Author's response to reviews

Title: Comparison of orthopaedic manifestations of multiple epiphyseal dysplasia caused by MATN3 versus COMP mutations: a case control study

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Author's response to reviews: see over
Dear Dr. Sean McGee,

Please consider the enclosed revised manuscript entitled "Comparison of Orthopaedic Manifestation Of Multiple Epiphyseal Dysplasia Caused By MATN3 Versus COMP Mutations : a case control study" (MS 1230935567112090) for publication in the BMC Musculoskeletal Disorders.

We hope you agree that we have carefully considered editor’s and reviewers’ comments. A point-by-point response to the concerns of the reviewers is attached to this cover letter. The main authors’ affiliation was changed from hospital to university.

We look forward to your favorable response.

Sincerely yours,

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Response to Reviewers

Reviewer #1: Michael Briggs

Reviewer's report:

General Points:

[abbreviated]

Whilst the focus of the study was to compare COMP-MED vs. MATN3-MED, I wonder whether it would be also possible to use these data to look at the variability within each gene group? For example, in MATN3-MED the majority of patients have the same p.R121W mutation, so do the clinical/radiographic features for that mutation differ from the other MATN3 mutations combined? Does p.R121W produce a consistent phenotype? These investigations might shed new light on the variability within MED and support the hypothesis of genetic modifiers (see further comments below). The authors have the data to hand and could quickly perform these analyses, or at least exclude the possibility that there are any differences.

In the beginning of this study, we actually tried to figure out the characteristic phenotype of p.R121W mutation or difference between the p.R121W vs. non-p.R121W patients. However, we could not find any statistical difference between the two groups in any clinical or radiological parameters tested, at least in our series. We did not include these negative data because it does not seem to have enough power to confirm no difference.

I think that the final conclusion should just summarize the significant differences only between the two groups (i.e. p<0.05).

We revised the Conclusion section, stressing the differences having statistical significance (gait abnormality and hip dysplasia)(lines 220 – 223). Some other sentences were moved to the end of Discussion section.

Specific Points:

With respect to the relative proportion of COMP/COL9/MATN3 mutation in MED patients (page 3), the authors should really reference Jackson et al Hum Mut 2011, since it is the largest single study to date on the genetic basis of MED.
We included the article in the references (ref 9), and revised the proportion in Caucasian populations accordingly (lines 42-44).

Page 6 for Table 2

The reason for using a \( p < 0.05 \) value is to define what can be considered significant. It is therefore not appropriate to use the term ‘borderline significant’ for a \( p < 0.084 \). It is \textit{NOT} significant.

We agree with reviewer’s comment. It was described as “tended to be more common ….. although it was not statistically significant”, instead of “borderline significant” (lines 115-116).

I’m not certain that Pearson’s chi-square can be applied because the number of patients is below 10 for some analyses – this needs double-checking with a statistician. This comment is applicable to all tables.

We consulted a statistician on this issue and realized that Pearson's chi-square test was not appropriate in some analyses as you commented. Pearson’s chi-square test is generally acceptable when the expected frequency is above 5, and Fisher’s exact test is recommended when the expected frequency is less than 5. Hence, we applied Pearson's and Fisher's tests according to the above mentioned criteria. It was described at the last paragraph of Materials and Methods section (lines 94 - 95). Fisher's exact test was used in the analysis of frequency of the knee pain (Table 2). For analysis of Stulberg classification, linear by linear association test was newly applied because it involved a 2-by-k table containing zero value (lines 96 - 97, Table 3)

Page 6 for Figure 4

The authors state that 12/37 MATN3 patients were above average height and it would be useful for them to present more detail. For example, what percentile above average?

In compliance with your suggestion, the paragraph describing height of the subjects was revised, including the height centile values and z-value of those MATN3-MED patients whose height was above average. (lines 121-123).
Can the authors also comment on whether differences in height were more variable in the COMP vs. MATN3 patient groups. It seems from the figure that COMP-MED is more phenotypically variable (at least for height). In simple terms the errors bars are broader in COMP; is this important? More comprehensive figure legends, in particular figure 4. For example is 0 taken as average?

