Author’s response to reviews

Title: A Possible Founder Mutation in FZD6 Gene in a Turkish Family with Autosomal Recessive Nail Dysplasia

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RESPONSE TO REVIEWERS

We thank the reviewers for their comments. In response, we have adjusted the manuscript as discussed below.

Liesbeth Vossaert (Reviewer 1):

Abstract:

Background: make clear that there are already seven mutations reported within this FZD6 gene.

We agreed with the reviewer’s comment. We pointed out more clearly that there are only seven known mutations of FZD6 associated with nail dysplasia.

Results: also a sentence about the 3D modeling should be included - as a large part of the manuscript is dedicated to that; and not just mention it in the conclusion.
We mentioned that “the pathogenicity of this frameshift mutation is proposed to be caused by disturbing the C-terminal domain structure and hence interaction partners of FZD6” in the fourth sentence of the result part of the abstract.

Try to highlight was this study is adding to the already existing knowledge.

We mentioned that “this study contributes to the already existing knowledge by proposing the pathogenicity mechanism of the mutation” in the last sentence of the conclusion part of the abstract.

Main text:

1) Clinical information:

More clinical information can be given about this family. The index case was diagnosed at birth; are all nails affected, and if so, all similarly or are there differences? Are there any differences between her and her affected sisters?

We agreed with the reviewer’s comment and added more clinical information about this family in the Patients and Phenotypic Characteristics section.

If compared with the families described in the Kasparis et al. paper (2016), are all symptoms comparable? Did they also report on ocular tuberculosis?

The two Turkish family of Kasparis et al. have the same phenotypic characteriztics with our patients, except the uveitis in our index patient. We pointed out this fact in the second paragraph of the discussion.

Why was she only referred to genetic counseling for her second pregnancy?

This is actually her the first pregnancy. The pedigree of our manuscript was right, but there was an inconsistency in our Patients and Phenotypic Characteristics. We agreed with the reviewer’s comment and corrected this section.

Additionally, it might be good to transfer the clinical assessment to the Results section instead of the Methods part.
As suggested by the reviewer, the clinical assessment of the patients are removed from the methods part to the results-clinical findings section.

The first two sentences within the Results section fit more appropriately in the Methods section.

We agreed with the reviewer’s comment and transferred the first two sentences in the Results section to the Methods section.

2) Also more information could be included (in the Discussion) on whether there are any possible treatments or measures to be taken.

We agreed with the reviewer’s comment and added the treatment details of the index patient both in the discussion and Patients and Phenotypic Characteristics sections.

It is indicated in the abstract and the title that this is a possible founder mutation and that it definitely needs to be taken into account for the diagnosis of nail dysplasia - but this is not repeated in the main text, even though it would serve to make your point more clear.

As for this comment we repeated the possibility of the founder mutation in the second paragraph of the discussion section.

3) Different tools for pathogenicity estimations are mentioned in the Methods section, yet no results are shown for this. Why were all these different tools used?

Since each tool has several varying strengths and weaknesses, we always prefer to consider several pathogenicity predictors and meta-predictors. With the change we made in the variant filtration part, we tried to make this concept clearer. Besides, we added a new paragraph regarding the election process of FZD6 gene in the results section.

4) When explaining expression patterns of FZD6 (page 13-14), expand a bit more on the studies in mice that have been done before, which mutations were induced, how and where is FZD6 normally expressed, etc.

We have expanded the studies in mice and expression data in more detail in the seventh paragraph of the discussion section.
Are there any expression data available on FZD6 with specific frameshift mutation? Did the authors do any experiments for this?

There are no expression data available on FZD6 with this truncation mutation. We checked the expression status via the RFFlow program, but we did not get a statistically significant result. Hence, we decided not to add this to the manuscript.

5) As mentioned before, try to highlight sufficiently what this manuscript adds to the already existing knowledge.

We agreed with the reviewer and highlighted the contribution of this manuscript to the literature again in the conclusion section of the main text.

In support of the hypothesis that the change in structure this frameshift induces leads to altered interactions of FZD6 with other players in its pathway, a figure could be added more clearly showing in which pathway FZD6 is involved, and which interactions are thus influenced, so that is how nail dysplasia is established.

As for this comment we added a new paragraph in the discussion section to explain that FZD6 is known to interact with WNT family proteins. WNT pathway and innate immunity's link was also mentioned in this paragraph.

6) Figures:

Figure 1: There seem to be a few errors within that figure:

The supplementary table is correct, the figure is changed accordingly.

Figure 2: It is mentioned in the Methods section that the index case had given birth to an unaffected offspring before, so two children should be indicated for her.

This is actually her the first pregnancy. The pedigree of our manuscript was right, but there was an inconsistency in our Patients and Phenotypic Characteristics section.
References to figures does not seem correct.

As for this comment, the references to the figures were corrected.

A reference to Figure 1 on Page 4 first paragraph can be included.

We agreed with the reviewer’s comment and included a reference to Figure 1 on page 4 first paragraph.

In terms of language, grammar and style, a thorough review is advised to get a better flow in the text.

We thank the reviewer for this comment. Accordingly a comprehensive review was conducted in terms of language, grammar and style.

Saadullah Khan, PhD (Reviewer 2):

We thank the reviewer for his comments. Accordingly a comprehensive review was conducted in terms of language, grammar, style, and technical flaws.

The mutation is already reported, so I think no need of seq. chromatogram.

As for this comment, we remove the chromatogram figure in the results part.

Pedigree should be marked properly (generations and individuals in Roman and Arabic numerals respectively).

We agreed with the reviewer’s comment and pedigree corrected as requested.

Sulman Basit, PhD (Reviewer 3):
Mutations in FZD6 gene are known to cause Autosomal Recessive Nail Dysplasia. I wonder, why a direct sequencing of FZD6 has not been performed. Kindly explain.

Among the ten different types of NCDCs, only the associated genes are known for only five of them. Besides, the presence of ocular tuberculosis in the index patient suggested to us that there might be another syndromic condition. Hence, instead of directly sequencing of those five, we decided to do a WES analysis.