.

Nowadays, individualized therapies such as tailored and safe immunosuppression are urgently demanded for organ transplantation. Advanced molecular biological techniques, such as gene array, proteomics researches, mass spectrometry, and genome-wide association studies (GWAS), are discovering valuable biomarkers, including mRNA, miRNA, protein, small chemical molecules, and genetic signatures [single nucleotide polymorphism (SNP) SSR, CNV et al.] (Hernandez-Fuentes & Lechler, 2010; Offit, 2011; Rook & Rand, 2011). These objectively detectable or measurable molecules and genetic signatures are biologically or pathogenically involved, and might act as parameters for diagnosis and disease staging, as well as indicators or predictors for disease prognosis and clinical response (Hernandez-Fuentes & Lechler, 2010). The analysis of genetic characteristics of a patient would assist in interpreting his/her biological and immune response, and help to depict allograft rejection, so that damages to parenchymal tissues can be diagnosed in advance and prevented before irreversible (Hernandez-Fuentes & Lechler, 2010; Offit, 2011).

SNPs of cytokines and costimulatory molecules are associated with acute rejection (de Reuver et al., 2003; Hernandez-Fuentes & Lechler, 2010; Kim et al., 2010;); however, none of these findings has been introduced into identifying the risk of rejection. We wondered whether a quantitative risk assessment model could be deduced, by integrating critical biomarkers, such as genetic polymorphism, and other risk factors. And we expected, by using the model, recipients would receive optimized immunosuppression for individualizing their clinical cares.

In conclusion, we discovered SNPs of costimulatory molecule, *CD276*, as well as its ligand, *TREML2*, were associated with liver grafts acute rejection. Moreover, HBV (hepatitis B virus) infection was also statistically confirmed as a risk factor for acute rejection. Genetic polymorphism influenced the production of *CD276* mRNA. Moreover, by integrating these risk factors, we established a risk assessment model, which categorized recipients into low-, medium-, and high-risk groups.

2 | METHODOLOGY

2.1 | Population

The diagnoses of enrolled recipients included hepatocellular carcinoma, fulminant hepatitis, and decompensate liver cirrhosis (Table 1). Recipients with autoimmune hepatitis, or drug-induced hepatitis, or sclerosing cholangitis, or those underwent a second or subsequent liver transplantation, or multiple organ transplantation were excluded. In the retrospective study, 299 recipients who received liver grafts from 2006 to 2011 were enrolled for the clinical aspects analysis (Table 1). However, due to DNA sample quality and limitation of sequencing technology, we used 289 cases, with complete genotype information of total 11 SNPs, to analyze genetic association with acute rejection. The rest 10 cases which lacked genotype information of at least one SNP were excluded in the association analysis. While four of the 10 cases did not lack the genotyping results of rs2127015, rs6915083, and rs7754593, 293 cases were used in the following risk assessment model deduction. Another 89 recipients who received liver grafts from 2011 to 2012 were enrolled for further prospective validation of the risk assessment model. Among them, 11 recipients developed acute rejections. These two cohorts included 345 males and 43 females, aged from 21 to 69 (46.9 ± 9.5) years old.

All 388 recipients followed a routine triple combination of immunosuppressive regimen, including tacrolimus, corticosteroid, and mycophenolate mofetil. In brief, the minimum level of tacrolimus blood concentration was maintained at 10–12 ng/ml for the first month after transplantation, at 8–10 ng/ml later in the first year, and at 5–8 ng/ml thereafter. Mycophenolate mofetil was administered 1–2 g per day. Corticosteroid treatment was initiated with 1,000 mg prednisolone once during the operation, continued with gradually reduced methylprednisolone starting at 240 mg on day 1 and ending up at 2.5 mg before discontinuation after 2 months (Xu et al., 2011; Yu et al., 2011).

2.2 | Diagnosis of acute rejection

The diagnosis of acute rejection is confirmed by liver biopsy and graded by Banff criteria. Rejection occurred within 6 months was considered as acute rejection (Adeyi, Fischer, & Guindi, 2010; Neuhaus et al., 2002).

2.3 | Ethical Compliance

We followed the World Medical Association's Declaration of Helsinki. Written informed consents were obtained. The research procedure was approved and supervised by the Ethical Review Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University.

2.4 | DNA extraction and genotyping

Genome DNA was extracted from preoperative peripheral blood. Based on the data from Hapmap (http://www.hapmap.org), the selection of candidate SNPs of *CD276* and *TREML2* was in accordance with the rule that minor allele frequency and r^2 should be no less than 20% and 0.8, respectively. Genotyping was performed by SNaPshot (Applied Biosystems, CA). Data were collected by ABI3130xl Genetic Analyzer (Applied Biosystems, CA), and analyzed on GeneMapper 4.0 (Applied Biosystems, CA).

TABLE 1 Relevance of clinical aspects and characteristics of recipient with acute rejection

	Acute rejection group ($n = 43$)	Nonrejection group ($n = 256$)	p value	OR (95% CI)
Age	48.9 ± 10.2	46.5 ± 9.0	0.895	
Gender (male/female)	38/5	224/32	0.872	1.086 (0.40–2.96)
MELD score	19.1 ± 8.0	19.8 ± 9.4	0.776	
Blood type mismatch	8 (18.6%)	32 (12.5%)	0.277	1.600 (0.68–3.75)
Chronic HBV infection	32 (74.4%)	143 (55.9%)	0.0223	2.299 (1.11–4.76)
Primary disease				
Cirrhosis	36 (83.7%)	192 (75.0%)	0.2137	1.714 (0.73–4.04)
Fulminant Hepatitis	7 (16.3%)	50 (19.5%)	0.6154	0.801 (0.34–1.91)
HCC	16 (37.2%)	102 (39.8%)	0.7436	0.895 (0.46–1.74)
Comorbidities				
Ascites	20 (46.5%)	106 (41.4%)	0.5304	1.231 (0.64–2.36)
Hepatorenal syndrome	6 (13.9%)	22 (8.6%)	0.2643	1.725 (0.66–4.54)
Hepatic encephalopathy	3 (6.9%)	15 (5.9%)	0.7756	1.205 (0.33–4.35)
Portal hypertension	2 (4.7%)	10 (3.9%)	0.8179	1.200(0.25–5.68)

2.5 | Detection of *CD276*/*TREML2* mRNA, and membrane *CD276* in PBMCs

Total RNA was extracted from peripheral blood of recipients within 6 months posttransplantation, and cDNA was synthesized by reverse transcription kit (Biorad, CA). We detected the transcripts of *CD276* and *TREML2* on ABI 7500fast (Applied Biosystems, CA) with iQ SYBR Green Supermix PCR kit (Biorad, CA). The primer pairs used in real-time PCR reaction were listed as follows; *CD276*, forward 5′-CTCCCTACAGCTCCTACCCTC-3′, reverse 5′-TGGTCTGTGTATCGCATCCTT-3′, based on *CD276* Genbank sequence (NM_001024736.2); *TREML2*, forward 5′-CCCACAGCCTCATAGATAAGACA-3′, reverse 5′-CCATATTGCTTTGTTCCCCTT-3′, based on *TREML2* Genbank sequence (NM_024807.4); and *GAPDH*, forward 5′-ATGGGGAAGGTGAAGGTCG-3′, reverse 5′-GGGGTCATTGATGGCAACAATA-3′, based on *GAPDH* Genbank sequence (NM_001256799.2). The relative expression of *CD276* and *TREML2* mRNA was calculated by ΔΔCT method. mRNA expression of both *CD276* and *TREML2* was detected three times in each cDNA sample.

