Title: Association of the mtDNA m.4171C>A/MT-ND1 mutation with both optic neuropathy and bilateral brainstem lesions

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

we carefully evaluated the helpful reviewers' comments and we here provide our point-by-point reply to their questions and critiques.

Reviewer 1: Dr. Ramon Marti

The manuscript reports the association of the m.4171C>A mutation (so far associated to pure LHON phenotype only) with a combination of LHON and Leigh-like phenotype in a male patient. The mother and one sister had presented only a mild and partially reverted optic phenotype in the past. In this family, the m.4171C>A mutation is associated with a synonymous change in MT-ND1, and with 3 non-synonymous transitions in MT-ND2 and MT-ND6, all of them in homoplasmy and in the haplogroup H. These variations are markers of different specific ancient mtDNA sub-haplogroups, but had never been reported in the haplogroup H. The main interest of this case report is the novelty of the overlapping LHON and LS phenotype in association to the m.4171C>A mutation, together with the mtDNA study. These observations suggest that the interaction of these variants in ND genes with the m.4171C>A mutation could contribute to the atypical phenotype in the proband. The report indicates that sequencing the complete mtDNA may help us understand the variability in the clinical presentation often observed in mitochondrial patients, as the authors note in their conclusions.

I only recommend some Discretionary Revisions:
1-I suggest eliminating or reformulating the sentence in the abstract indicating that in silico analysis supports the synergetic role of the variants. According to table 1, the conservation of the affected amino acids is only moderate, and the results obtained from the two software tools used are not fully consistent. Although reporting this information in the results is useful for the readers and it helps to discuss the findings, it is probably not justified to say in the abstract that these weak predictions support the role of the variants.

Author’s reply: we agree with Dr. Marti's recommendation and we simply erased the short sentence on the “in silico” predictions.

2-Table 1 presents results of conservation. While the method used to analyze the conservation of the contiguous amino acids has been referenced (ref. 7), the method used to obtain the conservations of the actual amino acids in Eukaryotes, Vertebrates and Mammals should be more detailed (i.e., how many species were included, or refer to a previous described method).

Author’s reply: we have now added the reference (ref. 7, former ref.12) for the evaluation of amino acids conservation, as well as the number of sequences aligned for the conservation analysis (Table 1).

3-Although some clinical laboratories still use mg/dl as units for many biochemical parameters, it is currently more common and probably more recognizable to express the lactic acid levels as mmol/l (or mM).

Author’s reply: we agree.
Author's reply: we converted all the lactic acid evaluations into mM.

Reviewer 2: Dr. Gavin Hudson

The authors present an interesting case, a patient with Leber's hereditary optic neuropathy who also suffers bilateral brainstem lesions. The conclusion is that the mitochondrial DNA mutation (4171C>A) is the cause of both phenotypes, similar to the effect seen in Leigh-like syndromes.

I have a couple of questions that need to be addressed:
1 - The authors state that the m.4171 variant is present in the proband and mother, as well as an "unaffected sister" - is the unaffected sister negative for LHON as well as the brainstem lesions - if so how do the authors account for the reduced penetrance given the homoplasmic nature of the variant?

Author's reply: Dr Hudson raises the question of incomplete penetrance of the homoplasmic m.4171 variant in this family. Assuming that our assessment of homoplasmic mutant mtDNA in blood samples reflects a similar situation in all tissues from the three individuals analyzed, we here face the same phenomenon that characterizes LHON. As Dr Hudson well knows for LHON, the same mutation may range from no phenotype to pure LHON, to LHON 'plus' phenotype, as in the current case. Our analyses took into account only the mtDNA genetic background by complete sequencing and we propose that certain combinations of polymorphic variants may account for part of this variability in phenotype and penetrance. However, nDNA variability, in combination with environmental factors, are both probably responsible for the incomplete penetrance in LHON, and our case should be considered as a LHON 'plus'. We did not explore the nDNA variability/environmental interactions here, because such analysis would be beyond the scope of the present report, and also because it would be poorly informative in a single family. We strongly feel that a comprehensive analysis of large series of such cases should provide some answers.

2 - On a similar theme the discussion states that the affected mother and sister only shows optic neuropathy, without brain pathology - again how can the authors account for this variable penetrance?

Author's reply: same considerations as before, except for the gender difference that we emphasized in the discussion, having some evidence that estrogens in females act as protective factor in LHON, thus alleviating the phenotype in female subjects.

3 - The brainstem lesions and resultant phenotypes are characteristic of a cerebellar based disorder, given the reduction in penetrance (the isolated proband phenotype is potentially autosomal recessive) it would be prudent to exclude common causes of ataxia etc before concluding that the phenotype is caused solely by mtDNA?

Given the frequency of LHON it is possible that the proband is merely unlucky and has coincidentally inherited two different syndromes.

Author's reply: the brainstem lesions observed in our proband are, to our knowledge, not reminiscent of a cerebellar-based disorder, but they are typical of a
Leigh-like disorder, as well documented with many other mtDNA mutations affecting ND subunits of complex I. In fact, Leigh-like lesions typically affect bilaterally the basal ganglia or other brainstem nuclei. Given this consideration, we interpreted, according to the well-known principle of parsimony in medicine, that we are facing one disease, possible due to the m.4171 mtDNA mutation. There is no reason for thinking that we have a double trouble in this case. However, Dr Hudson’s point is understandable in the sense that variants in any other nDNA gene may act as modifier in this male patient, but we go back to the discussion of the previous point. We did not feel that we had specific candidate nDNA modifying genes. In fact, there are so many of them potentially exerting a modifying role that we should have run exome sequencing or even better the whole genome sequencing. We thought this could be done only within the frame of a larger study in the future.

4 - It is interesting that idebenone appeared to reduce the brainstem lesions, however the authors do not indicate why the mother and affected sister were not treated - especially in light of the possible visual recovery seen in the proband?

Author’s reply: these patients live in Belarusia, where idebenone is not available, and we had the possibility to provide the drug on a compassionate use basis for rare disease only to the proband, who had the most severe expression of disease. Both the mother and sister have at the moment a fairly mild optic neuropathy with maintenance of visual function that did not justify therapy as compared to the proband’s clinical picture.

Reviewer 3: Dr. Gregory M Enns

Reviewer’s report:
La Morgia and colleagues have submitted a report on an interesting mitochondrial disease patient who carries the m.4171C>A mutation, and have broadened our understanding of the phenotypic spectrum associated with this specific pathogenic mutation. They also postulate that additional non-synonymous transitions affecting other ND genes may have contributed to the clinical phenotype. In addition, data on clinical course following idebenone supplementation is provided. The manuscript is interesting and well written. Discretionary Revisions
Consider adding idebenone to the key words, because there is a relative lack of data on outcomes following therapy.

Author’s reply: we agree with Dr. Enns suggestion and we have now added idebenone to the list of key words.

It might be helpful to explain the measurement related to visual acuity, as most will not be familiar with this. Similarly, in the figure legend related to visual field a sentence or two briefly summarizing the visual field data might be considered.

Author’s reply: we have modified the text for the two points raised by Dr. Enns, in order to make less technical and more understandable the data on visual function. In particular, we defined visual acuity as a measure of central vision function in the
main text, whereas we added a description of visual fields improvement and OCT results in the figure legend.

We hope that with our point-by-point answers to the reviewers’ questions we satisfactorily covered all the critiques and that our report is now suitable for publication.

Sincerely yours.

Dr. Chiara La Morgia, MD, PhD
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