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RESEARCH PAPER

Study of the association of seventeen single nucleotide polymorphisms and their haplotypes in the *TNF-α, IL-2, IL-4* and *IL-10* genes with the antibody response to inactivated Japanese encephalitis vaccine

Yufeng Yao[a], Xiuwen Xu[a], Yaheng Li[a], Xiaona Wang[a], Huijuan Yang[a,b], Jun Chen[a], Shuyuan Liu[a], Yan Deng[a,b], Zhimei Zhao[a,b], Qiongzhou Yin[a,b], Mingbo Sun[a,b], and Li Shi [a]

[a]Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, China; [b]Yunnan Key Laboratory of Vaccine Research, Development on Severe Infectious Disease, Kunming, China

ABSTRACT

To investigate whether the *TNF-α, IL-2, IL-4* and *IL-10* genes contribute to variations in vaccine-induced immune responses after immunization with the inactivated Japanese encephalitis vaccine (IJEV), a total of 369 individuals who received the IJEV were enrolled. Based on Japanese encephalitis virus (JEV) neutralization antibodies (NAbs), the individuals were divided into seropositive (SP) and seronegative (SN) groups. Then, 17 SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes were genotyped using the TaqMan method. Although there was no association of the *TNF-α, IL-2, IL-4* and *IL-10* genes with JEV seropositivity triggered by JEV vaccination when all the individuals in the SP and SN groups were compared, differences were observed in a subgroup analysis. In the male group, rs2243291 in the *IL-4* gene showed a difference between the JEV SP and SN groups with the overdominant model (P = .045), and the C/G genotypes conferred more JEV seropositivity (OR = 1.87; 95% CI: 1.01–3.49); the CT genotype of rs3093726 in the *TNF-α* gene showed higher JEV NAbs geometric mean titer (GMT) than the TT genotype (P = .018, CT: 1.677 ± 0.144 vs TT: 1.271 ± 0.039). Furthermore, the rs1800629 genotype in the *TNF-α* gene and the rs1800896 genotype in the *IL-10* gene exhibited a trend of association with JEV seropositivity in the female group, but the difference was not significant. The present study suggested that the polymorphisms in the cytokine genes could be associated with sex-specific JEV NAbs seroconversion. However, more samples should be studied, and further functional verification should be performed.

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Introduction

Vaccination is an efficient method for controlling infectious diseases. However, there is interindividual variation in immune responses to vaccines. For example, the seroconversion rate and hepatitis B and measles neutralization antibody levels were different after vaccination with the hepatitis B vaccine (HBV) and the measles vaccine,[1,2] which indicated that host genetic polymorphisms may play an important role in the efficacy of vaccines.

Japanese encephalitis (JE) is one of the most serious mosquito-borne infectious diseases.[3] To date, four different types of JE vaccines (inactivated mouse brain-derived, live attenuated cell culture-derived, inactivated cell culture-derived, and genetically engineered live attenuated chimeric vaccine) are available in different countries. After immunization, the serum neutralizing antibody positive conversion rates ranged from 64.4% to 93.3% for the inactivated or live attenuated vaccines.[4,5] The variations in the positive serum conversion rates indicate that host genetic polymorphisms could play a key role in the efficacy of JE vaccines.

Recently, human leukocyte antigen (HLA) alleles and several single-nucleotide polymorphisms (SNPs) in cytokine genes, such as the pro-inflammatory cytokine *TNF-α* gene, subsets of the Th1-promoting cytokine *IL-2* gene, and the Th2 cytokines *IL-4* and *IL-10* genes, were investigated to be associated with hypo- or nonresponsiveness and the variable antibody levels in immune responses to different vaccines. The variation of *IL-2* gene has been investigated in association with measles vaccine and hepatitis B vaccine (HBV) induced antibody response,[6–9] the variation of *IL-4* genes were in association with HBV, diphtheria, tetanus, and combined pneumococcal conjugate and polysaccharide vaccines,[8,10-12] and the variation of *IL-10* genes were in association with diphtheria, tetanus, and measles vaccine.[6,7,11] In 2009, Yucesoy et al. investigated the association between cytokine or cytokine receptor gene polymorphisms and the immune response to childhood vaccines (HBV, 7-valent pneumococcal conjugate, and diphtheria, tetanus, acellular (DTaP) pertussis vaccines) and found that SNPs in the *TNF-α, IL-12B, IL-4Rα*, and *IL-10* genes were associated with vaccine-specific immune responses (P < .05).[13] Moreover, SNPs in the *IL-1β, TNF-α, IL-2, IL-4, IL-10, IL-4Rα*, and *IL-12B* genes were associated with serum immunoglobulin (IgG, IgA, and IgM) levels (P < .05).[13] All studies suggested that genetic variations in cytokine genes can influence vaccine-induced immune responses, which in turn may influence vaccine efficacy. As

