Annals of Clinical and Medical Case Reports

Case Report

ISSN 2639-8109 | Volume 10

A Novel Frameshift Mutation in GPT2 Gene Found by Whole Exome Sequencing Causing Mental Retardation and Seizures

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Received: 03 Nov 2022
Accepted: 21 Nov 2022
Published: 29 Nov 2022
J Short Name: ACMCR

Keywords:

Whole Exome Sequencing; GPT2; Novel deletion; Mental retardation

Citation:

Omarmeli V, A Novel Frameshift Mutation in GPT2 Gene Found by Whole Exome Sequencing Causing Mental Retardation and Seizures. Ann Clin Med Case Rep. 2022; V10(4): 1-4

1. Abstract

1.1. Objective: Some studies reported GTP2 gene missense mutations are associated with neurodevelopmental delay with spastic paraplegia and microcephaly. Here, we report a new frameshift mutation of this gene with new clinical presentations in an Iranian family.

1.2. Methods: Whole Exome Sequencing (WES) was performed for a 26-year-old woman following by confirmation of the new frameshift in her sister and brother through Sanger sequencing method. Clinical manifestations were compared with the previously reports of GTP2 gene mutations.

1.3. Results: WES analysis indicated that a novel frameshift mutation (homozygous) of GTP2 gene (GPT2: NM_133443: exon10: c.1260delG:p.T420fs) in the case. Also, Sanger sequencing for confirmation of her sibs signified heterozygosity of the mutated allele for her sister and homozygosity of normal allele for her brother. Her parents was certainly both heterozygotes for this mutation.

1.4. Conclusion: Overall, our results can be broadened the previously reported phenotypes of GTP2 gene mutation including symptoms of hypotension, spasticity of the extremities, difficulties in walking, along with mental and developmental delays. Noteworthy, navigation problems and aggressive behaviors were seen for the first time in this case. Moreover, we designed modeling and superimposition bioinformatics analyses for the new frameshift mutation of GTP2 gene and found certain truncation in the structure.

2. Introduction

Glutamate Pyruvate Transaminase 2 (GTP2) contains 12 exons with cytogenetic location in 16q12.1 [1]. The GPT2 protein is a nuclear-encoded mitochondrial enzyme which catalyzes the reversible addition of an amino group from glutamate to pyruvate, producing alanine and alpha-ketoglutarate. GPT2 protein is specially localized in the mitochondria, within which its role is in regulating metabolic procedures including amino acid metabolism and the tricarboxylic acid (TCA) cycle. Also, it might be involved in neurotransmitter metabolism, because glutamate is the main neurotransmitter of excitation in the brain and the inhibitory neurotransmitter gamma-aminobutyric acid is produced from glutamate. According to a function in synaptic processes, GPT2 transcription is reported to be upregulated in the postnatal brain development [2]. Previous studies demonstrated main neurodevelopmental disorder with spastic paraplegia and microcephaly resulting from missense mutations in GPT2 gene. Also, they showed

that the studied patients had global development delay with severely impaired intellectual development, speech problems, microcephaly, and spastic paraplegia [2-5]. Here, we investigated a ??-year-old Iranian woman which showed mental retardation and history of seizures by WES test to find putative missense mutation(s) and based on the results, mutation conformation by Sanger sequencing was done for her sibs. Complementary bioinformatics measurements were done to reveal the plausible changes in the mutant structure.

3. Case Presentation

A 25-year-old woman came to our genetics lab with her parents for genetic counseling. The woman's parents were married with a family history (cousin Consanguineous marriage) and had two daughters and a son. The case's brother and sister were both asymptomatic and healthy; While the patient had neuromuscular symptoms from infancy. These symptoms included neck hypotonia in the first year, severe seizures at age 1.5 after eating plums, and severe diarrhea leading to anesthesia. Other symptoms included difficulties in walking (hypotonia of leg muscles), stunted growth of the head, and delayed speech. What is interesting is that this patient was still suffering from muscle cramps at an older age, but the hypotension had improved during her growth and she now has no problem with walking. However, in some symptoms, the manifestations were new and more severe compared with prior signs, such as stuttering, especially in the expression of the letter R, lack of understanding of appropriate sentences for appropriate situations, difficulty in scheduling tasks, and most interestingly, severe problems of navigation and remembering the addresses. She even had a serious problem with remembering her home address. Aggressive behaviors and intolerance of approaching the patient were other interesting psychological aspects. Finally, it is notable that at a younger age, she was treated with Citicoline and Piracetam under the supervision of a physician and good effects were observed in her. Notably, the datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

4. Material and Methods

4.1. Molecular Investigations

To detect the plausible pathogenic mutations in the patient WES was utilized through these procedures: Genomic deoxyribonucleic acid (gDNA) was extracted from the patient's blood using a filter-based methodology and then quantified. A total amount of 1.0μg of gDNA was utilized as input substantial for the DNA sample refinement. Sequencing libraries were used by Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA) following manufacturer's instructions and x index codes were added to characterize sequences to sample. In brief, fragmentations were done through hydrodynamic shearing system (Covaris, Massachusetts, USA) to make 180-280bp fragment lengths. Residual overhangs were changed into blunt ends through exonuclease/polymerase functions and enzymes were then eliminated. Next after of 3' ends adenylation of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on each ends were selectively enriched in a PCR reaction. Captured libraries were enriched in a PCR reaction to increase index tags for hybridization preparation. Products were purified by AMPure XP system (Beckman Coulter, Beverly, USA) and quantified by the Agilent high sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. The qualified libraries were fed into NovaSeq 6000 Illumina sequencers. Finally, data quality control, analysis and interpretation were run on G9 generation of HP server through unix-based operating system. Conformation of the mutation was performed by Sanger sequencing technique on case's sibs. The datasets generated and analyzed during this study are available from the corresponding author on reasonable request.

4.2. Bioinformatics Investigations

For further investigations, the present report designed and performed the homology modeling and superimposition assessments for GPT2 protein in the novel mutation situation. Homology Modeling was carried out by SWISS MODEL, and also, validated by Molprobity, ProSA online software (available at https://prosa.services.came.sbg.ac.at/prosa.php), and ERRAT (available at https://servicesn.mbi.ucla.edu/ERRAT/). Modeled structures (Normal protein was PDB id: 3IHJ and modeled mutant with frameshift model) were visualized by the PyMOL Molecular Graphics System (Version 2.0 Schrödinger, LLC).

5. Results

5.1. Molecular Diagnosis

The results of WES analysis for the patient indicated a frameshift mutation in the 10th exon of GPT2 gene because of a nucleotide deletion in the Threonine 420 and causing a frameshift in open reading frame (ORF). Screening of CACNA1F showed genotype GA for woman, GA for her mother, G (Hemizygous) for her father, G hemizygote (normal) for three of her uncles, and A hemizygote (mutation) for two of her uncles (Figure 1). Carrier status of the other couple (husband) was evaluated for the reported variant(s) via Sanger sequencing and based on the results. There is no concern about the mentioned variants in this report. Prior professional genetic counseling is highly recommended for interpretation of test results or the decision to undergo genetic testing in the case of prenatal diagnosis

