Reviewer's report

Title: Influence of HRH2 promoter polymorphism on aberrant DNA methylation of DAPK and CDH1 in the gastric epithelium

Version: 1 Date: 20 August 2012

Reviewer: Lasse Sommer Kristensen

Reviewer's report:

The article by Nomura et al. named "Influence of HRH2 promoter polymorphism on aberrant DNA methylation of DAPK and CDH1 in the gastric epithelium" is rather well written and describes a correlation between the rs2607474 GG homozygote and an increased risk of age- and inflammation-related DAPK and CDH1 methylation in non-neoplastic gastric mucosa.

Comments to the authors:

Major Compulsory Revisions

1. It is claimed several times in the manuscript that the identified DNA methylation patterns are "aberrant". Nevertheless, a surprisingly high number of non-cancer subjects were found to be methylated for DAPK (201 individuals) and CDH1 (150 individuals). It would therefore be highly relevant to investigate whether the observed methylation has any influence on expression of these genes at the mRNA level (e.g. using RT-qPCR) and/or at the protein level (e.g. using immunohistochemistry).

2. It has previously been reported (by the same group) that CpG island methylation of both DAPK and CDH1 in non-neoplastic gastric mucosa corresponds to a risk of gastric cancer. It would therefore be of interest to investigate cancer tissue for DAPK and CDH1 methylation in a subset of the samples to show a correlation between the methylation patterns identified in non-neoplastic gastric mucosa and neoplastic cells from the same patient.

3. The polymorphism that is shown to influence the methylation status of DAPK and CDH1 is located in a gene on another chromosome. The influence is therefore expected to be indirect, possibly through an up-regulated action of histamines in the GG homozygotes. It would be relevant to study the functional role of the SNP on the expression of the HRH2 gene in order to provide evidence for such an indirect mechanism.

Minor Essential Revisions

1. The methylation studies were performed using previously published MSP primers, however, annealing temperatures and times were determined using DNA from peripheral blood of a young individual without H.pylori infection as negative control. These temperatures and times are not described in the manuscript and should be included.
2. It may be helpful for the reader if p-values are provided in the abstract where appropriate.

3. Some sentences and typos need to be corrected. For instance; line 12 on page 6 (“In addition, this report has been shown that rs2607474 is located in…”), and line 18 on page 8 (“Bisulfate…”).

4. Please provide more information on how the DNA was isolated. Was a commercial kit used?

5. It is interesting that the GG homozygote displayed no significant risk for DAPK methylation in subjects with GC (Table 4), please discuss this result further in the discussion section.

Discretionary Revisions

1. It would be of interest to investigate further whether the polymorphism has an effect on aberrant methylation patterns in general, or if the phenomenon is limited to the DAPK and CDH1 genes. It could, for instance, be interesting to include studies of CDKN2A methylation in the manuscript.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests