Author's response to reviews

Title: Further delineation of Loeys-Dietz syndrome type 4 in a family with mild vascular involvement and a TGFB2 splicing mutation

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Author's response to reviews: see over
Dear Editor,

thank you for the answer to our MS: 1673467268123181 “Further delineation of Loeys-Dietz syndrome type 4 in a family with mild vascular involvement and a TGFB2 splicing mutation”.

We accepted all the suggestions from the reviewers and we resubmit the revised version of the MS accordingly. We hope that our manuscript is now suitable for publication.

Yours sincerely,

Prof. Marina Colombi

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Point-by point answers to the reviewers:

Reviewer: Lut Van Laer

We thank the reviewer for her positive comments and helpful suggestions.

Minor essential revisions:

Comment 1. There exists some confusion regarding the numbering of the different LDS subtypes. According to OMIM, the following numbering should be applied: LDS type 1: TGFBR1 (609192, while 608967 no longer exists) LDS type 2: TGFBR2 (610168, while 610380 no longer exists) In Loeys et al. 2006 (NEJM), type 1 and type 2 were also introduced but then based on the phenotype (typical LDS versus more vascular Ehlers-Danlos syndrome like). However, these findings are now believed to be part of a continuum within the LDS spectrum of disease (Van Laer L, Dietz H, Loeys B. Adv Exp Med Biol. 2014;802:95-105). LDS subtype A and subtype B have never been defined. Please adjust the background on page 3 according to the OMIM recommendations.

Reply 1: We agree with the reviewer’s observation regarding the confusion about LDS classification and adjusted the entire background section accordingly. In this view, we added to the manuscript the following recently published papers:
Comment 2. Part of the sentence on page 8, line 13 (“and creates a new potential splice acceptor site 1 bp downstream the consensus site”) is not true. Please omit this part of the sentence!

Reply 2: As suggested by the reviewer we omitted this part of the sentence.

Comment 3. Page 9: The paradoxical activation of TGFβ signaling is only in the case of TGFBR1/2, SMAD3 and TGFβ2. In case of FBN1 there is indeed an activation, but this is not paradoxical (it is as expected). In case of ATS the exact mechanism is not exactly clear yet. Please reformulate. Easiest solution would be to omit “paradoxical”.

Reply 3: As suggested by the reviewer we eliminated the “paradoxical” term.

Textual:

Comment 4. In the title: Loeys-Dietz syndrome type IV.

Reply 4: We modified the title in “Further delineation of Loeys-Dietz syndrome type 4 in a family with mild vascular involvement and a TGFβ2 splicing mutation”.

Comment 5. Abstract, page 2, line 5: SMAD3 should be italic; Abstract, page 2, lines 16 and 17: splice site mutation; Abstract, page 2, line 18: PTC: no use of abbreviation in the abstract; Page 4, line 1: aneurysm; Page 4, line 2: at young age; Page 4, line 3: identified an additional gene; Page 4, line 19: presents at later onset; At several places: hyperextensible; Figure 3: p.Tyr99* should be blue.

Reply 5: We applied all changes accordingly and uploaded a new Figure 3.

Reviewer: Marcella Zollino

We thank the reviewer for her suggestions and reply point by point to her questions.

Comment 1. No clinical information are provided about both parents of the proposita. If they were alive, they should be tested for the mutation.

Reply 1: We are conscious that genetic testing of both proband’s parents, still alive and over 80-year-old, would have been useful, unfortunately both parents did not consent clinical evaluation and genetic testing. On the other hand, since the proband referred an unremarkable clinical history for both, we can suppose, with a high degree of confidence, that they are not affected and that the mutation arose de novo in the proband. We added a sentence about the proband’s parents in the Materials and Methods section.

Comment 2. The pathogenic role of the reported variant is convincing. However, in considering that it represents a novel gene mutation, caution is needed before transferring its significance into the clinics. A total of 200 alleles were analyzed to verify whether the observed variant can be a novel polymorphism. However they are not sufficient to test the hypothesis of a possible pathogenic role as rare variant with incomplete penetrance (several examples are provided in literature of well
tolerated loss-of-function gene variants). After proper genetic counseling, an extended family study could help, including healthy relatives.

Reply 2: We are sure that the identified variant is pathogenetic and has a complete penetrance, since it represents a loss-of-function allele, as the majority of the reported *TGFB2* mutations identified in LDS4 patients. The mutation was absent not only in our 100 control individuals, but also in the 1000 Genomes Project or NHLBI Exome Variant Server (ESP6500) databases. We added this information in the Material and Methods section. Other members of the family, including healthy relatives such as the proband’s parents, are not available for segregation study, as suggested by the reviewer. The absence of major vascular complications in the affected individuals of our family is not due to incomplete penetrance, but it is in agreement with the concept that LDS4 corresponds to the mildest end of the LDS spectrum, since aneurysms are usually, but not always, observed in fourth decade and the progression of the disease is slower than in the other forms. Furthermore, LDS4, as well as many other autosomal dominant connective tissue disorders with vascular involvement, for example Marfan syndrome, due to mutations in *FBNI*, and vascular Ehlers-Danlos syndrome, caused by *COL3A1* mutations, show a high degree of variable expressivity. Different genetic backgrounds involving possible modulator genes and environmental factors likely contribute to the inter- and intra-familial variability irrespective of the mutation type.

Comment 3. The possibility of a late-onset aneurysmatic vasculopathy should be more extensively discussed.

Reply 3. We were conscious that in the proband a late-onset aneurysmatic vasculopathy could occur, therefore a periodical follow-up with vascular imaging was already scheduled. Furthermore, MRA study was also highly recommended for her 34-year-old daughter, who actually did not consent this analysis. We added a sentence about this in the discussion section. Furthermore, we also informed the proband that her older daughter should undergo clinical evaluation and genetic testing in order to plan a correct follow-up and surveillance.

Comment 4. Several editing amendments are recommended.

The English text of the manuscript was edited by the Nature publishing group language editing (NPGLE) service. Few amendments were done also in agreement with the requests of Reviewer Lut Van Laer.