Author’s response to reviews

Title: Clinical features and genetic analysis of two siblings with Startle disease in an Italian family: a case report.

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February 13th, 2019

Dear Prof. Matteo Pasini,

Editor-in-Chief

As required, we have made the corrections and we have highlighted the revisions in red.
Reviewer 1:

Line 37 (30): we changed "mutations in other 4 genes" to "mutations in 4 other genes";

Line 45 (39): we changed "Center" to "center";

Lines 80-81 (73,74): the clinical evaluation of both probands was made when they were younger than 18. Genetic analysis was made when they were older than 18 and the consent has been given by themselves;

Lines 100-101 (91,92): we changed "all of the patients parents and probands were screened using Sanger sequencing" to "DNA samples from the probands and their parents were screened using Sanger sequencing";

Lines 116-.117 (104): the MAF for c.587C>A is not listed in the ExAC database. Anyway, we added the frequency as reported in TOPMED;

Lines 141-142 (129): we removed "compound heterozygote";

Lines 138-140 (135,136): we changed the sentence "GLRA1 accounts for about 80% of cases and pathogenic variants in another 4 genes" to “Although the diagnosis of HPX is based on clinical findings, pathogenic variants in five genes have been reported to cause HPX, of which GLRA1 accounts for about 80% of cases”;

Line 155 (144): we changed "Mother's patients" to "Patients' mother";

Line 162 (148-150): we changed “incomplete penetrance” to “variable expressivity”;

Lines 168-169 (157): we changed "compound heterozygosis mutation in GLRA1 is the same" to “compound heterozygous mutations in GLRA1 are the same”;

Line 182 (170): we changed "variable penetrance" to "variable expressivity".

Reviewer 2:

1. The paper was revised for language corrections;

2. Abstract section:
We cited the mutations after citing the methods; we stated the modes of inheritance of Startle disease; as suggested, we have rewritten the conclusions, focusing on the clinical heterogeneity in the family;

3. Background:

   Lines 35-37 (28): we added the sentence “To date, GLRA1 mutations have been reported as dominant missense (23%), recessive missense (39%) and recessive nonsense (38%)” in order to clarify the mode of inheritance due to mutations in GLRA1 gene;

4. Case presentation:

   - On the basis of data, reported in chronological way, in the patients’ medical file, we described first the younger patient, then the older one, and finally the mother and the father. As required, we reported the age of the patients at time of examination;

   - We added full name and url accession for all used database: ExAC, SIFT, Polyphen-2, TopMED, wANNOVAR, GRCh37/hg19, 1000 Genomes, gnomAD;

   - We reported in the text when the mutation p.R299X was described for the first time and its inheritance manner (lines 113-114);

   - To determine genetic causes of neurological diseases, routinely we apply a targeted gene panels using NGS technology, allowing us a dramatically reduction in terms of costs and time consuming;

   - As suggested, we reviewed the results of each experiments, adding the sentence “Molecular analysis of the GLRA1 gene revealed, in both parents and probands, previously described mutations as reported below: 1) the mother had the heterozygous c.895C>T or p.R299X (rs757488419), located in TM3 domain; 2) the father carried the heterozygous c.587C>A or p.D98E (rs199639315), located in ECD; 3) both probands showed these mutations in a compound heterozygous state, p.R299X inherited from the mother and p.D98E inherited from the father. Molecular variants identified are reported in figure 1” (lines 108-113);

   - the MAF for p.D98E is not listed in the ExAC database. Anyway, we added the frequency as reported in TOPMED;

   - The paper “A novel compound mutation in GLRA1 cause hyperekplexia in a Chinese boy- a case report and review of the literature” by Yang Zhiliang et al, published in BMC Med Genet. 2017, was already reported in the references (see reference number 8) and we referred to it in our paper. Anyway, taking into account the clinical phenotype of our family, we newly rely on this paper and reviewed our discussion.
We hope you’ll find our corrections suitable.

Sincerely yours,

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