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Genetic variation in the *LMP/TAP* gene and outcomes of hepatitis B virus infection in the Chinese population

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SUMMARY

Genetic polymorphisms of the *LMP/TAP* gene coded by the HLA-II region may be associated with outcomes of HBV infection. We conducted a case-control study to test the hypothesis, including a persistent group of 155 patients with chronic hepatitis B and 36 healthy carriers, a recovered group of 165 individuals spontaneously recovered from HBV infection, and an uninfected group of 278 healthy normal controls. Genotypes of eight polymorphisms of the *LMP/TAP* gene were analysed by PCR–RFLP. A logistic regression model was used to analyse statistical differences in polymorphisms or haplotypes in different groups. Of the eight polymorphisms, two (*TAP1* codon 637 and *LMP7* codon 145) were observed to have statistically significant association with outcomes of HBV infection ($P<0{\cdot}05$). The two-locus haplotype constructed with two such polymorphisms was analysed. The frequencies of haplotypes B (Asp-Lys), C (Gly-Gln), and D (Gly-Lys) were found to be increased significantly in the persistent group, compared to healthy controls (OR 2·26, 95% CI 1·62–3·15, $P<0{\cdot}001$; OR 2·37, 95% CI 1·69–3·32, $P<0{\cdot}001$; OR 4·38, 95% CI 1·78–10·77, $P=0{\cdot}001$, respectively). The prevalence of haplotypes B (Asp-Lys), C (Gly-Gln), and D (Gly-Lys) were also significantly higher in the persistent infectious group than in the recovered group (OR 2·68, 95% CI 1·81–3·98, $P<0{\cdot}001$; OR 2·40, 95% CI 1·62–3·55, $P<0{\cdot}001$; OR 3·03, 95% CI 1·22–7·55, $P=0{\cdot}017$, respectively). These findings indicated that genetic polymorphisms of the *LMP/TAP* gene might be an important factor in determining the outcome of HBV infection.

Key words: Hepatitis B virus, infection, *LMP/TAP* gene, outcome, polymorphism.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem worldwide, especially in China. The clinical features of HBV infection vary from clearance of the virus to fulminant hepatitis. Currently, the mechanism of susceptibility to chronic persistent HBV infection is not clear. The outcomes of HBV infection do not appear to be determined by virulence variations in viral strains. Instead, the course of the disease might be influenced by the host immune response [1, 2]. The hepatocellular injuries caused by HBV infection are predominantly immune-mediated.

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Immune attacks by the host against HBV are mainly mediated by a cellular response to small epitopes of HBV proteins, especially HBcAg, presented on the surface of liver cells. The human major histocompatibility complex (MHC) class-II region contains a cluster of genes whose products play an important role in processing intracellular proteins. Of these genes, *TAP1*, *TAP2*, *LMP2*, and *LMP7* are located between human MHC class-II DQB1 and DPB1 loci, and have been shown to be necessary in the MHC class-I antigen presentation pathway [3]. Therefore, genetic polymorphisms of *LMP/TAP* genes may also have a role in determining outcomes of HBV infection.

MHC class-I molecules are cell-surface glycoprotein, which bind intracellularly processed peptides and present them on the cell surface to cytotoxic T lymphocytes. Class-I molecules, therefore, play a key role in immune recognition of virally infected and transformed cells [4]. Two groups of proteins that participate in antigen processing are low-molecular-weight polypeptides (LMPs) and transporters with antigen processing (TAP). LMP2 and LMP7 are proteasome components that are able to enhance the proteolytic production of certain peptides [5, 6], while TAP1 and TAP2 form heterodimers and pump antigenic peptides into the lumen of the endoplasmic reticulum [7, 8]. Down-regulation of TAP1, TAP2, LMP2, and LMP7 was found to suppress MHC class-I molecule surface expression [9, 10].

Limited polymorphisms in the coding regions of the human *LMP/TAP* gene have been described in previous studies. It was reported that these polymorphisms were related to a number of immune diseases, including spondyloarthritis, juvenile rheumatoid arthritis (JRA), type I diabetes, and malignant diseases [11–14]. Other reports revealed that LMP and TAP proteins were strongly correlated with the immune response to viral infection such as hepatitis C virus (HCV), Epstein–Barr virus (EBV), measles virus (MV), and canine distemper virus (CDV) [15–17]. Based on the noticeable influence in immune diseases and virus infection, we hypothesized that *LMP/TAP* gene polymorphisms might be associated with different outcomes of HBV infection. In the current study we performed genotyping analyses to test the hypothesis for these single nucleotide polymorphisms (SNPs) in clinically well-defined groups of cases and controls from a Chinese population.

SUBJECTS AND METHODS

Study subjects

A total of 356 cases with a history of HBV infection were recruited between December 2006 and June 2009 from the Wuxi 101 Hospital and Wuxi Infectious Hospital. Controls were matched to cases by age (± 5 years), gender, and geographical area and were selected from Wuxi and surrounding regions, Jiangsu Province, China, during the same time period. Subjects were categorized into three different groups: (1) individuals who tested HBsAg negative and both anti-HBc and anti-HBs negative (uninfected group); (2) individuals who tested HBsAg negative and anti-HBs or anti-HBc positive but who had not been vaccinated for HBV (recovered group); and (3) individuals who tested HBsAg positive for at least 6 months by enzyme-linked immunosorbent assay (ELISA) (persistent group). The exclusion criteria for subjects included seropositivity for anti-HCV or anti-HIV, having other types of liver diseases, e.g. autoimmune liver diseases, alcoholic liver diseases, or metabolic liver diseases.

Each participant was scheduled for an interview after written informed consent was obtained, and a structured questionnaire was administered by trained interviewers to collect information on demographic data and environmental exposure history including tobacco smoking and alcohol consumption, etc. Those who had smoked <1 cigarette per day for <1 year during their lifetime were defined as non-smokers; otherwise they were considered as smokers. Those smokers who had quit smoking for >1 year were considered former smokers. Similarly, those that had consumed <3 alcoholic drinks a week for <6 months during their lifetime were defined as non-drinkers; otherwise they were considered as drinkers. Those drinkers who had quit for >1 year were considered former drinkers. After interview, a 5-ml peripheral blood sample was drawn from all participating subjects and stored at $-20\,^{\circ}C$ until assay.

Genotyping assays

Genomic DNA of each subject was extracted from peripheral blood leukocytes by sodium dodecyl sulphate (SDS) lysis and proteinase K digestion followed by standard phenol–chloroform purification as previously described [18]. The eight polymorphisms

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Table 1. *Primers used for* LMP/TAP *genotyping*

Gene	Position and substitution for the amino acid	PCR primers (forward/reverse)	Annealing temperature	Enzyme and fragments (bp)	SNP ID
TAP1	333 (ATC→GTC) Ile→Val	5-GCAGGTAACATCATGTCTCG-3 5-GACAGATTGTGGGGAGAAGC-3	60 °C	*Bcl*I (157+273)	rs1057141
	637 (ACG→GCG) Asp→Gly	5-CAGTAGTCTTGCCTTTATCC-3 5-ATGACTGCCTCACCTGTAAC-3	56 °C	*Acc*I (262+143)	rs1135216
TAP2	379 (GTA→ATA) Val→Ile	5-GAACGTGCCTTGTACCTGCG[C]^{a}-3 5-ACCCCCAAGTGCAGCAC-3	60 °C	*Bst*UI (20+192)	rs1800454
	565 (GCA→ACA) Ala→Thr	5-CCGGTTCTGTGAGGAACAACAG[T]^{b}-3 5-GGAGCAAGCTTACAATTTGT-3	54 °C	*Rsa*I (23+132)	rs2228396
	665 (ACA→GCA) Thr→Ala	5-GGTGATTGCTCACAGGCTGCC[G]^{c}-3 5-CACAGCTCTAGGGAAACTC-3	54 °C	*Msp*I (20+207)	rs241447
	687 (TAG→CAG) Stop→Gln	5-TGCAGAAGCTTGCCCAGCTC-3 5-CTGGAGACGCCCCTGAGAAGAG-3	67 °C	*Bfa*I (20+96)	rs241448
LMP2	60 (GCA→ACA) Arg→His	5-GTGAACCGAGTGTTTGACAAGC-3 5-GCCAGCAAGAGCCGAAACAAG-3	56 °C	*Hin*6I (39+213)	rs17587
LMP7	145 (CAG→AAG) Gln→Lys	5-TCATGGCGCTACTAGATGTATG-3 5-AACTCTTTGTCCTAACTTGCAC-3	54 °C	*Bsm*I (146+205)	rs2071543

