Format for ANSWERING REVIEWERS

December 11, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14930-review.doc).

Title: IL-21 gene polymorphisms and chronic hepatitis B virus infection in a Chinese population

Author: Jia-Yan Yao, Kang Chao, Min-Rui Li, Yan-Qing Wu, Bi-Hui Zhong

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14930

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) *Title: It's long and this study doesn't report an association between IL21 polymorphisms and CHB; the title proposed: interleukin-21 gene polymorphisms and chronic hepatitis B virus infection in a Chinese population*

The title had been revised as "IL-21 gene polymorphisms and chronic hepatitis B virus infection in a Chinese population"

(2) Introduction: ? Some recent studies investigated the serum level of IL21 in CHB which must be cited exp: - J Interferon Cytokine Res. 2014: Interleukin-21 Responses in Patients with Chronic

Hepatitis B - J Viral Hepat. 2014: Increased levels of IL-21 responses are associated with the severity of liver injury in patients with chronic activehepatitis B. ? the association between IL21 gene and CHB have been evaluated: - Viral immunom 2013: Association of IL-17, IL-21, and IL-23R gene polymorphisms with HBV infection in kidney transplant patients - Hum Immunol. 2013: IL21 and IL21R polymorphisms and their interactive effects on serum IL-21 and IgE levels in patients with chronic hepatitis B virus infection. This study explored IL21rs907715 and rs2221903 and IL21R T-83C and rs3093301 polymorphisms in 395 patients with chronic HBV infection, 75 HBV infection resolvers and 174 healthy controls. These results must be cited and discussed.

Recent studies Mentioned above had been cited and discussed

INTRODUCTION

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...Recent investigations have shown that serum level of IL-21 was increased in CHB patients and associated with severe liver inflammation[19]. The levels of IL-21 expression in the liver tissues were associated significantly with increased degrees of inflammation and fibrosis in CHB patients[20]. High serum IL-21 levels after 12 weeks of antiviral therapy predict HBeAg seroconversion in chronic hepatitis B[21]. IL-21 enhanced HBcAg-specific IFN-g+CD8+T cell proliferation, while treatment with anti-IL-21 inhibited antigen-specific IFN-g+CD8+Tcell expansion in vitro[22].

Collectively, these findings suggest that IL-21 might play a critical role in HBV infection. However, the precise mechanisms underlying the effects of IL21 on hepatitis B pathogenesis have not yet been clearly elucidated. Genetic polymorphisms in IL-21 have been explored to genetic susceptibility to chronic HBV-infected diseases. Literature report finds IL21 rs2221903 AG is less frequent in HBV patients than in HBV infection resolvers or in controls[23]. In kidney transplant patients with acute rejection, frequencies of TT homozygote genotype and T allele of IL-21-G1472T (rs2055979) polymorphism and CC homozygote genotype and C allele of IL-21-C5250T (rs4833837) polymorphism are higher in HBV-infected patients than in HBV-noninfected patients[24]. Accordingly, this study was conducted to replicate IL-21 rs13143866, rs2221903 and rs907715 gene polymorphisms with susceptibility to chronic HBV infection diseases in a Chinese population.

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DISCUSSION

In the present study, we analyzed allele and genotype frequencies at three SNPs (rs 13143866, rs2221903 and rs907715) of the IL-21 gene between 366 chronic HBV infected patients and 208 healthy controls in a Chinese population. The intronic SNPs may not be the actual risk mutation, but they are likely to be a surrogate marker for a mutation with functional consequences. The intronic SNPs may be in high linkage disequilibrium with a variant that associates with translation of the mRNA[25]. In other words, they may associate with protein expression. For example, synonymous SNPs have a substantial contribution to human disease risk and other complex traits[26]. Literature reports explored IL21rs907715 and rs2221903 and IL21R T-83C and rs3093301 polymorphisms in chronic HBV-infected patients, and IL-17rs2275913, IL-23Rrs10889677, IL-21rs4833837 and IL-21rs2055979 polymorphisms in kidney transplant patients with HBV infection[23, 24].

