Author’s response to reviews

Title: Association between polymorphisms of TAS2R16 and susceptibility to colorectal cancer

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Author’s response to reviews:

Dear Editor:

Please find enclosed the revised version of the manuscript entitled “Association between polymorphisms of TAS2R16 and susceptibility to colorectal cancer" by Barontini et al.,

We provide here a point by point response to the reviewer and editor queries.

The changes in the manuscript are highlighted using track change, while the changes in the supplementary tables are highlighted in yellow.

We wish that in the present form the manuscript would be acceptable for publication in BMC Gastroenterology.

Best regards

Stefano Landi

Reply to editor comments:

In the paper, authors tested the role of polymorphic variants in TAS2R16 gene in modulating the risk of colorectal cancer in a quite large set of cases and controls from 4 European cohorts.

The main results was rs1525489 that resulted significantly associated with increased risk of rectal cancer. For the other tagging SNPs no main associations were observed. In general, the manuscript is correct but there are some points that are not really clear and authors should consider.

Main:

I did not understand the approach chosen by authors in the selection of tagging SNPs. They reported a previous work by Campbell et al showing that a specific TAS2R16 SNP was
conferring sensitivity to salicin. Why the authors did not include also this functional polymorphism among the tested SNPs?

The polymorphisms reported by Campbell and colleagues that is mentioned in our study is monomorphic in Caucasians. We understand that nonincluding this information might be confounding for the reader. We have now written in the introduction this information.


We thank the editor for the suggestions we have modified the introduction section and cited the reviews she suggested.

Overall, I have noticed a general superficiality in presenting the data. There is no clear description of small but important details from the epidemiological point of view. For instance, for age is not specified whether it was age at sampling, at diagnosis etc.

We have added this information

The indication of the version of databases used is completely missed.

We have added this information

There are no details on the number of starting SNPs and details on the criteria of selection. This is important in view of a possible replication of data.

We have rewritten the section on SNP selection to be more clear. We are not sure to have understood what starting SNPs means. The SNP selection comprised 6 polymorphisms that were all genotyped successfully; we did not drop any SNP due to genotyping failures. The number of SNPs captured by our the selection is 21.
The main result is on rs1525489 but I would consider very careful these outcomes since the less frequent allele is particularly rare in the cohorts (see Table 2: only one individual with m/m genotype overall). I would consider the results obtained more cautiously, also considering that p-value obtained would not be significant if adjusted for multiple tests.

We do agree that the results should be interpreted with caution and indeed we conclude saying "our data suggest that polymorphisms of the TAS2R16 gene do not have a strong influence on colon cancer susceptibility, but a possible role in rectal cancer should be further evaluated in larger cohorts". However following also the suggestion of reviewer 2 we have changed the text saying that the association between the SNP and rectal cancer is borderline and not flat out significant. In addition considering also the comment on the differential expression of rectal and colon cancer (please see below a detailed answer) we have added in the discussion that the signal needs to be taken with caution since it comes from stratified analysis and therefore a smaller group of individuals.

Authors discussed the treatment of aspirin in inflammatory statuses; however, there are no info on the intake/use of aspirin or other NSAID drugs in the case-control cohorts included in the study. I think that this is a strong limitation in the study that limits the results obtained to simple speculations. Authors should at least mention this limitation in the discussion. Moreover, I found quite peculiar the attempt in the discussion to observe that rs1525489 was close to significance with p=0.084. Again, authors are trying to discuss about a very rare allele.

We agree that this a limitation that needs to be mentioned in the discussion section of the manuscript, regarding the association to which the editor is referring we do not say that it is close to significance we just say that it is the closer to significance.

Authors should report, if available, more data regarding the functionality/phenotypic effect of the SNPs selected.

We used three common bioinformatic tools, as written in the text, to assess, even if indirectly, the possible functional relevance of the SNPs. The results with all the software did not point to a functional effect of the SNPs.

Besides the already existing data on phenotypic effect of rs846664, authors did not even tell if the SNP investigated was in LD with the SNP investigated by Campbell et al.

The polymorphisms reported by Campbell and colleagues that is mentioned in our study is monomorphic in Caucasians. We understand that not including this information might be confounding for the reader. We have now written in the introduction this information.
I found quite strange the sentence on page 11 “This could be explained by a higher expression…” I think that this part of the discussion is conceptually wrong. From expression data you cannot evaluate the effect of a SNP unless you have genotype data (and then you can treat as eQTLs) or specific assay (CRISP/Cas or Luciferase). It is also incorrect that publicly available databases are not separating colon and rectal cancers: for example, TCGA keeps colon and rectal cancer separated (COAD and READ databases are for colon and rectal cancers, respectively).

The sentence was highly speculative and as the editor correctly states not supported by data analysis. We have checked the COAD and READ databases and we have also looked and another database ONCOMINE (www.oncomine.org). The results that we gathered from the databases do not seem to support any differential expression of the TAS2R16 gene in colon or rectal cancer. We would like to point out that we were referring the expression of the gene regardless of the genotyping and in normal tissue not in neoplastic one. However, since we cannot support our speculation we have decided to eliminate that sentence.

Also the discussion of rs1525489 MAF is quite strange: although the snps was rare it was included in the study for completeness but this is not mentioned in the material and methods.

The editor is right the sentence could be confusing. There was an error in the MAF threshold that we used we used (MAF)>0.05 and not (MAF)>0.01 as written in the manuscript. Now the error has been corrected. Rs1525489 has a low MAF around 6% in European 1000G. So if considering only the data from 1000G the frequency is included in the interval that we have chosen (MAF)>0.05 however in our populations combined the frequency is lower (3.1% as reported in supplementary table 1) and this explains why we have written that sentence. We agree with the editor anyway that it is not clear for the reader and therefore we have changed it accordingly.

