Author’s response to reviews

Title: Hereditary Hemorrhagic Telangiectasia associated with Cortical Development Malformation due to a start loss mutation in ENG

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Author’s response to reviews:

REVIEWER 1

Overall, the manuscript should more succinctly describe the Cases, with focus relevant details of history and findings. Indeed, previous publications/reports are few and should be better summarized in Background section and referenced in Conclusion section. Avoid speculation in Conclusion section that is not supported by your results.

Answer
We thank the reviewer for his/her careful revision.
The manuscript has been completely revised taking into account his/her suggestions.

Comment 1
Background:
Lines 11-16 - As written, these two sentences are confusion regarding whether stroke/abscess secondary to pAVM are the result of rupture vs shunting.

Answer
The two sentences regarding neurological manifestations of the disease have been rephrased.

Comment 2
Line 19 - suggest "meeting" rather than "matching". Also, this sentence is somewhat confusing regarding how # of criteria are related to diagnosis.

Answer
The sentence has been rephrased.

Comment 3
Line 27 McDonald et al 2015 [5] is not a reference for previous reports of cortical dev malformations in HHT. Did you mean [11]? Do previous reports support prevalence range of 5-10%? There are so few previous reports- worth summarizing these more specifically by way of background.

Answer
Please apologize for the mistake. We changed the reference and we provided additional details on the studies reporting cDM in HHT patients in Background and Discussion sections.

Comment 4

Answer
We extensively modified the paragraph.

Comment 5
Case Presentation:
Overall, suggest rewrite for improved organization of information and focus on pertinent history and evaluation. For example - could omit extraneous details of older brother's evaluation, but add relevant details of younger brother's.

Answer
The case presentation has been completely revised.

Comment 6
Discussion and Conclusion:
Line 2 Not sure what "novel familial case of HHT" means
Answer
We modified the sentence.

Comment 7
Line 6-18 This paragraph is also confusing and uninformative for the purpose of this case report in which affected tissue was not studied.

Answer
Taking into account comments by Reviewer 1 and 4, this paragraph was omitted in the revised version of the manuscript.

Comment 8
Line 20 cAVMs in "more than 30%" of HHT patients is higher number than supported by most previous publications. And no reference for this is provided.

Answer
Please apologize, the sentence has been corrected and a reference added.

Comment 9
Lines 20-34 Not relevant/priority info for this brief, case report.

Answer
We partially modified the indicated paragraph in Background.

Comment 10
Lines 35-60 This is the crux of what you have to say- and the Klostranec paper should have been summarized in Background to set the stage. Again [5] is not an appropriate reference for polymicrogyria/neuroradiologic findings in HHT patients.

Answer
We reorganized the Background section taking into account this helpful comment. Previous literature about cDM in HHT patients is commented in the Background and Discussion sections.

Comment 11
Line 6-11 (page 5) Best not to "speculate" findings/work presented here doesn't inform this conclusion.

Answer
We removed the paragraph in the revised version of the manuscript.

REVIEWER 2

Comment 1
Please correct the name of the gene ACVRL1 in the abstract.
Comment 2
Please add a sentence specifying which type of genetic analysis was performed (Sanger? NGS?) and which genes were analysed. Moreover, it is not clear if RNA analysis was performed on blood or on other tissues as ENG is mostly expressed in endothelium. Perhaps additional molecular information can be uploaded as supplemental material.

Answer
We added more details about molecular studies in the Case Presentation section and in the legend to Figure 2.

Comment 3
In the family three, in order to be more clear, I suggest to add the symbol of HHT also in the Patient's brother as having Epistaxis, pAVMs and an affected parent leads to HHT certain clinical diagnosis.

Answer
We modified Figure 2 according the suggestions provided by Reviewer 2 and other reviewers.