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Previous studies showed significant association between rs2221903 and systemic lupus erythematosus (SLE) in the Chinese population, European-Americans and African-Americans[25, 29, 30]. Although there was no statistically difference, our data showed that SNP rs2221903 was associated with an increased risk of chronic HBV infection. The frequency of allele C was 13.7% in the chronic HBV infection group and 10.3% in the control group, P=0.101. In the subgroup analysis, IL-21 rs2221903 genotype TC frequency in the HBV carrier group was higher than that in controls [29.7% *vs* 18.7%, P=0.035, OR (95%CI)= 1.809 (1.043~3.139)]. However, Li Na *et al* explored IL21rs907715 and rs2221903 and IL21R T-83C and rs3093301 polymorphisms in 395 patients with chronic HBV infection, 75 HBV infection resolvers and 174 healthy controls in a Chinese population and found rs2221903 allele C frequency in HBV patients was lower than that in controls (8.1% vs. 12.4%, ***P***= 0.023, OR (95%CI)= 0.625 (0.415 – 0.941) and TC genotype was less frequent in HBV patients than in controls (16.2% vs. 24.7%, ***P***= 0.017, OR (95%CI)= 0.589 (0.381 – 0.911)[23]. Several potential explanations need to be considered: firstly, the regulatory mechanisms of IL-21 intronic SNPs are still not very clear. Secondly, chronic HBV infection is a complex complication related to other genetic and environmental factors[3].

Our data found no significant association between rs907715 and chronic HBV infection,

which was in consistent with Li Na *et al*[23]. ...

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REFERENCES

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20 **Pan Q,** Yu Y, Tang Z, Xi M, Jiang H, Xun Y, Liu X, Liu H, Hu J, Zang G. Increased levels of IL-21 responses are associated with the severity of liver injury in patients with chronic active hepatitis B. *J Viral Hepat* 2014; 21(9):e78-e88 [PMID: 24611989 DOI: 10.1111/jvh.12242]

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22 **Li J,** Ren W, Ma W, Zhang J, Shi J, Qin C. Interleukin-21 Responses in Patients with Chronic Hepatitis B. *J Interferon Cytokine Res* 2014 [Epub ahead of print][PMID: 25243706]

23 **Hejr S,** Karimi MH, Yaghobi R, Kamali-Sarvestani E, Geramizadeh B, Roozbeh J. Association of IL-17, IL-21, and IL-23R gene polymorphisms with HBV infection in kidney transplant patients. *Viral Immunol* 2013; 26(3):201-206 [PMID: 23656167 DOI: 10.1089/vim.2013.0007]

24 **Li N,** Zhu Q, Li Z, Han Q, Chen J, Lv Y, Wang Y, Zeng X, Chen Y, Yang C, Liu Z. IL21 and IL21R polymorphisms and their interactive effects on serum IL-21 and IgE levels in patients with chronic hepatitis B virus infection. *Hum Immunol* 2013; 74(5):567-573 [PMID: 23354321 DOI: 10.1016/j.humimm.2013.01.005]

...

(3)Results: In the paragraph: Association of IL-21 Gene Polymorphisms and HBV infection subgroups: we must mentioned that distributions of genotype frequencies were significantly different between HBV carrier group and controls (P=0.031) with SNP rs13143866.

Result in the paragraph: Association of IL-21 Gene Polymorphisms and HBV infection subgroups, had been revised.

RESULTS

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Association of IL-21 Gene Polymorphisms and HBV infection subgroups

The distribution of genotypes and alleles of IL21 rs907715 polymorphisms showed no significant difference among HBV carriers, patients with CHB, patients with HBV-related cirrhosis and healthy controls. However, IL-21 rs13143866 and rs2221903 polymorphism was differently distributed between the HBV carrier group and controls. IL-21 rs13143866 genotype AA frequency in the HBV carrier group and controls was 5.9% and 1.0%, respectively. This rs13143866 genotype AA frequency in the HBV carrier group was higher than that in controls [*P*=0.019, OR (95%CI) = 6.280 (1.238~31.854)], and the effect of the recessive model [(AA *vs* GG+GA, OR (95%CI) = 6.494 (1.289-33.333)] was observed in the HBV carrier group. IL-21 rs2221903 genotype TC frequency in the HBV carrier group and controls was 29.7% and 18.7%, respectively. This rs2221903 genotype TC frequency in the HBV carrier group was higher than that in controls [*P*=0.035, OR (95%CI) = 1.809 (1.043~3.139)] .

