RESEARCH ARTICLE

Correlation between *TLR2*, *TLR3*, *TLR4*, and *TLR9* polymorphisms and susceptibility to and prognosis of severe hepatitis among the newborns

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Background: This study was aimed to explore how toll-like receptor 2 (*TLR2*), *TLR3*, *TLR4* and *TLR9* influenced the risk and prognosis of severe hepatitis among the Chinese newborns.

Methods: Altogether 135 newborns diagnosed with severe hepatitis and 140 healthy newborns were included in this study. Totally 12 single nucleotide polymorphisms (SNPs) within *TLR2*, *TLR3*, *TLR4*, and *TLR9* were chosen and genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the logistic regression. The univariate and multivariate analyses were used to analyze independent factors for prognosis of severe hepatitis among the Chinese newborns.

Results: The SNPs within *TLR2* [ie, rs1898830 (A>G) and rs3804100 (T>C)], *TLR3* [ie, rs1879026 (G>T)], *TLR4* [ie, rs2149356 (T>G)], and *TLR9* [ie, rs187084 (T>C), rs352139 (A>G), and rs352140 (C>T)] were significantly associated with modified risk of neonatal severe hepatitis (all $P < .05$). Furthermore, rs1898830, rs1879026, rs187084 and rs352139 were also demonstrated to modulate the prognosis [ie, aspartate aminotransferase (AST)/alanine transaminase (ALT) > 1.5] of newborns with severe hepatitis (all $P < .05$). Interestingly, the haplotype A-C-G-G-C-A-T were associated with higher susceptibility to neonatal severe hepatitis, and the newborns carrying haplotype A-C-G-G-C-A-T appeared to be correlated with more favorable prognosis (all $P < .05$).

Conclusions: Certain SNPs and haplotypes within *TLR2*, *TLR3*, *TLR4*, and *TLR9* can be considered as the potentially treatment targets for neonatal severe hepatitis.

KEYWORDS

neonatal severe hepatitis, prognosis, SNP, susceptibility, *TLR2*, *TLR3*, *TLR4*, *TLR9*

1 | INTRODUCTION

Perinatal infection was the main cause of neonatal liver damage, especially the fetal infection caused by TORCH syndrome (ie, toxoplasmosis, listerellosis, rubella virus, cytomegalovirus and herpes simplex virus). Human cytomegalovirus (HCMV) was widely located in the nature, and the positive rates of HCMV antibodies were, respectively, around 95% among pregnant women and 83.2% among children in

China.¹ Infection of HCMV could induce proportional changes of the subsets of T lymphocyte cells within infants, and determination of the cell subsets was of significance to judging the severity of active HCMV infection and guiding early-stage treatment.² Besides, liver not merely regulated the digestion, metabolism and endocrine systems, but also participated in the immune adjustment. For instance, there existed diverse immunoregulation-related cells within liver (eg, Kupffer cell, dendritic cell and lymphocyte), and the cells were responsible for antigen

presentation, engulf of pathogens, as well as secretion of cytokines and antibodies.³ To sum up, HCMV was vital for prediction of neonatal liver damage,⁴ and the genetic mechanisms underlying HCMV was hypothesized to partly explain the presence of neonatal liver damage.

Toll-like receptors (TLRs), which belonged to the pattern recognition receptor (PRR), were considered to count much in both innate immunity response and acquired immunity response.⁵ TLRs were expressed in diverse liver tissues, for example, TLR3 was principally expressed within Kupffer cells, hepatic cells and biliary epithelial cells, while TLR9 was mainly expressed within Kupffer cells and stellate cells.⁶ After human beings were infected with hepatitis virus, TLRs would activate immunity-related signaling pathway and induce inflammatory responses within liver cells, by virtue of identifying certain pathogen associated molecular patterns (PAMPs), including double stranded RNA (dsRNA), single-stranded RNA (ssRNA), or cytidylyl phosphate guanine (CpG).⁷ Owing to the fact that abnormal expressions of TLRs and high-level cytokines showed strong linkages with elevated susceptibility to viral hepatitis, liver cirrhosis, liver cancer and autoimmune hepatitis, and that TLR expressions were readily influenced by single nucleotide polymorphisms (SNPs) of TLRs, it was speculated that the SNPs within TLRs were also correlated with risk of certain liver disorders.

