Author's response to reviews

Title: Isolated brachydactyly type E can be caused by HOXD13 nonsense mutation: case report

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Version: 2 Date: 13 November 2011

Author's response to reviews: see over
RE: Case report: “Isolated brachydactyly type E caused by \textit{HOXD13} nonsense mutation: a case report”

November 12\textsuperscript{th}, 2011

Dear Editor,

We would like to thank you and the reviewers for your helpful comments and suggestions. We have revised the manuscript, and hope it is significantly improved. Please find the detailed responses to the reviewer’s comments below. This manuscript has not been submitted to any other journals, and the results have not been released in an abstract or conference presentation form.

With Kind Regards,
Aleksander Jamsheer, MD, PhD

\textbf{IMPORTANT: EXECUTIVE EDITOR MESSAGE}

\begin{quote}
Please can you confirm that the investigation of this case was carried out as part of standard care and that you have obtained consent for publication of the case from the mother and father your manuscript also contains details of their medical histories. Details of parental consent should be included in the manuscript.

\textbf{RE: A requested statement has been added at the end of the manuscript.}

We also ask that you include more detail about one the proband, and the background to the case. We also ask that you include a separate discussion section, and discuss your findings and their implications in more depth.

\textbf{RE: We have added more details on both patients and the background, as well as discuss our findings in more depth. Separate discussion section has been now introduced.}
\end{quote}

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.
We look forward to receiving your revised manuscript by 5 November 2011. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=2132390225553137. You will find more detailed instructions at the base of this email.

Please don't hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,

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On behalf of Dr Anne Slavotinek

Reviewer's report
Title: Isolated brachydactyly type E can be caused by HOXD13 nonsense mutation: case report
Version: 1 Date: 20 August 2011
Reviewer: Georg Schwabe

Reviewer's report:
Jamsheer and colleagues describe a novel heterozygous nonsense HOXD13 mutation in a Polish female and her father with an isolated brachydactyly type E (BDE). Whereas HOXD13 alanine repeat expansions are associated with synpolydactyly, recently two missense mutations located in the HOXD13 homeodomain have been reported to lead to an overlapping BDD/BDE phenotype. The new aspect presented here by Jamsheer et al. is the heterozygous HOXD13 nonsense mutation that is predicted to result in a loss of the HOXD13 homeodomain leading to BDE. Beyond the description of this interesting novel mutation, the presentation of the phenotype and the molecular data needs some refinement. In addition, only sparse background information on homeobox genes and limb development/malformation is offered. Furthermore, the authors need to better delineate their findings to previous data on HOXD13 mutations. Finally, the authors fail to discuss their findings in a broader, more functional and developmental context.

Major Compulsory Revisions
1. Phenotypic description
The phenotypic description of the limb malformation should be worked out in more detail and compared to the BDD/BDE phenotype, which is associated with missense mutations in the HOXD13 homeodomain. In addition to the fifth digit also other phalanges of the by Jamsheer et al. presented individual with BDE seem slightly deviated in the photographs and X-ray. Is a broad thumb
characteristic for other forms of BDE or for patients with previously described HOXD13 mutations? In addition, it should be mentioned, that unlike in many other BDE cases, the metacarpals of the fourth digit of the individuals described by Jamsheer et al. are not particularly shortened.

RE: The phenotypic description has been now extended and compared to the BDD/BDE phenotype caused by missense mutations in the HOXD13 homedomain.

2. Molecular analysis
2.1. PCR conditions should be listed. Primer sequences (and the size of PCR products) should be presented in the methods section, rather than being referred to only upon request.
RE: This has been introduced in the form of table (table 1).

2.2. In the chromatogram the predicted protein sequence and numbering of the codons should be added.
RE: This has been added in figure 1g.