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(4) *In this study, authors investigated the relationship between three single-nucleotide polymorphisms (SNPs) in IL-21 gene and chronic hepatitis B virus (HBV) infection in the Chinese population. Their found that rs13143866 A allele increase the risk of HBV carrying and ATA haplotype (rs13143866, rs2221903 and rs907715) increase the risk of HBV-related hepatocellular carcinoma. This paper is acceptable for publish. But authors ought to increase the analytical results for the non-HCC subgroups, although there were no significant differences in the frequencies of all alleles and genotypes among some subgroups.*

Results for the non-HCC subgroups were detailed in Table 3 (attached sheet at the end of the letter)

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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Table 3 Comparison of IL-21 polymorphismamang between non-HCC subgroups and controls

		Carrier (n=101)	CHB (n=76)	Cirrhosis (n=95)	Control (n=208)	Carrier vs Control		CHB vs Control		Cirrhosis vs Control	
						P	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)
rs13143866											
Allele frequency	Allele G	170(84.2)	128(84.2)	156(82.1)	363 (87.3)		Referance		Referance		Referance
	Allele A	32(15.8)	24(15.8)	34(17.9)	53 (12.7)	0.294	1.289 (0.802-2.073)	0.348	1.284(0.761-2.166)	0.095	1.493(0.933-2.388)
Genotype frequency	GG	75(74.3)	53(69.7)	64(67.4)	157 (75.5)		Referance		Referance		Referance
	GA	20(19.8)	22(28.9)	28(29.5)	49 (23.5)	0.600	0.854(0.474-1.539)	0.345	1.330(0.736-2.403)	0.227	1.402(0.811-2.424)
	AA	6(5.9)	1(1.3)	3(3.1)	2 (1.0)	0.019[a]	6.280(1.238-31.854)	1.000[a]	1.481(0.132-16.666)	0.156[a]	3.680(0.601-22.544)
	HWE	0.010	0.440	0.977	0.391						
Recessive model	GG+GA	95(94.1)	75(98.7)	92(96.8)	206 (99.0)		Referance		Referance		Referance
	AA	6(5.9)	1(1.3)	3(3.2)	2 (1.0)	0.017[a]	6.494 (1.289-33.333)	1.000[a]	1.373(0.123-15.367)	0.330[a]	3.359(0.552-20.441)
Dominant model	GG	75(74..3)	53(69.7)	64(67.4)	157 (75.5)		Referance		Referance		Referance
	AA+GA	26(25.7)	23(30.3)	31(32.6)	51 (24.5)	0.816	1.067(0.618-1.843)	0.330	1.336(0.746-2.392)	0.142	1.491(0.875-2.540)
rs2221903											
Allele frequency	Allele T	172(85.1)	130(85.5)	167(87.9)	373 (89.7)		Referance		Referance		Referance
	Allele C	30(14.9)	22(14.5)	23(12.1)	43 (10.3)	0.105	1.513(0.918-2.495)	0.172	1.468(0.846-2.547)	0.517	1.195(0.697-2.046)
Genotype frequency	TT	71(70.3)	56(73.7)	75(78.9)	167 (80.3)		Referance		Referance		Referance
	TC	30(29.7)	18(23.7)	17(17.9)	39 (18.7)	0.035	1.809(1.043-3.139)	0.324	1.367(0.729-2.598)	0.926	0.971(0.516-1.825)
	CC	0(0.00)	2(2.6)	3(3.2)	2 (1.0)	-[b]		0.575[a]	2.982(0.410-21.668)	0.330[a]	3.340(0.547-20.405)
	HWE	0.080	0.705	0.121	0.868						
Recessive model	TT+TC	101(100.	74(97.4)	92(96.8)	206 (99.0)		Referance		Referance		Referance
	CC	0(0.0)	2(2.6)	3(3.2)	2 (1.0)	-[b]		0.577[a]	2.784(0.385-20.120)	0.330[a]	3.359(0.552-20.441)
Dominant model	TT	71(70.3)	56(73.7)	75(78.9)	167 (80.3)		Referance		Referance		Referance
	CC+TC	30(29.7)	20(26.3)	20(21.1)	41 (19.7)	0.052	1.721(0.996-2.973)	0.232	1.455(0.787-2.689)	0.787	1.086(0.596-1.979)
rs907715											
Allele frequency	Allele G	115(56.9)	89(58.6)	105(55.3)	234 (56.2)		Referance		Referance		Referance
	Allele A	87(43.8)	63(41.4)	85(44.7)	182 (43.8)	0.873	0.973(0.693-1.366)	0.624	0.910(0.625-1.326)	0.820	1.041(0.737-1.470)
Genotype frequency	GG	33(32.7)	24(3.16)	28(29.5)	70 (33.7)		Referance		Referance		Referance
	GA	49(48.5)	41(53.9)	49(51.6)	94 (45.2)	0.715	1.106(0.645-1.896)	0.425	1.272(0.704-2.298)	0.352	1.303(0.746-2.277)
	AA	19(18.8)	11(14.5)	18(18.9)	44 (21.1)	0.800	0.916(0.465-1.806)	0.443	0.729(0.325-1.634)	0.950	1.023(0.507-2.064)
	HWE	0.914	0.331	0.674	0.238						
Recessive model	GG+GA	82(81.2)	65(85.5)	77(81.1)	164 (78.8)		Referance		Referance		Referance
	AA	19(18.8)	11(14.5)	18(18.9)	44 (21.2)	0.632	0.864(0.474-1.573)	0.210	0.631(0.307-1.296)	0.659	0.871(0.473-1.606)
Dominant model	GG	33(32.7)	24(31.6)	28(29.5)	70 (33.7)		Referance		Referance		Referance
	AA+GA	68(67.3)	52(68.4)	67(70.5)	138 (66.3)	0.864	1.045(0.630-1.733)	0.742	1.099(0.626-1.929)	0.471	1.214(0.717-2.055)