It has been demonstrated that SNPs within *TLR-2* and *TLR-9* were associated with congenital HCMV infection.^{8,9} Since HCMV infection was one major account for neonatal liver damage, it was hypothesized that the mutations of *TLR-2* and *TLR-9* might also lead to altered risk of neonatal liver damage. As for other molecules of the TLRs family, *TLR4* and *TLR9* were expressed remarkably higher in hepatic cells within patients with primary biliary cirrhosis and those with hepatitis C.¹⁰ Nevertheless, the studies of Morita et al. and Kikuchi et al. did not suggest an association of SNPs within *TLR4* and *TLR9* susceptibility to primary biliary cirrhosis.^{11,12} Moreover, it seemed that males carrying G allele of *TLR7* or C allele of *TLR8* were more liable to suffer from hepatitis C.¹³ An investigation targeting the Arabs also manifested that rs1879026 (A/C) could modify presence of hepatitis B.¹⁴ As a result, the SNPs of TLRs were quite possible to alter the risk of liver damage, but limited studies were concentrated on the neonatal liver damage among the Chinese newborns. In addition, previous study results differed on the association of SNPs within TLRs with susceptibility to liver damage, so further studies investigating similar contents were in demand.

All in all, this retrospective study was aimed to explore the associations of SNPs within *TLR2*, *TLR3*, *TLR4*, and *TLR9* with susceptibility to and prognosis of liver damage among the newborn, which might assist in finding potential therapy targets for congenital liver damage.

2 | MATERIALS AND METHODS

2.1 | Subjects

Totally 135 newborns with severe hepatitis were enrolled from the Central Hospital of Zhoukou City during the period between January 2013 and December 2016. They were confirmed with chronic severe viral hepatitis B, according to the diagnostic criteria of Prevention and

Treatment of Viral Hepatitis.¹⁵ Another 140 healthy newborns were randomly selected from the Central Hospital of Zhoukou City, and their hepatitis B virus markers were all indicated as negative. All the newborns were unrelated individuals of Han ethnicities. Besides, the newborns that suffered from malignant tumors or autoimmune diseases and those who were currently using immunosuppressive agents were excluded from this study. All the legal guardians of the newborns have signed informed consents. And this study was approved by the Central Hospital of Zhoukou City and the ethics committee of the Central Hospital of Zhoukou City.

2.2 | Genotyping

About 3 mL venous blood was extracted from each individual, and was then placed in the anti-coagulant blood vessels that contained ethylene diamine tetraacetic acid (EDTA). The blood was preserved in the -70°C refrigerator for extracting purified genomic DNAs, and multigelation was avoided.

Around 5 μ L genomic DNA was mixed with the proliferation system (50 μ L) that included 3.0 mM Mg²⁺, 200 μ M dNTPs, 2U Taq DNA polymerase, 1 μ M Pf, and 1 μ M PR. The mixture was transferred to GeneAmp9600 for proliferation of DNAs. The primers were designed based on the primer 5 software, and were synthesized with assistance of Shanghai Invitrogen Biotechnology Co. Ltd (Table S1). The reaction conditions were arranged as: (i) pre-denaturation at 94°C for 5 min; (ii) 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 30 sec; and (iii) extension at 72°C for 5 min. The 10 μ L amplified products were arranged for agarose gel electrophoresis for 10 min, and the remaining products were added into the GIA quick purification cartridge for purification after the quality of products was appraised. The genotyping was conducted with assistance of CEQ 8000 sequencer, and the results were compared with the standard sequences within GenBank.

