Author's response to reviews

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

Authors:

Frank Roels (frank.roels@ugent.be)
Patrick Verloo (patrick.verloo@ugent.be)
François Eyskens (Francois.Eyskens@pcma.provant.be)
Baudhuin François (baudouin.francois@skynet.be)
Sara Seneca (Sara.Seneca@uzbrussel.be)
Boel De Paepe (boel.depaepe@ugent.be)
Jean-Jacques Martin (jean-jacques.martin@ua.ac.be)
Valérie Meersschaut (vmeersschaut@hotmail.com)
Marleen Praet (marleen.praet@ugent.be)
Emmanuel Scalais (Scalais.Emmanuel@chl.lu)
Marc Espeel (marc.espeel@ugent.be)
Joël Smet (Joel.Smet@ugent.be)
Gert Van Goethem (gert.vangoethem@ua.ac.be)
Rudy Van Coster (rudy.vancoster@ugent.be)

Version: 5 Date: 11 March 2009

Author's response to reviews:

ANSWERS TO THE REFEREES

REFEREE 1:

With respect to the splicing question in patient 2:
We also sequenced DNA of both parents, and the father carried the A467T mutation only, whereas the splice site mutation was present in the mother. This finding confirms that both mutations are located on different alleles and it suggests that the combination of these mutations is very likely to cause the patients phenotype.

Furthermore, the novel mutation alters an evolutionary conserved splice site and is located in the splice donor GT sequence, which is functionally important in the splicing process. Online tools predict that the donor site cannot be recognized as such because of this sequence alteration (e.g. http://bioinfo.itb.cnr.it/oriel/splice-view.html). Consequently, we conclude that the c.3643+2T>C mutation is a genuine novel pathogenic mutation in POLG.

This is now included in Results and Abstract.

REFEREE 2

1- Confusion between COX mosaics and heteroplasmy:
We agree with the referee that the relationship between a mosaic staining, and
the presence of different mtDNA populations in the same cells, is not proven by all publications we refer to. In particular, reference 6 (Karppa, Herva, Moslemi, 2005) describe a heteroplasmic mutation as well as COX negative muscle fibers; but it does not find a correlation between both phenomena. Hudson et al (2008) do demonstrate such a correlation in their fig. 2e.

Also, it is not our purpose to give evidence of such a relationship. So we now mention the heteroplasmy described by many authors in the Discussion only, as a possible mechanism for mosaics.

Moraes et al (1991) found a correlation between the degree of mtDNA depletion and residual COX activity; this is added to the discussion.

However we do not understand the referee when he says that a mosaic pattern after COX staining in muscle has “a very low frequency”. We now mention 15 publications about such patients, and not all of them; in the older ones the genetic background is not yet investigated but the mosaic is there.

The Abstract-Background now reads as follows: “In muscle COX negative fibers (mitochondrial mosaics) have often been visualized; this was not fully explained”.


2- Is liver more informative than muscle in some cases? Publication by Sarzi et al:

We now refer to this paper, as follows (Discussion):

“In 4 unrelated children with AHS, there was respiratory chain enzyme deficiency in liver, but not in muscle (Gauthier-Villars 2001). In a large series of patients with mtDNA depletion and mutations in DGUOK, POLG, MPV17 or TK2, 17 cases had lowered respiratory enzymes in liver but normal activities in muscle (Sarzi 2007)”. 

So our own patients fit into the findings of Gauthier-Villars and Sarzi. However we do not agree with the referee that the latter authors had already published what we describe in our ms. Indeed, Sarzi et al have not included any microscopic pathology or COX staining, and such is a major novelty of our paper. Referee 2 himself writes about our ms: “This is very interesting because for the first time this pattern (mosaic) is associated with Alpers syndrome”.

Referee says our comparison between liver and muscle is not complete in each patient. However we have complete microscopic and histoenzymatic data on all (not all are shown in the figures!), and biochemistry on either liver or muscle. In a clinical setting the availability of material from infants is a limiting factor.

3- With respect to the priority for mtDNA analysis as requested by the referee: clinicians confronted with a very sick child need to start treatment immediately, and give answers as soon as possible. Although a genetic diagnosis will be
included (if an inherited disorder is suspected), the results of microscopy are available much faster (48 hrs at the latest), and less expensive too.

So, for the clinician, the sequence of investigations is always:

history (from the parents and prior examinations), physical examination, blood & urine analysis; -->imaging; -->skin biopsy; -->muscle biopsy; muscle biochemistry; -->liver biopsy; -->liver biochemistry; -->molecular biology.

DNA analysis can be the priority in a different context, for example in a genetic research project on a selected group of diseases. But even then, patients data should first be complete, including biochemistry or microscopy, or both.

Finally, in order to understand the pathogenesis and possibly the prognosis, the loss of COX activity is an essential finding, whether or not a DNA alteration has been discovered.

Last year, the Mitochondrial Medicine Society’s Committee on Diagnosis proposed this same approach, and strongly recommend a liver biopsy for microscopy (and they are not yet aware of the usefulness of the COX reaction). They underline that “DNA studies may take up to two months whereas biopsy can produce histological results in a few days” (Multiauthored publication, Mol Genet Metab 2008, 94:16-37).

4- Several references have been added, including the recent publication by Stewart et al (2009) on POLG1 mutations in patients with AHS of whom 9 show a COX mosaic in muscle. In the Discussion on the possible mechanisms that cause mosaics, several paragraphs were revised, as follows:

“How can the cell- and tissue heterogeneity of COX activity be explained?

"Many disorders have been linked to heteroplasmic alterations in mtDNA: in muscle (6), liver (31), cardiomyocytes and other tissues. Clinical syndromes associated with heteroplasy are several types of myopathy (6; Chinnery 2003); myoclonus epilepsy and ragged red fibers (MERRF) (Moslemi 1998); progressive external ophthalmoplegia, childhood optic atrophy, followed by deafness and ataxia later (Hudson[8]; dilated cardiomyopathy (Arbustini 1998; multiple lipomas (Holme[11]; Kearns-Sayre syndrome (MIM 530000)(McKelvie 1991; Larsson 1990), focal segmental glomerulosclerosis (Hotta [12]; early onset diabetes mellitus, optic atrophy and deafness (Wolfram syndrome) (Rötig 1993[13]; Alpers-Huttenlocher-like disease (Uusimaa[9]; and Pearson syndrome with Kearns-Sayre encephalomyopathy (McShane 1991 [14].

The histochemical COX staining in individual muscle fibers appears to be linked to the expression of a mutation in mtDNA (Moslemi 1998), and heterogeneous defects in COX activity may be due to differing populations of wildtype and mutant mtDNA (Matsuoka 1991). Hudson et al (2008) showed that COX-positive vs. negative fibers in muscle are correlated with their level of mtDNA deletion. In patient 1…",etc.

“Chinnery (2003) thinks it is likely that the progression in time of the COX defect in muscle ‘is due to clonal expansion of mutant mtDNA'.

```
"How can one explain a mitochondrial mosaic linked to a nuclear mutation? Comparing muscle of two patients Moraes et al [48] found a correlation between the degree of mtDNA depletion and COX activity. Since the reduction of mtDNA in cell cultures results in gradual loss of respiratory rate and COX activity (Rocher[49]), the question can be re-phrased as follows: why is the level of depletion varying between individual mitochondria, individual cells, and tissues?" etc.