Author's response to reviews

Title: Hepatic mitochondrial dysfunction in Friedreich Ataxia

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Author's response to reviews: see over
Dear Editor,

thank You very much for reviewing our manuscript “Hepatic mitochondrial dysfunction in Friedreich Ataxia”

We would like to address the comments as follows (added parts are marked yellow):

Comments of the editor:

1. I am concerned that there are two subjects where the diagnosis of Friedreich ataxia is not proven by molecular analysis. If this cannot be done, I suggest these two individuals are removed from the analysis. Were the other 14 homozygous for the GAA expansion? If so this should be stated.

ad 1: As stated in the first sentence of the methods part the diagnosis “Friedreich ataxia” was genetically confirmed in all16 patients. However, exact GAA repeat lengths in the FXN gene were available only in 14 of the 16 FRDA patients (see line 7 in the methods section). In two patients the exact repeat length was not given in the report from molecular testing, but it was stated that the patients are homozygous for the mutation. Genetic testing was not a part of the study. Our inclusion criteria was “genetically confirmed FRDA”, thus we were not able to perform a new molecular testing in the remaining two patients.

We added: 16 patients all with genetically confirmed Friedreich ataxia and homozygous for the GAA expansion were recruited from the Department of Neurology.

2. If there were compound heterozygotes, I suggest these be removed as the pathogenesis of disease may be different in these individuals.

ad 2: None of the 14 patients was compound heterozygote, for the remaining two patients, this is not mentioned in the report.

3. You have given doses for all medications taken except antioxidants which in my view are the most important to know how much medication the subjects were taking as this could impact hepatic mitochondrial function. In addition, I am interested to know if there was a difference in the breath test results between those taking antioxidants and those who were not.

ad 3: The dosage of antioxidants were added in the text. In addition we rephrased this sentence in order to point out, that one patient took three different antioxidants and only three patients were on antioxidants:

Three FRDA patients got one antioxidants; one took L-carnitine 1500 mg, coenzyme Q(10) 400 mg and vitamine E 1000 mg, one idebenone 2250 mg and one L-carnitine 1500 mg.

Statistical analysis revealed no difference between patients on antioxidants and those were not. We added in the results section:
4. Finally there are two papers that I suggest you reference that are relevant to the study. Koutnikova et al Nat Genet 16:345,1997 shows that there is high expression of FXN in the liver and König et al Epilepsia 40:1036,1999 describe liver failure in an individual with Friedreich ataxia treated with valproate."

Thank You very much for the reference. We added: Frataxin mRNA was found to show a broad expression pattern, including tissues with a high metabolic rate, like liver, kidney, brown fat and heart [3]. König et al was already cited.

Reviewer 1:
1. The authors used [methyl-13C]-methionine as substrate for the breath-test. Transmethylation of methionine results in the removal of the labelled methyl group if [methyl-13C]-methionine is used as substrate. The labelled methyl group may in part be used for the synthesis of sarcosine which is oxidized to formaldehyde and finally CO2 in mitochondria. I am not aware that this pathway is impaired in FRDA that this method has been used in other mitochondrial encephalomyopathies to evaluate hepatic mitochondrial dysfunction.

ad 1: As we stated in the introduction, iron-sulfur clusters (ISCs) serve as prosthetic group in several enzymes of the mitochondrial energy metabolism including aconitase and complexes I, II and III of the respiratory chain that are impaired in FRDA. Excess of methionine methyl groups is metabolised via sarcosine (N-methylglycine) and mitochondrial oxidation to CO2. All patients received an “excess” - amount of methionine in this established test, which makes an oxidation to CO2 necessary. CO2 oxidation occurs exclusively in the krebs cycle, by using the respiratory chain enzymes. To our knowledge there are no other studies available from other neurological disease, such as mitochondrial encephalomyopathies.

2. Two patients are without molecular diagnosis. It seems useful to specify if they fulfilled the Harding criteria for clinical diagnosis.

ad 2: the diagnosis “Friedreich ataxia” was genetically confirmed in all16 patients.

3. The use of concomitant medications represents the major drawback of the study. A scatterplot of the patients with or without medication might be sent to the referees.

ad 3: FRDA patients without any medication are very rare. Thus we were not able to recruit only patients without medication in this PILOT study.
In order to make this more transparent, we added at the conclusion:
Taken together, the data of our pilot study using the MeBT in FRDA indicate that FRDA patients exhale significantly smaller amounts of $^{13}$CO$_2$ compared with healthy controls indicating a subclinical liver affection in FRDA.

Moreover no differences were observed between drug-free FRDA patients and patients on medication. An impairment of mitochondrial metabolism is not described for the listed drugs.

We are happy to provide a scatteplot of the patients with (1) or without (2) medication for cPDR90 and PDRmax.

Reviewer 2:
We thank reviewer 2 for his comment.

1. At the end of the Discussion section the authors should state that measuring of hepatic mitochondrial activity by 13C-methionine-breath-test does not seem to be a good biomarker on the follow-up of natural history of the disease of the response to drugs in clinical trials.

ad 1. We added:
Thus, measuring of hepatic mitochondrial activity by MeBT does not seem to be a good biomarker on the follow-up of natural history of the disease and the response to drugs in clinical trials.

We deleted from the Conclusion:
Thus MeBT might be useful to identify potentially drugs and predict clinical benefit in the treatment of FRDA.

2. References style should revised to fit that of BMC Neurology if not.
ad 2. we did this.

We hope we addressed the comments sufficient. We thank You in advance for Your efforts.

We are looking forward to Your answer,

Yours sincerely

Carsten Saft