Original Article

Gene Variations of Sixth Complement Component Affecting Tacrolimus Metabolism in Patients with Liver Transplantation for Hepatocellular Carcinoma

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Abstract

Background: Orthotopic liver transplantation (OLT) improves the prognosis of patients with hepatocellular carcinoma (HCC). Moreover, the complement system is a powerful immune effector that can affect liver function and process of liver cirrhosis. However, studies correlating the complement system with tacrolimus metabolism after OLT are scarce. In this study, the role of single nucleotide polymorphisms (SNPs) associated with the sixth complement component (*C6*) in tacrolimus metabolism was investigated during the early stages of liver transplantation.

Methods: The study enrolled 135 adult patients treated with OLT for HCC between August 2011 and October 2013. Ten SNPs in *C6* gene and rs776746 in cytochrome P450 3A5 (*CYP3A5)* gene were investigated. The tacrolimus levels were monitored daily during 4 weeks after transplantation.

Results: Both donor and recipient *CYP3A5* rs776746 allele A were correlated with decreased concentration/dose (C/D) ratios. Recipient *C6* rs9200 allele G and donor *C6* rs10052999 homozygotes were correlated with lower C/D ratios. Recipient *CYP3A5* rs776746 allele A (yielded median tacrolimus C/D ratios of 225.90 at week 1 and 123.61 at week 2), *C6* rs9200 allele G (exhibited median tacrolimus C/D ratios of 211.31 at week 1, 110.23 at week 2, and 99.88 at week 3), and donor *CYP3A5* rs776746 allele A (exhibited median C/D ratios of 210.82 at week 1, 111.06 at week 2, 77.49 at week 3, and 85.60 at week 4) and *C6* rs10052999 homozygote (exhibited median C/D ratios of 167.59 at week 2, 157.99 at week 3, and 155.36 at week 4) were associated with rapid tacrolimus metabolism. With increasing number of these alleles, patients were found to have lower tacrolimus C/D ratios at various time points during the 4 weeks after transplantation. In multiple linear regression analysis, recipient *C6* rs9200 group (AA vs. GG/GA) was found to be related to tacrolimus metabolism at weeks 1, 2, and 3 ($P = 0.005$, $P = 0.045$, and $P = 0.033$, respectively), whereas donor *C6* rs10052999 group (CC/TT vs. TC) was demonstrated to be correlated with tacrolimus metabolism only at week 4 ($P = 0.001$).

Conclusions: Recipient *C6* gene rs9200 polymorphism and donor *C6* gene rs10052999 polymorphism are new genetic loci that affect tacrolimus metabolism in patients with HCC after OLT.

Key words: *C6*; Cytochrome P450 3A5; Hepatocellular Carcinoma; Liver Transplantation; Single Nucleotide Polymorphism; Tacrolimus Metabolism

Introduction

Hepatocellular carcinoma (HCC) ranks fifth among all the malignancies and third among cancer deaths worldwide.[1] Orthotopic liver transplantation (OLT) increases survival in HCC patients. However, immune suppressants are required in appropriate amounts to prevent transplant rejection. Tacrolimus, which is the first-line immunosuppressant after organ transplantation, reduces the incidence of rejection and improves graft and recipient survival.[2] Merely, tacrolimus has a narrow therapeutic interval, with varying pharmacokinetics. Standard doses of tacrolimus administered postoperatively are not effective in all

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patients equally.[3] Therefore, tacrolimus treatment requires monitoring for efficacy and safety. Nonetheless, it is a challenge to tailor individualized interventions during early postoperative period. Previous studies suggested that some gene polymorphisms are associated with drug metabolism.[4-6] Pharmacogenomics focuses on the relationship between host genetics and drug metabolism. Most previous studies focused on pharmacogenomics regardless of pathogenesis. Patients with HCC were treated with different therapeutic regimens, which are associated with complications and poor prognosis. Therefore, the identification of markers to facilitate individualized tacrolimus therapy, especially in patients with HCC undergoing transplantation, is imperative.

Tacrolimus is mainly metabolized by cytochrome P450 (CYP) 3A isoenzymes.[7] A cytochrome P450 3A5 (*CYP3A5*) gene variation involving an A-to-G transition at position rs776746 within intron 3 was significantly associated with CYP3A5 expression. Tacrolimus is poorly metabolized in *CYP3A5* rs776746 GG carriers.[8] However, the effects of *CYP3A5* rs776746 do not explain the total individual differences in tacrolimus metabolism. Therefore, additional markers can play a role in individual variation.

The complement system affects liver function and process of liver cirrhosis and eliminates virus-infected and cancer cells as well.[9,10] The sixth component of the complement system (C6) is involved in the formation of a membrane attack complex (MAC) with other components (C5–C9) following activation of the complement cascade.[11] The MAC was reported to mediate transplant rejection, C6 is an acute phase protein, which was studied in a rat heart transplantation model.[12] Interestingly, we demonstrated that patients with *C6* rs9200 GA presented with HCC recurrence following OLT.[13] However, to the best of our knowledge, no research has yet found the association between *C6* gene polymorphisms and tacrolimus pharmacokinetics. Therefore, we initially hypothesized that *C6* gene polymorphisms might regulate MAC function and mediate tacrolimus metabolism. This study was to investigate the association between tacrolimus metabolism and *C6* single nucleotide polymorphisms (SNPs) in a large cohort of patients undergoing OLT for HCC and to evaluate the possible role of tacrolimus as an immunotherapeutic intervention tailored to individual patients during early postoperative period.

Methods

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Institutional Review Board of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine. Informed written consent was obtained from all patients before their enrollment in this study.

Patients

The adult patients treated with OLT for HCC between August 2011 and October 2013 at the Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine were enrolled in the study. Patients with etiologically different liver disease (e.g., hepatic failure and autoimmune hepatitis) were excluded from the study. In addition, patients who were not treated with tacrolimus-based immunosuppressive regimens were excluded from the study. All the patients underwent immunosuppressive therapy using tacrolimus (Prograf, Astellas Pharma, Japan) administered orally twice daily, starting with a preliminary dose of 0.06 $mg \cdot kg^{-1} \cdot d^{-1}$. The dose was modified during the 1st month posttransplantation based on the target blood levels (7–10 ng/ml).

Determination of tacrolimus concentration

Tacrolimus levels were assayed using apron-TRAC TM II Tacrolimus ELISA kit (Diasorin, USA) and microparticle enzyme immunoassays (ELx 800NB analyzer, BioTek, USA). The concentration/dose (C/D) ratio was determined by dividing the trough concentration by the weight-adjusted daily dose ($mg \cdot kg^{-1} \cdot d^{-1}$). C/D ratios were determined at 4 weeks posttransplantation. The mean C/D ratios at weeks 1–4 were used to monitor weekly changes in tacrolimus metabolism.

Genomic DNA isolation and genotype determination

Recipient (ethylenediaminetetraacetic acid-anticoagulated whole blood) and donor (fresh-frozen liver tissue) genotypes were determined separately. Both the recipient and the donor DNA were determined using the AllPrep DNA/RNA Mini kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions. Genotype variations were determined using a Sequenom MassARRAY SNP genotyping platform (Sequenom, San Diego, CA, USA).[14] Quality control was performed to exclude individual SNPs or samples with genotype efficiency call rates <95% and SNP assays with poor-quality spectra/cluster plots, using LDR-PCR sequencing technology for further verification.

Statistical analysis

Hardy-Weinberg equilibrium and allele frequency were analyzed using SHEsis online version (http://analysis.bio-x.cn/myAnalysis.php). The patients' age and weight and tacrolimus C/D ratios are expressed as median (interquartile range). Quantitative variables among the groups were compared using nonparametric tests. Mann-Whitney *U*-test was used to compare differences between two groups, and Kruskal-Wallis test was used to analyze the differences among several groups. All the analyses were two sided and performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

Genotype and allele distributions

Initially, 135 adult patients treated with OLT for HCC between August 2011 and October 2013 were enrolled in the study. Fifty-five patients with etiologically different liver disease and nine patients who were not treated with tacrolimus-based immunosuppressive regimens were excluded from the study. A total of 71 patients were

included in final analysis. All the patients were of Chinese Han ethnicity. The patients' median age was 46.0 (21.0) years and median weight was 62.8 (23.2) kg. The average GPT and GOT levels at weeks 1, 2, 3, and 4 were 245.50, 54.91, 34.91, and 37.22 U/L and 341.79, 49.31, 35.66, and 38.25 U/L, respectively. Compared with standard values for GPT (5–40 U/L) and GOT (8–40 U/L), the patients' hepatic function was normal around 3-week posttransplantation.

