Reviewer's report

Title: Loss of heterozygosity at thymidylate synthase locus in Barrett's metaplasia, dysplasia, and carcinoma sequences

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Reviewer: Tom G Paulson

Reviewer's report:

The manuscript by Kuramochi, et al, reports an analysis of loss of heterozygosity at the thymidylate synthase locus in patients with Barrett's esophagus or esophageal adenocarcinoma. They find LOH at this locus, using markers both 5' and 3', in patients with BE, dysplasia and cancer, but not in those with GERD alone. They also report no differences in TS mRNA expression based upon genotype. This is a potentially interesting study, particularly given the use of 5FU in treatment for esophageal adenocarcinoma, and since LOH and copy number alterations on chromosome 18 have been reported in a number of studies in BE. However, there are some methodological concerns about the study that need to be addressed before the conclusions can be evaluated appropriately.

Major compulsory revisions: It is unclear exactly how this study was carried out. The abstract and methods mention 100 patients were part of this study, but when the various subgroups are added up (37 GERD, 29 IM, 13 dysplasia, 44 BA), it comes to 123. I suspect that the larger number is actually the number of samples examined, but I can't determine this from the manuscript. If it is really the number of samples examined, then the values for the number of patients with LOH at the TS locus needs to be recalculated. Without the correct data, it is difficult to make a final evaluation.

How many samples were taken per patient/tissue type? How big were the regions of BE in the esophagus? The authors found 2 examples of patients with LOH in their BA samples that did not have LOH in the surrounding tissue. If only a single sample was examined from a large region of surrounding tissue, it is possible that sampling error can explain those patients. This should be addressed by the authors.

What was the type of dysplasia? Were there any cases with dysplasia that didn't come from patients with BA? Did all of the IM have goblet cells? Providing more data on the patient population and samples obtained will allow evaluation of whether this finding is relevant to the general BE population or if it applies to a selected subset.

How was the patient population selected? Was it random or was there some underlying selection? This is relevant because the frequency of patients in the different genotypes doesn't quite fit a normal Hardy-Weinberg equilibrium. However, this may be due to the confusion over patients vs. samples as well, but
it is currently impossible to determine.

Minor essential revisions:

The authors did not find any association between TS genotype and expression; although they point this out in the discussion, it would be interesting to hear why this result may be at odds with the referenced papers – e.g., are there differences in the way the measurement was performed or the types of samples analyzed?

The manuscript would benefit from additional proof reading for grammatical errors.

**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