Underlined nucleotides were altered from germline sequence to create restriction sites at the SNP. SNP ID reference numbers are from the National Center for Biotechnology Information Single Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/SNP).

[a] Underlined nucleotide in primer TAP2-1 was changed from the germline T to G to create the *Bst*UI RFLP.

[b] Underlined nucleotide in primer TAP2-2 was changed from the germline T to G to create the *Rsa*I RFLP.

[c] Underlined nucleotide in primer TAP2-4 was changed from the germline A to C to create the *Msp*I RFLP.

of the *LMP/TAP* gene were detected by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis according to methods previously reported [19]. PCR was carried out in a 20-μl volume with 10 × buffer [500 mM KCl, 100 mM Tris–HCl (pH 8·8), 25 mM $MgCl_2$] to a final concentration of 1×, 1 pmol of each specific oligonucleotide primer (Table 1), 100 μM dNTPs, 50 ng genomic DNA, and 1 U *Taq* DNA polymerase (Boehringer Mannheim, Germany). PCR was performed using a MJ-PTC-200 Thermal Cycler (MJ Research Inc., USA) under the following conditions: 3 min at 95 °C; 30 rounds of denaturation at 95 °C for 40 s, annealing at 54–67 °C for 45 s (Table 1), extension at 72 °C for 50 s; final extension at 72 °C for 10 min. The amplified PCR products were digested using a specific restriction endonuclease (New England Biolabs, USA). The digested fragments were electrophoresed in a 2–3% agarose gel (Biowest agarose, Spain) according to the manufacturer's instructions, and visualized by ethidium bromide staining. The sizes of fragments were estimated by comparison with markers (Tiangen Biotech, China).

Statistical analysis

The difference in the distribution of gender, smoking, and drinking between cases and controls was analysed by χ^2 test, and age by one-way ANOVA. Hardy–Weinberg equilibrium was tested by χ^2 test. The haplotype frequencies were estimated from observed positive genotypes using Phase 1·0 software [20]. Odds ratios (ORs) of polymorphisms or haplotypes were estimated using unconditional logistic regression models. The ORs and 95% confidence intervals (CIs) were estimated using unconditional logistic regression analysis that was adjusted by gender, age, smoking, and drinking. The *P* value reported was two-sided and values of $P<0{\cdot}05$ were considered statistically significant. All analyses were performed with SAS software (version 9.1.3, SAS Institute, USA).

RESULTS

A total of 356 cases with history of HBV infection and 278 normal controls were enrolled in this study. The study groups consisted of 165 subjects (101 males,

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Table 2. *Distribution of selected variables and risk factors in each study group*

Variables	Persistent group ($n=191$) (%)	Recovered group ($n=165$) (%)	Healthy controls ($n=278$) (%)	*P* value
Age (mean ± S.D.)	41·01 ± 14·32	40·82 ± 11·36	40·15 ± 9·13	0·697*
Gender				
Male	128 (67·0)	101 (61·2)	168 (60·4)	0·319†
Female	63 (33·0)	64 (38·8)	110 (39·6)	
Smoking				
Non-smokers	119 (62·3)	94 (57·0)	203 (73·0)	
Former smokers	26 (13·6)	5 (3·0)	11 (4·0)	0·000†
Current smokers	46 (24·1)	66 (40·0)	64 (23·0)	
Drinking				
Non-drinkers	144 (75·4)	108 (65·5)	215 (77·3)	
Former drinkers	12 (6·3)	13 (7·9)	5 (1·8)	0·006†
Current drinkers	35 (18·3)	44 (26·7)	58 (20·9)	

S.D., Standard deviation.
* One-way ANOVA.
† χ^2 test.

64 females) in the HBV recovered group, 191 patients (128 males, 63 females) in the HBV persistent group, and 278 normal controls (168 males, 110 females) in the uninfected group. The baseline characteristics of cases and healthy controls are summarized in Table 2. The mean age (± S.D., years) was 41·01 ± 14·32 for the persistent group, 40·82 ± 11·36 for the recovered group, and 40·15 ± 9·13 for controls. There was no significant difference in the distribution of age and gender in the recovered group, persistent group, and healthy controls ($P>0·05$), suggesting that our frequency-matching was adequate. However, significant difference regarding smoking ($P<0·001$) and drinking ($P=0·006$) was found in all three groups. Of the 191 patients in the persistent group and the 165 subjects in the recovered group, the successful response rates for age of infection were both >85%. The mean age of infection (± S.D., years), defined as the time they were diagnosed as HBsAg positive for the first time, was 34·81 ± 13·85 for the persistent group ($n=163$) and 32·67 ± 10·96 for the recovered group ($n=142$). No significant difference was found between the two groups in age of infection (Student's $t=1·48$, $P=0·140$).

Eight polymorphisms in the coding regions of *LMP/TAP* genes, which were previously reported [11, 21–23], were detected. The genotype data were summarized in Table 3 for all study participants. The distribution of the observed genotypes of *LMP2*, *LMP7*, *TAP1*, and *TAP2* genes were not significantly different from the expected distribution according to Hardy–Weinberg equilibrium in the controls.

Comparison of the frequency of *TAP/LMP* genotypes between the recovered and control groups

Of the total eight polymorphisms of the *LMP/TAP* gene, no difference was found in the genotype frequency between cases and controls as revealed by multiple logistic regression analysis.

Comparison of the frequency of *TAP/LMP* genotypes between the persistent and control groups

Logistic regression analysis revealed that compared to the *TAP1* codon 637 Asp/Asp homozygote, subjects carrying the Asp/Gly heterozygote had a significant 1·95-fold increased risk of persistent infection (95% CI 1·31–2·90) and those carrying the Gly/Gly homozygote had a significant 3·55-fold increased risk (95% CI 1·57–8·02). Compared to the reference subjects with the *LMP7* codon 145 Gln/Gln homozygote, subjects with the Gln/Lys heterozygote had an increased risk of persistent infection (OR 2·04, 95% CI 1·36–3·05), and the risk for those with the Lys/Lys homozygote was also significantly increased (OR 2·69, 95% CI 1·27–5·69), as shown in Table 3.

Comparison of the frequency of *TAP/LMP* genotypes between the recovered and persistent groups

As shown in Table 4, the prevalence of the *TAP1* codon 637 Asp/Gly heterozygote or Gly/Gly homozygote was significantly higher in the persistent group than in the recovered group (OR 1·88, 95% CI 1·19–3·00, $P=0·007$; OR 2·76, 95% CI 1·09–6·89,

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Table 3. *Analysis of association between* TAP/LMP *polymorphisms and risk of HBV infection*

