Reviewer's report

**Title:** A cross-sectional study of global DNA methylation and risk of colorectal adenoma

**Version:** 1  **Date:** 27 April 2014

**Reviewer:** Ellen E Kampman

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This is an interesting and carefully conducted cross-sectional study which evaluated the association between LINE-1 methylation in normal appearing colon mucosa among those with and without adenomas in a screening colonoscopy population. The results contain important information, especially for aetiological research. The paper is well written. There are a few issues which need further attention or explanation.

**Major Compulsory Revisions**

. although very well written, the introduction is too long and could be shortened by at least one page.

. As the occurrence or prevalence of adenomas in this study population is not a rare disease, the OR may not provide the best estimate and may overestimate the association. Previous studies showed that for this reason it is preferable to estimate a prevalence risk ratio (see for instance Behrens T et al. Different methods to calculate effect estimates in cross-sectional studies. A comparison between prevalence odds ratio and prevalence ratio Methods Inf Med. 2004;43(5):505-9.), by for instance using a COX-model with time=0.

. why do the authors think differences are observed between men and women? This should be discussed in the Discussion section. Were the analyses planned to be conducted for men and women separately before data-analysis or was this decided based on the findings? Are sex-specific distributions necessary?

. the numbers for women, especially in the adenoma group are relatively low. This could also explain the non-significant findings. Maybe it would have been better to use tertiles instead of quartiles.

. in the genders combined, would it be possible to stratify for number of adenomas observed and size of adenomas?

. Is global hypomethylation indeed a useful marker of increased adenoma risk when it should be measured in the colon and the adenoma is already there?

. It is not clearly explained why two biopsies are used for the analyses. Is for those biopsies that are taken closer to the adenomas a stronger association expected? DNA methylation appears quite heterogeneous with a correlation coefficient of 0.66 between the two biopsies. What does that mean for the
reliability of this potential biomarker? This should be discussed in more detail in the Discussion section.

- The response rate is relatively low (61%). Could this have implications for the results as those who participated may be more health conscious?

- The fact that no association between blood LINE-1 methylation and colorectal adenomas is observed is very important for large epidemiological studies in which it is not possible to do endoscopies. Given the wide confidence intervals was this study large enough to evaluate this?

- Given the fact that in etiological studies in which suspected environmental and lifestyle factors are assessed the participants usually do not undergo a colonoscopy, the question is whether this is indeed a very useful biomarker in large epidemiological studies.

- I do agree that several potential confounding variables could be in the causal pathway and should not be adjusted for. However, why would age be different?

- Why is the LINE-1 methylation not assessed in the adenomas as well?

- Why was the normal appearing colon mucosa not histologically reviewed? I guess because no tissue was available anymore.

Minor essential revisions

- Abstract: include results blood LINE-1 and adenoma risk

- Introduction and Discussion: How small were the previous small observational studies?

- Explain why this age-group is used.

- Could a sensitivity analysis be conducted by including only those with a positive family history?

- Could a figure be added on the correlation between DNA-methylation in mucosa versus blood? Or between biopsy 1 and 2?

- LINE-1 is a proxy for global hypomethylation. It is a proxy for genome-wide DNA methylation.

- The number of words about the bisulfite conversion could be limited by adding a reference.

- reference 4: correct the website

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests.