Reviewer’s report

Title: Screening of the DNA mismatch repair genes, MLH1 and MSH2, in a Greek cohort of Lynch syndrome suspected families.

Version: 1 Date: 1 January 2010

Reviewer: Ignacio I Blanco

Reviewer’s report:

Dear Editor,

Georgia Thodi and colleagues presented a nice paper entitled “Screening of the DNA mismatch repair genes, MLH1 and MSH2, in a Greek cohort of Lynch syndrome suspected families”.

The aim of this study was to successfully identify Lynch syndrome families and to report the MLH1 and MSH2 mutational spectrum within Greek colorectal cancer families. Additionally, Authors did also aimed to develop an efficient DNA-based screening protocol for the Greek colorectal cancer patients’ cohort.

Authors analyzed data from the MLH1 and MSH2 germline analysis of twenty-four families fulfilling the Amsterdam or the revised Bethesda criteria, out of which twenty two of Greek, one of Cypriot and one of Serbian origin. Nine deleterious alterations were detected in eleven of the twenty-four families subjected to genetic testing. The authors concluded that the mutational spectrum of MLH1 and MSH2 genes it quite heterogeneous without any strong indication for the presence of a founder effect.

The question posed by the authors is well defined, the writing is acceptable, and the title and abstract accurately convey what has been found.

However, several questions can be raised that Authors should address before considering the publication of this paper.

Major Compulsory Revisions

One of the major comments is that Authors did not explain how these 24 families were selected for MLH1 or MSH2 germline analysis. Authors did not present data about microsatellite instability or Immunohistochemistry. They only present the clinical data and is hard to believe that only 7 patients fulfilling the Bethesda Criteria can be recruited from their area. Authors try to present their mutation detection rate and to compare it with the data reviewed in the literature. Again, the mutation detection rate depends on the selection criteria used and, nowadays, microsatellite instability or immunohistochemistry are considered the best screening methodology to improve the mutation detection rate (Vasen et al, Fam Cancer 2009). Additionally, Authors should comment why they did not perform the MSH6 or PMS2 germline analysis.

Another important aspect is related to one of the Authors’ aims. Authors claim that
they want to develop an efficient DNA-based screening protocol for the Greek colorectal cancer patients' cohort. It is surprising that they screened forty-two samples from twenty-four families for the presence of point mutations and large genomic rearrangements in either MLH1 or MSH2 genes. If this is true, more than one relative is screened from each family. We consider it not a good strategy. Usually, only the proband should be screened from each family. An important effort should be done in order to select the best proband. Only in big families where the Amsterdam criteria are still present when the analyzed proband is removed we considerer to analyze a second proband from the family. Analyzing the data presented by the Authors it is important to point out that 3 of the 11 detected mutations are deletions or gen rearrangements. I will recommend the Authors to modify their protocol starting with the MLPA analysis that is cheaper than the point mutation analysis. When the MLPA in normal I will proceed with the point mutation analysis.

Authors should distinguish between a recurrent mutation and a founder mutation. In order to clarify the possibility of founder mutations a Haplotype analysis should be performed.

Authors should include the sample size among one of the important limitations of their work. It is difficult to describe the mutational spectrum within Greek colorectal cancer families analyzing only 24 families. Authors included in the discussion the work done for another Greek group, however the paper from Kataki et al (Clin Genet 2006) is not comment. I will recommend a national wide study in order to clarify the real spectrum of mismatch repair gene mutations in Lynch Syndrome families, including MSH6 and PMS2 mutations.

Breast and Thyroid cancer are very common in the population. It is not uncommon to find non-carrier patients affected by breast cancer in big Lynch Syndrome families.

Minor Essential Revisions
In table 1 the term “Modified AMS” is used instead of “Bethesda Criteria”.

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

**Quality of written English:** Acceptable

**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