Sixty out of 71 liver transplantations were conducted in males and 11 in females, with median hospitalization period of 33.0 (21.5) days. In this study, 18 patients showed an association between *C6* polymorphisms and HCC recurrence after OLT. Genotype frequencies of the three SNPs are shown in Table 1. The distribution of allele A in *CYP3A5* rs776746 was 26.8% among recipients and 30.3% among donors. For *C6*, the rs9200 allele A (72.6% and 68.3%) and rs10052999 allele C (75.4% and 76.1%) represented the major alleles in both recipients and donors. All SNP frequencies were in accordance with Hardy-Weinberg equilibrium (all $P > 0.05$). No significant differences in the frequencies of alleles containing the three SNPs (rs776746, rs9200, and rs10052999) were found between donors and recipients ($\chi^2 = 0.384$, $P = 0.535$; $\chi^2 = 0.638$, $P = 0.424$; and $\chi^2 = 0.019$, $P = 0.890$, respectively). Statistically significant differences were found between *C6* genotype and tacrolimus metabolism in the recipient rs9200 group (AA vs. GG/GA) and donor rs10052999 group (CC/TT vs. TC), but no differences were obtained in other subgroups.

Table 1: Genotype frequencies of *CYP3A5* rs776746, *C6* rs9200, and rs10052999

Genotypes	Frequency of donors, *n* (%)	Frequency of recipients, *n* (%)
CYP3A5 rs776746		
GG	33 (46.5)	36 (50.7)
GA+AA	38 (53.5)	35 (49.3)
C6 rs9200		
AA	36 (50.7)	40 (56.3)
GA+GG	35 (49.3)	31 (43.7)
C6 rs10052999		
TC	30 (42.3)	31 (43.7)
CC+TT	41 (57.7)	40 (56.3)

Associations of cytochrome P450 3A5 rs776746, *C6* rs9200, and rs10052999 polymorphisms with tacrolimus concentration/dose ratios

The effect of recipient *CYP3A5* rs776746, *C6* rs9200, and rs10052999 polymorphisms on tacrolimus C/D ratios at 4-week posttransplantation is shown in Table 2. Recipient *CYP3A5* rs776746 allele A carriers yielded median tacrolimus C/D ratios of 225.90 at week 1 and 123.61 at week 2, whereas the median C/D ratios of non-A carriers were significantly higher (322.15, $P = 0.009$ for week 1 and 153.92, $P = 0.049$ for week 2). Recipient *C6* rs9200 allele G carriers exhibited median tacrolimus C/D ratios of 211.31 at week 1, 110.23 at week 2, and 99.88 at week 3, whereas the median C/D ratios of non-G carriers were significantly higher (292.93, $P = 0.025$ for week 1; 153.16, $P = 0.027$ for week 2; and 134.48, $P = 0.049$ for week 3). However, no significant differences in tacrolimus C/D ratios were found between recipient rs10052999 genotype TC carriers and non-TC carriers ($P > 0.05$). Therefore, *CYP3A5* rs776746 allele A and *C6* rs9200 allele G in the recipients were associated with rapid tacrolimus metabolism. The recipient *C6* rs9200 polymorphisms were highly correlated with tacrolimus concentration and dosage at week 1, week 2, and week 3 in the study.