Variance of height and the radiographic parameters were compared using F-statistics (Levene’s test), which was described additionally in Methods section (lines 98-99). The COMP group showed wider height distribution, although not statistically significant. It was added to the manuscript (lines 123-124). In the legend of figure 4, the following sentence was added, “Zero on the Y-axis denotes the average height” (lines 341-342).

Page 6 for Table 3
P values are not presented in the table.

P-value involves the whole table, and was described at the footnote. Instead of Pearson’s chi-square test, linear by linear association test was used in the revision, because it was a 2 x k table containing zero value in some cells. (Table 3)

Page 6-7 for Figure 5
Can the authors comment as to whether there is greater variability in the MED-COMP patient group (i.e. larger SEM) for the radiographic analyses?

Following the reviewer’s comment, we added the SEM values in Results section. (lines 131-135) SEM of all the parameters was larger in the COMP group than in the MATN3 group. Variance analysis using Levene’s test showed statistically significant difference in center-edge angle (p = 0.031), and femoral head coverage (p = 0.048). These findings were added to the manuscript (lines 135-138). And a sentence was added to the Discussion section (lines 167-168).

Discussion
Page 8: The sentence, “Hence, it is likely that COMP-pathy cases may constitute severe MED cases” doesn’t make sense. COMP mutations can also cause mild MED.
We agree that COMP mutation can also cause mild MED. Hence, the sentence was revised as “Hence, it is likely that COMP-MED constitutes a spectrum from mild to severe MED cases.” (lines 166-167)

Page 9, line 10: Do the authors mean Table 4?

It was corrected to Table 4 (line 148). Thank you.
Reviewer #2: Barbara Vertel

Reviewer's report:

[Abbreviated]

Major Compulsory Revisions

1. Fig 1F is shown as a MAT3-MED patient radiograph, but in the previous Kim et al 2011 publication from which it is reproduced, the same image is shown in Fig 3C as a COMP mutation patient radiograph. Please explain and correct this critical discrepancy.

=> Thank you very much for pointing out our mistake in selecting the radiograph. The previous Fig 1F was a radiograph of COMP-MED patient. It was replaced with a radiograph of MATN3-MED patient, and the legend was revised accordingly (lines 325-326).

2. Background paragraph 2. Since ethnic differences in the distribution of the causative genes for MED is a major rationale for these studies, it is really important to include the specific distributions (numbers) in Caucasian populations as a comparison for the numbers provided for Korean and Japanese populations so that this key point is properly supported by data.

=> We added the proportion of causative genes in the text as you recommended. (lines 40 – 44)

Minor Essential Revisions

3. Discussion Paragraph 2, sentence 5-It is written “the MATN3 mutation results in relatively mild phenotypes as compared with those of COMP mutation. Also written in the Conclusion- “MATN3-MED develops a relatively mild phenotype from an orthopedic point of view.” Are the phenotypes really relatively mild, or relatively milder in comparison to COMP-MED phenotypes? My suggestions are to change “relatively mild” to “relatively milder” in the Discussion, and to add “in comparison to COMP-MED” in the first sentence of the Conclusion.

=> We changed “relatively mild” to “relatively milder” at line 164. The Conclusion section was much revised, and now it starts with “In comparison between MATN-3 and COMP-MED…. ” (line 220).
4. Background- The sentence about severe Fairbank and mild Ribbing is a little distracting, and doesn’t add much to the manuscript. It could easily be deleted.

=> The sentence was deleted as you suggested.

5. Results Paragraph 1-beginning “In the MATN3 group…” since complaints of knee and joint pain did not differ statistically, it is more reflective of the data to equate them as the most common complaints rather than to include knee joint pain ahead of hip pain.

