Author's response to reviews

**Title:** Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population

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**Author's response to reviews:** see over
Dear Dr Edmunds,

Please find enclosed the revised original article MS 1321081475152364. Thanks to reviewers’ suggestions the MS has improved a lot, however I would ask if it is possible to Dr. Sichel (reviewer no. 2) to take a look at the CYP alleles nomenclature. I had actually changed it according to his suggestions, but for being safe that this is correct I would be grateful if you send him again the MS for checking out. Thanks for that.

Following the answers to each single reviewer’ comments:

**Reviewer 1 (Ladero)**

- Due to space constraints, the paper submitted on July included in the references the meta-analyses of both CYP2E1-RsaI and NAT2 polymorphisms and gastric cancer (Boccia S, Carcinogenesis, 2007; Boccia S, Ital JPH), instead of each single paper published (e.g., Nishimoto, Cai, Gao, Ladero, that you suggested). In fact, all these individual papers are incorporated in the mentioned meta-analyses. As for the Agudo paper, now it is included in the discussion. Lastly, Roth paper is focused on gastric cardia cancer, so due to space constraints I couldn’t add it, since I was forced in selecting references investigating the association of those SNPS with stomach cancer overall.

- Control group selection: it is true that as in every case-control study choosing the most appropriate control group is a daunting issue. Controls shouldn’t be chosen with the respect of their exposure status, as you say, and by choosing diseased controls as in our case, it might turn out in selecting more exposed
individuals. On the other hand, controls should be representative of the exposure distribution in the source population for the cases (Rothman; ‘Epidemiology an introduction’, 2002, chpt.4). So, the point is that the control population shouldn’t be selected among the general Italian population, but from those that give rise to cases; in other words, controls individuals should be people that would go the our hospital if they had a gastric cancer. In this sense, I do not find inappropriate our control selection (that actually include also around 15% of blood donors which is now reported), and I argue I shouldn’t acknowledge any limitation in the discussion about it, not because the SNPs frequencies are in HWE or are close to the Italian population, but for the mentioned reasons. So, I find very hard to figure out if by choosing very ‘healthy’ controls the validity of our study would improve.

- The gastrectomy interventions were performed with a curative intention in 100% of our cases, however it was actually curative only in around 82% of patients.

**Reviewer 2 (Sichel)**

- Thanks for pointing me out some mistakes. Now they are edited in the revised version. Also the CYP nomenclature has been modified according to the latest one (please check if correct, thanks).

- Point no. 1: I added in the discussion the weakness concerning missing data on *Helicobacter pylori* infection.

- Point no.2: instead of shortening the discussion, I added at the end of the 4th paragraph the limitation in interpreting results concerning CYP2E1*6 allele among ever-drinkers with respect to gastric cancer risk.

**Reviewer 3**
Point no. 1:

- We defined gastric cancer cases as including International Classification of Disease Ninth revision codes 151.0-151.9 (now specified in the methods section of the paper). In the ‘study population’ section of the methods the term ‘intestinal’ refers to the histotype of gastric cancer, according to the Lauren classification (ref.17). In the edited version of the MS I clearly specify it.

- According to the latest available statistics, gastric cancer is the second most common cause of mortality for cancer worldwide including males and females (http://www-dep.iarc.fr/globocan/database.htm). The Globocan statistics are in line with the reference of Veredecchia at el., 2003 (ref. no 1).

Point no. 2 (‘Study groups’)

- I agree with you about the fact that I cannot generalize the results of this MS to all the Italian population, so now I wrote elsewhere ‘this Italian population’, to make clear that results are referred to the studied population and not all Italians.

- Control group did not include cancer individuals, neither non-cancer gastric individuals.

- Study group is small especially for testing for gene-gene and gene-environment interactions, and this is acknowledged in the abstract, in pag. 12 second paragraph, and pag. 15 last paragraph.

Point no. 3: as for the ethical approval and informed consensus, on pag-6, last paragraph you can read that ‘The study was approved by the local review board and written informed consent was obtained from each subject’.

Point no. 4: Wine consumption was not collected according to the red or white one. By considering the overall wine consumption, an increased risk still
remains among consumers (adjusted OR for 0.5-1.5 glass/day versus 0 glass = 2.57 (1.43-4.61). So results do not change overall.

Point no. 5: Our results show that family history for cancer is more frequent among cases than controls, not really it confirms about ‘familiar clustering’. So I removed this sentence and just reported that confirmed previous reports on the increased risk in individuals with a positive family history for cancer. And I added a new the reference for that (ref. no. 28).

Point no. 6: Table 3 reports ORs for some gene-gene combinations adjusted by age and gender (those 2 are confounding variables—see Table 1). I argue the heading reflects the meaning of this.

All minor revisions are now implemented, thanks.

Reviewer 4 (Hsieh)

Your suggestions have been introduced in the revised MS.

Reviewer 5

All the minor revisions (points no. 1-4, 8, 11, 12) are now implemented in the revised version, thanks for that.

Specifically, point no. 5: gastric stump might also be called ‘gastric remnant’ but ‘gastric stump’ is the most used term to indicate the piece of the primordial stomach remained in a person after a previous surgical intervention.

Specifically, point no. 6: I added, in the appropriate section, that family history of cancer included also non-melanoma skin cancer.

Specifically, point no. 7: choosing to highlight the non-significant results for NAT2 slow (and not, e.g. CYP2E1 DraI), stems from that. i) the lower-bound CI for NAT2 slow is very close to 1 (0.88), anyway the closest to 1 respect to the other lower-
bound CIs estimates and that ii) it is computed on a relatively high percentage of individuals (59.8% of cases and 51.8% of controls) so it should be more accurate than other non-significant results.

Specifically, point no. 9: study group is small to test for gene-gene and gene-environment interaction analyses, I agree, in fact this is acknowledged in the abstract, in pag. 12 second paragraph, and pag. 15 last paragraph.

Specifically, point no.10: I changed the sentence, just highlighting the result of SULT1A1 His carriers among ever-smokers.

All authors have read and approved the revised manuscript.

With many thanks for your assistance,

Stefania Boccia