2.3 | Prognosis of the newborns with severe hepatitis

All the subjects were followed up for up to 6 weeks. The aspartate amino transferase (AST) and alanine aminotransferase (ALT) were detected with usage of the Reitman Frankel assay, and their normal values were all set as <40 units/L. The ratio of AST and ALT were regarded as the prognostic indicator for the newborns with severe hepatitis. It was considered as favorable prognosis when AST/ALT was ≤ 1.5 , and the nit would be deemed as poor prognosis when AST/ALT was > 1.5 .

2.4 | Statistical analysis

All the statistics were analyzed based on the SPSS 21.0 software. The enumeration data were analyzed with chi-square test and Fisher exact test. The measurement data were analyzed using t-test after homogeneity test of variances, and they were presented in the form of mean $\pm$ standard deviation (SD). It would be considered statistically significant when $P < .05$.

3 | RESULTS

3.1 | Baseline characteristics of the subjects

The newborns with severe hepatitis and healthy newborns were approximate with respect to their sex ratio and delivery mode (both $P > .05$). Besides, the mean weight of newborns with severe hepatitis was generally less than the healthy ones ($P < .05$), and the AST/ALT ratio of newborns with severe hepatitis was much higher than that of the healthy newborns ($P < .05$) (Table 1).

3.2 | Association of SNPs within TLR2-4 or TLR-9 with risk of neonatal severe hepatitis

The frequency distributions of genotypes and alleles regarding the 13 SNPs all abided by the Hardy-Weinberg equilibrium (HWE) law between healthy newborns and the newborns with severe hepatitis (Table 2). For *TLR2* rs1898830 (A>G), newborns with allele G possessed a lower risk of severe hepatitis than those with allele A (OR=0.68, 95% CI=0.48-0.98, $P=.038$). It was also manifested that the allele distributions of rs3804100 (T>C) also varied greatly between the newborns with severe hepatitis and the healthy ones (C vs T, OR=1.60, 95% CI=1.06-2.42, $P=.024$). Furthermore, the newborns carrying allele T of *TLR3* rs1879026 (G>T) were less probable to suffer from severe hepatitis than the carriers of allele G (OR=0.45, 95% CI=0.29-0.69, $P<.001$). When compared with the T allele of *TLR4* rs2149356 (T>G), its G allele was found to elevate the newborns' susceptibility to severe hepatitis (OR=2.62, 95% CI=1.76-3.92, $P<.001$). Moreover, concerning *TLR9* rs187084 (T>C), it was suggested that newborns with severe hepatitis had a significantly higher frequency of allele C than allele T (OR=2.01, 95% CI=1.39-2.91, $P<.001$). And a lower incidence of severe hepatitis was noticed among newborns carrying allele G of rs352139 than ones with allele A (OR=0.50, 95% CI=0.35-0.71, $P<.001$).

3.3 | Association of the SNPs within TLR2-4 or TLR-9 with prognosis of neonatal severe hepatitis

The prognostic analyses showed that AG carriers of *TLR2* rs1898830 (A>G) were less inclined to suffer from undesirable prognosis (ie, AST/ALT >1.5) than AA carriers (OR=0.38, 95% CI: 0.17-0.82, $P=.01$) (Table 3). In a similar manner, a smaller ratio of AST/ALT was noticed

within GT carriers of *TLR3* rs1879026, when compared with the GG carriers (OR=0.29, 95% CI: 0.11-0.75, $P<.01$). With regard to the gene *TLR9*, the homozygote CC of rs187084 was correlated with a larger ratio of AST/ALT in comparison with genotype CC (OR=7.12, 95% CI: 1.28-59.63, $P=.04$), whereas the heterozygote AG of rs352139 might protect the newborns with severe hepatitis from poor prognosis (OR=0.43, 95% CI: 0.20-0.92, $P=.03$).

