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Name of Journal: ***World Journal of Medical Genetics***

ESPS Manuscript NO: 26568

Manuscript Type: Case Report

May 23, 2016
Re: Manuscript NO 26568
Attn: President and Company Editor-in-Chief
World Journal of Medical Genetics

Dear Dr. Lian-Sheng Ma,

We have now received from you the response from three different reviewers regarding our manuscript entitled "Mutation in TNXB gene causes moderate to severe Ehlers-Danlos syndrome" (ESPS Manuscript NO: 26568). All three reviewers requested testing of other family members for the mutation status found in our patient. However, the patient does not have contact with her family, and she has essentially no information about them beyond the fact that they live in Israel. We have added a paragraph that provides an explanation for our inability to test other family members, which can be found near the end of the Discussion section in the revised manuscript. We recognize this as a potential limitation of the study, and have stated this in the manuscript.

Additionally, per the editor's suggestions found in the edited manuscript file, we added an institutional review board statement, informed consent statement, and comments section including case characteristics, clinical diagnosis, differential diagnosis, laboratory diagnosis, imaging diagnosis, pathological diagnosis, treatment, related reports, term explanation, experiences and lesson, and peer-review sections.

All changes made to the manuscript have been highlighted in the newest version.

Please find our other responses to the peer-review comments here:

Reviewer 1: "The author report a case of Ehlers-Danlos syndrome with a mutation in TNXB gene. The report is interesting, but some concerns exist. Which DNA is used? Does the sample come from skin? Is a deletion analysis of genes necessary? It seems overstatement that the mutation causes severe phenotype of Ehlers-Danlos syndrome based upon only one case. Is the family history necessary."

Response to Reviewer 1: DNA from a blood sample is used, and the manuscript has been edited to reflect this fact. Yes, a deletion analysis is necessary, and it was performed, as outlined in the description of the test ordered ("DNA sequencing panel and deletion/duplication analysis of 33 genes related to connective tissue disorders"). We do not believe we are overstating the proposed impact of this mutation, as we are not basing our report on only one case. As outlined throughout the manuscript, this same gene variant has been reported by a different research group in a second case study in a patient with similar phenotypic features to our patient. Additionally, in-depth analyses of the function and effects of mutations of this gene have been performed using computer simulation programs, and evidence by the literature is also outlined in the discussion section. Please see our explanation above about the inability to contact family members or obtain a detailed family history.

Reviewer 2: One important question: Is this mutation detected in other family members(affected or not), and in healthy individuals? I think the authors should make an effort to contact other family members. Minor comments: Text may be in the past tense throughout the manuscript

Response to Reviewer 2: Please see the discussion above about the inability to contact family members. This mutation has been detected in healthy individuals, but this does not alone prove that the mutation is not harmful or pathogenic. A variety of factors may cause differences in phenotype among people with this mutation, including variants of other genes that interact in the same pathways, individual differences in nutrition, environment, etc.

Reviewer 3: Drs Kaufman and Butler report a single adult patient with a phenotype resembling Ehlers-Danlos syndrome hypermobility type in whom they identified the heterozygous mutation c.[6074A>T] in TNXB. The mutation was identified with a NGS approach using a panel which included 34 genes related to known (systemic) hereditary connective tissue disorders. TNXB is a well-known gene related to a rare type of Ehlers-Danlos syndrome: the so-called TNXB-deficient EDS. This is an

autosomal recessive form of EDS, most common in The Netherlands, and sharing some features of classic EDS, from which is distinguished by the inheritance pattern. Novel features are actually under analysis by some research groups. Most patients has a homozygous common deletion or compound heterozygosity for the deletion and a point mutation. TNXB is deleted in both allele copies also in the contiguous gene deletion syndrome coupling EDS and congenital adrenal hyperplasia (due to mutation in the neighboring gene CYP21A2). Conversely, the literature concerning the Mendelian role of heterozygous TNXB mutations in a different genetic form of EDS more resembling the hypermobility type is still confusing. In light of this confusion, a more formal demostration of the Mendelian link between the identified mutation and the related phenotype is requested, especially (i) segregation study with extended clinical examination on the highest number of relatives available and (2) and the rate comparison with an adeguate sample of controls from the same genetic background.

Response to Reviewer 3: Please see the discussion above about the inability to contact family members. A rate comparison with an adequate sample of controls from the same genetic background would be ideal but is beyond the means of our research group at this time.

We hope the revised manuscript addresses the concerns raised by the reviewers. The revision process has strengthened the manuscript, and we feel it is now ready for publication in your journal.

Sincerely,

Merlin G. Butler, MD, PhD
Carolyn S. Kaufman
Department of Psychiatry & Behavioral Sciences
Department of Pediatrics
University of Kansas Medical Center