Author's response to reviews

Title: Neuromuscular abnormality and autonomic dysfunction in patients with cerebrotendinous xanthomatosis

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Version: 4 Date: 19 April 2011

Author's response to reviews: see over
To: The Editorial Board of BMC Neurology

Thanks very much for the valuable opinions and suggestions from the reviewers. In the literature, there are large differences in the findings of neuromuscular and ANS abnormalities in different reports, and these differences suggest that there is still a matter of debate on the subclinical features of CTX. The questions raised by the reviewers may not be answered easily, but we have tried our best to revise this manuscript as accordingly as possible. We know the limitations of our study, therefore, we have added a short statement in the “Conclusion” section as followings: This study is limited by its small case number, but it may shed light on further studies of the clinical and laboratory involvements of neuromuscular and autonomic systems in CTX.

<Reply to Reviewers>

To: Ettore Salsano

Major Compulsory Revisions:

1. In the Table 1, the absence or the presence of symptoms/signs due to peripheral and/or autonomic nervous system involvement should be reported. It is true that the involvement of both the PNS and ANS in CTX patients typically remains subclinical (without symptoms) even after a long history of disease. However, it may also be partially due to the fact that the signs/symptoms of PNS and ANS involvement are masked by the dominant presentations of other nervous system involvements such as dementia, pyramidal sign and cerebellar sign. The absence of S/S of PNS and ANS involvement is recorded in Table 1 in the revised manuscript.

2. The conclusions based on the nerve conduction studies should be carefully revised. According to the normal values reported by the authors in Table 2, it is hard to sustain that the patients I-1, I-2 and II-2 presented an axonal neuropathy, even if the sural nerve SAPs were always absent, and the neuropathy is defined as “mild” (see Results, NCS and EMG). Indeed, approximately all the CMAPs and SAPs reported in the Table 2 are in the normal range. For example, patient I-1 has peroneal CMAPs of 4.1 mV (right) and 4.5 mV (left) with a normal value > 2 mV, and tibial CMAPs of 5.5 mV and 9.6 mV with a normal value of > 4 mV. Are the authors sure that the normal values for peroneal and tibial CMAPs are correct? For example, the normal amplitude of peroneal CMAPs reported by Verrips et al. is > 4 mV (a value that is similar to that used in our laboratory).
Sorry for the misleading data. In the revised manuscript, we have cited normal data in healthy Chinese for a referential consideration (Lin KP, Chan MH, Wu ZA: Nerve conduction studies in healthy Chinese: correlation with age, sex, height and skin temperature. Chin Med Journal (Taipei) 1993; 52: 293-297).

3. **Medial plantar nerves may be easily damaged in non-pediatric patients; therefore, I believe that in adult patients the neurophysiologic study of medical plantar nerves is of scarce significance.**

   Thanks for your suggestion. We agree with your opinion; therefore, we have deleted the related data in the “Results” section and Table 2.

4. **A frank asymmetry (a difference #50% between left and right) is reported in the neurophysiologic findings in some cases (e.g., the SAP of median nerves in Case I-1 (47 uV versus 21 uV); the SAP of the ulnar nerves in Case I-2 (26 uV versus 13 uV); the distal latency, conduction velocity and CMAP of the peroneal nerves in Case II-1 (“absent” only on the right); the CMAP of the tibial nerves in Case II-2 (4.7 mV versus 2 mV): do the authors have some comments on this?**

   Clinically, the above-mentioned asymmetric involvement is not against the confirmative diagnosis of polyneuropathy (England JD, et al.: Distal symmetric polyneuropathy: a definition for clinical research. Report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Neurology 2005; 64: 199-207). Concomitant focal compressive neuropathy can be one of the possibilities responsible for the electrophysiologic asymmetries, but we cannot prove or disapprove it with present electrophysiologic data. Therefore, if the reviewer allows, we will not make a related discussion on this issue.

5. **The results of the autonomic nervous studies should be clarified/revised, and the absence of clinical dysautonomia (if so) should be stressed.**

   The absence of clinical dysautonomia has been listed in Table 1, and we have clarified the results of ANS in the “Results” section.

6. **R-R interval variation was decreased only in Case I-1, according to the normal value reported by the authors (that is “> 15”) (see Table 4, Autonomic Findings and Results of Autonomic Tests).**

   In the revised manuscript, we have corrected this error in the “Results” section.

7. **I believe that the “30:15 ratio” usually refers to the ratio of the R-R interval**
measured at 30 beats and at 15 beats after standing (not at 30 seconds and at 15 seconds as indicated in Patients and Methods, Autonomic Function Assessment)

It was our mistake in manuscript preparation and thank you for the correction. We have revised this as follows: “We used a “30:15 ratio” to assess this physiological response by measuring the ratio of the RR interval at beat 30 while standing to the RR interval at beat 15.”