3.4 | Correlation between haplotypes of significant SNPs and risk or prognosis of neonatal severe hepatitis

In the light of the haplotype analysis (Table 4), it was implied that the haplotype A-C-G-G-C-A-T were associated with higher susceptibility to neonatal severe hepatitis than other haplotypes (OR=4.11, 95% CI=1.61-10.54, $P=.002$). Besides, the newborns carrying haplotype A-C-G-G-C-A-T appeared to be accompanied by more favorable prognosis (ie, AST/ALT ≤1.5) in comparison to other haplotypes (OR=0.21, 95% CI=0.08-0.54, $P<.001$) (Table 5).

4 | DISCUSSION

Viral hepatitis was a chronic infectious disorder, and TLRs functioned to antagonize the viral infection through generating pro-inflammatory and anti-viral cytokines.^{16,17} Since the positioning of TLRs varied, *TLR4* mainly recognized the lipopolysaccharide of bacteria cell walls, while *TLR3* and *TLR9*, respectively, identified the nucleic acid constituent of virus and the double-stranded DNAs that were rich in unmethylated CpG.^{18,19} Their expressions were also featured by regional distinctions. To be specific, *TLR2*, *TLR3*, and *TLR4* were primarily expressed within human's oligodendrocytes, whereas *TLR9* was expressed within human's astrocytes.²⁰

Interestingly, they functional mechanisms were also diverse. For one thing, *TLR2* and *TLR9* functioned in a MyD88-dependent way to successively activate IRAK and TRAF6, with TIR-domain MyD88 as the adaptor protein. The phosphorylated TRAF6 would then induce transforming growth factor- β -activated kinase (TAK1) to mobilize NF- κ B, and to facilitate its translocation into the cell nucleus, which encouraged the production and release of such inflammatory factors as IL-1, IL-6, IL-8, IL-12, TNF- α , and TNF- γ .^{21,22} For instance, Arawalli et al. conducted the primary culture for the wide-type mice

TABLE 1 Baseline characteristics of the subjects

Characteristics	Case Group	Control Group	χ^2/t -test	P-value
Participants (n)	135	140		
Male (n)	84	88	0.01	.91
Female (n)	51	52		
Eutocia (n)	72	92	4.34	.04
Cesarean (n)	63	48		
Weight (g)	2936±483	3758±509	13.73	<.01
AST/ALT	1.52±0.48	1.13±0.52	6.47	<.01

AST, aspartate amino transferase; ALT, alanine aminotransferase.
The bold values mean a significant results.

TABLE 2 Association of SNPs within *TLR2-4* or *TLR-9* with risk of neonatal severe hepatitis