To detect membrane *CD276*, red blood cells were lysed with RBC lysing buffer (eBioscience) from whole blood; subsequently peripheral blood mononuclear cells (PBMCs) were pelleted by density-gradient centrifugation with Ficoll (Sigma). Cells were incubated with phycoerythrin-labeled anti-*CD276* antibody (R&D) and fluorescein isothiocyanate labeled anti-CD3 antibody (eBioscience) in 2% FBS containing PBS for 30 min at 4°C. A Mouse IgG1, κ (eBioscience) and IgG2a, κ (BD Pharmingen) were used as isotype controls for anti-*CD276* and anti-CD3 antibody, respectively. Finally, cells were quantified on a BD LSRII flow cytometry (BD Bioscience) using CellQuest (BD Bioscience), and data were analyzed by FlowJo (Tree Star, Stanford, CA). Membrane expression of *CD276* protein was detected three times in each PBMC sample.

2.6 | Comparison of mRNA or protein expression

Unpaired t test or one-way ANOVA test was used for comparison of two groups or more than two groups with Graphpad Prism 6.0 (Graphpad Software, CA). A two-tailed p value less than 0.05 was considered statistically significant.

2.7 | Association analysis and establishing risk assessment model

Analyses were performed to verify the association between genetic polymorphism and acute rejection by SNPStats (http://bioinfo.iconcologia.net/snpstats) or Haploview (http://www.broad.mit.edu/mpg/haploview). The relevance of clinical characteristics and acute rejection was confirmed by Fisher's exact test by Graphpad Prism 6.0. Variables considered to be statistically significant were subsequently analyzed by multivariable logistic regression using SPSS 20.0 (IBM, IL). The AUROC (area under receiver operating characteristic curve) evaluation was performed by SPSS 20.0 to assess the predictive value of variables and diagnostic accuracy of the model. AUROC value of 0.5 or 1 indicates a bad or good discrimination, respectively (Linden, 2006). A p value less than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | HBV infection risked acute allograft rejection

The overall incidence of acute rejection within the first half year postoperation was 14.4% ($n = 43$) in 299 recipients, who received their liver grafts from 2006 to 2009. We did not find any clinical relevance between acute rejection and age, gender, primary diseases, or comorbidities of recipients (Table 1).

We found recipients, positive with HBV infection, were at higher risk than those negatives (Table 1). However, the combination of HBV infection and other diagnoses, or combination between either two of the other diagnoses did not increase the risk of acute rejection (data not shown).

3.2 | Genetic polymorphisms of *CD276* and *TREML2* were both associated with acute allograft rejection

To illustrate the potential association between genetic polymorphisms of costimulatory molecules and acute allograft rejection, seven candidate SNPs of *CD276* and four of *TREML2* were investigated. Six SNPs of *CD276* located in intron, one in 3′ UTR; while two SNPs of *TREML2* located in intron, the others in 3′ UTR. We found both genetic polymorphisms of *CD276* and *TREML2* were associated with acute allograft rejection. Recipients carrying T allele at rs2127015 of *CD276*, or G allele at rs6915083 or rs7754593 of *TREML2* were at high risk of acute rejection (Table 2). Moreover, we performed linkage disequilibrium study to find out haplotype among the SNPs of *CD276* and *TREML2*, or between each other, and identified two haplotype blocks in *CD276* and one in *TREML2*, but none between these two genes (Figure 1). However, no association was observed between haplotypes and acute rejection (Table 3).

3.3 | rs2127015 genotype was associated with the expression of *CD276*

Since rs2127015, rs6915083, and rs7754593 located in either intron or 3′ UTR, these synonymous SNPs might affect mRNA

TABLE 2 Association results for SNPs of *CD276* and *TREML2* in acute rejection

SNP	Events	Genotype Count/frequency			*p* value	OR (95% CI)
CD276		A/A	T/A	T/T		
rs11072431	AR	7 (16.3%)	24 (55.8%)	12 (27.9%)	0.1071	
	NAR	75 (30.5%)	126 (51.2%)	45 (18.3%)		
CD276		A/A	G/A	G/G		
rs11574495	AR	2 (4.7%)	18 (41.9%)	23 (53.5%)	0.7931	
	NAR	18 (7.3%)	104 (42.3%)	124 (50.4%)		
CD276		C/C	C/T	T/T		
rs12593558	AR	5 (11.6%)	20 (46.5%)	18 (41.9%)	0.3265	
	NAR	42 (17.1%)	128 (52.0%)	76 (30.9%)		
CD276		G/G	G/T	T/T		
rs12594627	AR	22 (51.2%)	20 (46.5%)	1 (2.3%)	0.2439	
	NAR	113 (45.9%)	109 (44.3%)	24 (9.8%)		
		C/C	C/T	T/T		
	AR	2 (4.7%)	25 (58.1%)	16 (37.2%)	0.0733	
CD276	NAR	45 (18.3%)	130 (52.8%)	71 (28.9%)		
rs2127015		C/C	C/T+T/T			
	AR	2 (4.7%)	41 (95.3%)		0.0253	0.21
	NAR	45 (18.3%)	201 (81.7%)			(0.05–0.93)
CD276		C/C	C/T	T/T		
rs3816661	AR	24 (55.8%)	17 (39.5%)	2 (4.7%)	0.6933	
	NAR	124 (50.4%)	103 (41.9%)	19 (7.7%)		
CD276		A/A	G/A	G/G		
rs7176654	AR	14 (32.6%)	23 (53.5%)	6 (13.9%)	0.7775	
	NAR	79 (32.1%)	122 (49.6%)	45 (18.3%)		
TREML2		A/A	C/A	C/C		
rs4714431	AR	21 (48.8%)	19 (44.2%)	3 (7.0%)	0.2401	
	NAR	91 (37.0%)	121 (49.2%)	34 (13.8%)		
		A/A	G/A	G/G		
	AR	3 (7.0%)	26 (60.5%)	14 (32.6%)	0.0436	
TREML2	NAR	48 (19.5%)	104 (42.3%)	94 (38.2%)		
rs6915083		A/A	G/A+G/G			
	AR	3 (7.0%)	40 (93.0%)		0.0467	0.30
	NAR	48 (19.5%)	198 (80.5%)			(0.09–1.04)
		G/G	G/T	T/T		
	AR	23 (53.5%)	19 (44.2%)	1 (2.3%)	0.0887	
TREML2	NAR	101 (41.1%)	114 (46.3%)	31 (12.6%)		
rs7754593		G/G+GT	T/T			
	AR	42 (97.7%)	1 (2.3%)		0.0476	6.05
	NAR	215 (87.4%)	31 (12.6%)			(0.80–45.61)
TREML2		A/A	G/A	G/G		
rs9394767	AR	29 (67.4%)	13 (30.2%)	1 (2.3%)	0.3950	
	NAR	139 (56.5%)	97 (39.4%)	10 (4.1%)		

AR represents acute rejection, while NAR for nonacute rejection.