8. Results, Results of Autonomic Tests: I think that the significance/implication of abnormalities of some parameters that reflect the function of the cardiovascular autonomic system (i.e. the R-R interval variation, 30:15 ratio, and baroreflex sensitivity) should be briefly but clearly explained, as I suppose that many neurologists are quite unfriendly to these parameters. What is the meaning of the presence of RRIV, 30:15 ratio and BRS abnormalities, if any, why there is no change of the heart rate and/or blood pressure after tilting up?
The purposes of RRIV, 30:15 ratio and BRS measurement have been briefly explained in the “Patients and Methods” section.

It is difficult to explain the mismatches between the lab findings with the clinical presentations of BP and HR abnormalities. Therefore, we have added a statement on these mismatches in the “Discussion” section as follows: Despite these different degrees of laboratory abnormalities were demonstrated, they were not reflected fully in clinical blood pressure and heart rate changes in these four cases. In the meanwhile, there was also mismatch in the inter-lab data such as the finding of SSR and Q-sweat test. These mismatches need further verification by large-scale study.

9. Table 4 and Results. Results of Autonomic Tests: In the quantitative sudomotor axon reflex test (QSART) the total volume (in uL//cm²) is reported to be reduced at foot level in the Case I-1, in all four recorded sites (forearm, proximal and distal leg, and foot) in Cases I-2 and II-1, and at forearm level in Case II-2. However, in Case I-1, the sweat volume at forearm (0.15) is lower than in the Case I-2 (0.298), the sweat volume at proximal leg (0.134) is lower than in the Case II-1 (0.199), and the sweat volume at distal leg (0.183) is lower than in the Case II-1 (0.294). Could authors specify the normal values? Is a “relative” value used? If so, please specify when the sudomotor function is considered abnormal.

To date, there are no Chinese data available for the QSART; therefore, we listed
the normal values of Q-Sweat (not published) in Table 4 as follows (μl): median (max, min)

<table>
<thead>
<tr>
<th></th>
<th>Forearm</th>
<th>Proximal leg</th>
<th>Distal leg</th>
<th>Foot</th>
</tr>
</thead>
<tbody>
<tr>
<td>women</td>
<td>0.495 (0.02, 3.814)</td>
<td>0.505 (0.06, 4.49)</td>
<td>0.389 (0.015, 4.914)</td>
<td>0.203 (0.014, 0.802)</td>
</tr>
<tr>
<td>men</td>
<td>1.079 (0.02, 3.814)</td>
<td>1.091 (0.029, 2.734)</td>
<td>1.107 (0.043, 2.902)</td>
<td>0.585 (0.041, 1.718)</td>
</tr>
</tbody>
</table>

We have rewritten the study results of the Q-sweat test in the “Results” section as follow: “In the Q-Sweat test, compared with the data of normal control (Table 4), the sweat output showed a reduction of sweat volume in the four recorded sites of Cases I-1, I-2 and II-1 and in forearm of Case II-2. With a relative comparison, the sweat volumes were as follows: foot sweat volume < 1/3 of the forearm value in Case I-1; foot and proximal leg < 1/3 of the forearm value in Case I-2; and forearm and foot volume < 1/3 of the proximal leg value in Case II-1.”

10. **Table 4 and Results, Results of Autonomic Tests: The Sympathetic Skin Responses (SSRs) are normal despite an impairment of the quantitative sudomotor axon reflex.** I suggest to the authors to comment this divergence. The SSR measures a polysynaptic reflex with a spinal, a bulbar and a suprabulbar component, and the abnormality of this test can not differentiate the pre-ganglionic or post-ganglionic problems of sudomotor function. However the case number was too small to draw a conclusion; therefore, we have added a statement of these mismatches in the “Discussion” section as follows: “Despite these different degrees of laboratory abnormalities were demonstrated, they were not reflected fully in clinical blood pressure and heart rate changes in these four cases. In the meanwhile, there was also mismatch in the inter-lab data such as the finding of SSR and Q-sweat test. These mismatches need further verification by large-scale study.”

11. **Results, Quantification of Skin Innervations: The intradermal nerve fiber (IENF) density was clearly abnormal (3.19) only Case I-1; in Case I-2 was normal (6.14; n.v.>5.88) or mildly reduced (5.45 in Case II-1 e 5.13 in Case II-2). Therefore, the incidence of small fiber involvement in CTX is less than 100%, unlike the authors state in the Discussion. Moreover, it seems that the IENF densities does not reflect the QSART data at the distal leg (where the skin biopsy was performed). Indeed, the sweat volume was 0.183 in Case I-1, 0.108 in Case I-2, 0.294 in Case II-1, and 1.409 in Case II-1. I suggest to the authors to comment this discrepancy too.