Data are presented as n(%); [a]***P*** value for Fisher's exact test; [b]Chi-Square test can not be done for one cell of crosstabulation is zero; HWE: Hardy–Weinberg equilibrium; OR: odds ratio; 95 % CI: 95 % confidence interval; HBV: hepatitis B virus; non-HCC: chronic HBV infected patients without hepatocellular carcinoma; Carrier: HBV carriers; CHB: patients with chronic hepatitis B; Cirrhosis: patients with HBV-related cirrhosis.

Format for ANSWERING REVIEWERS

January 30, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14930-review.doc).

Title: IL-21 gene polymorphisms and chronic hepatitis B virus infection in a Chinese population

Author: Jia-Yan Yao, Kang Chao, Min-Rui Li, Yan-Qing Wu, Bi-Hui Zhong

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14930

The manuscript has been improved according to the suggestions of chief editor：

(1) *This study has an important methodologic issue that should be shown or discussed. There were no demographic data on each group. Progression from chronic hepatitis to cirrhosis,to HCC is highly dependent on patient's age. In addition, it can be modified by treatment or alcohol consumption. Male is known to develop HCC more frequently than female does. Therefore, simple comparison of allele frequency among several groups is meaningless. Clinical data on carriers, chronic hepatitis, cirrhosis and HCC should be presented and each group should be matched according to sex, age, alcohol, and/or history of treatment, etc. If the investigators cannot not match these variables, at least, it should be discussed as a weak point of this study, in the Discussion. Referance should be corrected into reference in table 1 and 2. Hardy-weinberg should be corrected into Hardy-Weinberg in table 1.*

Demographic datas on each group are shown in Table 1. Progression from chronic hepatitis to cirrhosis,to HCC is highly dependent on patient's age and male is known to develop HCC more frequently than female does. There were no statistical differences of gender and age among all groups in our study. All subjects including were exclused alcoholic liver disease. Referance is corrected into reference in tables. Hardy-weinberg should be corrected into Hardy-Weinberg in tables.

Table 1 Demographic datas for all groups

Groups	Gender (male/female)	P_c	P_x	Age (years)	P_c	P_x
HBV infection	263/103	0.672		48.3±11.8	0.657	
HCC	64/30	0.713	0.345	48.8±12.2	0.935	0.589
non-HCC	199/73	0.473		48.1±11.7	0.544	
Carrier	71/24	0.416		47.9±11.3	0.570	
CHB	58/18	0.310		46.5±11.0	0.151	
Cirrhosis	70/31	1.000		49.4±12.6	0.621	
Control	146/62			48.7±11.3		

P_c : P value compare with control group; P_x : P value between HCC group and non-HCC group; HBV: hepatitis B virus; HCC: chronic HBV-infected patients with hepatocellular carcinoma; non-HCC: chronic HBV-infected patients without hepatocellular carcinoma; Carrier: HBV carriers; CHB: patients with chronic hepatitis B; Cirrhosis: patients with HBV-related.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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