Author's response to reviews

Title: Autonomic neuropathy in Fabry disease: a prospective study using the Autonomic Symptom Profile and cardiovascular autonomic function tests

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Author's response to reviews: see over
Dear Miss Ilievska,

Please find enclosed a revised version of our manuscript entitled “Autonomic neuropathy in Fabry disease: a prospective study using the Autonomic Symptom Profile and cardiovascular autonomic function tests” by M. Biegstraaten, I.N. van Schaik, W. Wieling, F.A. Wijburg and C.E.M. Hollak.

We have revised our manuscript in accordance with the comments raised by the reviewers. A point-by-point response is attached.

We hope that you will find the revised manuscript acceptable for publication in BMC Neurology.

Yours sincerely,

Marieke Biegstraaten

List of contents included in the submission:

- List of comments by the referees in italic and our response (attached)
- Article including figure legend and Tables 1, 2a, 2b and 3 with and without changes marked
- Figure 1
List of comments by the referees in italic and our response

Referee 1

Comment 1: *The healthy control group has to be better described. How were they selected?*

Response 1: We agree with the reviewer and have changed the following sentence (page 4, line 21, changes in italic):

Scores obtained from our patients were compared with these scores and with scores obtained from a sample of 48 *sex- and age-matched* healthy hospital workers who were *asked for their co-operation by one of the authors (MB)*.

Comment 2: *Table 1: please separate clearly for each group those on ERT and those without. We need to know what were for example the MSSI for ERT and non-ERT. We need to know the age of ERT vs. non etc. The idea is really to account as much as possible for possible confounding effect of ERT.*

Response 2: We agree with the reviewer and have added treatment-subanalyses in Table 1 (page 15), Table 2a (page 16) and Table 2b (page 17).

Furthermore, we added a paragraph entitled “Role of ERT” in the Results section (page 7, line 6):

**Role of ERT**

Two of the 15 male study patients were untreated. They were similar to ERT treated patients with respect to age and disease severity. Female patients who were treated with ERT were older and more severely affected compared with untreated females (see Table 1). No difference in ASP sum score was found between ERT treated and untreated patients (p = 0.30), while secretomotor problems and sleep disorder were more frequent and more severe in ERT treated than untreated patients (see Tables 2a and 2b).

Twenty-five of the 36 patients (69%) who underwent autonomic cardiovascular function tests received ERT. Patients on ERT had lower heart rate variability than untreated patients. All three females and three males with an abnormal heart rate variability test result were treated with ERT. The 14-year-old girl was untreated. Altogether, ERT was not associated with less severe disease, lower ASP scores or less frequent cardiovascular autonomic function abnormalities.

Finally, the following sentences were added in the Abstract (page 2, line 15):

Enzyme replacement therapy was not associated with less severe disease, lower ASP scores or less frequent abnormal cardiovascular function test results.

and in the Discussion (page 9, line 18):
Besides, the cross-sectional character of the study precludes definite conclusions on the long term effect of enzyme replacement therapy in individual patients. However, our results suggest that patients with relatively severe disease are more often on ERT and that the severity of autonomic dysfunction is not influenced by ERT.

**Comment 3:** Table 2 and the text: I don’t see the usefulness of reporting the percent of patients with non-zero score. What is important is the absolute (raw) score in Fabry vs. healthy controls. Otherwise it looks like the healthy are not that healthy. It is surprising to me for example that healthy individuals have as many people with bladder dysfunction complaints. In this table too please separate the ERT from the non-ERT.

**Response 3:** We agree with the reviewer that raw scores can be important and have added a table (Table 2a, page16) with median ASP scores. We calculated medians and ranges as ASP scores were not normally distributed. In this case this often results in median scores of zero. In our view, the percent of patients with non-zero scores add valuable information and we therefore suggest presenting the original table as well (Table 2b, page 17). In the original paper of Suarez et al, results were expressed by means and percent of patients with a score of more than zero.

**Comment 4:** I agree with the conclusion that the abnormalities described are due to end organ dysfunction rather than to the neuropathy. However, this conclusion was reached by our group already in a number of primary (non-review) papers:

- **Sweath:** Schiffmann et al Muscle and Nerve 2003
- **Cerebral vasculature:** Moore et al. Circulation 2003 and Moore et al. J Magn Reson Imaging, 2004

**Response 4:** We agree and have changed the reference on sweat function in the Background section (page 3, line 13) and the Discussion (page 8, lines 2 and 18 ) from Schiffmann R, Acta Neurol Belg 2006, 106: 61-65 to Schiffmann R et al, Muscle and Nerve 2003, 28: 703-710.

Furthermore, we added the following sentence (page 8, line 16):

More likely, these symptoms and signs are caused by end-organ failure which has been suggested before by others [22,23] and is supported by findings from previous studies [7,21].