CONTACT Mingbo Sun smb@imbcams.com.cn; Li Shi shili.imb@gmail.com Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, Yunnan 650118, China

Japanese encephalitis vaccine be considered, our previous study investigated the association of *HLA-DRB1, HLA-DPB1*, and *HLA-DQB1* with the humoral immune response elicited by the inactivated Japanese encephalitis vaccine (IJEV) and showed that HLA-DQB1*02:01 was significantly associated with JEV seropositivity ($P < .05$), while HLA-DQB1*02:02 was significantly associated with JEV seronegativity ($P < .05$).[14] In addition, we found that certain *HLA-DRB1* and *HLA-DPB1* alleles were associated with higher geometric mean titers (GMTs) than others.[14] The association study of cytokine gene variations with vaccine antibody response are particularly important in developing countries where JE is still a major health issue, because it may provide a clue for vaccine efficacy evaluation and new vaccine development.

In the current study, we evaluated the association between polymorphisms of the cytokine genes (*TNF-α, IL-2, IL-4*, and *IL-10*) and vaccine-induced antibody responses following immunization with the IJEV in a Mongolian Chinese population. Our results suggested that the genetic variation in cytokine genes may play a role in the immune response to the JE vaccine. However, the SNP association needs to be verified by function studies in the future.

Materials and methods

Ethics statement

All the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (the Institutional Review Board of the Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College) and with the Helsinki Declaration of 1975, which was revised in 2008. Informed consent was obtained from all subjects included in the study.

Subjects

Our previous study reported that a randomized, double-blinded, positive-control, noninferiority IJEV trial was implemented in the Inner Mongolia autonomous region of China from August 2012 to September 2013.[14] A total of 1,200 individuals, aged 8 months to 12 years, were enrolled to receive two doses of IJEV on a 0- and 7-day schedule. Considering the blood sample value limitation and the consistence of the test, only 369 individuals of 3 to 12 years old who were negative for JEV-neutralizing antibodies (NAbs) before vaccination were selected for cytokine SNP genotyping in the current study. The positive serum conversion rate and GMTs were used as alternative markers of efficacy of JE vaccines, and a NAb titer of at least 10 has been established to correlate with protection against the JEV.[15,16] Based on the NAb titer, the enrolled individuals were divided into seropositive (SP) and seronegative (SN) groups.

Japanese encephalitis vaccine neutralization antibody detection

JEV-specific NAbs were determined by the National Institute for Food and Drug Control using the 50% plaque-reduction neutralization test according to the requirement of the Pharmacopoeia of the People's Republic of China[17] as previously reported.[14] As the NAbs at 50% plaque-reduction neutralization titer (PRNT50) of at least 10 have been established as a correlate of protection against development of JE disease in humans,[3] PRNT50 <10 or a PRNT50 increased less than fourfold after vaccination indicated negative seroconversion, while PRNT50 > 10 or a PRNT50 with at least a fourfold increase after vaccination indicated positive seroconversion.

Genotyping of the TNF-α, Il-2, IL-4 and IL-10 genes

Genomic DNA was extracted from peripheral lymphocytes using the QIAamp Blood Mini Kit (Qiagen, Hilden, German). Genotyping of three SNPs (rs1800629, rs3093668, and rs3093726) in the *TNF-α* gene, four SNPs (rs11932411, rs11575812, rs2069762, and rs4833248) in the *IL-2* gene, seven SNPs (rs2243247, rs2243248, rs2243250, rs2070874, rs2227284, rs2243291, and rs2243292) in the *IL-4* gene and three SNPs (rs1800872, rs1800871, and rs1800896) in the *IL-10* gene was performed using a TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). To determine the accuracy of SNP genotyping by the TaqMan assay, some samples were randomly selected for sequencing to confirm the TaqMan genotyping results.

Statistical analysis

The age and sex of the subjects were compared using Student's t and chi-square test, respectively. Hardy–Weinberg equilibrium (HWE) was assessed using the Guo and Thompson method. Linkage disequilibrium analysis (LD) was calculated, and a D' value greater than 0.80 was considered to be in linkage disequilibrium. The haplotypes were constructed based on the genotyping results by the expectation-maximization algorithm.[18,19] The χ^2 test was used to determine differences in the allele, genotype, and haplotype frequencies between the responder and nonresponder groups, and the Bonferroni correction was used for multiple comparisons. The association between each genotype and the immune response was assessed using the inheritance model analysis in the SNPstats software.[20] The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were calculated to determine the best fit inheritance model, which possesses the smallest AIC and BIC values. The association between the SNPs in the *TNF-α, IL-4* and *IL-10* genes and the antibody levels was analyzed through one-way ANOVA, and Tukey's correction was used for multiple comparisons. P values < .05 were considered statistically significant. The genotype and allele for each SNP and haplotype-specific risk analysis were calculated, and the odds ratios (OR) and the associated 95% confidence intervals (CIs) were also calculated for allele-specific or haplotype-specific risks.

Results

Subject characteristics

Table 1 lists the characteristics of the enrolled subjects. All NAbs were negative before vaccination. After vaccination, 160 individuals with PRNT50 > 10 were included in the SP group,

Table 1. Demographic characteristics of the IJEV NAbs SP and SN group.

	Seropositive group (n = 160)	Seronegative group (n = 209)	*P* value
Ages (years)	8.099 ± 2.53	7.62 ± 2.439	.066
Sex (M/F)	74/86	107/102	.346
Antibody level			
1:10	86		
1:20	31		
1:40	20		
1:80	17		
>1:80	6		

while 209 individuals with PRNT50 < 10 were included in the SN group. There was no significant difference in sex or age between the SP and SN groups (P = .066 and P = .346, respectively) (Table 1). There was no significant difference in age between the NAbs seropositive and seronegative responses in the male group and female group (P = .336 and P = .128, respectively). In the NAbs seropositive responders, the distribution of antibody levels showed no differences between males (GMT: 1.293 ± 0.338) and females (GMT: 1.238 ± 0.330) (P = .301).

Association of the 17 SNPs in the TNF-α, IL-2, IL-4 and IL-10 genes with the JEV NAbs response triggered by IJEV vaccination

The allele and genotype frequencies of 17 SNPs in the *TNF-α, IL-2, IL-4*, and *IL-10* genes are presented in Table 2. The genotype frequencies for the SNPs were in HWE (P > .05). The allele and genotype distributions of the SNPs showed no association with JEV seropositivity triggered by JEV vaccination (P > .05). For the subgroup analysis, when sex was compared, the GG genotype of rs1800629 in the *TNF-α* gene showed a higher trend in the SP group than in the SN group among females (0.872 VS 0.775, OR = 0.504, 95% CI: 0.230 ~ 1.104); however, the difference was not significant (P = .083). Similarly, the C allele of rs1800896 in the *IL-10* gene showed a higher trend in the SP group than in the SN group (0.157 VS 0.098, OR = 1.713, 95% CI: 0.924 ~ 3.178) with no significant difference (P = .085) in the female group (Table 3).

The other alleles and genotypes of the SNPs were not associated with JEV seropositivity in the female group (P > .05) (data not shown). In the male group, no SNPs associated with JEV seropositivity were observed (P > .05) (data not shown).

Model of inheritance analysis of the 17 SNPs in the TNF-α, IL-2, IL-4 and IL-10 genes with the JEV NAbs response triggered by JEV vaccination

In the current study, no significant differences in the SNPs were found between the SP and SN groups in the model of inheritance analysis (P > .05) (data not shown). For the subgroup analysis, rs2243291 in the *IL-4* gene showed a significant difference between the JEV SP and SN groups with the overdominant model (P = .045), and the C/G genotype conferred more JEV seropositivity (OR = 1.87; 95% CI: 1.01–3.49) in the male group (Table 4). In addition, rs1800896 in the *IL-10* gene showed a trend of a difference between the SP and SN groups with the log-dominant model in the female group; however, the difference was not significant (P = .086) (Table 5). No significant differences in the other SNPs were found between the SP and SN groups in the model of inheritance analysis in either the male or female groups (P > .05) (data not shown).

