Author’s response to reviews

Title: Exome sequencing reveals a novel TTC19 mutation in an autosomal recessive spinocerebellar ataxia patient

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Version: 4 Date: 16 November 2013

Author’s response to reviews: see over
Dear Mr. Rodis;

Thank you for your email of October 17, 2013, regarding our manuscript, “Exome sequencing reveals a novel TTC19 mutation in an autosomal recessive spinocerebellar ataxia patient,” as well as the valuable comments of the two reviewers. I have attached our revised manuscript, along with a point-by-point response to the reviewers’ comments.

We feel that the revised manuscript reflects a suitable response to the comments, and has been significantly improved over the initial submission. We believe that it is now suitable for publication in the BMC Neurology. Thank you in advance for your kind consideration of this paper.

Yours sincerely,

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Changes that we made with your suggestions are as follows:

Reviewer 1

Major revisions:
1- Interestingly all the identified mutations are nonsense mutations. In the previous papers on TTC19 no protein was detected in samples from TTC19 patients and the TTC19 transcript level was markedly reduced.

The authors suggest that the position of their substitution (more distal than the previous ones) could account for the milder clinical symptoms of the Japanese patient. If no biological material (fibroblasts?) is available for the detection of the protein, the authors should at least perform a quantitative PCR (using leukocytes for instance) to see if mRNA decay is present or if their mutation has a different behaviour compared to the other TTC19 mutations.
The level of TTC19 expression and its potential reduction is an important point, as suggested. However, the patient was transferred to another hospital several years ago, thus we could not obtain any additional clinical specimens other than genomic DNA unfortunately. Please see page 11, lines 1-3.

2- The four candidate genes remained after filtering have to be listed and a sentence should be added to explain why the other candidates were not taken into account. In consanguineous families we can’t exclude an unlikely but possible presence of two pathogenic mutations.

We have included the detail of the candidates on page 9 (line 13) and in Supplemental Table 1.

3- To be useful, the discussion needs to contain clear statements on the signs (i) that are common to all the TTC19 mutant patients described till now (for instance the involvement of the inferior olives at MRI) and that probably represent the hallmark for this genetic condition; (ii) that are typical of the Japanese patient and that could help to expand the phenotypic spectrum associated with TTC19 mutations.

In the first sentences of the discussion there are statements not clearly recognizable either on already published patients or on the Japanese case.

As suggested, we have clearly distinguished the signs that are common or typical of our patient. Please see page 10, lines 5-13.

Minor points:
1. What type of relationship has the parents, reported as “consanguineous”? A pedigree of the family could be useful.

We have added the relationship of the patients, but the pedigree chart could not be obtained. Please see page 6, line 13.

2. The exact values of lactate and pyruvate should be added, instead of saying normal and elevated levels.

We have included the exact values of lactate and pyruvate. Please see page 6, line 17 to page 7, line 1.

3. The software for functional prediction, reported in the Methods section, are never cited in the Results and can be removed
We have included the result of the functional prediction in Supplementary Table 1 and mentioned in the Discussion as a method for evaluating the probability of variant candidates. Please see page 12, lines 5-11.

4. Some info on the controls is missing: the origin of the “normal” control (are they Japanese?) and the meaning of “disease-controls”. What clinical presentation showed the heterozygous sample

We have added the details of the control samples. Please see page 8, lines 9-10 and page 10, lines 1-2

5. How many controls of Japanese origin are present in the cited open databases? (for instance in ESP5400 there are only subjects with Afro-American or European origins)

There are no Japanese subjects in ESP5400, as pointed out by the reviewer. The 1000 genomes database we used contains 89 Japanese subjects, and too many genetic variations of the Japanese population have been submitted to dbSNP. See page 11, line 6.

6. How many genes are present in the regions identified by IBD?

We have included the number of genes as suggested. Please see page 8, line 18.

Discretionary Revisions:
The meaning of some statements is not fully clear. Please modify, remove or improve the following sentences:
Page10: The therapeutic approach for mitochondrial disease is different from usual SCAs; therefore, we should carefully diagnose this disease.
What therapeutic approaches you refer to?

We have deleted the sentence as suggested. Please see page 11, line 3.

Page11: The clinical differences among mitochondrial abnormalities may be important to clarify the pathogenesis of MRC dysfunction.
What mitochondrial abnormalities?

We have improved the sentence as suggested. Please see page 12, line 18.
...the mutation we identified may help to elucidate the pathology of mitochondrial disorders.

How?

We have made some changes to this content. Please see page 13, lines 7-9.

Reviewer 2

I suggest minor changes:

The authors should indicate that other more common causes of SCAR have been ruled out.

We ruled out the other causes of SCAR as suggested, and there were no candidates in the known SCAR genes. Please see page 9, lines 11-12.

The authors should explicit the other genomic region IBD do not present known genes associated with SCAR.

There are SCA3 and SCA14 genes in the IBD regions but no associated genes where SCAR is located. Please see page 9, lines 1-2.

The authors should indicate the clinical category of the so termed “145 population-matched disease control sample”. One of the 145 cases harboured the Q277* mutation. Did they look for gene deletions/duplications?

As suggested, we have described the category of the heterozygous sample, and evaluated the copy number variants. Please see page 10, lines 1-2.

The authors should tell us why they did not test TTC19 protein levels at least in skin fibroblasts.

Thank you for your suggestion. Unfortunately, we could not obtain skin fibroblasts, because the patient was transferred to another hospital several years ago. Please see page 11, lines 1-3.