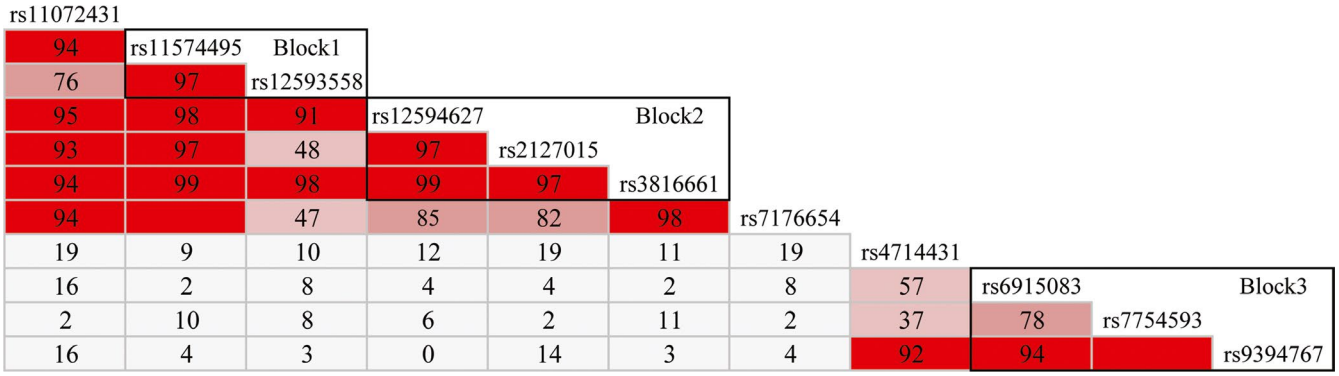

FIGURE 1 LD plots of *CD276* and *TREML2*. We identified two haplotype blocks, block 1 and block 2, in *CD276*, and one haplotype block, block 3, in *TREML2*

transcription or processing. We detected the expression of both *CD276* and *TREML2* mRNA in PBMCs, and found recipients carrying T allele in rs2127015 with a higher *CD276* mRNA expression (Figure 2). Subsequently, we verified membrane *CD276* expression by flow cytometry, and found membrane *CD276* expressed on both *CD3*- and *CD3* + PBMCs. The percentages of *CD276* + cells were positively correlated in the two subpopulations (correlation coefficient = 0.381, $P < 0.01$). Recipients carrying T allele at rs2127015 were with a higher percentage of *CD276* + *CD3*- cells in PBMCs (Figure 3).

3.4 | Prediction of acute rejection by combining genetic polymorphisms and HBV infection

Since SNPs of *CD276* and *TREML2*, and HBV infection as well, increased the risk of acute rejection, we performed multivariable logistic regression analysis to verify the possibility of genetic polymorphisms and HBV infection as independent risk factors for acute rejection. Then a risk assessment model of acute rejection was established by the combination between genotype of

TABLE 3 Association results of haplotypes in acute rejection

			Haplotype			
Block	**Haplotype**	**Events**	**Carrier**	**Noncarrier**	***p* value**	**OR (95% CI)**
Block 1	GT	AR	54	32	0.3084	1.27
		NAR	280	212		(0.79–2.04)
	AC	AR	20	66	0.3025	0.754
		NAR	141	351		(0.44–1.29)
	GC	AR	10	76	0.4896	0.7802
		NAR	71	421		(0.38–1.58)
Block 2	GTC	AR	55	31	0.1258	1.44
		NAR	271	221		(0.90–2.32)
	TCT	AR	19	67	0.2093	0.70
		NAR	141	351		(0.40–1.21)
	GCC	AR	8	78	0.3370	0.68
		NAR	64	428		(0.31–1.48)
	TCC	AR	2	84	0.7142	0.7571
		NAR	15	477		(0.17–3.33)
Block 3	GA	AR	63	23	0.1040	1.52
		NAR	316	176		(0.91–2.54)
	TG	AR	16	70	0.2927	0.73
		NAR	117	375		(0.40–1.31)
	TA	AR	7	79	0.3000	0.65
		NAR	59	433		(0.28–1.47)

AR represents acute rejection, while NAR for nonacute rejection.

