Validating the meta-analytical results on MDM2, CASP8, XRCC3 polymorphisms and breast cancer risk: examination of Hardy-Weinberg Equilibrium

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Validating the meta-analytical results on MDM2, CASP8, XRCC3 polymorphisms and breast cancer risk: examination of Hardy-Weinberg Equilibrium.

Running title: Hardy-Weinberg Equilibrium and breast cancer.

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Dear Editor,

Following the valuable methodological remarks by Mao et al. [1] regarding our meta-analysis on glutathione-S-transferase polymorphisms and breast cancer risk [2], we decided to present elaborate sensitivity analyses on Hardy-Weinberg Equilibrium (HWE) in our three previous meta-analyses examining MDM2 SNP309 [3], CASP8 [4] and XRCC3 Thr241Met [5] polymorphisms and breast cancer risk.

Sensitivity analyses were performed excluding studies whose allele frequencies in controls exhibited significant deviation from HWE, given that the deviation may denote bias [6]. For the assessment of the deviation from HWE, the appropriate goodness-of-fit chi-square test was performed [6, 7].Of note a deviation from HWE in a mixed control population was allowed as the underlying assumptions of HWE are not fulfilled [8]. For the evaluation of the goodness-of-fit chi-square test, statistical significance was defined as $p<0.05$.

Concerning MDM2 SNP309 polymorphism only one study [9] included controls deviating from HWE. Significant deviation from HWE was noted in the controls of the Chinese part in the study by Haiman et al. [10] regarding CASP8 -652 6N del polymorphism; no deviation was noted regarding studies on CASP8 D302H polymorphism. With respect to XRCC3 Thr241Met polymorphism, deviation was noted in three individual case-control studies [11-13], as well as the Madrid study of the article published by the Breast Cancer Association Consortium [14].

We are happy to report that the sensitivity analysis replicated all the results pertaining to MDM2 SNP309 and XRCC3 Thr241Met. Concerning CASP8 -652 6N del

polymorphism, it should be declared that pooling of Chinese studies was no more feasible at the sensitivity analysis, as only one study remained. Of note, no sensitivity analysis was needed regarding CASP8 D302H in the light of no deviation. Detailed results are presented in the Table.

Taken as a whole, this Letter supports the validity of our previously published results as they proved robust enough to persist at the sensitivity analysis presented herein.

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Table: Pooled Odds Ratios (ORs) as initially published in the meta-analyses regarding MDM2 SNP309, CASP8 -652 6N del, CASP8 D302H and XRCC3 Thr241Met polymorphisms, as well as pooled ORs after the exclusion of studies whose controls significantly deviated from Hardy-Weinberg Equilibrium. Bold cells denote statistically significant ORs.

