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June 24, 2017

Ze-Mao Gong
Science Editor
Baishideng Publishing Group

Dear Ze-Mao Gong,

We thank you and referees for reviewing our Unsolicited Manuscript titled "**Association of INDEL polymorphisms with Colorectal Cancer risk and clinical features**" by Marques et al. for publication in the World journal of Gastroenterology (The manuscript ID: 34257).

We revised the manuscript based on the Reviewer's comments and in compliance with the guidelines of Revision Instruction (please see the point-by-point responses to the comments of the reviewer). We have made the requested changes in the "Methods" (i.e. incorporating "Polymorphism selection" section.) and in the Supplementary table. Finally, the manuscript was reviewed by native English speakers.

Please find attached our revised and reformatted manuscript. The revised portions are highlighted by colored text in the revised manuscript. We hope the revised manuscript is now acceptable for publication in the WJG.

With kind regards,

Vivian Nogueira Silbiger
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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 34257

Title: Association of INDEL polymorphisms with Colorectal Cancer risk and clinical features

Reviewer's code: 00069015

Reviewer's country: China

Science editor: Ze-Mao Gong

Date sent for review: 2017-04-27

Date reviewed: 2017-05-09

COMMENTS TO AUTHORS

The manuscript entitled "Association of INDEL polymorphisms with colorectal cancer risk and clinical features"; the author conducted this study between 140 patients with CRC and 140 normal controls based on 16 INDEL polymorphisms, and found significant associations of INDEL variations in ACE, TYMS, IL4, NFKB1, CASP8, TP53, HLAG, UGT1A1, and SGSM3 with CRC risk and clinical features. The data would be helpful to relative studies, thus some revision should be made: 1. The sample size is very small. Please calculate the statistical power. 2. This work analyzed 16 INDEL polymorphisms, but criteria for SNP selection were not shown. Please show this in the "Materials and Methods" section. 3. The case and control groups differ significantly in terms of gender, alcohol consumptions, and tobacco consumptions. How did these factors affect the colorectal cancer risk? 4. Use of English language and grammar requires some minor revision.

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Answer To Reviewer #1:

1. The sample size is very small. Please calculate the statistical power.

Thank you, this comment has been well taken. As a result, we consider statistical power from 10.000 simulations (Please, see Table Supplementary 02).

2. This work analyzed 16 INDEL polymorphisms, but criteria for SNP selection were not shown. Please show this in the "Materials and Methods" section.

We agree, and thus, this issue has been addressed in the "*Background*" and "*Materials and Methods*" section of the revised manuscript.

Background:

The polymorphisms investigated in this study exhibit common features, given they are all functional polymorphisms that alter the expression of genes participating in metabolic pathways associated with carcinogenesis. Also, these genes are associated with different types of cancer with high incidence in the Brazilian population, such as stomach and CRC.

Materials and Methods:

Polymorphism selection

Recently, INDELs have been the focus of multiple investigations [21-25]. This type of polymorphism presents interesting features as genetic markers: (1) INDELs are spread throughout the human genome; (2) INDELs derive from a single event (they do not present homoplasy); (3) since the allele frequencies of many INDELs are significantly different in separated populations; (4) small INDELs can be analyzed using short amplicons, which improves the amplification of degraded DNA and facilitates multiplexing; and (5) INDELs can be easily genotyped with a simple dye-labeling electrophoretic approach. Furthermore, all these genes evaluated in the present study show potential activity in pathway and may contribute to the carcinogenesis process (Table 1). Their genetic variations could contribute to: (1) risk of developing CRC; (2)

impact on treatment response; or (3) in prognosis.

3. The case and control groups differ significantly in terms of gender, alcohol consumptions, and tobacco consumptions. How did these factors affect the colorectal cancer risk?

Thank you for this question. Several studies have reported the association gender, alcohol consumptions, and tobacco consumptions with carcinogenesis process, including colorectal cancer [1–6].

Exposure to estrogens has long been recognized as a risk factor for developing a variety of human cancers [7]. Estrogens contain a benzene ring in their structure, and benzene and poly-cyclic aromatic hydrocarbons [8]. Most of them, approximately 95%, are metabolically activated to electrophilic forms that react covalently with the nucleophilic groups of DNA, RNA and protein [9–11].

Regarding tobacco and alcohol, they are environmental carcinogens responsible for the release of exogenous compounds, including reactive oxygenated intermediates represented by benzo[a]pyrene N-nitrosamines, heterocyclic amines and polycyclic aromatic hydrocarbons. These compounds are metabolically activated in electrophilic forms before interaction with DNA, and they generate adducts and contribute to tumor initiation [12]. The chronic consumption of alcohol and tobacco can increase adduct generation and reactive oxygen species [4,13–15].

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4. Use of English language and grammar requires some minor revision.

The original manuscript has now been reviewed by native English speakers and changes have been made. No language certificate is provided.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 34257

Title: Association of INDEL polymorphisms with Colorectal Cancer risk and clinical features

Reviewer's code: 01588319

Reviewer's country: Taiwan

Science editor: Ze-Mao Gong

Date sent for review: 2017-04-27

Date reviewed: 2017-05-10

COMMENTS TO AUTHORS

1. The authors should perform a functional assay of this Indel polymorphism to verify the true effects not just statistical association. 2. The "huge" supplementary materials also should be more concise.

Answer To Reviewer:

1. The authors should perform a functional assay of this Indel polymorphism to verify the true effects not just statistical association.

Thank you for this observation. We addressed this topic by incorporating the "Polymorphism selection" sub-section in the "Materials and Methods" section (please, see Table 1).

2. The "huge" supplementary materials also should be more concise.

Thank you. We agree, and thus, have addressed this in the manuscript text. We represented the genotype frequency of all INDEL polymorphism in the Supplementary Tables 5-6.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 34257

Title: Association of INDEL polymorphisms with Colorectal Cancer risk and clinical features

Reviewer's code: 02533618

Reviewer's country: Turkey

Science editor: Ze-Mao Gong

Date sent for review: 2017-04-27

Date reviewed: 2017-05-18

COMMENTS TO AUTHORS

1. Please check for syntax errors and language. 2. Table 1; why did the authors categorize the cases and controls into 2 groups who are younger and older than 45 years of age? 3. Table 1; what is the frequency of "eventual" and "frequent" alcohol consumption, please define.

Answer To Reviewer #3:

1. Please check for syntax errors and language.

The original manuscript has now been reviewed by native English speakers and changes have been made. No language certificate is provided.

2. Table 1; why did the authors categorize the cases and controls into 2 groups who are younger and older than 45 years of age?

Thank you for this observation. The main purpose was to observe the regional incidence

of colorectal cancer in each age group, to develop future studies.

3. Table 1; what is the frequency of "eventual" and "frequent" alcohol consumption, please define.

Thank you, this was an important question. This definition was included in "Definitions" sub-section in the "Materials and Methods" section.