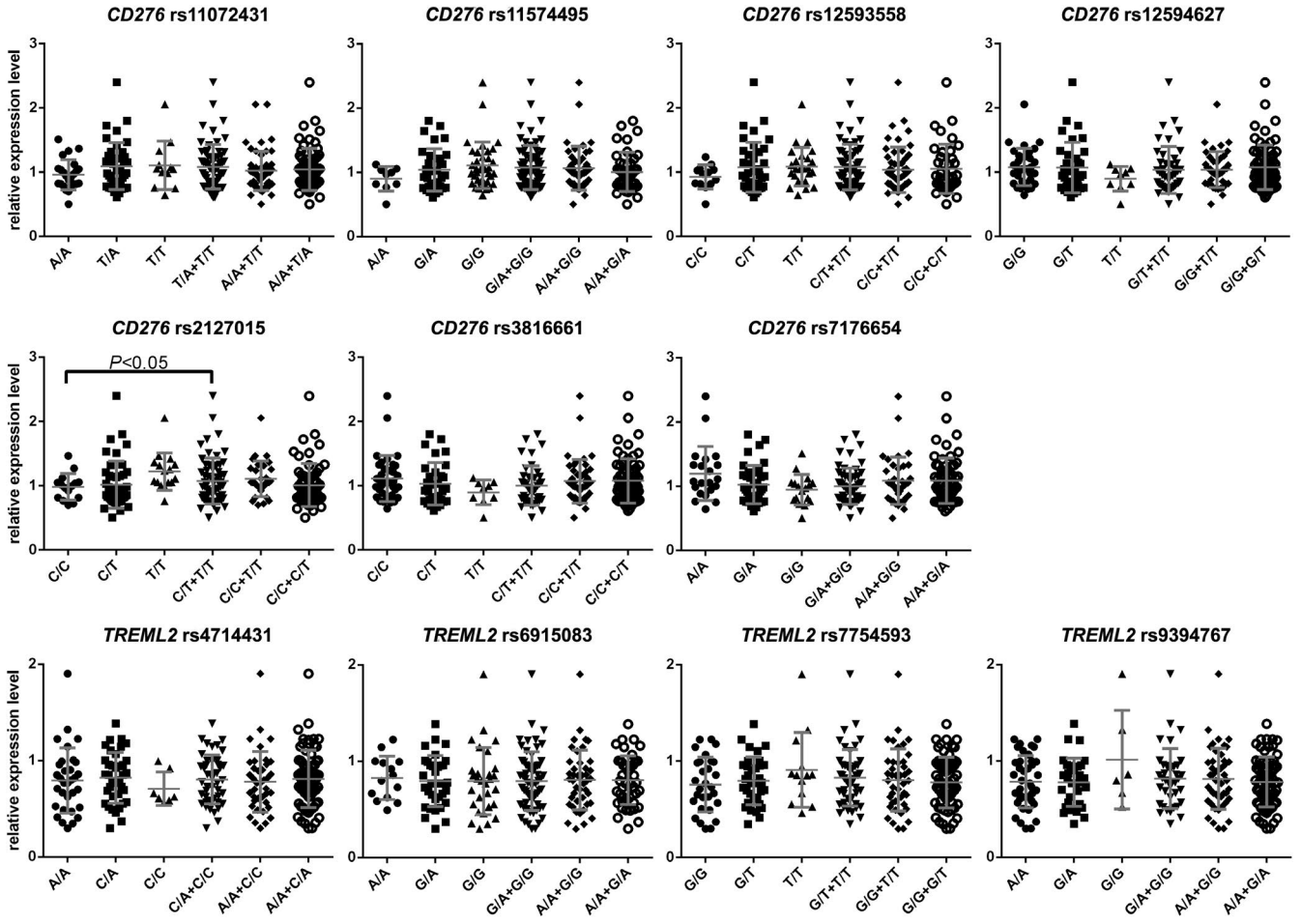

FIGURE 2 *CD276* and *TREML2* mRNA expression in PBMCs. Plots represented relative expression level of mRNA of each individual recipient. The result indicated that T allele carriers of rs2127015 expressed more *CD276* mRNA than the others ($p < 0.05$). Bar represented mean ± standard deviation of the scatter plots

rs2127015 and HBV infection. The other two associated variables, rs6915083 and rs7754593, which failed the test, were not included. In this model, the risk score was equal to -1.122 + 1.493 × rs2127015 + 0.817 × HBV (for rs2127015, T/T and C/T carrier was equal to 1, while C/C was equal to 0; for HBV, HBV infection was equal to 1, otherwise it was equal to 0).

The model exhibited a sensitivity of 69.8%, specificity of 54.7%, and an area under the curve of 0.634 (Figure 2). Subsequently, each recipient could be categorized into three groups: the score > 1, 1 > the score > 0, and the score < 0, which could be considered as high-, medium-, and low risk, respectively. According to the model, the incidence of acute rejection was 20.8% ($n = 30$) in high-risk group ($n = 144$), 11.2% ($n = 11$) in medium-risk group ($n = 98$), and 4.3% ($n = 2$) in low-risk group ($n = 51$).

3.5 | Validation of the prediction significance of the model by a prospective study

To validate the prediction significance and precision of the assessment model, we performed a prospective cohort study with a population of 89 recipients who received their liver grafts from 2010 to 2011. According to their scores, 89 recipients were divided into three groups (high-risk group with the score > 1, $n = 47$; medium-risk group with 1 > the score > 0, $n = 21$; low-risk group with the score < 0, $n = 21$), then all the recipients were followed up for more than 6 months after transplantation. Finally, 17.0% ($n = 8$) recipients developed acute rejection in the high-risk group, while that was 9.5% ($n = 2$) and 4.8% ($n = 1$) in the medium- and low-risk group, respectively.

4 | DISCUSSION

In our current study, we identified several acute rejection-associated risk factors, including genetic polymorphisms and HBV infection. And, we also provided a semiquantitative risk assessment model, which would facilitate the individualized immunosuppressive therapy for recipients according to the risk group which he/she belonged to. Recipients categorized into the high-risk group would be suggested a more optimal

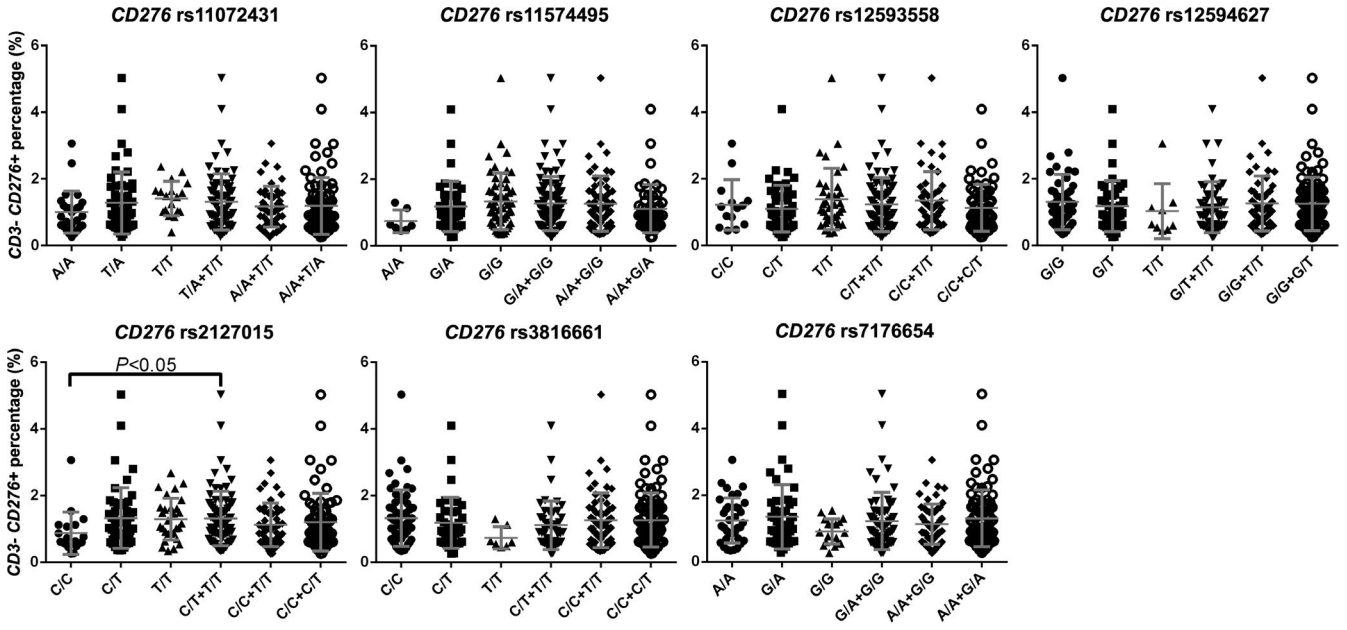

FIGURE 3 Membrane *CD276* expression in *CD3*- PBMCs. Plots represented the percent of *CD276* positive cells in *CD3*- PBMCs of each individual recipient. The result indicated that T allele carriers of rs2127015 possessed more *CD276* + cell in *CD3*- peripheral blood ($p < 0.05$). Bar represented mean ± standard deviation of the scatter plots

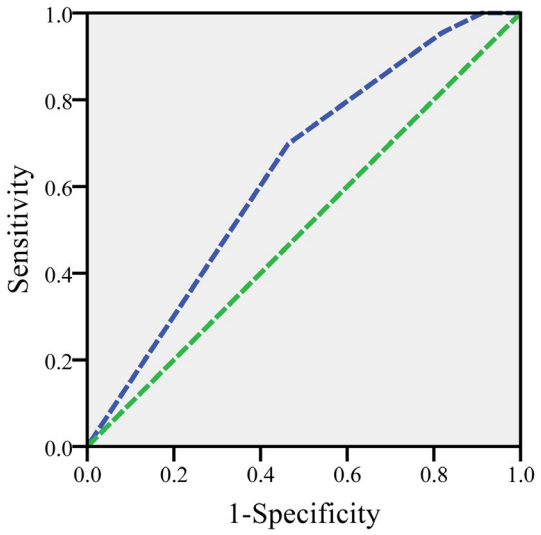

FIGURE 4 Acute rejection model assessment with ROC curve

regimen and frequent surveillance of immunosuppression, while those in the low-risk group should avoid excessive immunosuppression.