Association of the haplotypes in the TNF-α, IL-2, IL-4 and IL-10 genes with the JEV NAbs response triggered by JEV vaccination

The SNPs were considered to construct haplotypes when D'≥0.800 in the LD analysis. After the haplotypes were constructed, there were no differences between the SP and SN groups after Bonferroni correction (P > .05) (data not shown). For the subgroup analysis, the haplotypes of the SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes were not associated with JEV seropositivity in the male and female groups after Bonferroni correction (P > .05) (data not shown).

Association between JEV NAbs GMTs and 17 SNPs in the TNF-α, IL-2, IL-4 and IL-10 genes

A total of 160 individuals in the SP group were included in the analysis of the association between the SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes and the JEV NAbs GMTs. However, there was no significant difference in the GMTs among the different SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes (P > .05) (data not shown). For subgroup analysis, rs3093726 in the *TNF-α* gene showed significant GMTs among the TT and CT genotypes (P = .018), and the CT genotype showed higher JEV neutralization antibody GMTs than the TT genotype (CT: 1.677 ± 0.144 vs TT: 1.271 ± 0.039) in the male group. The other SNPs were not associated with the JEV neutralization antibody GMTs in the male group (P > .05) (data not shown). In addition, no SNPs were associated with the JEV Nab GMTs in the female group (P > .05) (data not shown).

Discussion

Cytokines play central roles in the regulation of the Th1/Th2 balance in response to vaccine antigens.[21–23] Genetic polymorphisms in the genes encoding cytokines representing both Th1 (IL-2) and Th2 (IL-4 and IL-10) subsets showed that the genetic polymorphisms of cytokines were associated with hypo- or nonresponsiveness and variations in antibody levels in the immune responses to different vaccines.[6–13] In the present study, we did not identify an association of SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes with JEV seropositivity triggered by JEV vaccination when all individuals in the SP and SN groups were compared, but we investigated sex-specific association in the subgroup analysis.

TNF-α is a pro-inflammatory cytokine that is associated with the regulation of cellular immune responses.[24] Several SNPs in the *TNF-α* promoter regions have been shown to directly affect gene transcription.[24,25] In 2012, Ovsyannikova et al. performed a study of the association between

Table 2. Allelic and genotypic distribution of 17 SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes in the IJEV NAbs SP and SN group.