As shown in Table 3, donor *CYP3A5* rs776746, *C6* rs9200, and rs10052999 polymorphisms affected tacrolimus C/D ratios 4-week posttransplantation. At weeks 1, 2, 3, and 4, donor *CYP3A5* rs776746 allele A carriers exhibited median C/D ratios of 210.82, 111.06, 77.49, and 85.60, whereas the median C/D ratios of non-A carriers were significantly higher (360.74, $P < 0.001$ for week 1; 144.22, $P = 0.018$ for week 2; 148.31, $P < 0.001$ for week 3; and 143.96, $P = 0.001$ for week 4). At weeks 2, 3, and 4, donor *C6* rs10052999 genotype TC carriers exhibited median C/D ratios of 167.59, 157.99, and 155.36, whereas the median C/D ratios of non-TC carriers were significantly lower (107.39, $P = 0.011$ for week 2; 86.21, $P = 0.003$ for week 3; and 90.15, $P = 0.001$ for week 4). As shown in Table 3, there was no significant difference in tacrolimus C/D ratios between donor *C6* rs9200

Table 2: Effects of recipient *CYP3A5* rs776746, *C6* rs9200, and rs10052999 polymorphisms on tacrolimus concentration/dose ratios

Genotypes	Week 1			Week 2			Week 3			Week 4		
	C/D ratio	*U*	*P*	C/D ratio	*U*	*P*	C/D ratio	*U*	*P*	C/D ratio	*U*	*P*
CYP3A5 rs776746		6.816	0.009		3.868	0.049		0.127	0.721		1.376	0.241
GG ($n = 36$)	322.15 (305.21)			153.92 (129.19)			119.71 (114.16)			129.35 (174.85)		
GA + AA ($n = 35$)	225.90 (216.77)			123.61 (98.59)			110.43 (104.99)			99.75 (79.50)		
C6 rs9200		5.059	0.025		4.903	0.027		3.884	0.049		0.716	0.397
AA ($n = 40$)	292.93 (301.27)			153.16 (115.80)			134.48 (130.72)			119.04 (172.01)		
GA+GG ($n = 31$)	211.31 (249.07)			110.23 (98.42)			99.88 (94.85)			130.39 (97.08)		
C6 rs10052999		0.751	0.386		0.001	0.982		0.011	0.917		0.077	0.782
CC+TT ($n = 40$)	276.97 ± 267.84			133.10 (108.10)			113.63 (121.88)			124.37 (143.02)		
TC ($n = 31$)	247.25 ± 227.44			126.75 (132.99)			114.27 (101.99)			119.62 (105.41)		

The data are shown as median (IQR). C/D: Concentration/dose; IQR: Interquartile range.

Table 3: Effects of donor *CYP3A5* rs776746, *C6* rs9200, and rs10052999 polymorphisms on tacrolimus concentration/dose ratios

Genotypes	Week 1			Week 2		
	C/D ratio	*U*	*P*	C/D ratio	*U*	*P*
CYP3A5 rs776746		12.202	<0.001		5.585	0.018
GG (*n* = 33)	360.74 (364.92)			144.22 (135.36)		
GA+AA (*n* = 38)	210.82 (205.46)			111.06 (109.80)		
C6 rs9200		2.100	0.147		1.719	0.190
AA (*n* = 36)	286.09 (250.90)			137.84 (142.61)		
GA+GG (*n* = 35)	227.19 (312.24)			120.86 (111.06)		
C6 rs10052999		3.398	0.065		6.388	0.011
CC+TT (*n* = 41)	226.55 (237.69)			107.39 (87.00)		
TC (*n* = 30)	317.84 (318.74)			167.59 (135.47)		
Genotypes	**Week 3**			**Week 4**		
	C/D ratio	***U***	***P***	**C/D ratio**	***U***	***P***
CYP3A5 rs776746		13.104	<0.001		11.645	0.001
GG (*n* = 33)	148.31 (91.33)			143.96 (174.98)		
GA+AA (*n* = 38)	77.49 (90.17)			85.60 (70.91)		
C6 rs9200		0.460	0.497		0.331	0.565
AA (*n* = 36)	121.02 (99.98)			129.35 (140.12)		
GA+GG (*n* = 35)	104.66 (120.89)			120.60 (99.78)		
C6 rs10052999		9.086	0.003		11.856	0.001
CC+TT (*n* = 41)	86.21 (78.04)			90.15 (66.34)		
TC (*n* = 30)	157.99 (113.41)			155.36 (194.37)		

The data are shown as median (IQR). C/D: Concentration/dose; IQR: Interquartile range.

allele G carriers and non-G carriers ($P > 0.05$). Therefore, donor *CYP3A5* rs776746 allele A and *C6* rs10052999 homozygote represented statistically significant markers of rapid tacrolimus metabolism.