Although the development of acute rejection is complicated, its initiation requires both allorecognition and T-cell activation (Ingulli, 2010; Wood & Goto, 2012). Genetic polymorphisms of MHC, costimulatory molecules, and cytokines genes, which involve in the allorecognition and the activation, have been proved to be associated with acute rejection in liver and renal transplantations (Bitetto et al., 2012; Jiang et al., 2007; Tapirdamaz et al., 2006; Zhu, Huang, Liu, & Xie, 2012).

The fact that *CD80*, *CD86,* and their ligands involving in acute rejection (Marder et al., 2003; Marin et al., 2005), imply *CD276* and *TREML2* would be worth studying as well. Unlike other *B7*s, *CD276* expresses in a wide range of human organs both transcriptionally and translationally. Recent studies also proved an upregulation of *CD276* in different types of cancers and tumor-infiltrating blood vessels, including colon cancer, prostate cancer, pancreatic cancer, and liver cancer (Sun et al., 2010; Sun et al., 2012; Yuan et al., 2011; Zhao et al., 2013), suggesting *CD276* as an attractive and desirable target for cancer immunotherapy (Seaman et al., 2017). In addition, *CD276* promotes invasion and tumor progression (Wang, Kang, & Shan, 2013). *CD276* normally expresses on APC cells, and is considered as both a positive and a negative costimulatory molecule (Hofmeyer, Ray, & Zang, 2008). It promotes T cell-mediated immune responses and development of acute/chronic rejection (Wang et al., 2005), however, diverse functions and contrasting roles of *CD276* remain being explored. Thus, we focused on *CD276* and its ligand, *TREML2*, which could provide an intact signal cascade of positive costimulation (Hashiguchi et al., 2008). In this work, we proved that genetic polymorphisms of both *CD276* and *TREML2* were associated with acute rejection of liver transplantation. The evaluations of mRNA and/or protein expression of *CD276* and *TREML2* suggested that the association between SNPs and graft rejections might be due to the expression of *CD276* in

CD3- PBMCs. It seemed that individuals with T alleles at rs2127015 would possess higher *CD276* mRNA level in PBMC, which might lead to more membrane *CD276* expression. We also confirmed a membrane expression of *CD276* in *CD3* + PBMC, where its ligands normally express. However, *TREML2* did not show any mRNA expression difference among alleles. Our results implied the involvement of positive costimulation via *CD276* and *TREML2* in acute rejection.

The diverse functions and contrasting roles of *CD276* may be mainly due to its multiple counterparts on different cells or in different signal pathways. According to the dual role of *CD276* in both positive and negative costimulation effects, *CD276* might also inhibit acute immune reaction posttransplantation probably. Mouse transplantation model ought to facilitate the studies with gene-edited mouse. However, some studies suggested that *TREML2* might not be a ligand for murine *CD276* (Leitner et al., 2009; Yan et al., 2013), which makes it more complicated to elucidate the molecular and immune functions of *CD276*.

Meanwhile, the regulation of *CD276* protein production is also obscure. Three kinds of isoforms of *CD276* protein have been reported, transmembrane 4Ig-*CD276*(4Ig-*B7-H3*) (Steinberger et al., 2004), transmembrane 2Ig-*CD276*(2Ig-*B7-H3*) (Chen, Hou, Li, Xiong, & Liu, 2011), and soluble *CD276*(s*B7-H3*) (Zhang et al., 2008), with different in vivo or cellular distribution patterns, and various biological functions. Meanwhile, soluble 2Ig-*CD276* isoform could be released in serum by matrix metalloproteinase cleavage of 4Ig-*CD276* (Zhang et al., 2008). At least two forms of alternative spliced *CD276* mRNAs have been found, one translated into soluble *CD276* in hepatocellular carcinoma cells (Chen et al., 2013), another translated into intracytoplasmic 2Ig-*CD276* in monocytes (Yoon et al., 2016). Thus, it is possible that several spliced *CD276* mRNAs exist and might be translated into various *CD276* isoforms with different biological functions. Besides mutations in UTRs or exons, SNPs in introns were also proved as alternative splicings and fold changes of certain mRNA forms (Suhy et al., 2014; Wang & Sadee, 2016). And in this study, we observed an association of increased expression of *CD276* mRNA in rs2127015 T allele carriers. Further studies are needed to elucidate how rs2127015 influences the mRNA expression and whether rs2127015 would lead to alternative splicings.

Several studies confirmed that HBV infection could decrease the incidence of liver graft acute rejection (Crespo, Marino, Navasa, & Forns, 2012; Farges et al., 1996; Neuberger, 1999; Samuel & Kimmoun, 2003); however, the molecular mechanism still remains undiscovered. Generally, to prevent the reinfection of HBV postoperation, most centers applied a long-term administration of high-dose intramuscular hepatitis B immunoglobulin (HBIG) (Samuel & Kimmoun, 2003), while we have developed a safe and efficient substitution therapy which combines lamivudine and low-dose intramuscular HBIG in our center (Zheng et al., 2006). Recent studies suggested that the treatment with immunoglobulin might lead to a decreased risk for rejection and promote allograft acceptance (Bucuvalas, Anand, & Studies of Pediatric Liver Transplantation Research, 2009; Tha-In, Metselaar, Bushell, Kwekkeboom, & Wood, 2010). The effect of immune tolerance might result from the inhibition of dendritic cells and provoking *CD4* + *FoxP3* + T cells (Kwekkeboom et al., 2005; Tha-In et al., 2010). According to these observations, HBIG administration seemed able to benefit the liver transplant recipients with a better immunosuppression and a reduced acute rejection incidence. However, some researchers claimed that further examinations and studies will be needed to draw the conclusion of the survival benefits from HBIG (Ni & Chang, 2006).