Genes	SNPs	Allele/ Genotype		Seropositive group n (Fre.)	Seronegative group n (Fre.)	*P* value	X^2	OR
TNF-α	rs1800629	Allele	A	33(0.103)	48(0.115)	.614	0.254	0.886[0.554 ~ 1.417]
			G	287(0.897)	370(0.885)			
		Genotype	AA	1(0.004)	0(0.000)	.376	1.954	
			AG	31(0.194)	48(0.230)			
			GG	128(0.800)	161(0.770)			
	rs3093668	Allele	C	16(0.050)	15(0.036)	.343	0.897	1.414[0.688 ~ 2.905]
			G	304(0.950)	403(0.964)			
		Genotype	CG	16(0.100)	15(0.072)	.333	0.938	1.437[0.688 ~ 3.002]
			GG	144(0.900)	194(0.928)			
	rs3093726	Allele	C	8(0.025)	6(0.014)	.293	1.104	1.761[0.605 ~ 5.126]
			T	312(0.975)	412(0.986)			
		Genotype	CT	8(0.050)	6(0.029)	.289	1.126	1.781[0.605 ~ 5.239]
			TT	152(0.950)	203(0.971)			
IL-2	rs11932411	Allele	T	320(1.000)	418(1.000)			
		Genotype	TT	160(1.000)	209(1.000)			
	rs11575812	Allele	A	293(0.916)	387(0.926)	.609	0.261	0.869[0.508 ~ 1.488]
			G	27(0.084)	31(0.074)			
		Genotype	AA	134(0.838)	178(0.852)	.505	1.365	
			AG	25(0.156)	31(0.148)			
			GG	1(0.006)	0(0.000)			
	rs2069763	Allele	G	155(0.484)	199(0.476)	.823	0.050	1.034[0.772 ~ 1.384]
			T	165(0.516)	219(0.524)			
		Genotype	GG	39(0.244)	46(0.220)	.815	0.408	
			GT	77(0.481)	107(0.512)			
			TT	44(0.275)	56(0.268)			
	rs2069762	Allele	A	208(0.650)	269(0.644)	.856	0.033	1.029[0.759 ~ 1.395]
			C	112(0.350)	149(0.356)			
		Genotype	AA	67(0.419)	86(0.411)	.982	0.036	
			AC	74(0.463)	97(0.464)			
			CC	19(0.119)	26(0.124)			
	rs4833248	Allele	A	113(0.353)	150(0.359)	.872	0.026	0.975[0.720 ~ 1.322]
			G	207(0.647)	268(0.641)			
		Genotype	AA	20(0.125)	27(0.129)	.987	0.026	
			AG	73(0.456)	96(0.459)			
			GG	67(0.419)	86(0.411)			
IL-4	rs2243247	Allele	G	320(1.000)	418(1.000)			
		Genotype	GG	160(1.000)	209(1.000)			
	rs2243248	Allele	G	27(0.084)	34(0.081)	.882	0.022	1.041[0.614 ~ 1.764]
			T	293(0.916)	384(0.919)			
		Genotype	GG	1(0.006)	2(0.010)	.891	0.231	
			GT	25(0.156)	30(0.144)			
			TT	134(0.838)	177(0.847)			
	rs2243250	Allele	C	84(0.263)	123(0.294)	.341	0.906	0.854[0.616 ~ 1.183]
			T	236(0.738)	295(0.706)			
		Genotype	CC	11(0.069)	16(0.077)	.569	1.126	
			CT	62(0.388)	91(0.435)			
			TT	87(0.544)	102(0.488)			
	rs2070874	Allele	C	83(0.259)	122(0.292)	.329	0.954	0.850[0.613 ~ 1.178]
			T	237(0.741)	296(0.708)			
		Genotype	CC	10(0.062)	15(0.072)	.569	1.129	
			CT	63(0.394)	92(0.440)			
			TT	87(0.544)	102(0.488)			
	rs2227284	Allele	G	53(0.166)	85(0.203)	.193	1.697	0.778[0.532 ~ 1.136]
			T	267(0.834)	333(0.797)			
		Genotype	GG	5(0.031)	7(0.033)	.328	2.229	
			GT	43(0.269)	71(0.340)			
			TT	112(0.700)	131(0.627)			
	rs2243291	Allele	C	238(0.744)	297(0.711)	.317	1.003	1.182[0.852 ~ 1.642]
			G	82(0.256)	121(0.289)			
		Genotype	CC	88(0.550)	102(0.488)	.493	1.416	
			CG	62(0.388)	93(0.445)			
			GG	10(0.062)	14(0.067)			
	rs2243292	Allele	T	320(1.000)	418(1.000)			
		Genotype	TT	160(1.000)	209(1.000)			
IL-10	rs1800872	Allele	G	124(0.388)	152(0.364)	.507	0.441	1.107[0.820 ~ 1.495]
			T	196(0.613)	266(0.636)			
		Genotype	GG	23(0.144)	30(0.144)	.620	0.957	
			GT	78(0.487)	92(0.440)			
			TT	59(0.369)	87(0.416)			
	rs1800871	Allele	A	196(0.613)	266(0.636)	.507	0.441	0.903[0.669 ~ 1.220]
			G	124(0.388)	152(0.364)			
		Genotype	AA	59(0.369)	87(0.416)	.620	0.957	
			AG	78(0.487)	92(0.440)			
			GG	23(0.144)	30(0.144)			
	rs1800896	Allele	C	42(0.131)	47(0.112)	.437	0.605	1.193[0.765 ~ 1.860]
			T	278(0.869)	371(0.888)			
		Genotype	CC	2(0.013)	2(0.010)	.730	0.630	
			CT	38(0.237)	43(0.206)			
			TT	120(0.750)	164(0.785)			

Table 3. Allelic and genotypic distribution of the rs1800896 in IL-10 and rs1800629 in *TNF-α* gene in the IJEV NAbs SP and SN groups in females.

SNP				Seropositive group (n = 86)	Seronegative group (n = 102)	*P* value	X^2	OR
IL-10	rs1800896	Allele	C	27(0.157)	20(0.098)	.085	2.964	1.713[0.924 ~ 3.178]
			T	145(0.843)	184(0.902)			
		Genotype	CC	2(0.023)	1(0.010)	.227	2.964	
			CT	23(0.267)	18(0.176)			
TNF-α	rs1800629	Allele	A	11(0.064)	23(0.113)	.376	0.784	1.320[0.713 ~ 2.443]
			G	161(0.936)	181(0.887)			
		Genotype	AG	11(0.128)	23(0.225)	.083	2.999	0.504[0.230 ~ 1.104]
			GG	75(0.872)	79(0.775)			

Table 4. Inheritance model analysis of the rs2243291 in *IL-4* gene in the IJEV NAbs SP and SN group in males.

