LETTER TO THE EDITOR

Overall corrections and assessments of "Correlations between TLR polymorphisms and inflammatory bowel disease: a meta-analysis of 49 case-control studies"

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Dear Editor,

Wang [1] recently published an article on the association between toll-like receptor (TLR) polymorphisms and inflammatory bowel disease (IBD). Their valuable study is one of the first works to attempt a meta-analysis of TLR polymorphisms on the risk of IBD. As a part of their main finding, overall and ethnic subgroups analyses revealed significant associations between the risk of IBD and TLR1 rs5743611, TLR4 rs4986790, TLR4 rs4986791, and TLR6 rs5743810 polymorphisms. Moreover, "the TLR9 rs352140 polymorphism was significantly associated with the risk of IBD in Africans." However, the results and conclusion described by authors are driven from some errors in their genotyping models, meta-analysis, sample sizes, and results interpretation. Here, we would like to discuss these issues and suggest some corrections.

Both dominant and recessive models presented by the authors are inaccurate, for instance, allelic comparison for TLR1 rs5743611 was based on G vs. C, so the dominant model should be GG+GC vs. CC. Authors inversely calculated GG vs. CC+GC as dominant and CC vs. GG+GC as recessive models. This error is also the case for other polymorphisms included in this study.

Second point is about repeating the number of controls when stratifying populations according to the type of disease. They finally analyzed the data of overall and different ethnic groups based on repeated numbers, which resulted in wrong interpretation. Therefore, all of the overall and ethnic subgroup analyses require correction. For instance, "Henckaerts 2007," "Kim 2012," and "Pierik 2006" control groups were repeatedly analyzed in the overall meta-analysis of TLR1 rs5743611 polymorphism association with IBD. Analysis of Caucasian population must also be performed according to only two genotypes whiles authors included four genotypes in their analysis.

Another point is missing for some genotyping data in their meta-analysis, it means that the weight of some genotypes was zero and their odds ratio and meta-analysis results were missed. For instance, "Kim 2012" and "Meena 2015" studies did not have any effect on the results of TLR1 rs5743611 meta-analysis. In these studies, GC and CC genotypes were zero and weighted zero which is missing in the results and should be corrected.

Moreover, as a result of previous errors, sample sizes in their meta-analysis are inaccurate, and some mentioned analyses were not possible for this study. For instance, African subgroup for TLR2 rs5743708 which was actually comprised of only one study and there was no possibility to consider meta-analysis on mentioned data. In contrast, some analysts were missed by mistake, for instance, comparison of recessive model for TLR2 rs121917864. Here, we applied corrected analysis and also mentioned number of included genotypes for each meta-analysis along with the sample size, which helps clearness and better understanding of our (correct) meta-analyses. The results are shown in Table 1.

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Table 1 Corrected meta-analysis for the association between TLR polymorphisms and IBD