Genotype	Healthy control $n=278$ (%)	Recovered group $n=165$ (%)	P*	OR (95%CI)*	Persistent group $n=191$ (%)	P*	OR (95%CI)*
TAP1-333							
Ile/Ile	182 (65·5)	98 (59·4)	—	1·00 (reference)	117 (61·3)	—	1·00 (reference)
Ile/Val	87 (31·3)	61 (37·0)	0·250	1·28 (0·84–1·96)	64 (33·5)	0·742	1·07 (0·71–1·61)
Val/Val	9 (3·2)	6 (3·6)	0·545	1·40 (0·47–4·21)	10 (5·2)	0·268	1·72 (0·66–4·49)
TAP1-637							
Asp/Asp	169 (60·8)	97 (58·8)	—	1·00 (reference)	78 (40·8)	—	1·00 (reference)
Asp/Gly	98 (35·3)	60 (36·4)	0·829	1·05 (0·69–1·59)	96 (50·3)	0·001	1·95 (1·31–2·90)
Gly/Gly	11 (4·0)	8 (4·8)	0·452	1·46 (0·54–3·92)	17 (8·9)	0·002	3·55 (1·57–8·02)
TAP2-379							
Val/Val	216 (77·7)	117 (70·9)	—	1·00 (reference)	140 (73·3)	—	1·00 (reference)
Val/Ile	56 (20·1)	44 (26·7)	0·120	1·45 (0·91–2·32)	46 (24·1)	0·537	1·16 (0·73–1·83)
Ile/Ile	6 (2·2)	4 (2·4)	0·696	1·30 (0·35-4·89)	5 (2·6)	0·518	1·49 (0·44-5·01)
TAP2-565							
Ala/Ala	228 (82·0)	134 (81·2)	—	1·00 (reference)	146 (76·5)	—	1·00 (reference)
Ala/Thr	49 (17·6)	29 (17·6)	0·935	1·02 (0·60–1·73)	43 (22·5)	0·192	1·37 (0·85–2·19)
Thr/Thr	1 (0·4)	2 (1·2)	0·242	4·41 (0·37–53·22)	2 (1·0)	0·592	2·00 (0·16–24·85)
TAP2-665							
Arg/Arg	100 (36·0)	55 (33·3)	—	1·00 (reference)	84 (44·0)	—	1·00 (reference)
Arg/Cys	140 (50·3)	86 (52·1)	0·627	1·12 (0·72–1·73)	86 (45·0)	0·091	0·70 (0·47–1·06)
Cys/Cys	38 (13·7)	24 (14·5)	0·509	1·24 (0·66–2·31)	21 (11·0)	0·135	0·62 (0·33–1·16)
TAP2-687							
Thr/Thr	102 (36·7)	52 (31·5)	—	1·00 (reference)	75 (39·3)	—	1·00 (reference)
Thr/Ala	127 (45·7)	85 (51·5)	0·278	1·28 (0·82–2·01)	90 (47·1)	0·754	0·94 (0·62–1·42)
Ala/Ala	49 (17·6)	28 (17·0)	0·564	1·19 (0·66-2·15)	26 (13·6)	0·218	0·70 (0·39-1·24)
LMP2							
Arg/Arg	192 (69·1)	118 (71·5)	—	1·00 (reference)	132 (69·1)	—	1·00 (reference)
Arg/His	81 (29·1)	43 (26·1)	0·566	0·88 (0·56–1·38)	49 (25·7)	0·469	0·85 (0·56–1·31)
His/His	5 (1·8)	4 (2·4)	0·728	1·28 (0·33–5·01)	10 (5·2)	0·065	2·88 (0·94–8·83)
LMP7							
Gln/Gln	163 (58·6)	104 (63·0)	—	1·00 (reference)	78 (40·8)	—	1·00 (reference)
Gln/Lys	100 (36·0)	51 (30·9)	0·214	0·76 (0·49–1·17)	94 (49·3)	0·001	2·04 (1·36–3·05)
Lys/Lys	15 (5·4)	10 (6·1)	0·816	0·90 (0·38–2·14)	19 (9·9)	0·010	2·69 (1·27–5·69)

OR, Odds ratio; CI, confidence interval.
* Logistic regression model, adjusted by gender, age, smoking and drinking.

$P=0·032$, respectively). Similarly, the prevalence of the *LMP7* codon 145 Gln/Lys heterozygote or Lys/Lys homozygote was more significantly found in the persistent infectious group than in the recovered group (OR 2·60, 95% CI 1·62–4·17, $P<0·001$; OR 2·65, 95% CI 1·12–6·24, $P=0·026$, respectively).

