Reviewer's report

Title: Cardiac involvement in beagle-based canine X-linked muscular dystrophy in Japan (CXMDJ): electrocardiographic, echocardiographic, and morphologic studies

Version: 3  Date: 30 September 2006

Reviewer: Luca Ferasin

Reviewer's report:

General
I would like to thank the Authors for taking the time to review their manuscript. The Authors have satisfactorily addressed many of my comments and made the appropriate changes. However, there are still some changes that, in my opinion, would be required before considering this manuscript acceptable for publication.

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

Introduction
The Authors explained in better details their justification for the study in the introduction. However, the sentence “dogs are large” is still present in the abstract and should be changed. Furthermore, the new sentence “In addition, we have gotten beagle bitches and male dogs in Japan, who have clear origins” is confusing and does not add any useful information.

Methods
Although I appreciate that the Authors changed the title of the paragraph “Electrocardiography”, the method description is still confusing. Presumably, the measured PQ and QRS intervals did not change between leads, so the word “lead III” could be omitted. Q/R ratio was calculated in the three limb leads and in one augmented lead (aVF). However, in Discussion, the Authors refer to “peculiar ECG finding in DMD characterized by deep and narrow Q-waves in limb leads I and aVL and left precordial leads V5-6 [13]”. Why was the Q/R ratio analysis made in aVF if the purpose of the study was to identify a suitable animal model which resembles DMD? I would suspect that the Authors have calculated the ratio in all augmented lead but found a significance difference only in aVF. If that was the case, it should be mentioned in the description of the methods. I would suggest the Authors to rewrite the entire paragraph.

During my first revision, I omitted to request clarifications on why histopathology of the right ventricular myocardium was not performed. A short explanatory sentence would be appropriate.

Results
The paragraph “echocardiographic findings” is rather confusing and should be rewritten. I would also recommend the Authors to double check the sequential list of events and lesions in the different subjects. a) The normal value of fractional shortening in dogs is reported as >30%; however this should be referenced. The same comment applies to the legend of figure 2A.

b) The Authors state the hyperechoic lesion was first detected in III-302MA at 12 months. However, they mention later that similar lesions were detected in III-D08MA and III-303MA at 5 and 6 months but not in other dogs at 6-7m. This is confusing. Furthermore, under Methods, the Authors state that echocardiographic examination was performed just before euthanasia, but the chronology does not match with the results and table 2. I would recommend rewrite echocardiographic methods and correct table 2.

c) The fact that clinical signs were not present does not imply that echocardiographic changes were mild. They were mild based on subjective evaluation of a mild regional hypokinesis and near-to-normal FS (although I would still consider 27% a normal value for FS in dogs)

Discussion
The hyperechoic lesions are mentioned only for the dog III-D08-MA. What about the other two individuals?

The Authors imply that CXMDj dogs may represent a better animal model when compared to GRMD. Although early ECG changes are well documented, other cardiac lesions appear to develop in a milder fashion at a later stage in the CXMDj dogs. In my opinion, this may represent a disadvantage, since a longer period of time is needed to obtain significant changes in diseased animal. Other important limitations that
the Authors should acknowledge are the low number of animals used in this study, the lack of biochemical monitoring of myocardial damage (i.e. serum troponin-I levels), and the fact that the progression of the disease was not assessed over a longer period of time.

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

Figure 3A
Please report a reference for >30% as normal FS value in dogs

Figure 4
Figure 3B and figure 4 might be merged. Alternatively, the Authors should consider reporting only the pictures of dogs with the observed echocardiographic lesions.
Please explain in the text the meaning of the arrowhead (i.e. hyperechoic lesions).

Figure 5
Figure 5 is very elegant. However, the Authors should also report the sample origin for III-D55MA and III-D02MA (or at least explain it in the legend)

Figure 6
Figure 6 implies that 100% of CXMDj dogs would present hyperechoic lesions and fibrotic changes at 20 months of age. This does not match with the results presented in this study. Please verify and correct. Furthermore, the significance of the dot-line is not explained.

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Discretionary Revisions (which the author can choose to ignore)

There are some grammatical imperfections that need to be corrected before the editorial review. Line numbering would have helped.
A short list of suggested changes is reported below:
Page 3, Methods, 2nd line: “were” instead of “was”
Page 4, Conclusion, 1st line: “…are milder and have slower progression than those described in GRMS dogs”.
Page 5, 2nd paragraph, line 6: “…In DMD patients, the electrocardiogram (ECG) may show tall R waves…”
Page 6, line 8: “…the reason why the posterobasilar…”
Page 6, last line: the first letter of common breed names (such as Beagles) should be written in capital font.
Page 8, line 5: “…dogs were recently described [25]”. (remove “in our recent paper”)
Page 8, line 13: “unnecessary instead of excessive
Page 9, under ECG and Echocardiography: “just before euthanasia” instead of “just before autopsy”
Page 9, ECG, last line: “calibration”, instead of “voltage standardization”
Page 9, Echocardiography, line 5: standard abbreviation for left posterior ventricular wall is LVPW (instead of PW)
Page 9, Echocardiography, line 7: fractional shortening is abbreviated as %FS. Later in the manuscript is abbreviated as FS. Please choose one or the other.
Page 10, 3rd line: “…underwent cardiac histological…”
Page 10, last line: “none of the dogs in the present study showed…”
Page 11, 1st line: please change “symptoms” into “clinical signs”.
Page 11, line 5: Please remove “on the other hand”
Page 11, line 5: “Serum CK levels in the CXMDj dogs ranged from 12,500 to 138,000 IU/l. These vales were significantly different…”
Page 11, line 8: Please remove “(Table 1)”
Page 11, ECG findings, 1st line: “intervals” instead of “interval”
Page 11, ECG findings, 1st line: “different from those recorded from”
Page 11, ECG findings, 2nd line: “littermates” rather than “littermate”
Page 11, ECG findings, 3rd line: “15 months of age”
Page 11, ECG findings, 4th line: “intervals” instead of “interval”
Page 11, ECG findings, 5th line: “were” rather than “was”
Page 11, ECG findings, 5th line: “correlated both in normal and affected dogs”
Page 11, ECG findings, 7th and 9th line: “littermates” instead of “littermate”
Page 13, Discussion, 1st line: Please remove (PR)

What next?: Accept after minor essential revisions
Level of interest: An article whose findings are important to those with closely related research interests

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

Statistical review: No

Declaration of competing interests:
'I declare that I have no competing interests'