Gene	Rs number	Allele Change	Genotype and Models	Case Group	Control	OR (95% CI)
TLR2	rs1898830	A>G	AA	70	57	Ref.
			AG	54	64	0.69 (0.60-0.78)
			GG	11	19	0.47 (0.33-0.66)
			Allelic model			0.68 (0.48-0.98)
			Dominant model			0.64 (0.40-1.03)
			Recessive model			0.56 (0.26-1.24)
	rs3804100	T>C	TT	4	9	Ref.
			TC	40	54	1.67 (0.75-3.68)
			CC	91	77	2.66 (1.25-5.66)
			Allelic model			1.60 (1.06-2.42)
			Dominant model			2.25 (0.68-7.49)
			Recessive model			1.69 (1.04-2.76)
	rs5743708	G>A	GG	43	44	Ref.
			GA	51	58	0.90 (0.76-1.06)
			AA	41	38	1.10 (0.91-1.33)
			Allelic model			1.06 (0.76-1.48)
			Dominant model			0.98 (0.59-1.63)
			Recessive model			1.17 (0.69-1.97)
TLT3	rs5743305	T>A	TT	57	59	Ref.
			TA	61	64	0.99 (0.87-1.12)
			AA	17	17	1.04 (0.77-1.39)
			Allelic model			1.01 (0.71-1.43)
			Dominant model			1.00 (0.62-1.61)
			Recessive model			1.04 (0.51-2.14)
	rs5743311	G>A	GG	91	96	Ref.
			GA	39	40	1.03 (0.89-1.18)
			AA	5	4	1.32 (0.52-3.32)
			Allelic model			1.07 (0.69-1.66)
			Dominant model			1.05 (0.64-1.75)
			Recessive model			1.31 (0.34-4.98)
	rs5743312	C>T	CC	95	101	Ref.
			CT	36	36	1.06 (0.92-1.23)
			TT	4	3	1.42 (0.43-4.63)
			Allelic model			1.10 (0.70-1.75)
			Dominant model			1.09 (0.65-1.84)
			Recessive model			1.39 (0.31-6.35)
	rs1879026	G>T	GG	98	73	Ref.
			GT	34	56	0.45 (0.39-0.52)
			TT	3	11	0.20 (0.08-0.49)
			Allelic model			0.45 (0.29-0.69)
			Dominant model			0.41 (0.25-0.68)
			Recessive model			0.27 (0.07-0.98)
TLR4	rs11536889	G>C	GG	102	103	Ref.
			GC	31	34	0.92 (0.79-1.08)
			CC	2	3	0.67 (0.13-3.58)
			Allelic model			0.89 (0.55-1.46)
			Dominant model			0.9 (0.52-1.55)
			Recessive model			0.69 (0.11-4.18)

(Continues)

TABLE 2 (Continued)

Gene	Rs number	Allele Change	Genotype and Models	Case Group	Control	OR (95% CI)
TLR9	rs2149356	T>G	TT	4	17	Ref.
			TG	38	64	2.52 (1.27-5.02)
			GG	93	59	6.70 (3.46-12.96)
			Allelic model			2.62 (1.76-3.92)
			Dominant model			4.53 (1.48-13.83)
			Recessive model			3.04 (1.85-4.99)
	rs4986791	C>T	CC	90	96	Ref.
			CT	40	39	1.09 (0.95-1.26)
			TT	5	5	1.07 (0.47-2.44)
			Allelic model			1.07 (0.69-1.66)
			Dominant model			1.09 (0.66-1.81)
			Recessive model			1.04 (0.29-3.67)
	rs187084	T>C	TT	8	21	Ref.
			TC	49	67	1.92 (1.28-2.89)
			CC	78	52	3.94 (2.64-5.88)
			Allelic model			2.01 (1.39-2.91)
			Dominant model			2.80 (1.20-6.57)
			Recessive model			2.32 (1.43-3.76)
	rs352139	A>G	AA	66	41	Ref.
			AG	57	69	0.51 (0.45-0.59)
			GG	12	30	0.25 (0.18-0.34)
			Allelic model			0.50 (0.35-0.71)
			Dominant model			0.43 (0.26-0.71)
			Recessive model			0.36 (0.17-0.73)
	rs352140	C>T	CC	13	21	Ref.
			CT	58	67	1.40 (1.03-1.90)
			TT	64	52	1.99 (1.45-2.72)
			Allelic model			1.41 (0.99-2.01)
			Dominant model			1.66 (0.79-3.46)
			Recessive model			1.53 (0.94-2.47)

SNP, single nucleotide polymorphism; TLR, Toll-like receptor.

