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: Shireen R Lamande

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This paper uses custom CGH arrays to search for genomic deletions/duplications in 12 patients with clinically diagnosed UCMD or BM (collagen VI-related disorders) who were either negative for mutations in COL6A1, COL6A2 and COL6A3, or who had single mutations that failed to fully explain the clinical phenotype. The patients had been previously screened for mutations in COL6A1, COL6A2 and COL6A3 by genomic PCR and sequencing. The arrays included probes for the 5 human collagen VI genes as well as 11 candidate genes encoding extracellular matrix proteins or receptors. Copy number variations were identified in three patients. Two were near integrin genes and were found in healthy controls. One potential mutation was identified in a COL6A2 intron.

Major compulsory Revisions

1. Patient screening for collagen VI mutations by genomic PCR and sequencing should be described in the methods. The genomic DNA mutation in BM1 is described in the first section of the results but the underlying genomic mutation in UCMD2 that produces the p.V117A substitution is not described or shown anywhere in the manuscript. This should be addressed. It should be acknowledged that while the V117A substitution is not a common polymorphism (wasn’t found in 200 control chromosomes) this does not mean it is pathogenic. Non-pathogenic amino acid changes in the A domains of collagen VI are common. The description of the array design in the first part of the results should come after the description of the patient group that was screened to maintain the logical flow of this section.

2. The CGH data did not identify a 2kb deletion as written on page 4. The analysis indicates a deletion detected by two overlapping probes. This could be a deletion as small as 80-100 bases. The exact position of these two probes should be described. The deletion is a maximum of ~2kb based on the position of the two flanking probes that have normal hybridization levels. This incorrect description needs to be corrected. Real time PCR was used to confirm that the region was deleted. The probe and primer set needs to be described and the data shown. Where were the real time primers relative to the deleted probes? Could additional primer/probe sets be used to delineate the deletion? How many control DNA samples were analysed?

3. The authors attempted to PCR amplify across the deletion. They state in the
results that the primers were within the predicted deletion boundaries – presumably they mean the primers were outside of the maximum theoretical deleted region. This may be an example of difficulties with translation into English. Again the PCR primers and methods used for this are not described in the methods section and it is not clear why the PCR was unsuccessful. Was the product from the normal allele amplified? If so, was it sequenced to exclude the possibility that the mutant allele was also amplified but contained a deletion too small to detect by gel electrophoresis? If the normal allele wasn’t amplified, were different PCR primers and conditions tried?

4. The evidence that this deletion is pathogenic is not yet convincing. It seems clear from the RT-PCR and sequencing that only one COL6A2 allele is transcribed but a direct connection between the deletion and lack of transcription has not been established. Are fibroblasts from the father available for RT-PCR analysis to determine if one or two alleles are expressed? Some data directly linking the deletion with the lack of transcription is required.

5. Muscle biopsy staining is shown in Figure 3. Antibodies to collagen VI, laminin b1 and collagen IV are shown for the patient but only the collagen VI staining is shown for the control. The controls for the other two antibodies need to be shown particularly as it is concluded that laminin b1 staining is reduced in the patient – this judgement can’t be made without a control to compare it to.

6. The interpretation of the fibroblast immunostaining for collagen VI is problematic. There is clearly reduced staining in patient fibroblasts. However, it is not obvious that “the texture is coarser than normal with the thinner fibrils less represented”. The fibrils look thinner if anything in the patient cells. Could this reflect the fact that the patient matrix has less total collagen VI than the control matrix? Interpretation of the organisation probably requires electron microscopy.

7. The collagen VI antibodies used on the western blots are not described in the methods. The methods only refer to MAB1944 which is the a3(VI) specific monoclonal antibody used for immunostaining. The antibodies are not described in reference 31 either.

8. The authors should clarify what they mean by the statement at the bottom of page 7 “The reported patient also support the observation that BM recessive mutations, differently from classical dominant cases, affect collagen VI expression both in muscle and fibroblasts.” I don’t understand what is mean by “differently from classical dominant cases” and reference 3 (referred to several times in the paper) is not yet available on line for clarification. If the authors are suggesting that collagen VI biosynthesis in skin fibroblasts is different to collagen VI biosynthesis in muscle fibroblasts then this is quite a radical suggestion that needs to be supported by experimental data.

Minor Essential Revisions

The manuscript would benefit from editing for English expression. Some places to concentrate on are the last paragraph of the discussion on page 8, "increased sensibility" should be "increased sensitivity" on page 7, the description of the
immunofluorescence on page 6.

Discretionary Revisions
None

**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