Haplotype analysis between the recovered and persistent groups

Of the eight polymorphisms, two (*TAP1* codon 637 and *LMP7* codon 145) were observed to have statistically significant association with outcomes of HBV infection. Since LMP7 and TAP1 are located next to each other, the extended two-locus haplotypes were constructed by Phase 1.0 software to cover SNPs in the *LMP7* codon 145 and *TAP1* codon 637. A total of four haplotypes (A, B, C, D) were constructed and found in healthy control, recovered, and persistent groups. The distribution of different haplotypes in the three groups is shown in Table 5. The sum of A, B, C, and D haplotype frequencies corresponded to 100% genotypes in the three groups. By using the logistic regression model analysis adjusted by interferential factors, haplotypes B (Asp-Lys), C (Gly-Gln), and D (Gly-Lys) were shown to present significant differences between the healthy control and persistent groups (OR 2·26, 95% CI 1·62–3·15, $P<0·001$; OR

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Table 4. *Comparison of frequencies of* TAP/LMP *genotype frequencies between the persistent and recovered groups*

Genotype	Persistent group ($n=191$) (%)	Recovered group ($n=165$) (%)	P*	OR (95% CI)*
TAP1-333				
Ile/Ile	117 (61·3)	98 (59·4)	—	1·00 (reference)
Ile/Val	64 (33·5)	61 (37·0)	0·303	0·78 (0·49–1·25)
Val/Val	10 (5·2)	6 (3·6)	0·874	0·91 (0·29–2·86)
TAP1-637				
Asp/Asp	78 (40·8)	97 (58·8)	—	1·00 (reference)
Asp/Gly	96 (50·3)	60 (36·4)	0·007	1·88 (1·19–3·00)
Gly/Gly	17 (8·9)	8 (4·8)	0·032	2·76 (1·09–6·98)
TAP2-379				
Val/Val	140 (73·3)	117 (70·9)	—	1·00 (reference)
Val/Ile	46 (24·1)	44 (26·7)	0·375	0·79 (0·48–1·32)
Ile/Ile	5 (2·6)	4 (2·4)	0·960	1·04 (0·26–4·09)
TAP2-565				
Ala/Ala	146 (76·5)	134 (81·2)	—	1·00 (reference)
Ala/Thr	43 (22·5)	29 (17·6)	0·389	1·27 (0·73–2·21)
Thr/Thr	2 (1·0)	2 (1·2)	0·715	0·68 (0·08–5·57)
TAP2-665				
Arg/Arg	84 (44·0)	55 (33·3)	—	1·00 (reference)
Arg/Cys	86 (45·0)	86 (52·1)	0·090	0·66 (0·41–1·07)
Cys/Cys	21 (11·0)	24 (14·6)	0·205	0·63 (0·31–1·28)
TAP2-687				
Thr/Thr	75 (39·3)	52 (31·5)	—	1·00 (reference)
Thr/Ala	90 (47·1)	85 (51·5)	0·340	0·79 (0·49–1·28)
Ala/Ala	26 (13·6)	28 (17·0)	0·465	0·78 (0·40–1·52)
LMP2				
Arg/Arg	132 (69·1)	118 (71·5)	—	1·00 (reference)
Arg/His	49 (25·7)	43 (26·1)	0·969	1·01 (0·61–1·67)
His/His	10 (5·2)	4 (2·4)	0·179	2·36 (0·68–8·25)
LMP7				
Gln/Gln	78 (40·8)	104 (63·0)	—	1·00 (reference)
Gln/Lys	94 (49·3)	51 (30·9)	<0·001	2·60 (1·62–4·17)
Lys/Lys	19 (9·9)	10 (6·1)	0·026	2·65 (1·12–6·24)

* Logistic regression model, adjusted by gender, age, smoking and drinking.

