Author's response to reviews

Title: The TGFBR1*6A allele is not associated with susceptibility to colorectal cancer in a Spanish population: a case-control study

Authors:

Adela Castillejo (acastillejo@umh.es)
Trinidad Mata-Balaguer (trini_mata@hotmail.com)
Paola Montenegro (paolacmb@yahoo.es)
Enrique Ochoa (enrique.ochoa@hospital2000.net)
Rafael Lázaro (lazaroraf@gva.es)
Ana Martínez-Cantó (martinez_anacan@gva.es)
María I Castillejo (micastillejo@gmail.com)
Carla Guarinos (carlaguarinos@gmail.com)
Víctor M Barberá (barberavicua@gva.es)
Carmen Guillén-Ponce (carmenguillenponce@yahoo.es)
Alfredo Carrato (acarrato@telefonica.net)
José L Soto (jlsoto@umh.es)

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Author's response to reviews: see over
Dear Editors,

We thank you for your prompt response to our submission. The reviewers’ comments were constructive and useful for improving our manuscript. We addressed all aspects covered by the reviewers and revised the manuscript as follows.

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**Major compulsory revisions**

**Reviewer #1: Boris Pasche**

1. *MSI status was assessed in tumor DNA from 120 patients using only one marker, BAT-26. The authors should justify why only one marker was used when standard international guidelines recommend the use of several markers to assess microsatellite instability. Most studies use at least BAT-25 in addition to BAT-26 and this reviewer is not aware of any report in which only BAT-26 was used to assess MSI status.*

We agree with the reviewer. We performed the analysis using four other mononucleotide markers (BAT25, NR21, NR24 and NR27). These markers, together with BAT26, have been shown to have sufficient specificity and sensitivity for diagnosis of microsatellite instability status in tumours without the need for individual normal DNA counterparts (Buhard et al., 2006; Xicola et al., 2007). We use this set of markers in routine clinical screening of families suspected of having Lynch syndrome, as does the Reference Laboratory for the Genetic Counseling in Cancer program of the Comunidad Valenciana (Spain). In our experience, in the Spanish population, the BAT26 marker is sufficiently informative to enable microsatellite instability assessment in selected cases of sporadic colorectal cancer (unpublished results). In fact, results obtained in the present subseries of 120 cases when
the four extra microsatellite markers were added were identical to those obtained using BAT26 alone.

The relevant paragraph in the Methods section for microsatellite instability was modified as follows.

“A subset of colorectal tumour DNAs from 120 patients was screened for MSI status using five mononucleotide markers (BAT26, BAT25, NR21, NR24 and NR27) and multiplex PCR as previously described by Buhard et al. (2006) [15].”

2. While the study is powered to detect an OR of 2.0 for *9A/*6A heterozygous individuals and an OR of 3.0 for *6A homozygous individual with 80% power, this should be considered markedly under powered based on the previous studies sided by the authors. Indeed, the OR for heterozygotes has ranged between 1.15 and 1.20 and the OR for homozygotes has not exceeded 2.0. Hence, the study is under powered to detect differences between cases on controls based on the published literature. The authors should acknowledge this weakness.

We agree with the reviewer. We discussed the contradictory results in the literature, the limited sample size and the missing information on case–control matching in some of the studies. Being aware of this limitation, we acknowledged this weakness in the Discussion.

“In association studies, contradictory conclusions may arise from population stratification or inappropriate sample size”…. “Because of the limitation that sample size exerts on association studies, we attempted to extract extra information from our data to elucidate the susceptibility effect of the minor allele of this polymorphism”.

In accordance with the reviewer’s recommendation, we added the following sentence to the Discussion.

“There was little information on which to base the sample size for our study because information about this polymorphism in our population is limited and contradictory results were reported for another population. The results show that our study was under-powered for detecting weak associations between the TGFBR1*6A allele and CRC. The data indicate that a sample size of 10,000 cases and 10,000 controls
is necessary to detect an OR of 1.15 with 80% power using a two-sided test with an alpha level of 5%.”