Sorry, it was a mistake in manuscript preparation. In the revised manuscript, we have corrected this as follows: “In this study, IENF density measurement revealed
a relatively high incidence (75%, 3/4) of small fiber involvement in CTX, and this figure of incidence is as high as those neuropathies with a small-fiber sensory neuropathy.”

The recording site of distal leg part in the Q-Sweat was at the medial side (5 cm above the medial malleolus) and the site of skin biopsy was at the lateral side (10 cm above the lateral malleolus) of the distal leg. This difference in sites may give us a different study results in each test, however, so far we can not prove or disapprove this and the exact cause needs further study for verification. Therefore, we have made a statement in the “Discussion” section for this discrepancy as follows: “But again, as shown in this study, there is a discrepancy between the finding of IENF density measurement and Q-Sweat test. This mismatch also needs further large scale study for verification.”

12. In the work of Arpa et al. cited in the Discussion, the authors found that “cardiovagal and adrenergic functions were preserved”, while “sweat gland density was […] reduced in the foot”, Hence, they found an abnormality which might suggest a “postganglionic cholinergic failure” limited to the sudomotor system alone. Please, change the text according to this comment.

In the study (single case report) of Arpa et al., the only description of ANS study in the “CASE REPORT” SECTION was as follows: Cardiovagal and adrenergic function were preserved. Sweat gland density was normal in the hand and reduced in the foot. This isolated abnormality was consistent with postganglionic cholinergic failure accompanying somatic peripheral neuropathy. The study methodology and findings were too simply described in the report of Arpa et al. and no raw data were shown in that report. We do not doubt their findings, however for scientific reason, if the reviewer can allow, we would prefer not to change our text according to the comments of Arpa et al.

Minor Essential Revisions:
1. Abstract, methods, First Paragraph:: “……all received a nerve conduction study”. I believe that the term “electromyography” should be preferred, as EMG studies were also performed.

We agree with the reviewer’s opinion. In this manuscript, both terms (NCS and EMG) are needed. Therefore, we ask the reviewer’s permission to let us keep these two terms in the text.

2. Results, NCS and EMG, First paragraph: “Lower amplitude”: the amplitude should be “low” or “lower” than something else”: please specify.
In the revised manuscript, we have cited normal data of Chinese for a referential comparison; therefore, we have changed “lower amplitude” to “decreased amplitude”.

3. Results, NCS and EMG, First Paragraph: “Prolonged late response”: do the authors mean “prolonged F-waves”?
Thank you for your reminder, we have corrected it to delayed F response.

Discretionary Revisions:

1. The thesis that the peripheral neuropathy associated with CTX is predominantly axonal is interesting, as one hypothesis upon the pathogenesis of the central nervous system dysfunction in CTX is based on the replacement of the cholesterol normally present in the myelin by cholestanol, thus suggesting that CTX may be primarily a myelinopathy. What about the presence of cholestanol in peripheral nerves (axons, Schwann cells)? Could the authors discuss this point in more detail than reported in the Discussion?
The presumption that CTX is a disorder of myelinopathy may not be wholly true. In our recent report (Chang et al.: Multi-parametric neuroimaging evaluation of cerebrotendinous xanthomatosis and its correlation with neuropsychologic presentations. BMC Neurol 2010; 10:59), we examined the white matter change by using FA and MD findings in DTI studies and by reviewing the pathologic findings reported by Soffer et al. and Pop et al., and we found that demyelination, axonopathy and tissue atrophy all occur in CTX. Axonal neuropathy, demyelinating neuropathy and mixed-type neuropathy have all been reported in the literature, as listed in Table 5 of our manuscript. Because we made a similar discussion in our previous paper as mentioned above, we did not perform any pathological studies of the peripheral nerve. If the reviewer will allow, therefore, we would prefer not to have such a discussion in this manuscript.

2. A MIBG scintigraphy may be performed to study the parasympathetic innervations of the heart, in order to definitely investigate the presence of a post-ganglionic cholinergic failure.
Thanks very much for your valuable suggestion. For several limitations, we did not perform heart MIBI-scintigraphy for these CTX patients; therefore, we can not offer the related data in this manuscript.

3. In the discussion, I suggest to remark- for each investigated tissue (i.e. peripheral nerve, muscle, autonomic system)-first the results of the work, then the papers in
agreement with that findings, and finally the papers with discordant data. Thanks for this suggestion. We have revised the “Discussion” section accordingly.