The bold values mean a significant results.

transfected with herpesvirus hominis and ones deficit in TLR2, discovering that TLR2 was indispensable for CXCL-9 and CXCL-10 expressions.²³ For another, the signal transductions of TLR3 and TLR4 were in progress based on the TIR domain-containing adaptor (TRIF) in a MyD88-independent manner. Somewhat distinct from TLR3 that led the expressions of IFN- β and pro-inflammatory genes (eg, IL-6, interferon and TNF- α) via activating interferon regulatory factor 3 (IRF3),^{24,25} TLR4 guided the secretion of inflammatory factors through activation of NF- κ B.²⁶

Considering the role of TLR2, TLR3, TLR4, and TLR9 in the development of hepatitis, the SNPs that determined their expressions were hypothesized to be associated with susceptibility to multiple inflammations and infectious diseases. For instance, *TLR3*-1377C/T (ie, rs3775290) was intimately correlated with the inflammatory reactions derived from gastric carcinoma among a Chinese population.^{27,28} As far as *TLR3* rs1879026 was concerned, the frequency of its T allele

was significantly lower among the Saudi Arabians with hepatitis than among the healthy ones, and it seemed that haplotype GCGA could protect the people against hepatitis.¹⁴ Besides, the homozygote AA of *TLR4* rs4986790 might increase the risk of chronic hepatitis to 1.28 fold among the Caucasian males, while this trend was not applicable to the females.²⁹ Agundez et al. also found that rs2149356 located within *TLR4* was associated with altered risk of hepatitis and liver cancer.³⁰ Moreover, the findings of Xie et al. assumed that heterozygote CT of *TLR2* rs3804099 and rs3804100 served as the advantageous genotypes against primary hepatic carcinoma, yet hardly any associations were observed between a couple of SNPs within *TLR9* (ie, rs352139 and rs352140) and the cancer.³¹ A bit distinct from the aforementioned studies, the current study showed that rs352139 (A>G) and rs352140 (C>T) could serve as the biomarkers for the onset of severe hepatitis among the Chinese newborns. The distinctions were speculated as that the studied populations were different, and

TABLE 3 Association of the SNPs within *TLR2-4* or *TLR-9* with prognosis of neonatal severe hepatitis

Gene	Rs number	Allele Change	Genotype	AST/ALT≤1.5	AST/ALT>1.5	OR (95% CI)	χ^2	P-value
TLR2	rs1898830	A>G	AA	38	32	Ref.		
			AG	41	13	0.38 (0.17-0.82)	6.17	.01
			GG	7	4	0.68 (0.18-2.53)	0.34	.56
	rs3804100	T>C	TT	3	1	Ref.		
			TC	37	3	0.24 (0.02-3.12)	1.35	.25
			CC	46	45	2.94 (0.29-29.29)	0.92	.34
	rs5743708	G>A	GG	27	16	Ref.		
			GA	31	20	1.09 (0.47-2.51)	0.04	.84
			AA	28	13	0.78 (0.32-1.93)	0.28	.59
TLT3	rs5743305	T>A	TT	36	21	Ref.		
			TA	38	23	1.04 (0.49-2.19)	0.01	.92
			AA	12	5	0.71 (0.22-2.31)	0.32	.57
	rs5743311	G>A	GG	58	33	Ref.		
			GA	24	15	1.10 (0.51-2.38)	0.06	.81
			AA	4	1	0.44 (0.05-4.10)	0.55	.46
	rs5743312	C>T	CC	64	31	Ref.		
			CT	20	16	1.65 (0.75-3.62)	1.58	.21
			TT	2	2	2.07 (0.28-15.36)	0.52	.47
	rs1879026	G>T	GG	56	42	Ref.		
			GT	28	6	0.29 (0.11-0.75)	6.93	<.01
			TT	2	1	0.67 (0.06-7.60)	0.11	.74
TLR4	rs11536889	G>C	GG	64	38	Ref.		
			GC	20	11	0.93 (0.40-2.14)	0.03	.86
			CC	2	0	0.36 (0.02-7.17)	1.17	.28
	rs2149356	T>G	TT	3	1	Ref.		
			TG	35	3	0.26 (0.02-3.30)	1.23	.27
			GG	48	45	2.81 (0.28-28.05)	0.84	.36
	rs4986791	C>T	CC	56	34	Ref.		
			CT	27	13	0.79 (0.36-1.74)	0.33	.56
			TT	3	2	1.10 (0.17-6.91)	<0.01	.92
TLR9	rs187084	T>C	TT	7	1	Ref.		
			TC	40	9	1.58 (0.17-14.46)	0.16	.69
			CC	39	39	7.12 (1.28-59.63)	4.1	.04
	rs352139	A>G	AA	36	30	Ref.		
			AG	42	15	0.43 (0.20-0.92)	4.83	.03
			GG	8	4	0.82 (0.22-3.01)	0.09	.76
	rs352140	C>T	CC	8	5	Ref.		
			CT	40	18	0.72 (0.21-2.51)	0.27	.61
			TT	38	26	1.10 (0.32-3.72)	0.02	.88

