Reviewer's report

Title: Clinical and genetic analyses of three Korean families with hereditary hemorrhagic telangiectasia

Version: 2 Date: 11 July 2011

Reviewer: LUISA-MARÍA Botella

Reviewer's report:

Major Compulsory Revisions

Dear Dr. Guian Paolo Declaro and Dr. David-Alexandre Tregouet,

I have revised with pleasure the new version of the manuscript MS: 9286819105268473 by Mi-Jung Kim, Seon-Tae Kim, Hyoung Doo Lee, Kyu-Yong Lee, Jiyoung Seo, Jae-Bom Lee, Young Jae Lee and S. Paul Oh entitled:

“Clinical and genetic analyses of three Korean families with hereditary hemorrhagic telangiectasia”

In general the manuscript has greatly improved due to the accurate answers to the questions raised by the reviewers, particularly the Reviewer 1.

This reviewer 2 appreciates and recognises the great effort made by the authors to answer the major point, however the concern remains, but it may be solved.

The authors have tried to respond the comment via an alternative means. Instead of using protein analysis, they have made the RNA analysis which may be a perfect tool, was the analysis made in the accurate way.

The main point was to sustain the pathogenicity of the ENG. -127 c.1 -127 C>T mutation by demonstrating that in vivo, HHT patients, carrying this particular mutation, have only half of Endoglin expression compared to non-HHT probands of the family.

Authors have made a restriction fragment analysis on genomic DNA since the change of normal C by T, creates a new BtsC1 restriction site, specifically on the mutant DNA. The results on the genomic analysis are complementary to the genomic sequencing showing clearly that at DNA level, the affected samples have an allele mutated with the new restriction site coincident with the C by T change. This is beautifully illustrated on the left side of Figure 4. However, the main point is the level of expression of ENG.

Unfortunately, this point is not answered, and the figure 4 on the right side may lead to a mistaken interpretation. The RT-PCR (from F2-R2 PCR) leads to higher levels of cDNA ENG in the affected sample compared to the unaffected, which is misleading and contradictory.

We understand that the picture shows a qualitative result, and therefore the
intensity of the bands we should not be taken into account.

We understand that the bands which should correspond to the mutant allele, are hardly visible, then the interpretation of early mediated decay of the mutant RNA is correct. But, what is worrying is the increase in the wild type RNA in the affected sample. If this was the real case, instead of having haploinsufficiency of ENG as a cause of the pathology, we would have an “excess” of RNA (the result of a kind of compensatory mechanism operating on the mutated sample). This excess is in contradiction with the in vitro results, presented in Figure 3, where the luciferase expression (used as reporter), present is much lower in the case of the mutant than in the wild type case.

Therefore, this reviewer thinks there is a technical problem with the results shown on the right side Figure 4, and recommends the authors the following strategy.

First of all, when doing the Reverse Transcription on the total RNA use random primers instead of an specific ENG exon 3 primer. If early nonsense mediated decay of RNA is present as shown by the authors, then using the primer from exon 3 could be preferentially annealed to wild type messengers, and therefore this will originate a “bias” in the cDNA population resulting from the reverse transcription.

Second, perform a real time PCR to quantify the amount of RNA using a reference unchanged RNA as control in affected and unaffected samples, such as 18S RNA, or GADPH.

If real time PCR was not technically available, even a semiquantitative PCR using reference endogenous RNA levels to quantify the endoglin RNA population could be enough.

In conclusion, the recommendation is to perform these experiments to show clearly that the mutant samples have half of the Endoglin RNA.

2. Minor details.

On page 7 in section Methods, in the penultimate line from Human subjects, change systemic by "systematic"

Level of interest: An article of importance in its field

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'