In addition, none of the eight *C6* SNP genotypes including rs3805712 ($P_{min} = 0.053$, donor at week 3, AG vs. AA/AG), rs3805715 ($P_{min} = 0.059$, recipient at week 4, AA vs. GG/AG), rs3805716 ($P_{min} = 0.068$, donor at week 4, AA vs. AT/TT), rs6865420 ($P_{min} = 0.478$, donor at week 3, CA vs. CC/AA), rs7443562 ($P_{min} = 0.089$, donor at week 2, AA vs. GG/GA), rs121917779 (all genotypes were AA), rs138105385 (all genotypes were CC), and rs150358068 (all genotypes were CC) was associated with tacrolimus metabolism.

Combined polymorphisms and tacrolimus concentration/dose ratios

CYP3A5 rs776746 genotypes (GA and AA) and *C6* rs9200 genotypes (GA and AA) of recipient and donor *CYP3A5* rs776746 genotypes (GA and AA) and *C6* rs10052999 genotypes (CC and TT) were associated with rapid tacrolimus metabolism. All the genotypes were further analyzed to determine the role of SNPs in tacrolimus metabolism. We divided the patients into three groups based on those genotypes: patients carried zero or one related genotypes were defined as poor metabolizers, named Group 1; patients carried two or three related genotypes were defined as intermediate metabolizers, named Group 2, whereas patients carried four related genotypes were defined as junior extensive metabolizers, named Group 3. As shown in Figure 1, in Kruskal-Wallis test, with increasing number of genotypes

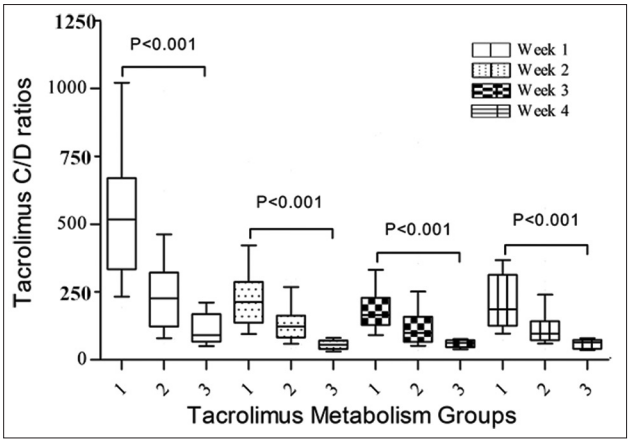

Figure 1: Combined effect of four related genotypes on tacrolimus concentration/dose ratios in various groups during the first 4-week posttransplantation ($n = 20$ in Group 1, $n = 46$ in Group 2, and $n = 5$ in Group 3).

associated with rapid metabolism, patients exhibited lower tacrolimus C/D ratios at all the time points during the first 4-week posttransplantation ($H = 26.842$, $P < 0.001$ for week 1; $H = 20.875$, $P < 0.001$ for week 2; $H = 18.214$, $P < 0.001$ for week 3; and $H = 21.200$, $P < 0.001$ for week 4, respectively).

Metabolism factors associated with tacrolimus concentration/dose ratios in the multiple linear regression analysis

To have a better understanding about the associations of these genotypes with tacrolimus metabolism during early

postoperative period, stepwise multiple linear regression analysis was performed. To avoid unnecessary errors from patient characteristics, age and gender were also considered in the statistical analysis. Notably, final regression models were established to predict the C/D ratios of tacrolimus during the first 4-week posttransplantation [Table 4], recipient *C6* rs9200 group (AA vs. GG/GA) was found to be related to tacrolimus metabolism at weeks 1, 2, and 3 ($P < 0.05$), whereas donor *C6* rs10052999 group (CC/TT vs. TC) was demonstrated to be correlated with tacrolimus metabolism only at week 4.

