Author’s response to reviews

Title: OPA1-related dominant optic atrophy is not strongly influenced by mitochondrial DNA background

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Version: 2 Date: 13 April 2009

Author’s response to reviews: see over
Dear Dr. Scott Edmunds,

We have carefully considered the different reviewer’s comments and changed several parts of our manuscript as well as data set.

The purpose of this study is to put in light, a possible correlation between some mitochondrial haplogroups and the ADOA, this type of correlation has been extensively studied for other optic atrophy (for review: Carelli et al. 2007 and Han et al 2006). A striking influence of mtDNA haplogroup J on atrophy optic expression has been demonstrated, suggesting that it can increase the penetrance of optic atrophy.

We agree with the reviewers that haplogroups may modulate the phenotypic expression of the ADOA, but that was not our purpose. Such a study needs, as it is mentioned by the reviewers, more details on the patient’s phenotype (age of onset, visual loss, etc.) and a large number of patients. However, in the previous studies they were not able to conclude on the phenotypic expression also.

The major criticism of the reviewer is the numbers of patient and their selection. Indeed at the beginning of the study, we have selected 72 patients with clearly defined mutations in OPA1 gene. However, some of these patients were relatives and we have chosen to select only one patient per family. Nevertheless, the reviewer’s comments on the inheritance of OPA1 gene is fully justified and there were no reasons to exclude the fathers who carry the mutations in a family. This new strategy has the advantage to increase the numbers of studied patients (41/32).

Nonetheless, we keep only one OPA1 carrier by mitochondrial lineage. This selection is justified by the fact that a global study on all patients with relatives would create a bias by a stochastic over representation of some lineage (with the same haplogroup) more studied by some clinicians. It is also for this reason that we did not refer to the publication Hudson et al. 2006 as suggested by Dr. Carelli. Indeed, Hudson et al. studied a greater number of patients but related in smaller number of lineage (33 for the mutation 14484, and 36 for the mutation 3460). Therefore if we select only one mutation carrier by mitochondrial lineage in Hudson et al., the number of patient is lower than in our study. More generally, in studies on rare diseases, the choice between the number of patients and the number of lineage is crucial, and we have chosen to work on a lower number. This strategy has the disadvantage to be less sensitive in the case of minor relation between haplogroup and pathology, but it has the advantage to be more reliable and produce less “false positive results”. It is why our conclusion avoids only a strong relation between OPA1 and haplogroup. In addition, the other studies in the literature, which have demonstrated a strong relation with LHON, were done on such low number of patients. However, we have changed the conclusion in order to take into account this weakness.
In order to simplified and clarified in the text, this selection of patients, we have
(1) Removed the reference to the 72 starting patients that was confusing
(2) Corrected the conclusion.
(3) Corrected the minor points

MINOR POINTS

Eduardo Ruiz-Pesini minor points

Page 3, line 3: They should change neuropathie by neuropathy.
        >>> we have corrected that point.

Page 3, last line: Why do they not use the article from Richard et al, 2007 In this article, the authors analyze 1385 French individuals.
        >>> We agree and in the new version, we use Richard et al, 2007 as reference. This does not change the results.

Page 4, line 24: They should change “…AluI amplification enzyme…” by “…AluI Enzyme of an amplicon…”.
        >>> we have corrected that point.

Page 6, line 17: The meaning of this sentence (This image ….. French people) is not clear.
        >>> We agree and we have deleted this sentence

Page 8, line 20: It is true, the clinical expression of the m.3460G>A pathologic mutation is not influenced by the mtDNA haplogroup J but it has been recently shown that another mitochondrial genetic background, haplogroup Uk, is over-represented in patients with this pathologic mutation (Hudson et al, 2007).
        >>> We have answered before. Use relative patients could create a bias by a stochastic over representation of some lineage (with the same haplogroup) more studied by some clinicians.

Page 18, Table 1: The control region sequence and RFLP analysis is not enough to characterize the patient 1742 as a member of the haplogroup U5. He could also be a member of U4.
        >>> We agree and we have noted « U5 or U4 »

Valerio Carelli’s Minor points

Page 4: The last sentence of the PATIENTS paragraph is unclear
        >> We agree and we have corrected: « Among this cohort of individuals diagnosed as carriers of the OPA1 mutations, we have listed 41 distinct maternal lineages. »

Page 6: Again the sentence “This image takes sub-haplogroups into account……..” is unclear.
        >>> We agree and we have deleted this sentence. (same point as Ruiz-Pesini)

Conclusion, this study has a major weakness in the low number of patients analyzed, thus the conclusions should consider the preliminar nature of these results.
        >>> We agree and we have corrected: “These data argue to (allow to) conclude that OPA1 should be considered as a “severe mutation”, “influence of mitochondrial background should be (could be) excluded for OPA1 mutation”,
In the discussion, I would downplay the indication that OPA1-negative DOA are influenced by mtDNA haplogroups.

>>> We agree and we have corrected: “Interestingly it has been proposed by Han et al. that the expression of OPA1-negative ADOA could remain dependant of additional negative effect of mtDNA background. However, his study was based on an insufficient number of patients and the patients may have a heterogeneous genetic basis, given that multiple loci associated with DOA still await for other genes to be discovered.”

Needs some language corrections before being published

>>> English native does it.

Bernd Wissinger Minor points

(1) Identity of OPA1 mutations and phenotype/genotype correlations are all but straight in OPA1-associated ADOA the genotype information is essential in studies that aim to define modifying factors.

>>> As say before, “We agree with the referees that haplogroups may modulate the phenotypic expression of the ADOA but again it was not our purpose, such study need, as it is mention in by referees, more details on the patient’s phenotype (age of on set, visual loss, etc) and a large number of patients. However, in the previous studies they were not able to conclude on the phenotypic expression also.”

(2) Figure 2 is inadequate for an original paper that addresses a very specific question.

>>> We do not agree, the aim of this figure it to highlight the specific relationship between OPA1 mutations and haplogroups. “The absence of strong influence of mtDNA background on OPA1-related ADOA expression suggests that, similarly to G3460A mtDNA mutation, the deleterious effect of OPA1 mutations could be responsible itself of the pathology and do not need additional mitochondrial factor”

(3) Ellison and co-workers (Am J Hum Genet 83: 254-260) recently proposed that the association of the np14484 mutation with haplogroup J might be the result of an elevated specific mutation rate. The findings of that study and its interpretation might be worth to be discussed in the paper.

>>> We agree, with this point, however our data doesn’t allow increasing or refuting this hypothesis. We have add in the text page 8 line 12 : “However, we could not exclude that the association of the T14484C mutation with haplogroup J might be the result of an elevated specific mutation rate as proposed by Elliott and co-workers”

(4) >> We also corrected theses sentences.

- Abstract : … Mutations in the OPA1 gene ...
- Abstract : … LHON mutations require an additional factors to express ...
- background: … is associated with one of the three main primary ...
- background: … Until now, four loci have been identified for ADOA.
- background: … Most are localized in the GTPase domain and the N-terminus of the protein, whereas the C-terminus is largely spared.
- background: … We compared the distribution of mtDNA haplogroups between
Yours sincerely,

Thierry Letellier
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