Author's response to reviews

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

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Author's response to reviews: see over
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RE: MS # 9286819105268473

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

Dear Dr. Tregouet:

We are submitting the revised version of manuscript 9286819105268473. We thank the editorial board and reviewers for the positive and constructive reviews on our work. Both reviewers suggested valuable comments to improve our manuscript, and we have made changes accordingly. All changes are marked by red fonts. Specific changes in response to editor’s and reviewers’ comments are as follow:

Editor’s comment:
“As suggested by one reviewer, at least one additional in vivo experiment (from the patient sample) would be very useful to give more strength to the reported findings (non-sense mediated mRNA decay, allele specific quantification, etc).”
We have performed allele-specific expression analysis with a restriction fragment length polymorphism (RFLP) analysis and direct sequencing of genomic DNA- and RT-PCR products using templates isolated from blood samples of unaffected and affected family members. Results from both methods show that the transcripts from the mutant allele were significantly diminished in affected family members, indicating that the mutant transcripts are subject to non-sense mediated mRNA decay. The data is summarized in new Figure 4, and detailed method and results were described.

Reviewer 1:
“I would add other references concerning the mutation rate of ENG and ACVRL1 in HHT (For example, Lesca et al., Genet Med 2006).”
>> More references [12, 13] were added in the background section.

“The date of the last update of the HHT database should be mentioned.”
>> The last update of the database is 09/19/2009. The date was added to the reference [14].

“Recent data by the team of S Bailly strongly suggesting the role of BMP9 as a (the) major ligand of ALK1 and Endoglin should also be mentioned.”
>> A sentence “Recent biochemical studies have suggested that BMP9 is the physiological ligand of ALK1 [18-20], was added to the background section.

“I think “incomplete penetration” should be replaced by “incomplete penetrance””
>> The change was made.

“I would replace “affected immediate family member” by “affected first degree family member”.”
The change was made.

“The number of patients having telangiectases seems surprisingly low (1 patient!), in contradiction with the Background section. Were all the family members carefully examined by a trained clinician?”

“Which methods (MRI, X ray, angiography…) were used for clinical evaluation of AVMs?”

“Were patients screened for Hepatic AVMs?”

Telangiectasia and other AVMs were not systemically screened. Only symptomatic lesions were evaluated by appropriate methods such as X-ray, CT, and MRI. We included sentences of “Only obviously detected symptoms are listed. Since systemic screening was not employed, visceral AVMs and skin telangiectasia present might have been missed from some patients.” at the method section.

“Were the PAVMs found in patients due to clinical symptoms (embolism, infarction, dyspnea...) or were some of them found by systematic screening?”

PAVMs of the proband in family 1 was suspected because of the brain abscess and were detected by chest X-ray and CT. The first daughter of the proband (I-4) was screened for PAVM because of low oxygen saturation. However, asymptomatic mutation carriers have not been screened for PAVM, CAVM, and other visceral AVMs.

“For family 1, it is mentioned that “two children of the affected proband’s sister (I-3) were diagnosed free from HHT”. Does it mean that they had no symptoms or that they did not bear the family mutation?”

We revised the sentence as “No mutation was found in two children from the affected proband’s sister (I-3).”

Reviewer 2:

“Major compulsory revision: to make a protein analysis of endoglin in family 2, the reviewer recommends to measure it by flow cytometry on macrophages from the proband and a non affected HHT relative, just to prove that half of the protein is present in the mutant. This will reinforce the in vivo analysis of this new mutation.”

We agree with this comment. To accommodate this suggestion, however, we will need to collect fresh blood from patients and family; and also it requires addendum in the existing IRB. For the sake of time and other technical reasons, we chose to respond to this comment via alternative means suggested by the editor as described above.

We hope you find this revised manuscript acceptable for the publication in BMC Medical Genetics.

I look forward to hearing from you soon. Thank you very much for your consideration.

Sincerely,

S. Paul Oh, Ph.D.
Associate Professor of Physiology