Discussion

Drug resistance is a potentially fatal challenge post-OLT for HCC. Pharmacogenomics provides effective methods to investigate the mechanisms underlying interactive gene networks. An understanding of these interactions may contribute to the development of individualized drug therapy. The risk of early organ rejection is the highest immediately posttransplantation.[15] Tacrolimus concentrations during the 1st week after transplantation determine the extent of rejection.[16] Therefore, the identification of key predictors of tacrolimus concentrations in the early stage after liver transplantation is clinically valuable. Pharmacogenetic dose algorithms for tacrolimus based on genotypes and clinical variables may facilitate the development of stable therapeutic doses. In the current study, researchers investigated the associations of CYP3A5, interleukin (IL)-6, IL-10, and IL-18 genetic polymorphisms with tacrolimus metabolism.[4-6] Based on our findings, we propose the existence of a gene interaction network that affects tacrolimus pharmacogenomics in patients with HCC after OLT.

Tacrolimus is extensively metabolized by the CYP system. *CYP3A5* rs776746 allele A noncarriers produce a truncated, nonfunctional CYP3A5 enzyme due to a splicing defect; therefore, these noncarrier patients metabolize tacrolimus more slowly than carriers.[8] In the current study, the *CYP3A5* rs776746 genotype in donors altered tacrolimus C/D ratios during the 4-week posttransplantation, which was consistent with earlier studies.[17] In contrast, in this study, the recipient *CYP3A5* rs776746 genotype was found to influence tacrolimus levels only at weeks 1, which was consistent with a recent meta-analysis and systematic review.[18] In the final combination analysis, an additional Kruskal-Wallis test including *CYP3A5* polymorphisms alone revealed significantly lower tacrolimus C/D ratios in patients with rapid-metabolizing genotypes at 4-week posttransplantation ($P < 0.001$, $P = 0.004$, $P = 0.007$, and $P = 0.001$, respectively, data not shown). Thus, we hypothesized that *CYP3A5* rs776746 polymorphisms play a key role in tacrolimus pharmacokinetics although donor *CYP3A5* polymorphisms have a more significant influence.[19] A limited number of clinical markers have been associated with tacrolimus pharmacokinetics.[20] However, these markers or genetic polymorphisms do not account for all the observed variations. Interestingly, in this study, variations in tacrolimus C/D ratios among individual patients were partially correlated with *C6* polymorphisms.

Recipients of liver grafts are known to be at increased risk of ischemia-reperfusion injury (IRI), immunological injury, and drug toxicity during the posttransplantation period. The complement system includes a series of complex biochemical and immunological pathways that eliminate foreign components from an individual. Complement mediates inflammation and injury after ischemia. Complement inhibition, therefore, is a potential therapeutic strategy to reducing IRI.[21] MAC inhibition facilitates early complement activation triggering cytokine production and hepatocyte proliferation.[22] Wu *et al*.[12] reported that C6-deficiency inhibits a late step in complement activation resulting in delayed xenograft rejection. Furthermore, C6-deficiency in rats ameliorates IRI.[23] Activation of the complement system triggers apoptosis and opsonization.[24] However, complement activation also mediates liver regeneration.[25] Thus, the MAC can induce transplant rejection and also accelerates the restoration of liver function.

The complement system is involved in several cytolytic activities. Surface expression of complement regulatory

Table 4: Metabolism factors associated with tacrolimus concentration/dose ratios in the multiple linear regression analysis

Factors	Unstandardized coefficients	Standardized coefficients	*P*	Determination coefficients
Week 1				
Donor *CYP3A5* rs776746 (1 = GG, 2 = GA/AA)	−188.848	−0.380	<0.001	0.385
Recipient *CYP3A5* rs776746 (1 = GG, 2 = GA/AA)	−171.655	−0.346	0.001	
Recipient *C6* rs9200 (1 = AA, 2 = GA/GG)	−142.139	−0.285	0.005	
Week 2				
Recipient *C6* rs9200 (1 = AA, 2 = GA/GG)	−60.012	−0.239	0.045	0.057
Week 3				
Donor *CYP3A5* rs776746 (1 = GG, 2 = GA/AA)	−78.841	−0.249	0.031	0.126
Recipient *C6* rs9200 (1 = AA, 2 = GA/GG)	−78.604	−0.247	0.033	
Week 4				
Donor *C6* rs10052999 (1 = CC/TT, 2 = TC)	79.145	0.383	0.001	0.223
Donor *CYP3A5* rs77674 (1 = GG, 2 = GA/AA)	−49.929	−0.243	0.027	

