Author’s response to reviews

Title: Mitochondrial mosaics in the liver of 3 infants with mtDNA defects

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

In the title “patients” has been substituted by “infants”.

The abstract was formatted as requested by the editorial office. Both Background and Conclusions are now more detailed, without exceeding 350 words.

Methods and Conclusions have been incorporated into the body of the manuscript. Conclusions contain the essential elements of the experience in the three patients, findings from others suggesting a tentative explanation of mitochondrial mosaics in the liver and confirming their importance, as well as a recommendation for pediatricians and pathologists.

Ethical issues: written informed consent by the parents was obtained including their agreement with publication.

A paragraph was added under Methods, as follows:

“Ethical issues: all tests and investigations reported in this paper were carried out for diagnostic purposes in the interest of the patients, and under the authority of the university hospitals involved. In particular the parents gave written approval for the muscle and liver biopsies, as well as for publication”.

Under Acknowledgements we added: “Written consent was obtained from the patients parents for publication of the study”.

Authors contributions have been added; all authors have approved the revised version.

With respect to the referees remarks:

Referee 1:
1. Depletion was not assayed in liver and muscle of patient 1, instead a mutation in the mtDNA was searched for (and found): indeed the clinical diagnosis of Pearson syndrome where mutations previously had been described, justifies this approach. Biochemistry was not performed on muscle of patient 1 because muscle pathology and histoenzymatic stains had already shown normal patterns.

Biochemical assays were not performed on the liver of patient 2 because not enough fresh tissue was available; for this same reason depletion was not examined in this liver.

The explanation given for the slightly reduced activity of complex II in patient 2 are the small amount of tissue available and a weak complex II band in the control as well.

This is indicated in the legend of fig 4.

2. The order of presentation of the figures was carefully taken into consideration. They are ranged in accordance with the sequence of the results of which they are a part. This sequence largely reflects the actual diagnostic history of the patients. For example, brain imaging was done early, while DNA analyses came last after enough indications had been gathered.

Figure markings: arrows and asterisks were added to figures 6a; 7a,b; 8; 10; 11; 12; 13. The legends were modified accordingly. Legends of fig 16a,b were detailed.

Quality of figs: fig 2: the contrast of some MRI images was improved. Fig 15 (electron microscopy) has improved sharpness.
Table 1 gives, in addition to fig. 4, also the spectrophotometric data and mean values of a large number of controls. So table and figure supplement each other.

3. The effect of the mutation on POLG1 mRNA splicing, and on the exon composition of the mRNA is an interesting research goal, and it is likely to be carried out soon by our collaborators. However we feel this issue should be addressed in a journal on human molecular genetics, while our novel results on morphology are most appropriate to a journal of clinical pathology.

4. In the Discussion the respective values of liver vs muscle biopsies vs DNA analysis are now phrased more clearly, as follows: “The COX deficiency in liver directed subsequent analysis towards blue native electrophoresis of the OXPHOS proteins and the mtDNA”..../

In a recent series of 10 children with mutations in POLG1, 5 had normal enzymes in muscle (Koch et al, 2008); the authors conclude that the assay of mitochondrial enzymes in muscle alone is not effective. Mutations in DGUOK causing mtDNA depletion nearly always result in normal muscle enzymes, and the study of liver is recommended (Freisinger et al, 2008). The patients with the mutation in PEO1 had a severe reduction of OXPHOS complexes in the liver and not in muscle (Sarzi et, 2007).

In the Conclusions: “Histoenzymatic COX staining of a liver biopsy is fast and yields crucial data about the pathogenesis; it indicates whether mtDNA should be assayed. Each time muscle data are non-diagnostic, a liver biopsy should be recommended.” We submit that finding a mutation in itself does not explain pathogenesis unless a defective activity of a crucial enzyme is demonstrated. At variance of the referees suggestion, a liver biopsy was necessary since no abnormalities were found in muscle that might point to a gene defect.

Minor comments:
-spelling errors: were corrected (correct are: glycogen; cryostat).
-technical factors in the muscle stain of patient 2 are now phrased as “the delay between sampling and staining”.
-the reference to a case with a homoplastic mutation of mtDNA is now phrased as follows: “A mosaic of COX activity in muscle has also been observed in a case of a homoplastic mutation of mtDNA (ref); this is more difficult to explain”.
-normal enzyme activities in fibroblasts: we cite several publications, including an additional one (Freisinger et all, 2008) that underline this phenomenon, which we have confirmed. The following sentence is now added to the Discussion: “A possible explanation is that cultured cells originate from mothercells that have wild-type mtDNA while the mutated cells die or do not proliferate”.

Referee 2:
The referee is mistaken in stating that our three patients are all affected by a mtDNA depletion. Indeed, patient 1 has a deletion (mutation) in the mtDNA.
The paper by Müller-Höcker et al from 2002 reported but a single case with less complete data, and the origin of the depletion was not determined. This detail is now added under Background.
The question how a mitochondrial mosaic can be caused by a nuclear mutation is indeed a very important one. We do not try to fully resolve it within the Discussion in this paper; but we propose a likely mechanism based on findings of others, to which we now add most recent data on POLG1 (Galassi et al, 2008; Freisinger et al, 2008;
Koch et al. 2008). The key novelty of this manuscript is to describe in detail mitochondrial mosaics observed by microscopic pathology in several infants whose disease is fully diagnosed. Our data will be of genuine practical value in the first place to pediatric pathologists and pediatricians.

We agree that heteroplasmy should be better defined, and we have now added the definition given by the referee. The relationship between heteroplasmy and a mosaic seen after COX staining is clarified, as follows:

(Abstract) “Mitochondrial disorders may be accompanied by heteroplasmy. This can be detected by analysis of mtDNA, but has also been revealed by use of cytochrome oxidase staining of muscle”.

(Background) “Mitochondrial heteroplasmy i.e. the presence of two mtDNA molecule populations in the same cell, has repeatedly been visualized in muscle by mitochondrial heterogeneity after cytochrome oxidase staining (refs)”. 

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