**Comment 5:** The HRV abnormalities in 19 boys and its correction with ERT is actually described in Ries et al. 2006.
Response 5: We have added this reference in the Background section (page 3, line 22) and the Discussion (page 8, line 10).

Comment 6: I do not understand the separate influence of the hypertrophy as an explanation of the abnormal HRV rather than the effect of the small-fiber neuropathy. Rather, it is likely that the end organ that is relevant here is the conduction system not the hypertrophy per se. Decreased HRV is seen in children without LVH (see Ries et al. 2006 above). This is also relevant to the last sentence of the first paragraph of page 10. ...the disease itself. It is all due to the disease itself.

Response 6: We agree with the reviewer and have changed the following sentence (page 8, line 8) from:

However, all 6 boys had an increased left ventricular cardiac muscle mass. It is known from studies on HRV in patients with myocardial infarction, that a reduction in parasympathetic cardiac control can be found in patients with cardiac pathology [21]. Cardiac pathology in Fabry disease could thus have influenced the abnormalities observed.

to:

Studies on heart rate variability (HRV) in pediatric Fabry patients revealed significantly different results between boys and both girls and controls, with significant improvement of heart rate variability in boys upon ERT [10,11]. However, it is likely that cardiac pathology (i.e. left ventricular hypertrophy and/or conduction system pathology) has influenced the abnormalities observed in these patients.

Furthermore, the last sentence of the first paragraph of page 10 ...the disease itself has been removed.

Comment 7: Regarding patients on ERT: we need to know their ages and how long they were on ERT. Do the authors think the ERT plays any therapeutic role vis a vis the parameters they measured or if not is ERT just a marker for relatively severe disease?

Response 7: See also Response 2: We have added treatment-subanalyses in Table 1, a new paragraph Role of ERT and sentences as indicated in Response 2. Treatment duration was already given in the first version of the manuscript (page 5, line 27).

Referee 2

Comment 1: The authors propose that in view of the low frequency and degree of abnormalities for autonomic symptoms and cardiac autonomic testing, some of the observed autonomic dysfunction may represent end-organ damage and not necessarily autonomic fiber involvement. However, they provide no objective data on this cohort for the presence or absence of abnormal end-organ function and its relation to autonomic symptoms or
abnormal autonomic testing. Additional objective data supporting the absence of presence of end-organ
dysfunction would be needed to support this argument or it should be modified.

Response 1: We have described the cardiac involvement due to Fabry disease in the results section in relation to
the cardiovascular autonomic function tests and have shown that damage to the heart is most likely the cause of
abnormal test results. We investigated specifically the influence of cardiac disease on cardiovascular function
tests (see Figure 1). However, we propose to make some modifications to clarify this inherent uncertainty.
Therefore the following sentences have been changed:

Page 2, line 19:
from:
It is likely that end-organ damage plays a key role in the development of symptoms and signs in Fabry patients.
to:
Possibly, end-organ damage plays a key role in the development of symptoms and signs in Fabry patients.

Page 7, line 22:
from:
This raises the important question whether end-organ failure or autonomic neuropathy plays the most prominent
role in Fabry signs and symptoms.
to:
This raises the important question of whether autonomic neuropathy plays a prominent role in Fabry symptoms
and signs.

Page 8, line 15:
from:
Thus, symptoms and signs compatible with autonomic dysfunction in Fabry patients are very likely to be due to
end-organ disease and not to autonomic neuropathy.
to:
Altogether, our results indicate that symptoms and signs compatible with autonomic dysfunction in Fabry
patients are probably not due to autonomic neuropathy. More likely, these symptoms and signs are caused by
end-organ failure which has been suggested before by others [22,23] and is supported by findings from previous
studies [7,21].
Comment 2: Since most patients were mildly or moderately affected based on the MSSI, it is possible that autonomic neuropathy is more prevalent in severely affected patients. The question of severity should be addressed by the authors.

Response 2: We agree with the reviewer and have added the MSSI scores to the patient data in the Results section (page 6, line 26, changes in italic):

Three female patients aged 25, 50 and 53 years old and with MSSI sum scores of 13, 24 and 17 showed abnormal results of the forced breathing test. They scored respectively 12, 9 and 9 beats per minute indicating a decreased HRV. The 53-year-old woman suffered from cardiomyopathy and the ECG of the 50-year-old woman showed signs of left ventricular hypertrophy (LVH). Three males aged 25, 52 and 63 years old and with MSSI sum scores of 13, 27 and 47 had an abnormal initial heart rate response to standing up from supine position. The latter was known with cardiomyopathy, while the other two males did not have cardiac function abnormalities. They scored respectively 17, 11 and 11 beats per minute which are abnormally low scores in relation to their age. One 14-year-old girl with an MSSI sum score of 2 had a persistent fall of 11 mmHg in diastolic pressure 3 minutes after standing up.