=> We evaluated the frequency of chief complaints, which brought the patient to medical attention, and separately the frequency of all clinical manifestations detected during interview and physical examination. This paragraph described the frequency of chief complaints, while Tables 1 & 2 described the frequency of all clinical manifestation. It may be confusing, but we believe it is worthwhile to separate them. In order to distinguish the chief complaint from clinical manifestations, a phrase describing the latter was added in the paragraph (line 109-110).

6. Figure 3 legend- Please provide a more detailed description of the findings, as has been done for Figure 1 and 2 legends.

=> We added detailed description of the radiographic findings to the legend of Figure 3 (lines 336 - 339).

7. Discussion Paragraph 4 is rambling and unfocused. Please focus, tighten and clarify this discussion of the clinical findings and their implications. Sentences 1 and 2 are particularly difficult to understand. As written, “more guarded” and the point about MED in relationship to Legg-Calve-Perthes disease lack meaning. Please reorganize and rewrite the paragraph.

=> We realized that the paragraph was mixed with two issues – hip problems in MED and differentiation from AVN. Hence, we divided it into two paragraphs. Hip problems in MED was discussed in the former paragraph (lines 178-191), and differentiation from AVN was discussed in the latter paragraph (lines 192-205).
8. Although there is a separate Conclusion section, the Discussion would be improved by ending it with a stronger, more specific and focused paragraph.
   => Encouraged by your comment, a paragraph was added at the end of Discussion section (before Conclusion), saying the main point we would like to stress (lines 212-217).

9. Conclusion, last sentence-Suggest rewriting as follows to better clarify the message-“These differences in clinical manifestation and prognosis justify distinguishing between the COMP or MATN3 molecular subtypes of MED in orthopedic patients.”
   => We revised the last sentence as you suggested, except “in orthopedic patients” because it seems to a repeat of the previous sentence (lines 222-223).

The following are spelling, grammar and editorial suggestions and corrections.
   => We appreciate very much for your detailed correction. They are corrected as you suggested. Their location in the revised manuscript was denoted in parentheses.

10. Background Paragraph 1, sentence 5-Mutations of the genes encoding cartilage (line 32)
11 & 12. Background Paragraph 2, sentence 1-ethnic differences; last sentence-Type IX collagen (line 47)
13. Background Paragraph 3, sentence 2-each genotype of (lines 49 - 50)
14. Background Paragraph 3, sentence 4-clinical characteristics (line 54)
15. Methods sentence by itself before last paragraph-Type and purpose…..were categorized (line 92)
16. Results last line-should refer to Table 4 and not Table 3. (line 148)
17. Discussion Paragraph 2, sentence 2- should add “and” so that it reads “and early onset osteoarthritis” (line 159)
18. Discussion Paragraph 2, sentences 4 and 5-corrected as follows: “The MATN3 group demonstrated a significantly lower incidence of gait abnormality throughout the follow-up period, and patients were less likely to have complaints of hip pain and limitation of daily activity at the latest follow-up. Accordingly, our findings suggest…..”
(lines 161 - 163)
19. Discussion Paragraph 3, sentence 2- angular (spelling) (line 170)
20. Discussion Paragraph 3, last sentence- better as “Due to the multicenter nature of this study” (lines 176)
21. Discussion Paragraph 3, sentence 4, under 5) compatible (spelling) (line 200)
22. Discussion Paragraph 3, sentence 5- “abnormal hp development which is not…..” (line 201)
23. Discussion Last paragraph, sentence 2- should be “on an all-or-none basis” (line 207)
24. Conclusion, sentence 2- hips not hip (line 214)
25. Figure 2 legend- Resorption (spelling) (line 333)
26. Figure 1- major issue queried above under Major Compulsory Revisions; also, remove the random letter “G” on panel D (Figure 1)
27. Figure 1- remove the random letter “R” on panel A (Figure 1)