AST, aspartate amino transferase; ALT, alanine aminotransferase; SNP, single nucleotide polymorphism; TLR, Toll-like receptor.
The bold values mean a significant results.

the investigated diseases were also discrepant, making the two study results less comparable.

All in all, this study firstly explored whether *TLR2*, *TLR3*, *TLR4*, and *TLR9* could contribute to regulate the risk of severe hepatitis among the Chinese newborns, however, several limitations could

not be neglected. First, the size of newborns was relatively small, and the studied subjects were all of Chinese Han ethnicities, so the results might not be well applicable to other groups. Second, the follow-up period was a bit short, and a comprehensive evaluation about the long-term prognosis of neonatal severe hepatitis

TABLE 4 Correlation between haplotypes of significant SNPs and risk of neonatal severe hepatitis

Haplotype	Case Group Number (%)	Case Group Number (%)	OR (95% CI)	χ^2	P-value
ACGGCAC	9 (0.07)	4 (0.03)	2.43 (0.73-8.08)	2.21	.14
ACGGCAT	21 (0.15)	6 (0.04)	4.11 (1.61-10.54)	9.86	<.01
ACGGCGT	9 (0.07)	5 (0.04)	1.93 (0.63-5.91)	1.36	.24
ACGGTAT	7 (0.05)	4 (0.03)	1.86 (0.53-6.50)	0.97	.32
ACGTCAT	4 (0.03)	3 (0.02)	1.39 (0.31-6.35)	0.19	.67
ATGGCAT	5 (0.03)	2 (0.02)	2.65 (0.51-13.92)	1.43	.23
GCGGCAT	8 (0.06)	4 (0.03)	2.14 (0.63-7.29)	1.55	.21

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.
The bold values mean a significant results.

TABLE 5 Correlation between haplotypes of significant SNPs and

prognosis of neonatal severe hepatitis

Haplotype	AST/ALT $\leq$ 1.5 Number (%)	AST/ALT $>$ 1.5 Number (%)	OR (95% CI)	χ^2	P-value
ACGGCAC	4 (0.05)	6 (0.13)	0.35 (0.09-1.31)	2.62	.11
ACGGCAT	8 (0.1)	16 (0.32)	0.21 (0.08-0.54)	11.64	.00
ACGGCGT	4 (0.05)	5 (0.1)	0.43 (0.11-1.68)	1.55	.21
ACGGTAT	4 (0.04)	2 (0.04)	1.15 (0.20-6.50)	0.02	.88
GCGGCAT	4 (0.05)	4 (0.08)	0.55 (0.13-2.30)	0.69	.41

AST, aspartate amino transferase; ALT, alanine aminotransferase; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.
The bold values mean a significant results.

needed to be further investigated. Finally, only the superficial correlation between the SNPs and susceptibility to severe hepatitis was studied, the underlying molecular mechanisms have not been derived.

In conclusion, certain SNPs within *TLR2* (eg, rs1898830 and rs3804100), *TLR3* (eg, rs1879026), *TLR4* (eg, rs2149356) and *TLR9* (eg, rs187084, rs352139, and rs352140) could serve as biomarkers for risk and prognosis of neonatal severe hepatitis among a Chinese population, yet a large-scale study was further in demand.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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