Model	Genotype	Seropositive group (n = 74)	Seronegative group (n = 107)	OR (95% CI)	*P* value	AIC	BIC
Codominant	C/C	46 (62.2%)	52 (48.6%)	1	.130	246.9	256.5
	C/G	23 (31.1%)	49 (45.8%)	1.88 (1.00–3.56)			
	G/G	5 (6.8%)	6 (5.6%)	1.06 (0.30–3.71)			
Dominant	C/C	46 (62.2%)	52 (48.6%)	1	.071	245.6	252
	C/G-G/G	28 (37.8%)	55 (51.4%)	1.74 (0.95–3.18)			
Recessive	C/C-C/G	69 (93.2%)	101 (94.4%)	1	.750	248.8	255.2
	G/G	5 (6.8%)	6 (5.6%)	0.82 (0.24–2.79)			
Overdominant	C/C-G/G	51 (68.9%)	58 (54.2%)	1	.045	244.9	251.3
	C/G	23 (31.1%)	49 (45.8%)	1.87 (1.01–3.49)			
Log-additive	–	–	–	1.41 (0.85–2.32)	.170	247.0	253.4

Table 5. Inheritance model analysis of the rs1800896 in IL-10 gene in the IJEV NAbs SP and SN group in females.

Model	Genotype	Seropositive group (n = 86)	Seronegative group (n = 102)	OR (95% CI)	*P* value	AIC	BIC
Codominant	T/T	61 (70.9%)	83 (81.4%)	1	.230	262.3	272.0
	C/T	23 (26.7%)	18 (17.6%)	0.58 (0.29–1.16)			
	C/C	2 (2.3%)	1 (1%)	0.37 (0.03–4.15)			
Dominant	T/T	61 (70.9%)	83 (81.4%)	1	.092	260.4	266.9
	C/T-C/C	25 (29.1%)	19 (18.6%)	0.56 (0.28–1.10)			
Recessive	T/T-C/T	84 (97.7%)	101 (99%)	1	.460	262.7	269.2
	C/C	2 (2.3%)	1 (1%)	0.42 (0.04–4.67)			
Overdominant	T/T-C/C	63 (73.3%)	84 (82.3%)	1	.130	261.0	267.5
	C/T	23 (26.7%)	18 (17.6%)	0.59 (0.29–1.18)			
Log-additive	–	–	–	0.58 (0.31–1.09)	.086	260.3	266.8

cytokines and smallpox vaccination and found that Caucasian individuals demonstrated significant associations between rs3093726 and rs30936687 in the *TNF-α* gene and secreted IL-1β.[26] In the current study, rs3093726 showed significant GMTs between the TT and CT genotypes, and the CT genotype showed higher JEV neutralization antibody GMTs than the TT genotype (CT: 1.677 ± 0.144 vs TT: 1.271 ± 0.039) in the male group. For another SNP, rs1800629, the GG genotype was significantly associated with high levels of hepatitis B neutralizing antibodies induced by HBV vaccination.[13] In 2009, Yucesoy et al. reported that rs1800629 may be associated with changes in the amount and function of the gene products, which in turn affects the processing and presentation of antigens by antigen-presenting cells. However, Macedo et al. found that there was no correlation between the rs1800629 polymorphism and the hepatitis B vaccine response in children approximately 1-year-old.[27] In the present study, the genotype of rs1800629GG in *TNF-α* genes showed a trend of association with a higher positive seroconversion rate after the IJEV in the female group, although the difference was not significant. Thus, rs1800629, which is located in the 5′-flanking region of the *TNF-α* gene, influenced the expression of the *TNF-α* gene, potentially affecting the immune response to vaccines.

