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: Robert Weiss

Reviewer's report:

Numbers of patients with collagen VI-like phenotypes, but lacking identified mutations in the COL6A genes are accumulating. Efforts to identify a molecular cause in such cases are becoming increasingly important. Bovolenta et al. present a novel intronic mutation associated with Bethlem Myopathy identified using a custom CGH array, that adds to the spectrum of mutational mechanisms described for these phenotypes.

Major Compulsory Revisions

1. Design of the custom CGH chip includes genes with functional relations to collagen VI in addition to the COL6A1, COL6A2, COL6A3, COL6A5, and COL6A6 genes. Since identification of genetic heterogeneity in this case is dependent on the selection of which genes are included on the array. It would be helpful if a more thorough review of the rationale for selection of these genes was included.

2. In regards to the utility of CGH technology in identification of “gross rearrangements” referenced in the abstract and elsewhere, it is probably an overstatement that this technology can identify such changes, particularly as a rearrangement is proposed here, but not confirmed. It would be more precise to discuss CGH in terms of copy number rather than possible rearrangements.

3. In regards to the deletion mutation identified in patient BM1. While the deletion is maximal at 2094 bp, the deletion is also potentially much smaller. The authors describe attempts to amplify the segment without success. Particularly since the deletion is identified only on the basis of two probes in the CGH array, it would be helpful to include a more thorough evaluation of the mutation, including identification of the breakpoints if possible. In evaluating the possibility of a larger rearrangement, inclusion of some data on this point would greatly enhance the paper. At a minimum, a conventional high-resolution cytogenic study could be included. An explicit test involving long PCR combined with the real time PCR CNV analysis of exon 1 would support a promoter inversion. Speculation as to whether the SP1 binding site or other features are included in the deletion allele are premature in absence of data defining the exact extent of the deletion.

4. On p.5, the authors state: “Comparative sequence analysis revealed that the two deleted probes identify a genomic region highly conserved among vertebrates”, which is also shown in Figure 1 as the “Vertebrate Multiz Alignment
& Conservation (17 species)”. This block of conservation appears to be an artifact produced by the UCSC genome browser. If the authors carefully re-examine the UCSC browser’s Vertebrate Multiz track for 28 and 44 species, they will see that there is no support for a conserved sequence block in this region. Why the browser is depicting a block at this location on the Multiz 17 species track, is not clear.

5. Patient BM1 is noted to have been previously reported by Demir et al (ref11). While not a major point, it is interesting that this patient’s phenotype was called Ullrich Congenital Muscular Dystrophy in that report. The authors may want to address this difference in the syndrome named. As this report identifies a new mutation in this patient, it would be helpful to have in this report a short description of the phenotype (broader than what is in Table2), or specific reference to which patient this is in Demir et al. In a broader sense, it would be useful to define in the methods section the criteria used to include patients in the study as “clinically affected,” and thus eligible for inclusion.

6. Since the CGH approach failed to detect novel structural mutations (deletions/duplications) in 11 of 12 patients, a more conclusive statement may be needed in the discussion about what these negative results may mean in regard to the issue of allelic versus genetic heterogeneity. Are the authors willing to speculate on the percentage of mutations in UCMD and BM that are caused by large deletions or duplications?

Minor Essential Revisions

1. Page 3 line 1 refers to an article in press describing “for the first time” recessive mutations in Bethlem Myopathy. As this is an increasingly recognized mode of inheritance for BM, would recommend pulling phrase “for the first time.”

2. Several definitions are left off of Table 2 including SKM, nd, AD

3. Figure 2 should include annotation of the deletion allele as it is not straightforward to identify from the chromatogram, especially if presented in a small format.

4. Specific genomic information (NM and NP identifiers and chromosomal coordinates) about these genes included in Table 1 could be placed in a supplementary table rather that in the main text making the table more readable.

5. In general, the manuscript is well organized and written although it would benefit from English editing throughout.

Discretionary Revisions

1. Inclusion of the Real Time PCR experiments (mentioned on page 4 last line, but data not shown) from both the patient and father would be appropriate. In this regard, inclusion of the father’s data for the pseudohomozygosity would be appropriate as well as he would be expected to have a similar drop out of the deletion allele as the patient.

**Level of interest:** An article of importance in its field
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