3. Genotype-phenotype analysis
This part would benefit from improvement, including a more precise genotype-phenotype delineation and additional background information.
3.1. The BDE and the SPD phenotype and their underlying mutations should be delineated more clearly. A figure indicating the novel and previously described HOXD13 mutations, their functional consequences and the resulting phenotypes should be introduced.
RE: This has been introduced both in the text and in figure 2 and table 2.

3.2. Are there examples for homeobox genes other than HOXD13, indicating that mutations affecting the homeodomain and alanine repeats lead to distinct phenotypes?
RE: A paragraph on HOXA13 associated phenotypes was introduced.

3.3. PTHLH mutations leading to BDE are mentioned in the introductory remarks. This idea may be resumed again in the discussion. Is there a molecular connection between HOXD13 and PTHLH?
RE: This has been added at the end of the manuscript.

3.4. A more general description of the role of HOX genes in limb development is lacking at the beginning and / or end of the report.
RE: This paragraph has been introduced in the background section.

Minor Essential Revisions
1. It should be mentioned, whether other relatives without limb malformations exist. In addition, presentation of a pedigree, possibly together with the sequence chromatograms may be more illustrative.
RE: Corrected (pedigree in figure 1f has been added).

Discretionary Revisions
1. Figure legend: Letters A - D in the figure legend should be in bold. Use B, C instead of B&C.
RE: Corrected.

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.

Reviewer’s report
Title: Isolated brachydactyly type E can be caused by HOXD13 nonsense mutation: case report
Version: 1 Date: 14 September 2011
Reviewer: Eva Klopocki
Reviewer’s report:
Jamsheer et al. describe in their manuscript entitled “Isolated brachydactyly type E can be caused by HOXD13 nonsense mutation: case report” a family with two affected (father and daughter) who present with brachydactyly. They compare their findings to the previously published HOXD13 mutations associated with BDE.
Comments:
Major compulsory revisions
1. Case presentation: Previously reported mutations are associated with foot anomalies (“insertional duplication in the first web space” Kan et al 2003) which become apparent only on X rays and are not from the clinical pictures. Therefore the clinical pictures of the father’s feet and the statement “feet of the proband were clinically inconspicuous” are not sufficient to rule out the aforementioned foot anomalie. X-rays of the feet of both proband and her father should be provided in figure 1.
RE: We understand the need of clarifying clinical phenotype in feet with X-ray examination. Unfortunately, the patients decided not to undergo X-ray survey, thus, we are unable to provide a requested image. We believe that even clinical findings (without radiological support) may be still valuable to the medical community. Now, we are more cautious while describing the phenotype and emphasize lack of radiological confirmation.

2. Case presentation/figure 1: Description of the phenotype is fragmentary. Female proband and her father show contractures (compare to Johnson et al). Deviations of index and ring finger in the proband. Finger nail of finger V on the left hand of proband’s father appears hypoplastic (figure 1b). Thumbs (left more than right) appear shortened (figure 1b) – X-ray needed for clarification.
RE: The clinical description has been worked out in more detail. For the reason mentioned above, we could not provide an X-ray image.
3. Case presentation, last paragraph: hypothesis on the pathomechanism? Functional haploinsufficiency as postulated for the truncating mutations in contrast to missense mutations? Give an explanation for the rather mild phenotype.
RE: An explanation of milder phenotype of nonsense mutation in reference to missense has been added.

Minor essential revisions
1. Background: the authors refer to 3 out of 12 annotated HOXD13 mutations which result in premature stop codon. Please give appropriate references. What is the phenotype associated with these mutations?
RE: We extended the list of mutation to 24 and introduced a table of all known HOXD13 variants. Type of mutation, associated limb phenotype, and appropriate references have been added in the text and in table 2.

2. Case presentation: age of the female proband?
RE: Added.

3. comment on the recently described nonsense mutation E181X (Brison et al. 2011)
RE: Commented and discussed.

Discretionary revisions
1. Conclusions: this sentence does not read well. I suggest rephrasing.
RE: Corrected.

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