Reviewer's report

Title: Dystrophin deficiency in canine X-linked muscular dystrophy in Japan (CXMDJ) alters myosin heavy chain expression profiles in the diaphragm more markedly than in the tibialis cranialis muscle.

Version: 1 Date: 18 October 2007

Reviewer: Peter Reiser

Reviewer's report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors assert that the CXMDj model is the BEST model for studying muscular dystrophy. This is a judgment on their part, which may or may not be valid. A suggestion is to simply emphasize the relevance of the CXMDj model to human MD and the advantages of this model. Evidence is presented that indicates that the CXMDj is probably a better model in some respects. However, to declare CXMDj as the BEST model is not necessarily warranted or justified. This should be modified throughout the manuscript, including in the Conclusions paragraph on page 21.

2. There is an important difference, which the authors should consider discussing, in myosin expression in limb muscles between large mammals (including dogs and humans) and mammals with smaller body mass, especially rodents. The former do not express MHC-IIB in limb muscles, while it is abundantly expressed in the latter. Therefore, changes/adaptations in muscles of dogs with MD are likely to be more relevant to human DMD, than are changes in the mdx mouse. The authors should consider adding this to their paper, in the Introduction and the Discussion. An appropriate place in the Introduction is on page 6, near the sentence beginning with “Myofibers express various MHC isoforms...”. A reference for the lack of fibers expressing MHC-IIB in dog limb muscles is Snow, D.H., R. Billeter, F. Mascarello, E. Carpene, A. Rowlerson and E. Jenny. No classical type IIB fibres in dog skeletal muscle. Histochem. 75:53-65, 1982.

3. The authors should clarify what they mean by “embryonic MHC-negative populations of CXMDj muscles” near the bottom of page 3. Presumably, they are referring to muscle fibers that express embryonic MHC, which is a much simpler and direct statement. This change should be made throughout the manuscript when references to these fibers are being made. It some instances, it might be even clearer to refer to these fibers as “co-expressing embryonic MHC and other MHC isoform(s)".
4. The basis for distinguishing wild type and dystrophic littermates, especially at one-month of age, should be described at the beginning of the methods.

5. There is a concern about the strength of the quantitation of some of the results. The authors state, near the bottom of page 11, that there were “slightly fewer fast MHC fibers...”. First, it is not clear if they are referring to the numbers in part A of Figure 2. If so, then this should be noted on page 11. If not, then more complete quantitative data need to be included. Secondly, the authors should clarify from how many dogs the data shown in Figure 2 were obtained. Also, the number of dogs sampled for the analysis of MHC isoforms in single fibers should be stated in the paragraph beginning at the middle of page 14.

6. The authors need to describe the fiber type composition of the normal human diaphragm to validly assert that “the proportions of MHC isoforms in the diaphragms of healthy dogs are much closer to those of humans than those of mice”. There are numbers on page 810 of Reference 33 that would be useful. Including these numbers would be more meaningful than merely citing this paper.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Specify that it is the NUMBER of fibers expressing slow MHC that increases markedly and the NUMBER of fibers expressing fast MHC that decreases in the fourth-last and fifth-last lines on page 3. Without stating “numbers” it is not immediately and unambiguously clear what is changing.

2. It is not clear that NCL-MHCd is specific for embryonic MHC. Some previous reports suggest that this antibody recognizes both neonatal MHC and embryonic MHC (e.g., De-Doncker et al., J. Histochem. Cytochem. 50:1543-1553, 2002) while other reports suggest that it recognizes exclusively embryonic MHC (e.g., Waltraut et al., Experimental Eye Research, 84:670-679, 2007). Since the authors do not provide independent evidence from dog muscle that it is specific for embryonic MHC, it would be more correct to refer to fibers stained with this antibody as expressing “developmental” MHC and stating that this means neonatal and/or embryonic MHC. This would not change the main conclusions of the paper.

3. The greater cross-sectional area of slow fibers in the diaphragms of affected dogs might be due to hypertrophy in compensation for loss of fast fibers. This idea is somewhat implied in the Discussion, but it could be stated more explicitly with simple editing.

Discretionary Revisions (which the author can choose to ignore)

1. Middle of page 6: the authors refer to exercise as being an abnormality or impairment. Simple editing should correct this.
2. “contractive” should be changed top “contractile” in line 8 on page 19.


4. It appears that the phrase “slower to faster” should be changed to “faster to slower” in line 12 on page 19.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**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.