Reviewer’s report

Title: Identification of a deep intronic mutation in the COL6A2 gene by a novel custom oligonucleotide CGH array designed to explore allelic and genetic heterogeneity in Collagen VI-related myopathies.

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Reviewer: Lee-Jun Wong

Reviewer’s report:

Dr. Bovolental and colleagues identified a novel intronic deletion of ~2 kb in the intron 1A of the COL6A2 gene by using custom designed aCGH analysis in a patient with Bethlem myopathy, in whom a heterozygous in-frame deletion of 6 nt had been identified by sequence analysis. The novel intronic deletion detected by custom aCGH failed to be confirmed by PCR. They used qPCR, RT-PCR, immunohistochemical staining and Western blot analysis to support their claim of pathogenicity of the deletion. The general impression is that there is not sufficient evidence to support the pathogenicity of this 2kb intronic deletion.

Comments

1. The authors claimed that the custom array is validated by finding no CNVs in 6 normal samples. As a rule of thumb, negative results do not prove anything. A positive control (could be any CNV in the genes included in the array) should have been included. Thus, the validation process is not very convincing.

2. The evidence for the intronic deletion detected by this array in patient 1 is not convincing (only 2 probes showed copy number loss). The possibility of false signal is not explored. The failure to be confirmed by PCR suggests the possibility of false signal. In addition, if this region is full of repeat sequences, it is likely that CNVs of this repeat sequence may occur in other part of chromosomes, which would give false signal. Possibility of alternative splicing or exon skipping should be sought for.

3. The apparent homozygosity as shown by RT-PCR is not necessarily supporting the notion of intronic deletion associated pathogenicity. Intronic deletion like this may cause exon skipping or alternative splicing with or without transcribed messages. Primer mismatch may also cause PCR failure. The bottom line is that the link between the 2 kb intronic deletion and the disease is weak.

4. Immunohistochemical analysis of collage VI expression in the muscle biopsy did not have internal controls. Has the possibility of technical artifacts been excluded?

5. page 4, end of first paragraph, SIFT and PolyPhen prediction of the significance of V117A missense variant should be described.
6. line 10, page 5, what does patient 4 e 5 mean?

7. page 9, end of paragraph, last sentence is an incorrect statement. It should be revised to “...for three copies, the value would be approximately +0.6 (it is a log 2 scale not a linear scale) and for 4 copies, the value would be +1. The way it is described is confusing and misleading.

8. The full names of UCMD and BM should be spelled out in the abstract.

9. page 4, the first line of the first paragraph of the Results: it was mentioned that genomic regions of COL6A1, A2, A3, A5, and A6 gene. ... were included in the array, but COL6A5 gene is not listed in Table 1. Also, there are errors in the 3rd line of Methods.

10. page 9, the second to the last sentence of the first paragraph, heterozygosis and homozygosis, should be heterozygosity or heterozygote or homozygote.

Overall, this reviewer is not convinced that the 2 kb intronic deletion is related to the cause of the disease. It is more likely a CNV. This manuscript needs significant improvement in terms of supporting evidence and the English writing.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**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’