Author’s response to reviews

Title: A novel CHD7 variant disrupting acceptor splice site in a patient with mild features of CHARGE syndrome: a case report

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Author’s response to reviews:

Laura Pranckėnienė (Reviewer 1):

Generally the conclusions are supported by the data, however I have a few comments.

1. Page 10. Could you specify how frequent the variant at -1 position in the acceptor splice site and the first G nucleotide in the adjacent exon sequence based on your reviewed literature is?

Answer:

According to the analysis of LITGEN project data of 98 unrelated individuals performed in our department 142 substitutions c.-1G>A of 242499 total variants were identified. We also made the analysis of Human genome reference sequence (Ensembl data), which demonstrated that exon starts with G after AG in acceptor splice site in 271628 of 583835 exons (46.52 %).
Therefore, 142x0.4652≈66 variants in 98 individuals are at c.-1G>A position in the acceptor splice site and the first G nucleotide in the adjacent exon sequence.

We also analysed the literature where we found out few studies, in which the variant was in the acceptor splice site at -1 position and the first nucleotide of adjacent exon was G. For example, Nancy et al. (2013) presented c.5930-1G>A variant of NOTCH2 and the first nucleotide of 33rd exon was G. Pasmooij et al. (2007), Mukhopadhyay et al. (2006), Gelincik et al. (2015), and Simpson et al. (2007) also presented acceptor splice site variants at -1 position and the next nucleotide of adjacent exon was G.

2. The pictures B and D in the figure 2 should be of better quality. It is hard to analyse them.

Answer:

We thank the Reviewer for the remark and we apologize for the insufficient quality of these pictures. Figures B and D are the original photos generated using ClustalO tool and Pfam database, respectively. However, we took into account this remark and we reproduced figures of better quality.

María-Isabel Tejada, Ph.D (Reviewer 2):

The authors reported a female patient with clinical signs of CHARGE syndrome caused by a novel heterozygous CHD7 gene variant c.5535-1G>A, located in the acceptor splice site of intron 26.

The manuscript is complete, the case description is correct and the variant reported and its molecular effect on mRNA is relevant to the field. So it may be acceptable for publication as a case report.

Nevertheless, there are some changes that must be made before its publication.

1.- Clinical findings are very well described, but authors should clarify "mild" features (on the title, page 3, line 47 and page 10, line 19) of CHARGE syndrome, and "many" characteristic features of CHARGE syndrome (pag.2, line 14,15), while on page 8 (lines 14,15) they affirm that she fulfils minor and major criteria for CHARGE syndrome.

Answer:
We deleted “many characteristic” in abstract (pag.2, line 14,15) and clarified information in discussion (page 8, lines 14,15): The patient carrying this acceptor splice site variant fulfils major diagnostic characteristics of CHARGE syndrome outlined by Blanke et al. [36] and updated by Verloes [37]: the proband has three major characteristics (unilateral choanal atresia, facial palsy, temporal bone abnormalities) and three minor characteristics (delayed puberty, delayed myelostones, cardiovascular malformation).

We would like to leave “mild” features in the title, because the patient presents fewer major characteristics (no ocular coloboma) and has no ID, growth deficiency, oral clefts or TE fistula.

2.- Was the patient reassessed at 18?? (pag.4, line 52). Explain this please, or order it better, because after that, on page 5 line 9, the assessment at 16 years reappears.

Answer:

Yes, the patient was referred to a gynecologist after genetic assessment. We moved this information to the end of the last paragraph of Clinical findings section.

3.- Accession dates to databases should be written.

Answer:

We wrote all known information (including accession dates) about databases to the bibliography according to the example (Online database: Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.) provided by the journal of BMC medical genetics. It should be noted that some databases ask to cite particular publications (we included them to the bibliography as well). Moreover, not all databases provide all the necessary information (e.g. country, year), for this reason in some cases we wrote only title, URL, and date accessed.

4.- It would be better to shorten some parts of the manuscript. For example, PCRs and Sanger sequencing -except the exons in which the primers were designed- are no longer described. In addition, authors use valid but outdated methods such as agarose gel electrophoresis, so it's advisable to remove that.

Answer:
We agree with a Reviewer that it is not necessary to fully describe classical methods as PCRs, gel electrophoresis, and Sanger sequencing, so we shortened this part leaving only main information.

5.- And the variant found in the GJB2 gene is irrelevant for this patient and for the work presented here. Therefore it should be deleted from the manuscript (pag. 7, lines 9,10; pag 9, lines 45-62 and pag10, lines 1-10).

Answer:

The heterozygous pathogenic GJB2 variant is related with hearing loss. Our patient has hearing impairment, so we decided to analyse all the pathogenic variants found out by WES. However, as the variant found in the GJB2 gene was determined in its heterozygous state to be insufficient to cause hearing loss in the previously reported patient, this information can be irrelevant for our work. For this reason we agree to delete this additional information.

