Author’s response to reviews

Title: A case report of a mild form of multiple acyl-CoA dehydrogenase deficiency due to compound heterozygous mutations in the ETFA gene

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Author’s response to reviews:

Dear editor,

Please find enclosed the revision of our manuscript entitled “"A case report of a mild form of multiple acyl-CoA dehydrogenase deficiency due to compound heterozygous mutations in ETFA". A number of changes have been made to the paper, as indexed in this letter (in blue) and in red in the manuscript. Your comments have been very interesting, constructive and helpful to improve our paper. More specific responses are outlined below.
We are grateful for your time and your expertise. We hope we managed to revise this article in such a way to fulfil the aims of your journal.

Sincerely yours,

Pierre-Antoine FAYE

Editor comments:

(1) Provide additional details regarding sequencing and analysis methods; for example, describe the gene panel (genes, panel type, manufacturer) that was used.

This is indeed a relevant remark. We added the section below pg. 6 (Case presentation, line 3, pg. 6):

“Logically, genes involved in multiple acyl-CoA deshydrogenase deficiency (ETFA (NM_000126), ETFB (NM_001985) and ETFDH (NM_004453)) and in riboflavin transport and metabolism (SLC52A1 (NM_017986), SLC52A2 (NM_024531), SLC52A3 (NM_033409), SLC25A32 (NM_030780), FLAD1 (NM_025207) and RFK (NM_018339)) were analyzed using a Next Generation Sequencing (NGS) approach. Library was obtained using a custom panel (NimbleGen SeqCap EZ Technology (Roche)) targeting exons and exon-intron boundaries (+/-25 nucleotides; SeqCap EZ Choice Nimblegen, Roche with NextSeq500, Illumina) targeting exons and exon-intron boundaries (+/-25 nucleotides). Sequencing was performed on a NextSeq500 (Illumina) sequencer. Coverage was 100% at 30X depth and bioinformatic pipeline allows SNV and CNV detection. For the patient, the diagnosis of MADD was confirmed by the finding of two new heterozygous ETFA substitutions, c.354C>A (p.Asn118Lys) and c.652G>A (p.Val218Met). Sanger sequencing was performed to confirm pathogenic variants.”

Moreover, we removed “The diagnosis of MADD was confirmed by genetic analysis exhibiting two new heterozygous ETFA substitutions, c.354C>A (p.Asn118Lys) and c.652G>A (p.Val218Met)” and “(+/- 25 nucleotides; SeqCap EZ Choice Nimblegen, Roche with NextSeq500, Illumina)” pg.6 (Case presentation, line 3, pg. 6).
We also added “NGS: Next Generation Sequencing” in the list of abbreviations (List of abbreviations, line 32 pg. 11).

(2) Under declarations, please state whether research involving human subjects, human material, or human data was performed in accordance with the Declaration of Helsinki.

The sentence “and was performed in accordance with the Declaration of Helsinki” was added under the declaration section (Declaration, line 8, pg. 12).

(3) Also, on discussion, pg. 7, should 'live' be 'life’?

It is a mistake, we corrected the discussion in accordance to this remark, pg. 7 (“live” to life”) (Discussion and Conclusion, line 45 pg. 7).

Gerald Vockley (Reviewer 1):

“I assume that all of the metabolite studies presented are from a clinical laboratory, but this should be made explicit.”

Thank you for pointing out this point. We specified “samples at Clinical Biochemistry departments of Limoges and Lyon University Hospitals” (Cased presentation, line 22, pg. 5).

“There are no functional studies to verify that ETF is actually dysfunctional.”

This is indeed a very relevant remark, but no muscular, liver or skin biopsies were made on this young child in order to avoid invasive exams.

“The authors should clarify if they did any other genetic testing besides sequencing the ETFA gene.”
This is indeed a relevant remark. We added the section below pg. 6 (Case presentation, line 3, pg. 6):

“Logically, genes involved in multiple acyl-CoA deshydrogenase deficiency (ETFA (NM_000126), ETFB (NM_001985) and ETFDH (NM_004453)) and in riboflavin transport and metabolism (SLC52A1 (NM_017986), SLC52A2 (NM_024531), SLC52A3 (NM_033409), SLC25A32 (NM_030780), FLAD1 (NM_025207) and RFK (NM_018339)) were analyzed using a Next Generation Sequencing (NGS) approach. Library was obtained using a custom panel (NimbleGen SeqCap EZ Technology (Roche)) targeting exons and exon-intron boundaries (+/- 25 bp). Sequencing was performed on a NextSeq500 (Illumina) sequencer. Coverage was 100% at 30X depth and bioinformatic pipeline allows SNV and CNV detection. For the patient, the diagnosis of MADD was confirmed by the finding of two new heterozygous ETFA substitutions, c.354C>A (p.Asn118Lys) and c.652G>A (p.Val218Met). Sanger sequencing was performed to confirm pathogenic variants.”