I would suggest also that in view of completeness also the SNP found by Cambell et al would have been included.

The polymorphisms reported by Campbell and colleagues that is mentioned in our study is monomorphic in Caucasians. We understand that not including this information might be confounding for the reader. We have now written in the introduction this information.

The manuscript presents several weaknesses that should be discussed more carefully and consciously by the authors and more data on previous studies investigating TAS2R16 gene should be included.

We sincerely thank the editor for the comments and suggestion and we truly believe that addressing them has improved the manuscript.
Minor comments:

Abstract

Double check the “background” section: there is an Italian word appearing.

We carefully checked but we did not find any Italian word in the abstract.

Results

Revise the text since there are some type errors (see for instance the end of first paragraph on page 8 “..reported In supplementary..”)

We have thoroughly revised all the manuscript.

Discussion

Please rephrase the first sentence of the section since it is not understandable “to further… that show the role of genes polymorphic variants taste receptor genes in…”

We thank the editor for the comment and we have changed the sentence to be clearer.

Table 1

Authors did not tested whether there were significant differences among cases and controls in the distribution of covariates presented in Table1 both overall or among cohorts. For the Italian Case-control group for example there is a quite large difference of age (almost 15 years) between cases and control. Similarly, the gender distribution in controls is not reflecting the real distribution of the cancer among gender..

As the editor knows very well it is extremely difficult in a retrospective study to match cases and controls for epidemiologic parameters especially in disease of the elderly such as cancer. However, we are aware of the potential bias that this can introduce and this is the reason why we systematically adjust all the analyses for age and gender.
Reviewer reports:

Manuela Gariboldi (Reviewer 1):

Only one SNP (rs1525489) was significantly different, although with low significance, in two of the four populations analyzed. And overall it only showed a p-trend. The fact that the association was found in sub-populations from Lithuania and Spain and not in populations from Czech Republic and Italy, compensating the different origins in both groups, supports the possibility that it is a casual interaction.

A study aimed at evaluating the role of TAS2R16 in the context of colorectal adenomas has already been conducted, although on populations from different ethnicity (Schembre SM et al., Variations in bitter-taste receptor genes, dietary intake, and colorectal adenoma risk. Nutr Cancer. 2013; 65:982-90), and should be mentioned and discussed in the text. This study found non association of the SNP analyzed and presence of adenomas. The sentence "The study was carried out in a large cohort of European individuals and it is the first to evaluate the role of this receptor in the context of CRC carcinogenesis" should be modified accordingly.

The manuscript by Schembre and colleagues should was cited in our manuscript (it was reference 24 of the originally submitted version). We did not discuss it in detail because it was conducted in the multietnic PLCO cohort in which only a small part is constituted by "whites" as defined by the authors. In addition, in the work by Schembre only two SNPs have been investigated: one is monomorphic in Caucasian and the other one rs860170 (that we genotyped in our study) was not in HWE in any of the population studied by Schembre and colleagues. However, it is true that add some discussion on the work of Schembre and colleagues could help in putting in prospective ours and therefore we have added a text in the discussion that reads: "Schembre and colleagues have performed a study investigating two TAS2R16 polymorphic variants (rs846672 and rs846664) in relation two colorectal adenoma risk. Even though the study is rather small (914 cases of three different ethnic groups) their results are concordant with ours suggesting an overall no effect of the two variants in the disease risk. The rs846664 SNP is monomorphic in Caucasian and therefore was not typed in our study while rs846672 is in complete LD with rs860170 that was used in the present study (r2=1 in European Hapmap Ceu)". The sentence "The study was carried out in a large cohort of European individuals and it is the first to evaluate the role of this receptor in the context of CRC carcinogenesis" was instead removed from the manuscript.

The only new result emerging from here is the association of SNP rs1525489 with an increased risk of developing rectal cancer (p=0.007) in the whole series analyzed. Please add its association in each stratified population.
We have modified the supplementary tables and added all the stratified analyses requested by the reviewer.

To support the significance of rs1525489 SNP between colon and rectum, authors speculate that colon and rectum may have a different expression of TAS2R16. Despite what they say, separate gene expression profiles of rectal and colon cancer are available for example at TCGA portal and should be investigated and results should be added to the text, to confirm what they say. Nevertheless, it has been reported that there is no overall effect of aspirin on the risk of rectal cancer (Garcia-Albeniz X. and A.T. Chan. Aspirin for the prevention of colorectal cancer. Best Pract Res Clin Gastroenterol. 2011; 25: 461-472).

The sentence was highly speculative and as the editor correctly states not supported by data analysis. We have checked the COAD and READ databases and we have also looked another database ONCOMINE (www.oncomine.org). The results that we gathered from the databases do not seem to support any differential expression of the TAS2R16 gene in colon or rectal cancer. We would like to point out that we were referring the expression of the gene regardless of the genotyping and in normal tissue not in neoplastic one. However, since we cannot support our speculation we have decided to eliminate that sentence.

Paolo Peterlongo (Reviewer 2):

A well written and clear little piece of information adding to the field of CRC genetic predisposition.

We thank the reviewer for his comment

You may want to specify that, according to the Bonferroni correction you applied, the p-vale of 0.007 is suggestive of "marginal/borderline significance"

We have modified the results and the discussion section of the manuscript taking into account the reviewer comments. The results now read: Following the stratification by tumor site (colon / rectum) we found that rs1525489 showed a marginal/borderline significance with increased risk of rectal cancer at nominal level of (P_{trend}=0.0071), while the associations between the SNPs and colon cancer were similar to those considering the two strata together, while the discussion now is: This association showed a marginal/borderline significance after Bonferroni’s correction (P=0.0071).