McMaster University

Mohammad Yaghoobi, MD, MSc, AFS, FRCPC, FACG
Assistant Professor of Medicine
Division of Gastroenterology

1280 Main St. W.
Room 3V3
Hamilton, ON
Canada L8S 4K1

Phone 905-521-2100
Ext. 76380
Fax: 905-521-4958
Email: yaghoobi@mcmaster.ca

Hamilton Health Sciences

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Re: BPG 47608

Dear Dr. Lian-Sheng Ma,

Thank you for the opportunity to revise our manuscript. Please find below our responses to very valuable comments by the reviewers. Changes have been highlighted in the manuscript file.

Reviewer One

"This is an overall interesting meta-analysis and the paper generally well written. I have some requests for improving this. The Figure 1 funnel plot shows clearly unsymmetrical distribution, indicating publication bias. I recommend that the authors conduct another analyses focused on well-powered studies excluding those with low-powered studies that were biased. It is evident that there is influence of environment (including the microbiome) on cellular molecules and proteins, affecting cancer risk. The environment broadly include family history (embracing genetics and environment together) and the microbiome; these factors have been shown to affect cancer risk. It should be discussed more in detail. Related to the above point, the authors should discuss the recent trend of molecular pathological epidemiology (MPE). MPE is an emerging field that can link environment including family history, the microbiome, food, and lifestyle to molecular pathologies, often detected in cancers. MPE can contribute to biomarker research and precision medicine. Please discuss MPE. You can find relevant papers easily by net and pubmed search (eg, I see relevant ones such as Gut 2011, Annu Rev Pathol 2019, J Pathol 2019)."

Response

Many thanks for your interest in our paper and the valuable comment. We agree that there is asymmetry in the funnel plot presented in the paper, raising suspicion for the possibility of publication bias. We have performed an additional sensitivity analysis using the Trim and Fill method to account for the asymmetry caused by smaller studies included in the meta-analysis (Figure 3). The statistical analysis showed that despite the presence of asymmetries, our results are robust as they did not change significantly following this statistical test. Moreover, we also performed a sensitivity analysis where individual studies were excluded in turn to determine if any single study is strongly influencing the overall estimate of the risk ratio.

We also thank you for your suggestion of including molecular pathology epidemiology in the discussion. We have revised the discussion to include more information about this emerging field. Our revision is outlined in the original manuscript and is as follows: *Recent advances in cancer research has recognized*

the individual variability in biological markers in cancer patients, leading to the emergence of pathological molecular epidemiology [29,30]. According to this emerging field, it is possible that specific environmental factors such as dietary choices, physical activity and alcohol consumption contribute to the incidence and prognosis of specific forms of colorectal cancer categorized through the presence or absence of pathological molecular markers. For instance, it is well established that mutations within KRAS and BRAF oncogenes lead to an increased risk of developing colorectal cancer through the activation of the epidermal growth factor receptor. A recent case case-control study of 959 Chinese CRC cases found that one's mutational status is associated with variables such as sex, smocking status, serum carbohydrate antigen 19-9 and carcinoembryonic antigen [31]. According to the findings of this paper, colorectal cancer tumours with mutated KRAS or BRAF were associated with higher levels of serum carbohydrate antigen 19-9 and carcinoembryonic antigen which are considered to be indicative of poor prognosis and survival in CRC patients [31]. Moreover, another pathological molecular epidemiology study determined that having a first degree relative with CRC is significantly associated with having wild type KRAS [32]. Many of the studies looking at specific subsets of CRC patients are recent and still substantial variability between individual papers is present, making it exceedingly difficult to perform a meta-analysis with high clinical importance. Over the next decade, as newer studies in the field of molecular pathological epidemiology become available, an updated meta-analyses can potentially examine specific subsets of colorectal cancer, such as those with mutated KRAS and BRAF to further explore the role of family history as compared or in combination of other factors demonstrated by molecular epidemiology studies.

Reviewer Two

"This meta-analysis is of great relevance in the design of the preventive strategies in familial CRC. Although it is well designed according to the guidelines, I find two main drawbacks that limit the study. 1.The authors have included all the available studies. This is limiting the applicability of the results. Before 2000, the diagnosis of Lynch síndrome, or other forms of non-polyposis hereditary CRC (MUTYH associated CRC) was not posible. So, all the studies before 2000 could not exclude the hereditary predisposition that now we exclude in routine. In this sense, I recommend the authors to exclude all the studies published before 2000. 2.The conclusions the author produce could be improved. I would recommend them to analyze if the age at diagnosis and the number of FDR influence the RR of CRC detection."

Response

Thank you for your absolutely valuable comments on our paper. We totally agree that only recently the diagnosis of hereditary colorectal cancer has become more accurate. We believe that this is an extremely important point, However, we have already performed a sensitivity analysis (supplementary figure 4) to

determine if there is a significant statistical difference between studies published before and after the year 2000. To address the importance of your point, we have added the following section in the discussion outlining the results of our sensitivity analysis:

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Moreover, it is possible that studies published before the year 2000 included patients with hereditary conditions such as familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) due to lack of awareness or technological advances to detect those patients, therefore contributing to the overestimation of colorectal cancer risk in individuals with a positive family history. However, a sensitivity analysis did not show a significant difference in the overall risk in studies published before 2000 as compared to those published afterward.

Regarding your second point, we made an attempt to determine if the number of first degree relatives and age at diagnosis influence the relative risk of colorectal cancer detection, however, we were not able to reliably collect this information in the vast majority of the included papers and therefore were unable to make any conclusions on that. We believe that it is important that future research papers report this important information in their manuscript, but unfortunately, at this point, we are unable to make any changes.

Yours sincerely,

Mohammad Yaghoobi, MD, MSc (Epi), AFS
Assistant Professor of Medicine
Division of Gastroenterology
McMaster University and McMaster University Medical Center