

Letter to answer the reviewers' comments/suggestions

1- Title:

Screening of *UBE3A* gene referred for Angelman Syndrome followed by a genetic approach to identify *UBE3A* putative targets

2- Please provide point to point answer to all reviewers. Authors should revise their article according to the reviewers' comments/suggestions and provide point-by-point responses to each in a letter that is to accompany their resubmission.

Please check for several grammatical errors: checked and send to professional English language editing company

In the methods or results section, include a few sentences on how specifically these 50 were treated:

Added:

The patients were referred to different pediatric departments, due to unidentified etiology of severe mental retardation, epileptic seizures or abnormal EEG findings, severe speech impairment and dysmorphic facial features, and the genetic anomaly was confirmed for all of them.

Cytogenetic analysis demonstrated a 46,XY and 46,XX karyotype in all analyzed cells from the patients. The parent's karyotypes were found to be normal as well. After that, microsatellite marker PCR analysis was conducted using the conventional methodology, employing polymorphic markers situated within the 15q11q13 region. The purpose was to authenticate the duplication and ascertain the parental source of the duplicated chromosome 15. All our patients were normal. We performed methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) for diagnosis of AS associated with deletions, UPD15, or rare duplications. After all these tests, the deletion, UPD, and imprinted defects were excluded in all 50 patients.

Molecular analysis by direct sequencing (exons 7 to 16 and flanking exon/intron boundaries) of the *UBE3A* gene performed on all patients revealed negative results.

In the discussion session, implications of specific genes can be put together in a table for easy reading

Added:

The gene expression for the first family were detailed in **Table 6**.

The exome sequencing of the second patient in family 2 (**Figure 2**) gave different genes. The gene expression for the second family were detailed in **Table 6**.

In the discussion session, implications of specific genes can be put together in a table for easy reading. • You can discuss and highlight the importance of performing patient-derived iPSC modelling (PMID: 33370574; PMID: 35316126) from these individual mutation types for a better understanding of these disease phenotypes and better targeting.

Angelman syndrome still lacks a cure. It is possible to generate pluripotent stem cells (iPSCs) line derived from skin fibroblasts of AS patients. These iPSC models of genomic imprinting disorders will facilitate the investigation of AS disease processes and allow study of the developmental timing and mechanism of UBE3A repression in human neurons [17,18]. The rapid growth of iPSC technology has transformed these cells into multipurpose for basic and clinical research tools. Several studies have already developed this method, which may be very interesting to investigate for our patients. Future iPSC research will facilitate drug discovery, cell therapy, and new modes of diagnosis for neurogenetic disorders.

Peer-Review Report

==I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted.

==I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

==Before its final acceptance, please provide and upload the following important documents:

==Biostatistics Review Certificate, a statement affirming that the statistical review of the study was performed by a biomedical statistician;

== Institutional Review Board Approval Form or Document, the primary version (PDF) of the Institutional

==Review Board's official approval, prepared in the official language of the authors' country;

==Signed Informed Consent Form(s) or Document(s), the primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country;

==STROBE Statement, an important document related to manuscript writing of observational/case control/retrospective cohort studies.

==The quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, it is recommended that the authors provide the English Language Certificate issued by a professional English language editing company.

==Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

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