Moreover, we removed “The diagnosis of MADD was confirmed by genetic analysis exhibiting two new heterozygous ETFA substitutions, c.354C>A (p.Asn118Lys) and c.652G>A (p.Val218Met)” and “(+/- 25 nucleotides; SeqCap EZ Choice Nimblegen, Roche with NextSeq500, Illumina)” pg.6 (Case presentation, line 3, pg. 6).

We also added “NGS: Next Generation Sequencing” in the list of abbreviations (List of abbreviations, line 32 pg. 11).

“While the treatment reported is standard, there is no mention of its effectiveness in this patient.”

Perhaps it appeared too late in the manuscript, however, the treatment effectiveness was mentioned pg. 10 (Discussion and Conclusion, line 17, pg.10): “In this case, according to treatment compliance and a strict fasting avoidance, the 5-year-old patient exhibits a normal development and schooling without any other decompensation episode.”

We rebuilt the sentence as :

“In this case, with a good compliance and a fasting avoidance, the 5-year-old patient exhibited a normal cognitive development.” (Discussion and Conclusion, line 17, pg.10)

And we specified pg.6: “This seemed to be quite adequate, as the 5-year-old patient exhibited a normal development and schooling without any other metabolic crisis, during the following months.” (Case presentation, line 20, pg.6)
Nor is there any discussion about the effect of carnitine supplementation.”

It was difficult to answer due to duration between the initial treatment and the submission of this case report. The patient is always under carnitine and riboflavin supplementations at the concentrations described in the case report, with normal development and schooling without any decompensation episode. As mentioned in the manuscript, after L-carnitine supplementation (100 mg/kg/day), levels of free and total carnitine exhibited a significant increase; from 3 to 65 µmol/L and from 5 to 85 µmol/L respectively. Indeed, it seems important to maintain this supplementation. Moreover, Silmara de Moraes et al., showed that L-carnitine treatment had protective properties on DNA injury in long chain 3-hydroxyacyl-CoA dehydrogenase deficiency, medium chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiency [14]. It suggested that L-carnitine could be used as systematic supplementation in these pathologies. In addition, El-Gharbawy and Vockley showed that, during an episode of MADD, the effect of the treatment is not so clear. They advised to avoid fasting and MCT oil as supplementation and to use L-carnitine, glycine with a low-fat and protein diet [15]. According to the recent literature, L-carnitine supplementation could be interesting at different levels. Briefly, it induces a neuroprotective effect in central nervous system, as described on microglial and endothelial cells of mice with Parkinson's disease [16], in peripheral nerve injury, carnitine prevents sensory neuron death and accelerates regeneration [17], or in erythrocytes, carnitine exhibits an antioxidant activity [18].

So, we add in the manuscript (discussion and Conclusion, line 17, pg. 10):

“Silmara de Moraes et al., showed that L-carnitine treatment had protective properties on DNA damage in long chain 3-hydroxyacyl-CoA dehydrogenase deficiency, medium chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiency [14]. It suggested that L-carnitine could be used as systematic supplementation in these pathologies. In addition, El-Gharbawy and Vockley showed that during an episode of MADD, the treatment effect is not so clear. They advised to avoid fasting and MCT oil as supplementation and to use L-carnitine, glycine with a low-fat and protein diet [15]. According to the recent literature, L-carnitine supplementation could be interesting at different levels. Briefly, it induces a neuroprotective effect in central nervous system, as described on microglial and endothelial cells of mice with Parkinson's disease [16], in peripheral nerve injury, carnitine prevents sensory neuron death and accelerates regeneration [17], or in erythrocytes, carnitine exhibits an antioxidant activity [18].”
And references according to Vancouver reference style using by BMC Med Gen policy (References, pg. 14):


Brian Meyer (Reviewer 2):

This is a comprehensive and well written case report which merits publication based upon the somewhat milder phenotype associated with novel compound heterozygous mutations. The authors clearly explain the differential clinical and genetic diagnosis and present the case in a manner that is both of interest and value to future laboratory or clinical based researchers and physicians. Some minor English language edits are necessary prior to publication.

Thanks a lot for your comments. English language was corrected by Pr. Franck G. Sturtz.

Celia Nogueira (Reviewer 3):

“The paper is well written, although some minor revisions should be done”

Thank you for pointing out these mistakes.
Page 2:
- line 36 - the gene ETFA should be written in italic; corrected
- line 38 - the mutations should also be written at a protein level; (p.Asn118Lys) and (p.Val218Met) were added pg. 2.

Page 4:
- line 8 - remove "in" after "the previous treatment" corrected
- line 10 - the gene ETFDH should be written in italic; corrected
- line 13 - the gene ETFA should be written in italic; corrected

Page 5:
- line 50 - substitute "augmentation" by "increase" corrected

Page 8:
- line 6 - the gene ETFA should be written in italic; corrected

Page 10
- line 30 - the gene ETFA should be written in italic; corrected

Page 16:
- line 4 - substitute "liaisons" by "bonds" corrected, We also substituted "liaisons" by "bonds" in the main text pg. 7 (Discussion and Conclusion, line 50, pg. 7)