6.- CHD7 in italics is the gene. Therefore "gene" after CHD7 is wrong written unless you put "the": "the CHD7 gene". Please correct this throughout the text.

Answer:

We apologize for this inattentive mistake. We corrected the text according to the Reviewer’s suggestion.

7.- The analysis of the cDNA sequence is not a functional analysis. Functional analyses are those of mRNA and/or protein expression, minigenes, animal models, etc. So please, correct this throughout the text. The sentence: "the evaluation of its effect..." (pag.2, line 11,12) expresses well what this analysis is.

Answer:

We corrected the text according to the Reviewer’s comment.

8.- Discussion and bibliography about functional analysis in CHD7 should be updated (For example Villate et al., Front. Genet. 2018; Ogier et al., Sci.Report 2018).

Answer:
We updated the discussion and bibliography with suggested publications.

9.- In Figure 2A, the genomic DNA of the mutated sequence should be better explained because the change is only -1G>A and it is reflected also in the cDNA (use of new acceptor) which should be represented only in the cDNA representation.

Answer:

We thank this Reviewer for the remark. We corrected figure 2A and we hope that now it is more understandable.

Frédéric Bilan (Reviewer 3):

1. SEMA3E should not be considered as a CHARGE syndrome causative gene. The authors should discuss the potential role of EFTUD2 (and maybe KMT2D) instead.

Answer:

According to OMIM database there are only two genes directly related with CHARGE syndrome (MIM # 214800; https://urlzs.com/AFAvr ): CHD7 (MIM #608892) and SEMA3E (MIM #608166).

Based on OMIM database: “In a patient originally described by Martin et al. (2001) with CHARGE syndrome (214800) and a de novo balanced translocation involving chromosomes 2 and 7, Lalani et al. (2004) mapped the translocation breakpoints and identified the SEMA3E gene within 200 kb of the breakpoint on 7q21.11. Screening of patients with CHARGE syndrome for mutations in the SEMA3E gene revealed a de novo mutation in an unrelated patient (S703L; 608166.0001).” For this reason we decided to mention this gene in our manuscript and we would like to leave this information.

We added the information about two genes EFTUD2 and KMT2D suggested by the Reviewer.

2. The patient described in the case report is a typical CHARGE syndrome patient according to Verloes updated criteria (or Hale). The authors should discuss why WES was performed rather than a targeted molecular analysis (many NGS panels containing CHD7 are available).

Answer:
Due to our current technical possibilities and circumstances we have chosen to perform WES for this patient.

3. All the finding concerning GJB2 variant (non relevant for this case report) should be removed from the manuscript.

Answer:

This was suggested also by previous Reviewer M. I. Tejada. We agree with this suggestion and the information about GJB2 variant has been deleted.

4. Figure 1 is useless and should be replaced by the RT-PCR gel electrophoresis.

Answer:

Figure 1 shows aplasia of the semi-circular canals and cochlear nerve canal atresia thus giving an evidence of hearing impairment, which is characteristic for CHARGE syndrome. We would like to leave this figure as an illustration of clinical consequences of CHARGE syndrome.

PCR not RT-PCR has been performed in our study. According to previous Reviewer M. I. Tejada agarose gel electrophoresis is outdated and she advised to remove the information about this method. Moreover, after agarose gel electrophoresis no additional bands (corresponding to mutant PCR product) were visualized under UV light, as in our case a cryptic 3’ splice site has been activated only one nucleotide downstream from the pathogenic variant site. Thus PCR products of wild-type and mutant alleles were differing only one nucleotide in length and visualized as one band. For this reason, in our opinion, picture of gel electrophoresis is not informative in our case. However, if the Editor and Reviewers would decide overwise, we could include this picture to our manuscript.

5. As the CHD7 variation reported here should be classified "pathogenic" using the ACMG recommendation (no doubt about this !), the authors should explain why they perform a functional test.

Answer:

The detected NC_000008.11(NM_017780.4):c.5535-1G>A variant is novel and not recorded in the CHD7 database, HGMD, or other databases. So in order to reveal the possible effect on splicing mechanism, the cDNA analysis was performed and we successfully demonstrated that
this splicing variant disrupts the original acceptor splice site and is pathogenic. Furthermore, in silico we predicted it's possible effect at protein level.

If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English.

Answer:

The text has been corrected by English specialist before the submission. Reviewers L. Pranckėnienė and F. Bilan indicated that the quality of written English is acceptable, while Reviewer M. I. Tejada suggested that there is a need of some language corrections before being published. She indicated to correct "the CHD7 gene or just CHD7" throughout the text. We corrected the text according to her meaningful comment.