The ultimate aim of our risk assessment model was to predict the incidence of acute rejection in advance and individually. The association between risk factors and acute rejection created the opportunity to predict disease risk for principal concern (Kooperberg, LeBlanc, & Obenchain, 2010). Thus, primary diagnosis, genetic background, and some other preoperative variables that clinically related were considered in this work. Although the research community is making great progress in association studies, poor conducts have been discussed (Moons, Kengne, Woodward, et al., 2012). Most genetic association studies only proved a clinical relevance, but not a clue of when the symptom starts and how severe it develops (Bitetto et al., 2012; Jiang et al., 2007; Tapirdamaz et al., 2006; Zhu et al., 2012). It led to a statement for genetic risk prediction studies to strengthen and encompass works with genetic risk factors in translational medicine (Janssens, Ioannidis, van Duijn, Little, & Khoury, 2011). Some other works also advocated successive and consecutive steps for researches (Moons, Kengne, Grobbee, et al., 2012; Moons, Kengne, Woodward, et al., 2012).

So far, our model does not predict whether or when a single recipient will develop acute rejection, however, due to currently limited understanding of genetic factors and other molecules in the immune response of allograft acute rejections. Some works suggested how risk factors would be applied to risk assessment models which follow a procedure of association study, then statistical certification, and finally a formula deduction (Moons, Kengne, Grobbee, et al., 2012; Moons, Kengne, Woodward, et al., 2012). In this study, a "predictor selection strategy" was applied (Moons, Kengne, Woodward, et al., 2012), of which candidate factors that do not contribute usefully will be removed from the model. Then, due to the failure of passing statistical verification,

two SNPs of *TREML2*, which are both associated with acute rejection were not included in the model. The other risk factors, rs2127015 and HBV infection, each assigned to a statistically calculated coefficient, could categorize the recipients into three groups. Nevertheless, the incidence of acute rejection in high-risk group was as much as four to five times to that in low-risk group, while the incidence of medium-risk group was close to normal incidence. We also evaluated our model by internal validation. With this model, we would be allowed to treat recipients with different immunosuppressive regimen. Therefore, recipients categorized into the high-risk group will receive additional surveillance of immunosuppression both immunologically and pharmacodynamically (Sawitzki, Schlickeiser, Reinke, & Volk, et al., 2011), whereas recipients in the low-risk group should avoid excessive immunosuppression. To refine our model, integrating more risk factors, statistically or experimentally proved, should be proceeded.

In conclusion, by combining biomarkers, such as genetic polymorphism, with other risk factors, we deduced a semi-quantitative risk assessment model, which would benefit recipients with an individualized immunosuppression for clinical cares.

ACKNOWLEDGMENTS

This work was supported by Natural Science Foundation of Zhejiang Province (No. LY18H100003), Innovative Research Groups of National Natural Science Foundation of China (No. 81721091), Major program of National Natural Science Foundation of China (No. 91542205), National S&T Major Project (No. 2017ZX10203205 and 2018ZX10723204-006), and Zhejiang International Science and Technology Cooperation Project (No. 2016C04003).

RESEARCH INVOLVING HUMAN PARTICIPANTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants enrolled in the study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Shusen Zheng https://orcid.org/0000-0003-1459-8261
Lin Zhou https://orcid.org/0000-0001-5689-3512

REFERENCES

Adeyi, O., Fischer, S. E., & Guindi, M. (2010). Liver allograft pathology: Approach to interpretation of needle biopsies with clinicopathological correlation. *Journal of Clinical Pathology*, *63*(1), 47–74. https://doi.org/10.1136/jcp.2009.068254

Bitetto, D., Fabris, C., Falleti, E., Fornasiere, E., Avellini, C., Cmet, S., & Toniutto, P. (2012). Recipient interleukin-28B Rs12979860 C/T polymorphism and acute cellular rejection after liver transplantation: Role of the calcineurin inhibitor used. *Transplantation*, *93*(10), 1038–1044. https://doi.org/10.1097/TP.0b013e31824df7f3

Bucuvalas, J. C., Anand, R. & Studies of Pediatric Liver Transplantation Research, G (2009). Treatment with immunoglobulin improves outcome for pediatric liver transplant recipients. *Liver Transplantation*, *15*(11), 1564–1569. https://doi.org/10.1002/lt.21843

Chen, W., Hou, Z., Li, C., Xiong, S., & Liu, H. (2011). Cloning and characterization of porcine 4Ig-B7-H3: A potent inhibitor of porcine T-cell activation. *PLoS ONE*, *6*(6), e21341. https://doi.org/10.1371/journal.pone.0021341

Chen, W., Liu, P., Wang, Y., Nie, W., Li, Z., Xu, W., & Liu, H. (2013). Characterization of a soluble B7-H3 (sB7-H3) spliced from the intron and analysis of sB7-H3 in the sera of patients with hepatocellular carcinoma. *PLoS ONE*, *8*(10), e76965. https://doi.org/10.1371/journal.pone.0076965

Clarkson, M. R., & Sayegh, M. H. (2005). T-cell costimulatory pathways in allograft rejection and tolerance. *Transplantation*, *80*(5), 555–563. https://doi.org/10.1097/01.tp.0000168432.60022.99

Crespo, G., Marino, Z., Navasa, M., & Forns, X. (2012). Viral hepatitis in liver transplantation. *Gastroenterology*, *142*(6), 1373–1383 e1371. https://doi.org/10.1053/j.gastro.2012.02.011

de Reuver, P., Pravica, V., Hop, W., Boor, P., Metselaar, H. J., Hutchinson, I. V., & Kwekkeboom, J. (2003). Recipient ctla-4 + 49 G/G genotype is associated with reduced incidence of acute rejection after liver transplantation. *American Journal of Transplantation*, *3*(12), 1587–1594. https://doi.org/10.1046/j.1600-6135.2003.00261.x

Dell-Olio, D., & Kelly, D. A. (2010). Immunosuppressants: What's new? *Current Opinion in Organ Transplantation*, *15*(5), 594–600. https://doi.org/10.1097/MOT.0b013e32833d8936

Farges, O., Saliba, F., Farhamant, H., Samuel, D., Bismuth, A., Reynes, M., & Bismuth, H. (1996). Incidence of rejection and infection after liver transplantation as a function of the primary disease: Possible influence of alcohol and polyclonal immunoglobulins. *Hepatology*, *23*(2), 240–248. https://doi.org/10.1053/jhep.1996.v23.pm0008591847

Hashiguchi, M., Kobori, H., Ritprajak, P., Kamimura, Y., Kozono, H., & Azuma, M. (2008). Triggering receptor expressed on myeloid cell-like transcript 2 (TLT-2) is a counter-receptor for B7-H3 and enhances T cell responses. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(30), 10495–10500. https://doi.org/10.1073/pnas.0802423105

Hernandez-Fuentes, M. P., & Lechler, R. I. (2010). A 'biomarker signature' for tolerance in transplantation. *Nature Reviews Nephrology*, *6*(10), 606–613. https://doi.org/10.1038/nrneph.2010.112

Hofmeyer, K. A., Ray, A., & Zang, X. (2008). The contrasting role of B7-H3. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(30), 10277–10278. https://doi.org/10.1073/pnas.0805458105

Hubscher, S. G. (2009). Transplantation Pathology. *Seminars in Liver Disease*, *29*(1), 74–90. https://doi.org/10.1055/s-0029-1192057