Models of studied polymorphisms	**pooled OR (95% CI)**	**pooled OR (95% CI) after exclusion of studies deviating from HWE**	**pooled OR (95% CI)**	**pooled OR (95% CI) after exclusion of studies deviating from HWE**	**pooled OR (95% CI)**	**pooled OR (95% CI) after exclusion of studies deviating from HWE**
MDM2 SNP309	**Overall**		**non-Chinese**		**Chinese**	
Heterozygous (GT vs. TT)	**1.056 (1.000-1.115)**	**1.077 (1.002-1.158)**	1.042 (0.985-1.103)	1.055 (0.977-1.139)	**1.272 (1.025-1.578)**	no deviation from HWE
Homozygous (GG vs. TT)	0.981 (0.908-1.060)	1.053 (0.951-1.166)	0.950 (0.875-1.030)	1.005 (0.898-1.124)	**1.323 (1.034-1.694)**	no deviation from HWE
Dominant (GT and GG vs. TT)	1.036 (0.984-1.090)	1.068 (0.998-1.142)	1.021 (0.968-1.076)	1.043 (0.971-1.121)	**1.287 (1.048-1.579)**	no deviation from HWE
CASP8 -652 6N del	**Overall**		**Caucasian**		**Chinese**	
Heterozygous	0.899 (0.779-1.037)^{R}	no deviation from HWE§	0.949 (0.886-1.017)	no deviation from HWE	0.838 (0.510-1.379)^{R}	only one study remained
Homozygous	0.871 (0.740-1.025)^{R}	no deviation from HWE§	0.933 (0.860-1.013)	no deviation from HWE	0.641 (0.391-1.052)^{R}	only one study remained
Any carriers	0.884 (0.761-1.028)^{R}	no deviation from HWE§	0.944 (0.884-1.008)	no deviation from HWE	0.811 (0.492-1.338)^{R}	only one study remained
CASP8 D302H	**Overall**		**Caucasian**		**Chinese**	
Heterozygous	-	-	**0.889 (0.847-0.933)**	no deviation from HWE	-	-
Homozygous	-	-	**0.711 (0.606-0.833)**	no deviation from HWE	-	-
Any carriers	-	-	**0.874 (0.834-0.917)**	no deviation from HWE	-	-
XRCC3 Thr241Met	**Overall**		**non-Chinese**		**Chinese**	
Heterozygous (CT vs. CC)	1.010 (0.949-1.074)^{R}	1.004 (0.961-1.049)	1.010 (0.968-1.054)	0.999 (0.956-1.044)	1.143 (0.664-1.968)^{R}	no deviation from HWE
Homozygous (TT vs. CC)	**1.073 (1.010-1.140)**	**1.073 (1.008-1.143)**	**1.082 (1.018-1.150)**	**1.083 (1.016-1.153)**	**0.574 (0.336-0.979)**	no deviation from HWE
Dominant (TT and CT vs. CC)	1.020 (0.962-1.081)^{R}	1.021 (0.979-1.064)	1.026 (0.985-1.069)	1.018 (0.976-1.061)	1.102 (0.623-1.949)^{R}	no deviation from HWE
Recessive (TT vs CC and CT)	**1.064 (1.007-1.124)**	**1.066 (1.008-1.129)**	**1.072 (1.014-1.133)**	**1.075 (1.014-1.138)**	0.815 (0.580-1.147)	no deviation from HWE

OR: Odds Ratios; CI: confidence interval; HWE: Hardy-Weinberg Equilibrium; R: Random effects; §: in non-mixed studies.

Comments on: Validating the meta-analytical results on MDM2, CASP8, XRCC3 polymorphisms and breast cancer risk: examination of Hardy-Weinberg Equilibrium

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Dear Editor,

We read with great interest the article by Sergentanis et al [1]. The authors performed sensitivity analyses on Hardy-Weinberg Equilibrium (HWE) in their three previous meta-analyses and concluded that the results of their previous studies were robust. We thank them for accepting our suggestion on sensitivity analyses by excluding the HWE-violating studies, but we are concerned about the following issues.

First, the authors stated that deviation from HWE in a mixed control population was allowed as the underlying assumptions of HWE are not fulfilled. They cited the article by Yu et al as a reference [2]. However, this reference was not an original article for this issue. In addition, even if deviation from HWE in a mixed control population was allowed, it is more appropriate to consider the studies with a mixed control population as an independent group when performing subgroup analyses or sensitivity analyses. However, the author neglected this point when they performed subgroup analyses or sensitivity analyses. They just divided ethnicity into Chinese and non-Chinese in their two previous studies.

Second, for MDM2 SNP309 polymorphism, the OR with 95%CI for heterozygous changed from 1.056 (1.000-1.115) to 1.077 (1.002-1.158) when they performed sensitivity analyses by excluding HWE-violating studies. The results for this genetic model should be interpreted with caution.

Third, for CASP8 -652 6N del, CASP8 D302H and XRCC3 Thr241Met polymorphisms, the authors stated that significant heterogeneity was detected in some comparisons. As we know, heterogeneity is a potential problem that may affects the interpretation of the results. It is important to present the changes for heterogeneity when the authors performed sensitivity analyses by excluding HWE-violating studies. Maybe departure from HWE is the main factor contributing to substantial heterogeneity

Fourth, the authors published six meta-analyses on the journal of Breast Cancer Research and Treatment. They only presented data for three studies. Even if the results of these three studies are robust, it does not guarantee the robust results of the remaining studies. Take the article for GSTP1 polymorphism as an example [3], when sensitivity analyses were performed by excluding one HWE-violating study, the results for Chinese population were materially changed [4]. Furthermore, considering the influence of sample-sizes, heterogeneity and publication bias on the results of meta-analysis, it may not be appropriate for the authors to conclude that their previously published results were robust as proved by sensitivity analyses.

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