Polymorphisms	Population	Sample size	No.#	Dominant comparison OR(95%CI)	P value	No.	Recessive comparison OR(95%CI)	P value	No.	Additive comparison OR(95%CI)	P value	New Sample sizes*	No.	Allelic comparison OR(95%CI)	P value
TLR1 rs5743611	Overall	1829/1008	4	0.46(0.17–1.23)	0.12	4	1.01(0.77–1.33)	0.93	4	0.90(0.68–1.20)	0.48			0.95(0.74–1.21)	0.66
	Caucasian	1357/480	2	0.42(0.15–1.20)	0.11	2	1.02(0.77–1.34)	0.91	2	0.90(0.68–1.20)	0.47			0.95(0.74–1.22)	0.67
	CD	1054/658	3	0.42(0.15–1.19)	0.10	3	0.91(0.68–1.21)	0.50	3	1.02(0.76–1.36)	0.90			0.86(0.66–1.12)	0.27
	UC	775/1008	4	0.41(0.14–1.25)	0.12	4	1.32(0.91–1.92)	0.14	4	**0.65(0.44–0.97)**	**0.03**			1.16(0.83–1.62)	0.40
TLR2 rs5743708	Overall	3596/2793	9	0.69(0.22–2.19)	0.53	9	1.03(0.78–1.36)	0.83	9	0.95(0.72–1.25)	0.70			1.01(0.77–1.32)	0.95
	Caucasian	2780/1440	4	0.74(0.16–3.39)	0.70	4	1.04(0.78–1.37)	0.81	4	0.94(0.71–1.26)	0.69			1.01(0.77–1.34)	0.92
	East Asian	403/462	3	0.88(0.09–8.49)	0.91	3	0.88(0.18–4.38)	0.87	3	1.14(0.12–10.98)	0.91			0.88(0.24–3.26)	0.85
	African	*85/547*	*7*	*NA*	*NA*	*7*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	CD	2145/2279	7	0.86(0.64–1.17)	0.34	7	1.09(0.81–1.47)	0.57	7	0.87(0.64–1.18)	0.36			1.03(0.77–1.39)	0.82
	UC	1305/2441	7	1.24(0.86–1.77)	0.25	7	0.83(0.58–1.19)	0.31	7	1.22(0.84–1.75)	0.29			0.84(0.59–1.19)	0.33
TLR2 rs121917864	Overall	488/1003	4	0.57(0.08–4.08)	0.58	4	1.14(0.32–4.04)	0.84	4	0.78(0.17–3.59)	0.75			1.07(0.35–3.23)	0.90
	East Asian	403/462	3	0.88(0.09–8.49)	0.91	3	0.88(0.18–4.38)	0.87	3	1.14(0.12–10.98)	0.91			0.88(0.24–3.26)	0.85
	African	*85/547*	*7*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	CD	118/839	3	0.17(0.02–1.68)	0.13	3	0.48(0.10–2.46)	0.38	3	1.48(0.20–10.82)	0.70			0.41(0.10–1.67)	0.21
	UC	224/839	3	0.31(0.03–3.03)	0.32	3	0.72(0.17–3.00)	0.65	3	1.31(0.25–6.92)	0.75			0.70(0.20–2.48)	0.58
TLR4 rs4986790	Overall	9785/6728	36	**0.56(0.36–0.89)**	**0.01**	36	**0.73(0.66–0.82)**	**<0.0001**	36	**1.31(1.17–1.47)**	**<0.0001**	12089/8532	40	**0.77(0.67–0.89)**	**0.0003**
	Caucasian	8140/4411	25	0.63(0.38–1.07)	0.09	25	**0.70(0.62–0.79)**	**<0.0001**	25	**1.40(1.24–1.58)**	**<0.0001**	10444/6215	29	**0.74(0.63–0.88)**	**0.0004**
	West Asian	469/633	3	**0.17(0.039–0.78)**	**0.02**	3	0.88(0.51–1.53)	0.65	3	0.99(0.53–1.83)	0.98	469/633	3	0.80(0.49–1.32)	0.39
	African	213/617	2	0.32(0.01–9.47)	0.51	2	1.28(0.67–2.46)	0.45	2	0.72(0.36–1.43)	0.35	213/617	2	1.18(0.65–2.15)	0.58
	East Asian	*963/1066*	*6*	*0.86(0.17–4.30)*	*0.86*	*6*	*0.99(0.31–3.16)*	*0.99*	*6*	*0.88(0.19–4.12)*	*0.87*	*963/1066*	*6*	*0.94(0.36–2.47)*	*0.90*
	CD	6773/5675	30	**0.52(0.31–0.86)**	0.07	30	**0.70(0.62–0.80)**	**<0.0001**	30	**1.38(1.21–1.58)**	**<0.0001**	7614/7479	34	**0.74(0.64–0.86)**	**<0.0001**