3. The authors should cite a recent report by Zeng et al, Cancer Res 2009, 69:678-686, showing that constitutively decreased Tgfbr1 signaling is a potent modified of colorectal cancer development.

This is an interesting report that suggests that the germline Tgfbr1 haploinsufficiency plays a role in the development of CRC. We have mentioned it in the Background and included the reference.

“Recently, it has been suggested that a mouse model carrying a germline Tgfbr1 haploinsufficiency has constitutively reduced TGF-beta signalling that significantly enhances the development of colorectal cancer [4].”

4. The authors should clarify what they mean by "bystander effect" on ASE alterations on page 12, last line.

In the previous sentence, we discussed the strong effect that the ASE phenomenon appears to have in CRC risk and the linkage disequilibrium with the TGFBR1*6A allele.

“… the authors concluded that ASE of TGFBR1 is a major contributor to genetic predisposition for CRC (OR, 8.7; 95% CI, 2.6–29.1).... One of the putative mutations that causes ASE is probably in linkage disequilibrium with the TGFBR1*6A allele but is not itself causative of ASE.... The linkage disequilibrium between ASE and the TGFBR1*6A allele could explain the contradictory results in the literature on the association between this polymorphism and CRC.”

In this scenario, we think that it is possible that the previously reported potential CRC susceptibility conferred by the TGFBR1*6A allele may have been caused by linkage disequilibrium with the ASE alterations, which we consider a “bystander effect”.

As the message was conveyed clearly at the end of the Discussion, we deleted the sentence on the last line of page 13 to prevent misinterpretation.
Major compulsory revisions

Reviewer #2: Seyed Vahid V Hosseini

1. Material

This is a hospital-based case-control study with 800 subjects. The controls have been selected from the same hospital but without history of cancer. It is obvious that hospitalized controls are different from normal population and had other diseases. A question is which diseases they had and is there any association between the exposure of interest and those diseases.

The controls were patients who attended our emergency room and were selected on the basis of diagnoses considered unrelated to the exposures of interest (e.g., hernias, bone fractures, hydrocele and appendicitis). Because this information was not mentioned in the text, we added the phrase: “selected with diagnoses considered unrelated to the exposures of interest”.

2. Methods

I have a major concern with sample size calculation. It has been mentioned that matching has been done for age, sex and race/ethnicity. However, it is not clear whether the sample size was calculated for matched or unmatched study, and with what frequency of exposure among controls.

The sample size was calculated for matched controls, and the frequency of exposure among controls was estimated as 0.15 for heterozygous individuals and 0.0175 for homozygous individuals based on the previous results of our group (pilot study) and others.

The corresponding paragraph in the Discussion was modified accordingly.

“We determined a priori that our study with 400 sporadic CRC cases and 400 matched controls would enable us to detect an OR of 2.0 for 9A/6A heterozygous individuals (assuming a frequency of 0.15 in controls) and an OR of 3.0 for 6A/6A homozygous individuals (assuming a frequency of 0.0175 in controls) with 80% power (two-sided test, alpha level = 5%).”
Minors revisions
Reviewer #2: Seyed Vahid V Hosseini

Comments

1. *We cannot find MSI status just by one marker, it isn’t sufficient.*

   See response to Referee #1, Major compulsory revision 1.

2. *Please determine Chi square, p value if genotype distribution in case, control populations did not deviate from the Hardy-Weinberg equilibrium.*

   We have included the p values in the text (*p=0.295 for cases and p=0.033 for controls*).

3. *The p value = 0.024 shows the distribution of genotypes between proximal, distal and rectal not colon and rectal alone.*

   The p value of 0.024 refers to the distribution of genotypes between the proximal colon plus the distal colon versus the rectum.

References


We trust that you find the revised manuscript suitable for publication in the BMC Cancer journal.

Sincerely yours,

JL Soto, PhD
Elche University Hospital