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Answer to reviewers

Dear Dr. Ke Chen,

Science Editor, Editorial Office,

We are pleased to send you our reviewed version of the manuscript No. 36229. We would like to thank the reviewers and the editor for their time and attention given to our work. All questions and suggestions were considered and incorporated in the revision of the manuscript. In this document, we provide the answers to such questions and suggestions.

Comments made by the Reviewer #1: The authors evaluated the relation between 12 polymorphisms in different genes and the development of gastric cancer. This is a carefully done study and the findings are of considerable interest. For the benefit of the reader, however, a number of points need modifying. These are given below. 1. (Materials and Methods, Page 4, Line 24-26) How did authors confirm the condition of 475 cancer free individuals? Did all these people take upper endoscopy, colonoscopy and CT examination? 2. (Results and Discussion, Page 5, Line 18) The authors should separate Results and Discussion section. 3. (Results and Discussion, Page 6, Line 29- Page 7, Line 2) What is the percentage of H. pylori infection patients in these patients? H. pylori status seems to be more important for developing GC than genotype. 4. (Results and Discussion, Page 10, Line 1- 8) How does the joint presence of the alleles affect the development of GC and CRC? The authors should mention expected mechanism or previous reports.

Answers to Reviewer #1:

Thank you for taking the time to review our manuscript and for the compliments on the work. We have the following answers for the raised questions:

1. (Materials and Methods, Page 4, Line 24- 26) How did authors confirm the condition of 475 cancer free individuals? Did all these people take upper endoscopy, colonoscopy and CT examination?

Answer: We have included the following sentence in the text explaining the inclusion criteria in the cancer-free group (Materials and Methods, Page 7, Lines 15-17): "The cancer-free individuals didn't have personal or familial history of any kind of cancer and they didn't show any symptoms or signs of cancer".

2. (Results and Discussion, Page 5, Line 18) The authors should separate Results and Discussion section.

Answer: We have separated the Results and the Discussion sections. The Results section now starts at Page 8 (Line 14) and the Discussion section starts at Page 11 (Line 1).

3. (Results and Discussion, Page 6, Line 29- Page 7, Line 2) What is the percentage of *H. pylori* infection patients in these patients? *H. pylori* status seems to be more important for developing GC than genotype.

Answer: We do not have the information concerning *H. pylori* infection for these patients. Even though we recognize the importance of this infection in the development of GC, we do not believe it is determinant or more important than the genotype. Cancer development is a multifactorial process and genetics plays a major role in it. We have included the following excerpt in the text, supported by other references (Introduction, Page 6, Lines 6-12): "Carcinogenesis is a multifactorial process. Gastritis and colitis have been related to the development of GC [3,4] and CRC [5,6], respectively, but they are not determinant. The infection by *Helicobacter pylori*, one of the most common human infectious agents, is also very important to the development of gastritis and GC [7]. However, it should not be considered the only cause to the development of this type of cancer [8]. Genetics also plays a major role in the carcinogenesis and there are still a lot to be discovered regarding this subject".

4. (Results and Discussion, Page 10, Line 1- 8) How does the joint presence of the alleles affect the development of GC and CRC? The authors should mention expected mechanism or previous reports.

Answer: We performed the joint presence analyses of the alleles that were statistically significant when analyzed in homozygosis (one genotype in homozygosis vs. other genotypes): rs79071878 (*IL4* gene), rs3730485 (*MDM2* gene) and rs28362491 (*NFKB1* gene) in GC development; rs28362491 (*NFKB1* gene) and rs8175347 (*UGT1A1* gene) in CRC development. To the best of our knowledge, this is the first study investigating the association between the polymorphisms rs79071878 and rs3730485 and the development of GC, as well as between rs8175347 and the development of CRC. As for the polymorphism rs28362491 and the development of GC and CRC, it has been shown the positive association of the DEL/DEL genotype and the development of GC in a Japanese population, but some studies with other types of cancer in different populations obtained other results and it has been suggested that the effects of this polymorphism in the activity of *NFKB1* may be ethnic and cancer-type specific (Discussion, Page 12, Lines 12-25). Considering the role of *IL4* gene in inflammatory pathways and that the RP1 allele of the polymorphism rs79071878 has been associated with the increased risk of developing other types of cancer, it is likely that this

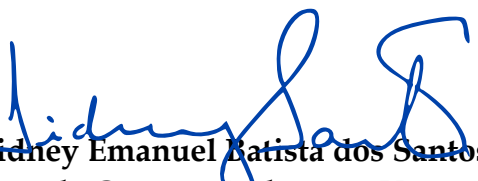
allele enhances the inflammatory activity of *IL4*, which is reinforced by the overlap of a higher frequency of this allele in the North population of Brazil and a high incidence of GC (Discussion, Page 11, Lines 15-27). In our study, the INS/INS genotype of the polymorphism rs3730485 was shown to reduce the risk of developing GC; in other studies, the allele DEL of this polymorphism was appeared to be related to increased chances of developing different types of cancer. Therefore, we believe that the INS allele of this polymorphism may reduce the activity of the oncogene *MDM2*, which could lead to an enhanced activity of the tumor suppressor *TP53*, due to the feedback relation of these genes, and reduce the chances of developing cancer (Discussion, Page 12, Lines 1-9). Lastly, the *UGT1A1* gene is involved in the metabolization of substances and the hepatic detoxification, but little is known about the rare alleles *36 and *37 of the rs8175347 polymorphism in this gene. In fact, some studies have reported that these alleles are extremely rare or even absent in different populations, but no association studies were found relating these alleles to cancer development (Discussion, Page 13, Lines 2-4). Nevertheless, considering our findings of a 13-times increase in the risk of developing CRC in the presence of *36 and/or *37, it is possible that these alleles induce a decrease in the activity of the *UGT1A1* gene, leading to a carcinogenic process.

Comments made by the Reviewer #2: I have read this manuscript with great interest. It is an interesting study. I recommend it for publication.

Answers to Reviewer #2: Thank you for taking the time to review our manuscript and for the compliments on the work.

Comments made by the Reviewer #3: Authors did a great study. There is nothing to comment.

Answers to Reviewer #3: Thank you for taking the time to review our manuscript and for the compliments on the work.



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