Table 5. *Frequencies of haplotypes constituted with polymorphisms of* TAP1-637 *and* LMP7 *in three groups*

Haplotype	Loci: [*TAP1*-637]-[*LMP7*]	Healthy controls ($n=556$) (%)	Recovered group ($n=330$) (%)	Persistent group ($n=382$) (%)
A	Asp-Gln	314 (56·5)	192 (58·2)	134 (35·1)
B	Asp-Lys	122 (21·9)	63 (19·1)	117 (30·6)
C	Gly-Gln	112 (20·1)	67 (20·3)	116 (30·4)
D	Gly-Lys	8 (1·5)	8 (2·4)	15 (3·9)

2·37, 95% CI 1·69–3·32, $P<0·001$; OR 4·38, 95% CI 1·78–10·77, $P=0·001$, respectively). Similarly, there were significant differences in the haplotype frequencies of B, C, and D between the recovered and persistent groups (OR 2·68, 95% CI 1·81–3·98, $P<0·001$; OR 2·40, 95% CI 1·62–3·55, $P<0·001$; OR 3·03, 95% CI 1·22–7·55, $P=0·017$, respectively). As shown in Figure 1, patients carrying [*TAP1*-637]-[*LMP7*-145] haplotype D showed the greatest risk of having persistent HBV infection.

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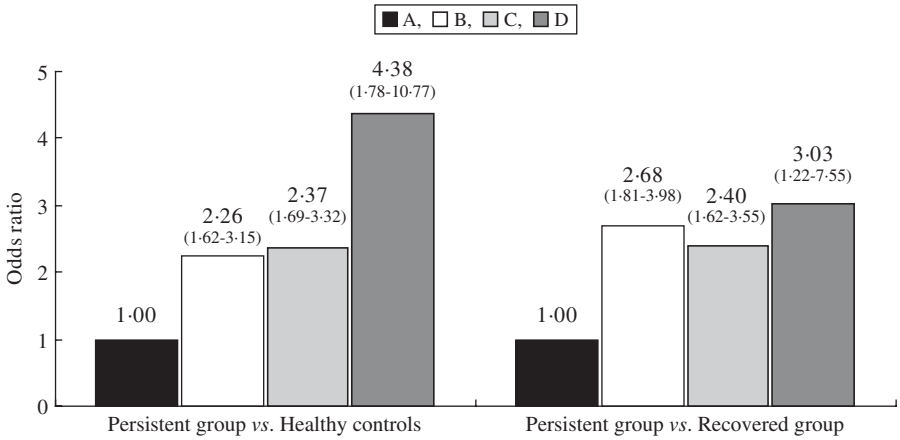

Fig. 1. Odds ratios for the risk of [*TAP1*-637]-[*LMP7*-145] haplotypes in patients with persistent HBV infection for A (Asp-Gln), B (Asp-Lys), C (Gly-Gln), and D (Gly-Lys).Odds ratio adjusted by logistic regression model for gender, age, smoking, and drinking. The odds ratio (OR 1·00) for the most common haplotype A (Asp-Gln) was used as the reference. All *P* values of odds ratios for haplotypes B, C, and D were $<0{\cdot}05$.

DISCUSSION

An estimated 350 million people worldwide are chronically infected with HBV. Host genetic and environmental factors are widely viewed as the common basis of the different outcomes of HBV infection. The HBV antigen recognition by cytotoxic $CD8^+$ cells is dependent upon a number of crucial steps in antigen processing, which include cleavage of antigen peptides by LMP2/LMP7, transportation into the endoplasmic reticulum by TAP1/TAP2, and binding to human MHC class-I molecule and β_2-microglobulin (β_2-m). In this process, the recognition of HBV antigen peptides, which are derived from intracellular processing and presentation on the liver cell surface by human MHC class-I molecules, leads to direct HBV elimination by human MHC class-I restricted $CD8^+$ cells [24]. Therefore, the *LMP/TAP* gene plays an important role in the immunological reaction to HBV infection.

A previous study showed that in the seven known polymorphisms of *LMP/TAP* genes in the Chinese population, the LMP7-145, TAP1-637, and TAP2-651 sites were associated with significantly increased risk of persistent infection of HBV [25]. The study by Dai *et al*. [21] suggested that in the two known polymorphisms of *LMP2/LMP7* genes in the Chinese population, the LMP7-145 site was associated with significantly increased risk of persistent infection of HBV. The current report further studied the association between genetic polymorphisms of the *LMP/TAP* gene and outcomes of HBV infection in the Chinese population. In this study, eight polymorphisms in the *LMP/TAP* gene were identified, two of which (*TAP1* codon 637 and *LMP7* codon 145) were associated with outcomes of HBV infection. The LMP7-145 and TAP1-637 sites were associated with significantly increased risk of persistent infection of HBV, which is in accord with previous studies [21, 25], suggesting that genetic variation in the *LMP/TAP* gene may play an important role in the development of HBV infection. However, the biological function of naturally occurring *TAP* polymorphisms is inconclusive [26]. The study conducted in human cells demonstrated no significant influence of *TAP* polymorphism on peptide selection [27]. Recently, *TAP1* polymorphism has been shown to influence peptide substrate specificity in human lymphoblastoid and tumour cell lines [28]. Nevertheless, an *in vitro* assay reported that different combinations of *TAP1* and *TAP2* allelic products did not result in an alternative peptide selection [29]. There are few documented studies determining the biological function of *LMP* polymorphisms. It has only been speculated that an amino-acid variation at *LMP7* codon 145 causing the electrical charge might have functional consequences [30]. In addition, a recent study showed that LMP7 could influence the structural features of 20S proteasomes, which in turn dramatically enhance the catalytic activity of LMP2 and MECL-1 prompting cleavage specificity. Therefore, LMP7 incorporation was of more functional importance for the generation of an HBV epitope CD8+ CTLs with cleavage specificity [31].

It is noteworthy that the [*TAP1*-637]-[*LMP7*-145] haplotype carries the *LMP7* codon 145 variation.

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Further experiments to characterize the functional impact of the [*TAP1*-637]-[*LMP7*-145] haplotype in antigen processing will be of interest. Recent studies have demonstrated that haplotype analysis might be superior in predicting the disease associations to polymorphism analysis [32–35]. Therefore, the present investigation was extended to analyse the haplotypes of *LMP7* codon 145 and *TAP1* codon 637 polymorphisms. Logistic regression analysis of each polymorphism showed that both polymorphisms were associated with the risk of HBV infection. These two polymorphisms within a haplotype might have an additional effect on the risk of persistent infection of HBV when considering: (i) the model incorporating both polymorphisms fitted much better than the model with *LMP7* codon 145 or *TAP1* codon 637 alone; (ii) the effect of the [*TAP1*-637]-[*LMP7*-145] haplotype on outcomes of HBV infection was greater than either *LMP7* codon 145 or *TAP1* codon 637 alone. In haplotype analysis, two haplotypes (B: Asp-Lys, C: Gly-Gln) showed statistical differences in the comparison of persistent *vs*. control or recovered groups. Furthermore, the subjects carrying [*TAP1*-637]-[*LMP7*-145] haplotype D showed an additive risk of having persistent HBV infection. The results suggest that these two polymorphisms might interact with each other during antigen processing and presentation of HBV.

Several limitations in our study need to be addressed. First, although this was a population-based case-control study, selection bias was inevitable and the subjects might not be representative of the general population. However, potential confounding factors were minimized by matching on age, gender, and residential area. In addition, other potential influencing factors to outcomes of HBV infection, e.g. age of infection, smoking, and drinking habits of the subjects, were also considered in this paper. Second, although we had 155 patients with chronic hepatitis B and 36 healthy carriers as a persistent group, 165 individuals spontaneously recovered from HBV infection, and 278 normal controls, the sample size of the current study might not be large enough to detect small effects from low penetrance genes. Finally, no biological and functional relevance can be assumed on the basis of this SNP approach, and additional studies are needed to identify the disease-causing SNPs.

In conclusion, *LMP*/*TAP* gene polymorphisms and their haplotypes may have a key influence on *LMP*/*TAP* activity and are significantly associated with outcome of HBV infection in the Chinese population. Thus our findings provid support for the concept that genetic factors (e.g. *LMP* and *TAP* genes) are, to some extent, associated with outcomes of HBV infection.

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DECLARATION OF INTEREST

None.

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