Furthermore, we added the following sentence in the Discussion (page 9, line 15):

Another limitation of the current study is that we included mainly mild to moderately affected patients. As we found a trend towards a correlation between the MSSI sum score and the ASP sum score and relatively high MSSI sum scores in 4 out of 6 patients with an abnormal heart rate variability test result, we cannot exclude that autonomic neuropathy is more prevalent in severely affected patients.

Comment 3: It is not clear why the authors only used non-parametric tests for statistical analyses and whether these were always appropriate. Additional information for this choice would be of interest.

Response 3: The choice for these tests was because of non-normal distribution of almost all data, due to the relatively small data set. However, some variables (i.e. results of cardiovascular autonomic function tests) indeed showed a normal distribution and therefore we have changed the following sentence (page 5, line 13) from:

All results are expressed by median and range. Differences between variables are calculated using the Mann Whitney U test.

to:

All results are expressed by mean and standard deviation or median and range where appropriate. Differences between variables are calculated using the unpaired t-test or Mann Whitney test and differences in proportions are tested using the Fisher’s exact test.

Results in text and tables have been changed accordingly.
We shortened the Methods section in order to keep up with the allowed word count (page 4, line 6):

from:

To measure the severity of Fabry disease in individual patients, the Mainz Severity Score Index (MSSI) was used [12]. The MSSI scoring system is composed of four sections that cover the general, neurological, cardiovascular and renal symptoms and signs of Fabry disease. For each symptom and sign, a single rating was assigned and the corresponding points were summed up to determine the total MSSI score. Patients with a total score of less than 20 are defined to be mildly affected, those with a score from 20-40 are moderately affected, and those with a score above 40 are severely affected [13].

to:

To measure the severity of Fabry disease in individual patients, the Mainz Severity Score Index (MSSI) was used [13]. Patients with a total score of less than 20 are defined to be mildly affected, those with a score from 20-40 are moderately affected, and those with a score above 40 are severely affected [14].

The 10 centimetre VAS score for most severe pain was replaced by an 11-point VAS score for most severe pain in the last 4 weeks as the latter was considered more appropriate due to the added time window of 4 weeks (page 4, line 8, changes in italic): Pain intensity was assessed on an 11-point visual analogue scale (VAS), anchored no pain (0) and worst possible pain (10). Patients were asked to score their most severe pain in the last 4 weeks [15].

We corrected the information on cardiac pathology in the male patients with an abnormal cardiovascular function test result as the left ventricular hypertrophy of the 25-year-old male was erroneously not mentioned (page 6, line 29, changes in italic):

Three males aged 25, 52 and 63 years old and with MSSI sum scores of 13, 27 and 47 had an abnormal initial heart rate response to standing up from supine position. The first was known with LVH and the latter with cardiomyopathy, while the 52-year-old male did not have cardiac pathology. They scored respectively 17, 11 and 11 beats per minute which are abnormally low scores in relation to their age. and (page 8, line 12, changes in italic):

As four of these six patients were known with LVH or cardiomyopathy, our findings could be partly explained by the underlying cardiac pathology.
• HSAN type 2 was removed from the Discussion. Literature research revealed that this type does not serve as a good example; thinly as well as thickly myelinated nerve fibres are affected in this type.

Page 8, line 28, changes in italic:

One other disease, hereditary sensory and autonomic neuropathy (HSAN) type 5, is known to cause selective loss of small myelinated nerve fibres. Autonomic function is usually spared in HSAN type 5; none of the reported patients had orthostatic hypotension, although anhydrosis has been reported in some [29].

• We added the sections “Competing interests” and “Authors’ contributions”:

**Competing interests**

MB received research support from Actelion Pharmaceuticals Ltd.

INvS received honoraria for lecturing and consultancy and research support from Actelion Pharmaceuticals Ltd. All consulting fees for INvS were donated to the Stichting Klinische Neurologie, a local foundation that supports research in the field of neurological disorders.

WW declares that he has no competing interests.

FAW received reimbursement of expenses and honoraria for lectures on lysosomal storage diseases, including Fabry disease, from Genzyme Corporation, Actelion Pharmaceuticals Ltd and Shire HGT.

CEMH received reimbursement of expenses and small honoraria for lectures on the management of lysosomal storage diseases, including Fabry disease, from Genzyme Corporation, Actelion Pharmaceuticals Ltd and Shire. All honoraria were donated to the Gaucher Stichting, a national foundation that supports research in the field of lysosomal storage disorders.

**Authors’ contributions**

MB participated in the design and coordination of the study, performed the statistical analyses and drafted the manuscript. INvS, FAW and CEMH conceived of the study and participated in its design and in drafting the manuscript. WW carried out the cardiovascular autonomic function tests and contributed to the interpretation of the results. All authors read and approved the final manuscript.