Ingulli, E. (2010). Mechanism of cellular rejection in transplantation. *Pediatric Nephrology*, *25*(1), 61–74. https://doi.org/10.1007/s00467-008-1020-x

Janssens, A. C., Ioannidis, J. P., van Duijn, C. M., Little, J., Khoury, M. J., &Group, G (2011). Strengthening the reporting of genetic risk prediction studies: The GRIPS statement. *Genetics in Medicine*, *13*(5), 453–456. https://doi.org/10.1097/GIM.0b013e318212fa82

Jiang, Z., Feng, X., Zhang, W., Gao, F., Ling, Q., Zhou, L., & Zheng, S. (2007). Recipient cytotoxic T lymphocyte antigen-4 + 49 G/G genotype is associated with reduced incidence of hepatitis B virus recurrence after liver transplantation among Chinese patients. *Liver International: Official Journal of the International Association for the Study of the Liver*, *27*(9), 1202–1208. https://doi.org/10.1111/j.1478-3231.2007.01553.x

Kim, H. J., Jeong, K. H., Lee, S. H., Moon, J. Y., Lee, T. W., Kang, S. W., & Chung, J. H. (2010). Polymorphisms of the CTLA4 gene and kidney transplant rejection in Korean patients. *Transplant Immunology*, *24*(1), 40–44. https://doi.org/10.1016/j.trim.2010.10.001

Kobori, H., Hashiguchi, M., Piao, J., Kato, M., Ritprajak, P., & Azuma, M. (2010). Enhancement of effector CD8 + T-cell function by tumour-associated B7-H3 and modulation of its counter-receptor triggering receptor expressed on myeloid cell-like transcript 2 at tumour sites. *Immunology*, *130*(3), 363–373. https://doi.org/10.1111/j.1365-2567.2009.03236.x

Kooperberg, C., LeBlanc, M., & Obenchain, V. (2010). Risk prediction using genome-wide association studies. *Genetic Epidemiology*, *34*(7), 643–652. https://doi.org/10.1002/gepi.20509

Kwekkeboom, J., Tha-In, T., Tra, W. M., Hop, W., Boor, P. P., Mancham, S., & Metselaar, H. J. (2005). Hepatitis B immunoglobulins inhibit dendritic cells and T cells and protect against acute rejection after liver transplantation. *American Journal of Transplantation*, *5*(10), 2393–2402. https://doi.org/10.1111/j.1600-6143.2005.01029.x

Leitner, J., Klauser, C., Pickl, W. F., Stockl, J., Majdic, O., Bardet, A. F., & Steinberger, P. (2009). B7-H3 is a potent inhibitor of human T-cell activation: No evidence for B7-H3 and TREML2 interaction. *European Journal of Immunology*, *39*(7), 1754–1764. https://doi.org/10.1002/eji.200839028

Linden, A. (2006). Measuring diagnostic and predictive accuracy in disease management: An introduction to receiver operating characteristic (ROC) analysis. *Journal of Evaluation in Clinical Practice*, *12*(2), 132–139. https://doi.org/10.1111/j.1365-2753.2005.00598.x

Marder, B. A., Schroppel, B., Lin, M., Schiano, T., Parekh, R., Tomer, Y., & Murphy, B. (2003). The impact of costimulatory molecule gene polymorphisms on clinical outcomes in liver transplantation. *American Journal of Transplantation*, *3*(4), 424–431. https://doi.org/10.1034/j.1600-6143.2003.00084.x

Marin, L. A., Moya-Quiles, M. R., Miras, M., Muro, M., Minguela, A., Bermejo, J., & Alvarez-Lopez, M. R. (2005). Evaluation of CD86 gene polymorphism at + 1057 position in liver transplant recipients. *Transplant Immunology*, *15*(1), 69–74. https://doi.org/10.1016/j.trim.2005.04.003

Moons, K. G., Kengne, A. P., Grobbee, D. E., Royston, P., Vergouwe, Y., Altman, D. G., & Woodward, M. (2012). Risk prediction models: II. External validation, model updating, and impact assessment. *Heart*, *98*(9), 691–698. https://doi.org/10.1136/heartjnl-2011-301247

Moons, K. G., Kengne, A. P., Woodward, M., Royston, P., Vergouwe, Y., Altman, D. G., & Grobbee, D. E. (2012). Risk prediction models: I. Development, internal validation, and assessing the incremental value of a new (bio)marker. *Heart*, *98*(9), 683–690. https://doi.org/10.1136/heartjnl-2011-301246

Neuberger, J. (1999). Incidence, timing, and risk factors for acute and chronic rejection. *Liver Transplantation and Surgery*, *5*(4 Suppl 1), S30–S36. https://doi.org/10.1053/JTLS005s00030

Neuhaus, P., Clavien, P. A., Kittur, D., Salizzoni, M., Rimola, A., Rimola, A., … Group, C. I. L. S (2002). Improved treatment response with basiliximab immunoprophylaxis after liver transplantation: Results from a double-blind randomized placebo-controlled trial. *Liver Transplantation*, *8*(2), 132–142. https://doi.org/10.1053/jlts.2002.30302

Ni, Y. H., & Chang, M. H. (2006). The ways paved for prophylaxis against de novo hepatitis B virus infection after liver transplantation: Still many stones left unturned. *Pediatric Transplantation*, *10*(4), 405–407. https://doi.org/10.1111/j.1399-3046.2006.00494.x

Offit, K. (2011). Personalized medicine: New genomics, old lessons. *Human Genetics*, *130*(1), 3–14. https://doi.org/10.1007/s00439-011-1028-3

Post, D. J., Douglas, D. D., & Mulligan, D. C. (2005). Immunosuppression in liver transplantation. *Liver Transplantation*, *11*(11), 1307–1314. https://doi.org/10.1002/lt.20614

Rook, M., & Rand, E. (2011). Predictors of long-term outcome after liver transplant. *Current Opinion in Organ Transplantation*, *16*(5), 499–504. https://doi.org/10.1097/MOT.0b013e32834a945d

Rothstein, D. M., & Sayegh, M. H. (2003). T-cell costimulatory pathways in allograft rejection and tolerance. *Immunological Reviews*, *196*, 85–108. https://doi.org/10.1046/j.1600-065X.2003.00088.x

Samuel, D., & Kimmoun, E. (2003). Immunosuppression in hepatitis B virus and hepatitis C virus transplants: Special considerations. *Clinics in Liver Disease*, *7*(3), 667–681. https://doi.org/10.1016/S1089-3261(03)00057-6

Sanchez-Fueyo, A., & Strom, T. B. (2011). Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. *Gastroenterology*, *140*(1), 51–64. https://doi.org/10.1053/j.gastro.2010.10.059

Sawitzki, B., Schlickeiser, S., Reinke, P., & Volk, H. D. (2011). Monitoring tolerance and rejection in organ transplant recipients. *Biomarkers*, *16*(Suppl 1), S42–S50. https://doi.org/10.3109/1354750X.2011.578754