	UC	3466/5043	26	**0.43(0.24–0.77)**	**0.005**	26	**0.71(0.62–0.82)**	**<0.0001**	26	**1.33(1.15–1.55)**	**0.0001**	4329/5851	28	**0.77(0.63–0.95)**	**0.01**
TLR4 rs4986791	Overall	4909/3467	19	0.75(0.38–1.48)	0.40	19	**0.72(0.62–0.84)**	**<0.0001**	19	**1.35(1.15–1.58)**	**0.0002**	6125/4457	22	**0.78(0.69–0.88)**	**0.0001**
	Caucasian	3624/2242	12	0.78(0.31–1.97)	0.61	12	**0.72(0.61–0.86)**	**0.0002**	12	**1.37(1.15–1.62)**	**0.0004**	4694/3068	14	**0.79(0.69–0.91)**	**0.0007**
	West Asian	376/507	3	0.60(0.18–2.00)	0.41	3	0.70(0.33–1.46)	0.34	3	1.35(0.73–2.53)	0.34	376/507	3	0.71(0.33–1.50)	0.37
	African	*128/83*	*1*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	East Asian	*617/635*	*3*	*0.93(0.10–9.01)*	*0.95*	*3*	*1.25(0.23–6.67)*	*0.80*	*3*	*0.65(0.08–5.34)*	*0.69*	*763/799*	*4*	*1.06(0.31–3.56)*	*0.93*
	CD	3022/3211	18	**0.70(0.34–1.44)**	0.32	18	**0.76(0.63–0.90)**	**0.002**	18	**1.30(1.09–1.56)**	**0.004**	3633/4037	20	**0.80(0.70–0.92)**	**0.002**
	UC	1723/2445	14	**0.37(0.15–0.94)**	**0.04**	14	**0.65(0.46–0.90)**	**0.01**	14	**1.47(1.07–2.02)**	**0.02**	2182/3081	15	**0.68(0.49–0.93)**	**0.02**
TLR6 rs5743810	Overall	1819/1010	4	0.95(0.73–1.23)	0.70	4	**1.27(0.996–1.61)**	**0.05**	4	**0.80(0.65–0.99)**	**0.04**	2327/1518	5	1.10(0.98–1.23)	0.11
	Caucasian	1347/482	2	0.95(0.73–1.23)	0.70	2	**1.29(1.01–1.64)**	**0.04**	2	**0.79(0.64–0.98)**	**0.03**	1855/990	3	1.10(0.98–1.24)	0.10
	CD	1042/660	3	1.00(0.76–1.31)	0.98	3	1.25(0.97–1.61)	0.08	3	0.83(0.66–1.04)	0.11	1550/1168	4	1.10(0.98–1.25)	0.10
	UC	777/1010	4	0.83(0.60–1.15)	0.26	4	1.32(0.97–1.79)	0.07	4	**0.70(0.53–0.92)**	**0.01**			1.05(0.86–1.27)	0.63
TLR9 rs352140	Overall	1248/1078	3	1.44(0.68–3.07)	0.34	3	1.01(0.66–1.56)	0.95	3	1.17(0.73–1.88)	0.52			1.14(0.80–1.61)	0.47
	Caucasian	*956/792*	*1*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	African	*244/239*	*1*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	CD	740/1031	2	1.21(0.45–3.23)	0.71	2	0.84(0.68–1.03)	0.10	2	1.38(0.62–3.10)	0.43			0.90(0.79–1.03)	0.13
	UC	508/1078	3	1.34(0.71–2.53)	0.37	3	1.12(0.71–1.78)	0.63	3	1.03(0.84–1.28)	0.76			1.18(0.81–1.71)	0.38
TLR9 rs5743836	Overall	2675/2123	7	0.74(0.46–1.18)	0.20	7	0.93(0.80–1.08)	0.34	7	1.05(0.90–1.21)	0.56			0.92(0.81–1.05)	0.23
	Caucasian	1957/1413	4	0.80(0.47–1.37)	0.42	4	0.96(0.82–1.12)	0.60	4	1.03(0.87–1.20)	0.76			0.95(0.83–1.1)	0.50
	East Asian	479/471	2	0.99(0.06–15.91)	1.00	2	0.67(0.10–4.30)	0.67	2	1.49(0.23–9.53)	0.67			0.67(0.11–4.29)	0.68
	African	*239/239*	*1*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	CD	1717/2123	7	0.71(0.42–1.18)	0.19	7	0.92(0.78–1.08)	0.32	7	1.06(0.89–1.25)	0.51			0.91(0.79–1.06)	0.22
	UC	958/1584	5	0.74(0.41–1.31)	0.30	5	0.96(0.79–1.16)	0.65	5	1.01(0.83–1.23)	0.89			0.94(0.80–1.11)	0.48