A previous study suggested that transcription of the *IL-4* gene is positively regulated by the coordination of multiple promoter and enhancer elements.[28] In 2011, Haralambieva et al. reported that rs2243248 was associated with measles virus-specific neutralizing antibody responses, and the AA genotype showed a high virus-specific neutralizing antibody titer.[6] In 2017, Youn Roh et al. reported that rs2227284G in the *IL-4* gene was significantly more frequent in nonresponder and lower-titer individuals than in high-titer responder individuals among 6-month-old subjects during the response to the HBV vaccine, and the rs2227284G frequencies were significantly different among the three subgroups.[29] In the current study, we found that rs2243291 in the *IL-4* gene showed a significant difference between the JEV SP and SN groups with the overdominant model in the female group. However, we did not observe the association of the rs2227284 allele and genotypes with the positive seroconversion rate after the IJEV.

As a Th2 type cytokine, IL-10 is a key immune modulating factor with anti-inflammatory activities that inhibits the activation of antigen-presenting cells, thereby influencing T cell

responses.[30] The polymorphisms located in the 5′-flanking region of the *IL-10* gene influence the expression of the *IL-10* gene, which is associated with the immune reaction. In 2005, Hohler et al. found that the rs1800896GG genotype was associated with strong anti-HBs responses after vaccination with HBsAg and Hepatitis A.[9] In 2008, Smith et al. reported that the rs1800896G and rs1800871T alleles were associated with low IL-10 production,[31] and rs1800871 was related to increased cellular and humoral immunity to measles vaccination.[7] Then, Yucesoy et al. reported that rs1800896GG was associated with higher antibody responses to HBV and DTaP vaccinations, and rs1800871CC was associated with higher antibody responses to PnPS serotypes.[13] In the current study, we found rs1800896GG showed a trend of association with a higher positive seroconversion rate after the IJEV in the female group, but the difference was not significant.

The above results suggested that sex may play a role in the antibody response after the IJEV. The different immune responses between males and females induced by vaccines have been investigated for several immunizations, including the hepatitis B, diphtheria, pertussis, pneumococcus, rabies, measles, malaria and human papillomavirus, influenza, herpes virus, smallpox and Td/T dap vaccines, for which high antibody responses against vaccine antigens were significant greater in females than in males.[32–34] These stronger adaptive humoral responses are characterized by higher basal and postvaccination IgG levels and increased B-cell numbers.[35] Further studies indicated that sex-based differences in the *HLA* genes and the *IL-4* and *IL-10* genes are associated with antibody responses against measles, mumps, tetanus, diphtheria, and hepatitis A vaccines.[36] Moreover, in mice, the cytokine response of CD4 + T cells differed between male and female mice, and females exhibited higher Th1 (i.e. IFN-r), Th2 (i.e. IL-4), and regulatory T cell (i.e. IL-10) responses than males;[37,38] in humans, peripheral monocytes isolated from males produced higher amounts of TNF-α, IL-1b, and IL-6 but lower amounts of IL-10 compared to cells from females.[39,40] Thus, we deduced that the variation in the SNPs in the *TNF-α, IL-4*, and *IL-10* genes may influence the expression of these cytokines, resulting in different JEV antibody responses between male and females.

In the present study, we did not identify an association of the SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes with JEV seropositivity triggered by JEV vaccination when all individuals in the SP and SN groups were compared. However, in the subgroup analysis, rs2243291 in the *IL-4* gene showed a significant difference between the JEV SP and SN groups with the overdominant model, and the C/G genotypes conferred more JEV seropositivity; rs3093726 in the *TNF-α* gene showed significant GMTs between the TT and CT genotypes, and the CT genotype showed higher JEV neutralization antibody GMTs than the TT genotype in the male group.

One limitation of the present study is that the relationship between these SNPs and the cytokine levels, or between the cytokine levels and the immune response triggered by JEV vaccination, has not been investigated. Moreover, the limitation of a smaller group of subjects may restrict the analytical power of the present study. Thus, more samples and functional studies are needed to better clarify and examine the association between these SNPs and antibody responses triggered by JEV vaccination.

Conclusions

In the current study, we evaluated the associations between 17 SNPs in the *TNF-α, IL-2, IL-4* and *IL-10* genes and the immune responses triggered by JEV vaccination in a Chinese Mongolian population. The results suggested that the polymorphisms in the cytokine genes could be associated with the JEV seroconversion rate after vaccination in the Chinese Mongolian population in a sex-specific manner. The present studies may provide some clue for future vaccine efficacy evaluation and new vaccine development. In the future, larger-scale studies are needed to better clarify and examine the association between cytokines and immune responses triggered by JEV vaccination.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Li Shi http://orcid.org/0000-0001-9508-7863

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