REVIEWER 3
This is a well written presentation of Hereditary Hemorrhagic Telangiectasia (HHT) in two siblings with CNS manifestations of the disease. This risk of severe manifestation of HHT is important to be aware of and in this sense the case report is an interesting educational case on HHT related acute cerebral ischemic events - especially in young patients with frequent epistaxis. In addition it sheds some light on the HHT related cerebral malformations in relation to CAVMs. The added new information is, however, somewhat limited. The mutation in ENG is a known HHT related variant and the CNS complication is likewise well known. It is, however, relative rare with cortical development malformations, though prevalence is probably not completely known since most HHT patients only have MRI of cerebrum performed if they have symptoms. The effect of the ENG variant is well studied and nicely presented in the figure.

Answer
We thank the reviewer for the positive comments.

Comment 1
Abstract - case presentation:
1st line: 'Here we describe the case of ..' can be changed to 'Here we describe a'

Answer
The sentence has been modified according reviewer suggestion.
24th line: The sentence 'We did detect the novel heterozygous ENG…' can be changed to 'A heterozygous ENG variant, c.3G>A (p.Met1Ile) was detected in the patient.'

Answer
Please apologize for the lack of novelty of the mutation identified. The sentence has been changed in the revised version of the manuscript.

Comment 3
Background:
2nd paragraph, line 29: 'six loci have been so far.' can be changed to "six loci have so far been..

Answer
As suggested by Reviewer 1 and 3, we extensively modified this paragraph.

Comment 4
3rd paragraph, line 42: 'in' instead of 'across'?

Answer
We corrected as suggested.

Comment 5
3rd paragraph, line 43: 'confirmed' instead of 'supported'.

Answer
We corrected as suggested.

Comment 6
3rd paragraph, line 44: '…by the identification of the ENG..' should be changed to "..by identification of a previously reported ENG c.3G>A substitution. In Human Gene Mutation Database (HGMD®) it is reported as disease causing (Alaa El Din (2015) PLoS One, PMID: 26176610, PM Tørring (2014) Clin Genet, PMID: 24001356, in supplementary files).

Answer
We corrected as suggested. Again, please apologize. We missed these previously published reports which are now included in References section.

Comment 7
Case presentation:
2nd paragraph, line 60: '..polymicrogyria and recurrent..' can be changed to '...polymicrogyria and had recurrent..' 

Answer
We corrected as suggested.

Comment 8
6th paragraph, page 4,line 29: 'A clinical diagnosis..' suggest rephrased to 'The mother of the patient had clinical HHT with XX and displayed pulmonary AVM but no cAVM'.

Answer
We revised the entire Case Presentation section.

Comment 9
Clinical diagnose of HHT should follow the Curaçao criteria, which consist of spontaneous and recurrent epistaxis, telangiectasia at characteristic sites, visceral AVMs and a first-degree relative with HHT. Fulfilling at least three criteria makes a definite diagnosis of HHT.

Answer
Taking into account reviewer indication, we modified the manuscript (Background).

Comment 10
7th paragraph, page 4, line 33: Add the age of the brother.

Answer
The age of proband’s brother is now included in the manuscript.

Comment 11
8th paragraph, page 4: This paragraph can more or less be omitted with reference to the published cases (see also https://arup.utah.edu/database/ENG/ENG_display.php) and Fig. 2. Some of the information might be available in text to the figure.

Answer
We modified the section and included the references which have previously reported the c.3G>A substitution (these references are not included in the ARUP database).

Comment 12
9th paragraph, page 4: Though the methods need not be presented in details in a case report, it should be indicated why and how quantitative RT-PCT was done (commercially available kit?). It might just be in the figure text.

Answer
As suggested by Reviewer 3 (and Reviewer 2), we added more details about molecular studies in the Case Presentation section and in the legend to Figure 2.

Comment 13
Discussion and Conclusions:
2nd paragraph, line 9: The sentence RT-PCR…' I think speculations on effect should be avoided/deleted. Instead it should be discussed that we regard the variant as 'first hit' and leading to - at least - functional haploinsufficency, but RT-PCT analysis showed normal transcript level supporting the NetStart suggested misstart as a likely explanation.

