Reviewer’s report

Title: NBS1 rs2735383 polymorphism is associated with an increased risk of laryngeal carcinoma

Version: 0 Date: 02 Jun 2017

Reviewer: Katarzyna Kiwerska

Reviewer's report:

In this paper, Hinmei Hu and colleagues performed a case-control study to detect the associations between two known SNPs in NBS1 gene in relation to the risk of laryngeal cancer. The authors shown that one of these polymorphisms, namely rs2735383C variant confers the increased risk of laryngeal carcinoma. The strong point for this study is that the authors demonstrated that GG and GC variants (together and separately) are accompanied by lower level of NBS1 mRNA.

The paper is mostly clearly written and the data are presented properly. However, there are lots of points that need to be explained and/or corrected during the major revision.

Comments

1. The manuscript should be generally improved in terms of bad English- there are lots of language errors throughout the whole paper.

2. There are ambiguities concerning studied population: in the Study subjects part the authors wrote: "we only recruited people whose ethnicity is unrelated Han or Zhuang", while from Table 1 we learn that only Han and Zhuang patients were included to the study group. Another term is used at page no. 4 (Chinese population of Guangxi of China). It should be unified.

3. One system concerning describing PCR mixture components should be accepted (page 6, line 15-17) - i.e. 5 mM MgCl2, 25 µl and so on… - there should be a space between the quantity and its measure.

4. There are numerous discrepancies concerning cited literature references, that has to be clarified. In this layout it does not generally support the selection of both polymorphisms to this study.

a) In the Background the authors maintain that "in recent years the incidence of laryngeal carcinoma around the world has been rising dramatically". The available reports, f.e. the newest „Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost,
Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015” (JAMA Oncol. 2017;3(4):524-548) indicate that the global incidence of larynx cancer decreases. Of course, laryngeal cancer is a large problem worldwide because of its complexity and heterogeneous nature and the searching for novel biomarkers or susceptibility genes rather comes from this characteristics and not necessarily from its common incidence.

b) The NBS1 polymorphic variants were previously analyzed in laryngeal carcinoma, therefore I suggest to change the cited paper in the Background, [14] from Lu et al 2009 to Ziolkowska et al 2007 [34].

c) In the paper cited on page 4, lines 30-31 [18] by Zheng et al 2011, the authors confirmed only the association between rs1805794 and NPC but not between rs2735383 and NPC. The same situation is for paper [19] (Jiang et al 2011) - no difference in the susceptibility to ALL was found concerning rs2735383 genotypes. Therefore, I suggest to exclude these citations from the Background.

d) Also during Discussion some cited papers are selected improperly, the authors cite Yao et al [24] (Tumor Biol 2013; page 11, line 17) after they wrote: "NBS1 rs1805794 polymorphism which has been frequently investigated showed that an association between the variant genotype and breast cancer risk". However, the cited authors outright suggests lack of NBS1 Glu185Gln polymorphism association with breast cancer risk in any populations. The same concerns Broberg et al [30] (Carcinogenesis 2005 (page 11, line 20)).

e) Discussion, page 11, lines 39-40 - authors claim, that in the Reference paper [32] (by Han J et al 2009) authors indicate association of rs2735383G>C in NBS1 with breast cancer. Indeed, they found a significant trend of increased risk with increasing numbers of risk alleles in the DSB-NHEJ pathway but the NBS1 gene (named NBN there) was included into the pathway of genes participating in DSB-HR pathway, which brought opposite results. Thus, the authors should carefully verify the available reports.

f) The same concerns "association rs2735383 G>C in NBS1 with lymphoid malignancies" - the cited authors (Rollinson et al 2006 [25]) indicated a lack of: "significant differences in allele or genotype frequency, global haplotype distribution between the cases and control, nor effect for individual haplotypes when analysed by unconditional logistic regression for either RAD50 or NBS1".

5. The authors should explain the origin of "major" and "minor" allele naming, reffering to adequate database. In the manuscript, the authors describe allele G for rs1805794 as major one and allele G for rs2735383 as minor one. According to dbSNP G alleles for both polymorphisms are the minor variants. On the contrary, the results collected in Table 2
indicate that G allele is the major allele for rs1805794 and rs2735383. This part of Genotyping analysis should be clarified.

6. In the Discussion, page 11, lines 25-26 - I suggest to replace the formulation "risk of CRC" with "risk of different tumors, including those of head and neck" - as this conclusion results from the cited paper.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

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