**Author's response to reviews**

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

**Authors:**
- Naoko Yugeta (yugeta@b-star.jp)
- Nobuyuki Urasawa (urasawan@amber.plala.or.jp)
- Yoko Fujii (fujii@dc4.so-net.ne.jp)
- Madoka Yoshimura (yoshimur@ncnp.go.jp)
- Katsutoshi Yuasa (yuasa@ncnp.go.jp)
- Akinori Nakamura (anakamu@ncnp.go.jp)
- Michiko R. Wada (wadarin@ncnp.go.jp)
- Masao Nakura (nakuramso@chugai-pharm.co.jp)
- Yoshiki Shimatsu (shimatsu@ncnp.go.jp)
- Masayuki Tomohiro (tomhromy@banyu.co.jp)
- Akio Takahashi (akio_tk@ncnp.go.jp)
- Noboru Machida (machida@cc.tuat.ac.jp)
- Yoshito Wakao (wakao@azabu-u.ac.jp)
- Shin'ichi Takeda (takeda@ncnp.go.jp)

**Version:** 3  **Date:** 20 September 2006

**Author's response to reviews:** see over
Ms. Iratxe Puebla  
Senior Assistant Editor  
BMC-series journals  

Dear Ms. Iratxe Puebla,

The comments of the two reviewers have been helpful in allowing us to revise our manuscript (MS: 1681285926109043) “Cardiac involvement in beagle-based canine X-linked muscular dystrophy in Japan (CXMDJ): electrocardiographic, echocardiographic, and morphologic studies”, by Naoko Yugeta et al, for publication in BMC Cardiovascular Disorders. We have attempted to address all the questions raised by the reviewers. We have prepared the reply letter to the reviewers and attached. The corrected parts in the manuscript have been indicated by red color.

Thank you for your consideration of the revised version in advance.

Sincerely yours,

Shin’ichi Takeda, M.D., Ph.D.  
Department of Molecular Therapy,  
National Institute of Neuroscience,  
National Center of Neurology and Psychiatry,  
4-1-1 Ogawahigashi, Kodaira,  
Tokyo 187-8502, Japan.  
Tel: +81-42-346-1720  
Fax: +81-42-346-1750  
E-mail: takeda@ncnp.go.jp
Reply to the reviewer #1, Dr. Dominic Wells:

Minor Essential Revisions
1. Table 1 has normal misspelled in the header for the first 4 columns.
   >The misspelling in Table 1 has been corrected.

2. Figure 1 is titled “Echocardiographic findings in CXMDJ”. Surely this should be Electrocardiographic?
   >We made an error in the title of Figure 1, therefore we corrected.

3. For the text relating to figure 1 the term “normal littermates” should be replaced with “the normal littermate” as data are only presented for one normal dog (page 11).
   >We have changed the phrase “normal littermates” to “the normal littermate” in the paragraph of ECG findings (p11 and 12) in response to the reviewer’s comment.

4. The author’s contributions section appears incomplete. Is FY actually YF? What was the role of MY?
   >We have changed FY to YF, and added the role of MY to the author’s contribution section (p18; line 1 to 2).

Discretionary Revisions
1. The authors discuss reasons for differences between the GRMD and CXMD on page 16 but do not appear to consider the effect of genetic background on the cardiac pathology. Although dog breeds are not as genetically uniform as many laboratory rodents, it seems quite possible that genetic differences between the two breeds may play some role in modifying disease progression.
   >We agreed to the suggestion pointed out by the reviewer. Therefore, we have added the sentence to the discussion that genetic difference between the two breeds may affect the phenotype (p16; line 9 to 11).

The sentence added in the Discussion:
The difference in the genetic background between GRMD, golden retriever and CXMD, beagle might also affect the disease progression.
Reply to the reviewer #2, Dr. Luca Ferasin:

Major Compulsory Revisions

1. Although the hypothesis of the study is relatively clear, the purpose should be explained in further detail. The Authors state that a valid animal model is already available (GRMD) but “dogs are large”. Do the Authors propose a different animal model because GRMD are more expensive to breed and maintain? Are there significant advantages in animal housing or welfare? Are Beagle-based dogs easier to examine? GRMD is very difficult to maintain because of its severe phenotypes. Mild phenotypes can be expected, if the size of dogs is small, as indicated by the cross-bred study by Valentine et al. (Ref. 20). Moreover, medium-sized beagle is easy to handle or raise than GRMD, therefore they have definite advantages in animal housing or welfare. In addition, we have gotten beagle bitches and male dogs in Japan, who have clear origins. We described those points at ‘Background’ (p6; line 19 to p7; line 3).

2. The Authors should also explain in detail the ECG recording technique used in this study. It is well documented that body position affects QRS morphology in dogs (Rishniw et al [2002] JVIM 16:69) and variations in recumbency may have been responsible for the observed changes. We have recognized that body position was very important in QRS morphology and we recorded all ECGs in the right lateral recumbency position. We added the sentence to the Methods (p9; line 4).