proteins (CRP) prevents cytolysis of cancer cells.[26] As well, cancers such as HCC overexpress CRPs including CD46 and CD59.[27] Increased CD46 expression in HCC represents an early step in disease progression.[28] Interestingly, upregulation of CD46, which is the cellular receptor for adenovirus type 35, may enhance the antitumor efficacy of oncolytic adenoviruses in HCC cells.[29] Tumor cells resist complement-mediated death through different mechanisms,[30,31] specifically, reduction of C6 and C7 expression levels in tumor tissues have been reported to enhance the capacity of tumors to escape complement-dependent cytotoxicity.[32] Otherwise, the MAC assembly by C6 and other complement components[33] induces cell lysis by generating pores in the cell membrane. Several human neoplasias are associated with genes located on chromosome 5,[34-36] and genes for *C6* and *C7* are closely linked on 5pl3.[11] In this study, the recipient *C6* rs9200 allele G and donor *C6* rs10052999 homozygote were linked with rapid tacrolimus metabolism after OLT in patients with HCC. Hence, C6 may play a decisive role in hepatocyte function in patients with HCC undergoing OLT.

Complement mediates hepatic injury and regeneration, as well as cancer immune surveillance. Antibody and complement play a key role in allograft rejection. Although the underlying mechanisms remain to be elucidated, complement activation products, including C6, play a critical role in inflammatory reactions, allograft rejection, and IRI. The results of this study indicated that both donor and recipient C6 might be closely related to tacrolimus metabolism after OLT. Liver is the primary site of C6 synthesis. In rats, extrahepatic synthesis of C6 has been reported during transplantation of livers from C6-deficient donors to normal recipients.[37-39] Therefore, according to the results of this study, we speculated that extrahepatic sites of C6 biosynthesis play a key role in tacrolimus metabolism immediately after OLT, and C6 biosynthesis in hepatocytes is restored after liver function recovery. Evidence suggested that reduced complement activity resulted from genetic variations in *C6*.[40] Further, a CAAT/enhancer binding protein site in the *C6* promoter has been found to be essential for normal C6 expression.[41] Thus, *C6* SNPs can regulate C6 synthesis.

In this study, *C6* and *CYP3A5* polymorphisms were further analyzed in stepwise multiple linear regression analysis. As shown in Table 4, the determination coefficients were 0.385, 0.057, 0.126, and 0.223 for the C/D ratios, respectively. It indicated that those regression models were bringing a discontent explanatory power, which just showed that amounts of genetic polymorphisms account for the C/D ratios were not in the models, and the individual differences of tacrolimus metabolism could be related to others. As such, studies in larger cohorts of patients with some more related genetic polymorphisms are required in the future.

The limitations of this study were as follows: First, the relatively small sample size comprising Chinese Han population precluded generalization of the results to different ethnic populations. Second, the complement system is a complex network, and the relationships between *C6* gene polymorphisms, C6 expression, and MAC activity require validation in further studies involving larger liver transplant cohorts of patients with HCC.

In conclusion, patients with OLT for HCC require different therapeutic regimens compared with patients with etiologically distinct liver disease. Specifically, this study found that recipient *C6* rs9200 allele G was associated with rapid tacrolimus metabolism during the first 3 weeks after transplantation, and donor *C6* rs10052999 homozygote represented a marker for rapid tacrolimus metabolism at weeks 2, 3, and 4 after OLT. Further, this study confirmed the association of *CYP3A5* rs776746 SNPs with tacrolimus C/D ratios. The combination of *C6* and *CYP3A5* polymorphisms exerted a greater effect on tacrolimus metabolism than individual SNPs and developed better equations that described the association between genotype and tacrolimus metabolism. The findings facilitated the design of appropriate and safe therapeutic regimens for the management of patients with OLT for HCC.

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Conflicts of interest

There are no conflicts of interest.

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