Answer
We thank the reviewer for this appropriate comment. We followed the suggestion and we modified the text (Case Presentation and Discussion) accordingly.

Comment 14
3rd paragraph, line 29: '..Patient...' should be changed to '...patient..'

Answer
We corrected as suggested.

Comment 15
4th paragraph, line 38: 'Index patient suffered from polymicrogyria: interestingly only ENG mutations (HHT1) have been associated with this peculiar radiologic evidence'. No clinical signs were mentioned so, change to 'Our index patient had polymicrogyria: interestingly only ENG variants (HHT1) have been associated with this radiologic findings'.

Answer
We modified the sentence taking into account reviewer suggestion.

Comment 16
4th paragraph, line 47: 'Authors...' changed to 'The authors..'

Answer
We corrected as requested.

Comment 17
4th paragraph, line 58: 'This conclusion fits with our patient where cAVMs are in close...' changed to ' This conclusion fits with the finding in our patient where cAVMs are seen in close...'

Answer
We rephrased the sentence, as suggested.

Comment 18
5th paragraph, page 6, line 12: It could also be unrelated to the ENG variant and HHT. This should probably be mentioned.

Answer
We rephrased the sentence, as suggested.

Comment 19
Last paragraph: As they usually have other signs of HHT it could be added ' especially when the patient report recurrent epistaxis.

Answer
We added the specification suggested by Reviewer 3.

Comment 20
Figure legend:
Fig 2a Instead of HHT (yellow symbol) indicate symptoms that led to the clinical diagnose (confirmed by the finding of the ENG variant).

Answer
We modified Figure 2 according the suggestions provided by Reviewer 3 and other reviewers.

Comment 21
If possible the electrograms should be enlarged in the figure.

Answer
We enlarged electropherograms in the revised version of the manuscript.

REVIEWER 4

The manuscript is interesting and reports a new phenotypic characteristic in Hereditary Haemorrhagic Telangiectasia, apparently related to the type of mutation found. However, some precisions and a protein control of the new mutation found, are necessary before to publish the manuscript.

Answer
We thank the reviewer for his/her interests in our work.

Comment 1
First of all: the title is misleading and inappropriate. I would suggest to change it to: Hereditary Hemorrhagic Telangiectasia associated to cortical development malformation due to a mutation of start loss in ENG

Answer
We changed the title in: “Hereditary Hemorrhagic Telangiectasia associated with Cortical Development Malformation due to a start loss mutation in ENG”.

Comment 2
page 5 line 6: the fact that Snellings et al (ref 9) found a second hit mutation in some of the skin telangiectases, doesn’t imply that it is a rule to find it in each patient. In the germinal line (sequencing from blood), it is not likely to find it. The idea would be to find it in some arteriovenous malformations. Therefore, the lines linwa 6 and 7 on the discussion, page 5 should be deleted.

Answer
We agree and we changed the paragraph accordingly.

Comment 3
Then, the following assumption, thta the c3G>A mutation exerts its effect at post-translational level, should be demonstrated. Authors should perform western blot study, either in macrophages derived from monocytes (which do express endoglin), and compare with wild type macrophages
from the family. Western blot may assess total amount, but, in order to test whether the loss of the
signal sequence of ENG, precludes it to migrate to the surface of the cells, leaving ENG trapped in
the cytoplasm or in vesicles, fluorescence microscopy is also necessary.

Answer
We totally agree this comment. The demonstration of the pathogenetic mechanism for the c.3G>A
change goes beyond the scope of this case report. The assumption about the post-translational
effect of the mutation was removed in the revised version of the manuscript.

Comment 4
Finally, please on Figure 2 A, don’t add colors for the clinical symptoms. You can
distinguish those with HHT with a shadowed square/circle, but in order to avoid confusion, use E
(por epistaxis), and cDM (for cortical development malformation).

Answer
We modified Figure 2 according the suggestions provided by Reviewer 4 and other reviewers.

Comment 5
On the Figure 2C to the right, add a line on top of the histograms with n/s (non significant).

Answer
We modified as requested.