Furthermore, at the end of the subheading “Clinical profiles of CXMD”, the Authors state that a dog “ran about wildly” during routine ECG and the “ECG monitor showed an idioventricular rhythm”. How could the ECG be recorded while the dog was running? Were these dogs permanently instrumented? The crucial situation of the dog has not been well documented. When we tried to record a routine ECG of the dog, the dog struggled to escape from recording and then ceased moving. Immediately afterwards, we recorded ECG and the monitor showed an idioventricular rhythm. We precisely described the situation in the Results (p11; line 8 to 11).
3. On multiple occasions, the Authors state that both PQ and PR intervals were measured. It is my understanding that PQ and PR intervals are the same thing (time between the onset of atrial depolarization [P wave] and the onset of ventricular depolarization [QRS complex], and it is measured from the beginning of the P wave to the first deflection of the QRS complex, whether this be a Q wave or an R wave) (Meek & Morris [2002] BMJ 324:470)

>As the reviewer pointed out, PQ and PR intervals have the same meaning. We measured PQ and PR intervals in lead III and always found Q waves in normal and affected dogs. We, therefore, preferred PQ interval and discarded PR interval. We changed the description in the Methods, Results, Discussion, and Figure 1 and 2.

4. The negative correlation between heart rate and PQ (PR) interval has been clearly demonstrated in normal Beagle dogs (Hanton & Rabemampianina [2006] Lab Animals 40:123) and it may be attributed to a parasympathetic input at the level of the AV node. The Authors should acknowledge the already available information.

> In response to the reviewer’s comment, we examined the correlation between HR and PQ interval in normal and affected dogs, and we found the negative correlation between two parameters even in affected dogs, although HR was increased in the affected dogs after 15 month. This indicates that the parasympathetic input was well maintained in affected dogs at AV node level. We have added a description to the Results and Discussion, and refer the paper indicated by the reviewer as Ref no. 29.

The sentence added in the Discussion (p13; line 15 to 19):
It has been reported that HR is negatively correlated with PQ interval in normal beagle dogs and it may be ascribed to a parasympathetic input at the level of the AV node [29]. The negative correlation between HR and PQ interval was also found in affected dogs, indicating the parasympathetic input was well maintained even in affected dogs at AV node level.

5. The Authors state that the Q/R ratio continuously increased from 6 to 21 months. However, this does not appear obvious from the data presented in figure 1C, where affected dogs presented a higher ratio but not clearly increasing over time (especially in lead aVF).
As the reviewer stated, Q/R ratio constantly remained high but not continuously increased. We, therefore, changed the expression in Discussion (p14; line 6).

6. The echocardiographic changes in figure 3B are convincing (increased echogenicity in the left ventricular posterior wall); however, the claimed hypokinesis in III-302MA, 21m is a subjective evaluation. Moreover, 27.3% fractional shortening is lower than that recorded in the other dogs in this study, but it can still be considered a normal value from a clinical point of view.

We recognized that hypokinesis was indeed subjective evaluation and that the decrease of FS in the affected dog was rather modest. We have added a new sentence to the result and discussion of echocardiographic findings as indicated below. We, then, thoroughly reevaluated the echocardiographic findings and found schematic overview of hyperechoic lesion was not correct in Figure 6. We, therefore, replaced it with new one.

The sentence added in the Results (p12; line 15 to 17):
The decrease in FS and hypokinesis were detected in the CXMD3 dog III-302MA, however there was no clinical cardiac symptoms, indicating that those changes were subtle.

The sentence added in the Discussion (p15; line 4 to 5):
The dysfunction found in the dog, however, was modest and the dog had no cardiac symptom.

7. It is my understanding that the Q wave represents the early depolarization of the interventricular septum and right ventricular subendocardium. Therefore, I find it difficult to believe that a fibrotic lesion of the posterobasal region of the left ventricle may cause deep Q waves. Altered ionic currents described by Perloff et al (ref n. 17) seem to explain this phenomenon more convincingly.

We agreed to the reviewer’s comment, since the well-distributed notion, fibrosis of postero-basal wall is the cause of abnormal Q waves, cannot explain the ECG changes. We have mentioned the possibility at the Discussion that altered ionic currents by lack of specific membrane proteins could affect the ECG changes, which Perloff et al suggested.
The sentence added to the Discussion (p17; line 1 to 3):
Perloff et al. suggested that the alteration of a particular ionic current by lack of specific membrane proteins associated with dystrophin might participate in the electrocardiographic changes.

8. I would imagine that data were not normally distributed. If so, the median (SE) may add useful information.
>Numbers of samples have been relatively limited in our statistical analysis, therefore we calculated $SE$ instead of $SD$ to verify the valiance of the data. We, therefore, revised Figure 2.

Minor Essential Revisions
1. Cephalic vein of a forefoot sounds redundant (cephalic vein is only on the forelimb)
> We have deleted the expression “of a forefoot” (p8; line 19).

2. Some paragraphs in the “Result” section report (or repeat) the description of the method. They should be moved to the “Methods” section or be removed.
> The same sentences that appeared again in the Results section have been deleted.

Discretionary Revisions
1. I would suggest to change the phrase “from 2-month old” into ‘from 2-months of age”
> We have changed the phrase “from 2-month old” to “from 2-months of age”.

2. I would suggest to change the phrase “almost comparable” instead of “roughly comparable”
> We have changed the phrase “roughly comparable” to “almost comparable” (p7; line 7).

3. In “Methods”, the subheading “electrocardiography” sounds rather confusing and should be rewritten.
> We have changed the subheading “electrocardiography” to “electrocardiographic
studies". 