Seaman, S., Zhu, Z., Saha, S., Zhang, X. M., Yang, M. Y., Hilton, M. B., … Tessarollo, L. (2017). Eradication of tumors through simultaneous ablation of CD276/B7-H3-positive tumor cells and tumor vasculature. *Cancer Cell*, *31*(4), 501–51 e508. https://doi.org/10.1016/j.ccell.2017.03.005

Snanoudj, R., Zuber, J., & Legendre, C. (2010). Co-stimulation blockade as a new strategy in kidney transplantation: Benefits and limits. *Drugs*, *70*(16), 2121–2131. https://doi.org/10.2165/11538140-000000000-00000

Steinberger, P., Majdic, O., Derdak, S. V., Pfistershammer, K., Kirchberger, S., Klauser, C., & Knapp, W. (2004). Molecular

characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. *The Journal of Immunology*, *172*(4), 2352–2359. https://doi.org/10.4049/jimmunol.172.4.2352

Suhy, A., Hartmann, K., Newman, L., Papp, A., Toneff, T., Hook, V., & Sadee, W. (2014). Genetic variants affecting alternative splicing of human cholesteryl ester transfer protein. *Biochemical and Biophysical Research Communications*, *443*(4), 1270–1274. https://doi.org/10.1016/j.bbrc.2013.12.127

Sun, J., Chen, L. J., Zhang, G. B., Jiang, J. T., Zhu, M., Tan, Y., & Zhang, X. G. (2010). Clinical significance and regulation of the costimulatory molecule B7-H3 in human colorectal carcinoma. *Cancer Immunology, Immunotherapy*, *59*(8), 1163–1171. https://doi.org/10.1007/s00262-010-0841-1

Sun, T. W., Gao, Q., Qiu, S. J., Zhou, J., Wang, X. Y., Yi, Y., & Fan, J. (2012). B7-H3 is expressed in human hepatocellular carcinoma and is associated with tumor aggressiveness and postoperative recurrence. *Cancer Immunology, Immunotherapy*, *61*(11), 2171–2182. https://doi.org/10.1007/s00262-012-1278-5

Tapirdamaz, O., Pravica, V., Metselaar, H. J., Hansen, B., Moons, L., van Meurs, J. B., & Kwekkeboom, J. (2006). Polymorphisms in the T cell regulatory gene cytotoxic T lymphocyte antigen 4 influence the rate of acute rejection after liver transplantation. *Gut*, *55*(6), 863–868. https://doi.org/10.1136/gut.2005.080937

Tha-In, T., Metselaar, H. J., Bushell, A. R., Kwekkeboom, J., & Wood, K. J. (2010). Intravenous immunoglobulins promote skin allograft acceptance by triggering functional activation of CD4 + Foxp3 + T cells. *Transplantation*, *89*(12), 1446–1455. https://doi.org/10.1097/TP.0b013e3181dd6bf1

Wang, L., Fraser, C. C., Kikly, K., Wells, A. D., Han, R., Coyle, A. J., & Hancock, W. W. (2005). B7-H3 promotes acute and chronic allograft rejection. *European Journal of Immunology*, *35*(2), 428–438. https://doi.org/10.1002/eji.200425518

Wang, L., Kang, F. B., & Shan, B. E. (2013). B7-H3-mediated tumor immunology: Friend or foe? *International Journal of Cancer*, *134*(12), 2764–2771. https://doi.org/10.1002/ijc.28474

Wang, D., & Sadee, W. (2016). CYP3A4 intronic SNP rs35599367 (CYP3A4*22) alters RNA splicing. *Pharmacogenetics and Genomics*, *26*(1), 40–43. https://doi.org/10.1097/FPC.0000000000000183

Wood, K. J., & Goto, R. (2012). Mechanisms of rejection: Current perspectives. *Transplantation*, *93*(1), 1–10. https://doi.org/10.1097/TP.0b013e31823cab44

Xu, X., Tu, Z., Wang, B., Ling, Q., Zhang, L., Zhou, L., & Zheng, S. (2011). A novel model for evaluating the risk of hepatitis B recurrence after liver transplantation. *Liver International: Official Journal of the International Association for the Study of the Liver*, *31*(10), 1477–1484. https://doi.org/10.1111/j.1478-3231.2011.02500.x

Yan, R., Yang, S., Gu, A., Zhan, F., He, C., Qin, C., & Feng, P. (2013). Murine b7-h3 is a co-stimulatory molecule for T cell activation. *Monoclonal Antibodies in Immunodiagnosis and Immunotherapy*, *32*(6), 395–398. https://doi.org/10.1089/mab.2013.0052

Yoon, B. R., Chung, Y. H., Yoo, S. J., Kawara, K., Kim, J., Yoo, I. S., & Lee, W. W. (2016). Preferential induction of the T cell auxiliary signaling molecule B7-H3 on synovial monocytes in rheumatoid arthritis. *Journal of Biological Chemistry*, *291*(8), 4048–4057. https://doi.org/10.1074/jbc.M115.680298

Yu, X., Xie, H., Wei, B., Zhang, M., Wang, W., Wu, J., & Zhou, L. (2011). Association of MDR1 gene SNPs and haplotypes with the tacrolimus dose requirements in Han Chinese liver transplant recipients. *PLoS ONE*, *6*(11), e25933. https://doi.org/10.1371/journal.pone.0025933

Yuan, H., Wei, X., Zhang, G., Li, C., Zhang, X., & Hou, J. (2011). B7-H3 over expression in prostate cancer promotes tumor cell progression. *Journal of Urology*, *186*(3), 1093–1099. https://doi.org/10.1016/j.juro.2011.04.103

Zhang, G., Hou, J., Shi, J., Yu, G., Lu, B., & Zhang, X. (2008). Soluble CD276 (B7-H3) is released from monocytes, dendritic cells and activated T cells and is detectable in normal human serum. *Immunology*, *123*(4), 538–546. https://doi.org/10.1111/j.1365-2567.2007.02723.x

Zhao, X., Li, D. C., Zhu, X. G., Gan, W. J., Li, Z., Xiong, F., & Zhao, H. (2013). B7-H3 overexpression in pancreatic cancer promotes tumor progression. *International Journal of Molecular Medicine*, *31*(2), 283–291. https://doi.org/10.3892/ijmm.2012.1212

Zheng, S., Chen, Y., Liang, T., Lu, A., Wang, W., Shen, Y., & Zhang, M. (2006). Prevention of hepatitis B recurrence after liver transplantation using lamivudine or lamivudine combined with hepatitis B Immunoglobulin prophylaxis. *Liver Transplantation*, *12*(2), 253–258. https://doi.org/10.1002/lt.20701

Zhu, C. L., Huang, Q., Liu, C. H., & Xie, F. (2012). Polymorphisms in the cytotoxic T-lymphocyte antigen 4 gene and acute rejection risk in transplant recipients. *Molecular Biology Reports*, *39*(9), 8701–8708. https://doi.org/10.1007/s11033-012-1727-4

How to cite this article: Yu X, Wei B, Su R, et al. A risk assessment model of acute liver allograft rejection by genetic polymorphism of *CD276*. *Mol Genet Genomic Med*. 2019;7:e689. https://doi.org/10.1002/mgg3.689