Italicized results indicate that values are different from Wang's [1] meta-analysis. Rows in bold were analyzed by mistake in Wang's meta-analysis. Underlined results were missed from Wang's meta-analysis

\# Number of included genotypes

*Sample sizes different from three other genetic models (due to including studies with only allele frequencies)
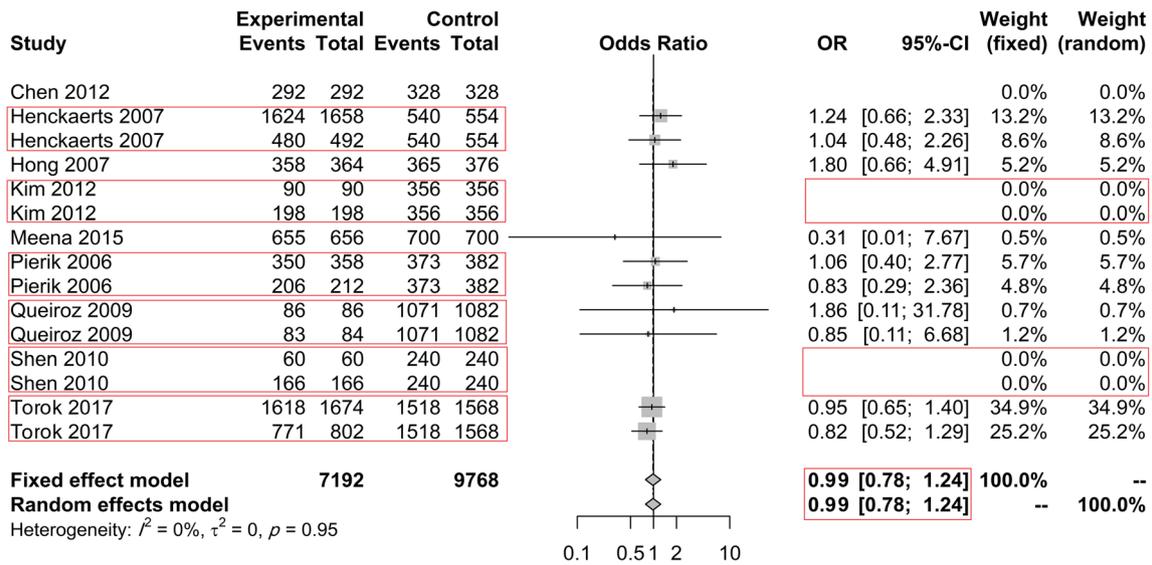
Fig. 1 Example of Wang's [1] incorrect meta-analysis (association between rs5743708 allelic model and IBD)

For allelic models, authors included allele frequencies of studies with non-available (NA) genotyping results. Accordingly, sample sizes for TLR6 rs5743810, TLR4 rs4986790, and TLR4 rs4986791 polymorphisms should be different from other genetic models. Also, the above-described mistake happened in the allelic model. For instance, for TLR6 rs5743810 polymorphism, "Kim 2012" was missed in the final meta-analysis; also "Henckaerts 2007" and "Pierik 2006" controls were repeated in their analysis (Fig. 1 shows inaccurate result and described mistakes by Wang [1] in TLR2 rs5743708 allelic model). Here, the corrected allelic model was shown (for all included polymorphisms) in Table 1 of our meta-analysis.

Further, authors missed to cite included articles in the meta-analysis (in their Table 1). We would recommend that the authors submit a corrigendum and include these references.

In conclusion, in contrast to Wang's [1] meta-analysis in overall and ethnic subgroup analyses, no significant association was observed between TLR1 rs5743611 and TLR9 rs352140 polymorphisms with IBD. Moreover, an African subgroup analysis was possible only on TLR4 rs4986790 polymorphism, while Wang analyzed six polymorphisms by

mistake. Based on basic mistakes in their meta-analysis, other results from our analysis (Table 1) were also different from their study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

Reference

1. Wang H, Zhou S, Zhang J, Lei S, Zhou J. Correlations between TLR polymorphisms and inflammatory bowel disease: a meta-analysis of 49 case-control studies. Immunol Res. 2019;67:142–50. https://doi.org/10.1007/s12026-018-9061-0.