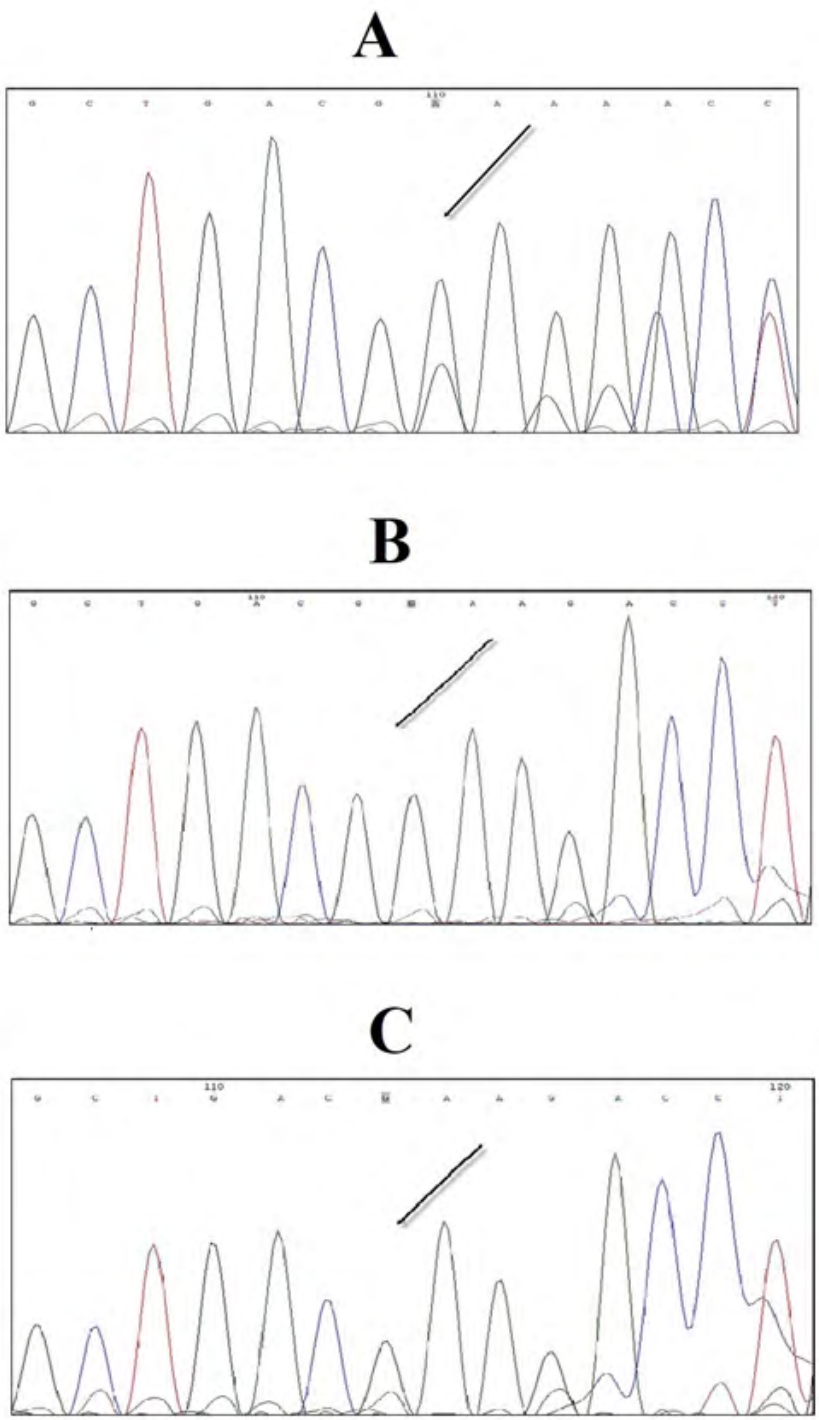

Figure 1: Mutation confirmation by Sanger sequencing in patient's parents (A and B) and herself (C). A shows heterozygosity in her mother and B indicates homozygosity for normal allele in her father.

5.2. In Silico Reports

Noteworthy, homology modeling was performed for mutant structure of GPT2 based on PDB ID: 3IHJ to compare the predicted changes. Ramachandran score was 98.06%for the frameshift model. Z-score for mutant model was -8.41. Moreover, ERRAT value was 97.8 as the overall quality factor and Verify 3D passed successfully. To find the putative changes in the mutant structure based on the deletion of codon nucleic acid and the frameshift occurred after 420th codon, superimposition of two structures (wild-type and mutant) was performed by PyMOL which showed a truncation in mutant model with the lack of three alpha-helix in the C-terminal (Figure 2).

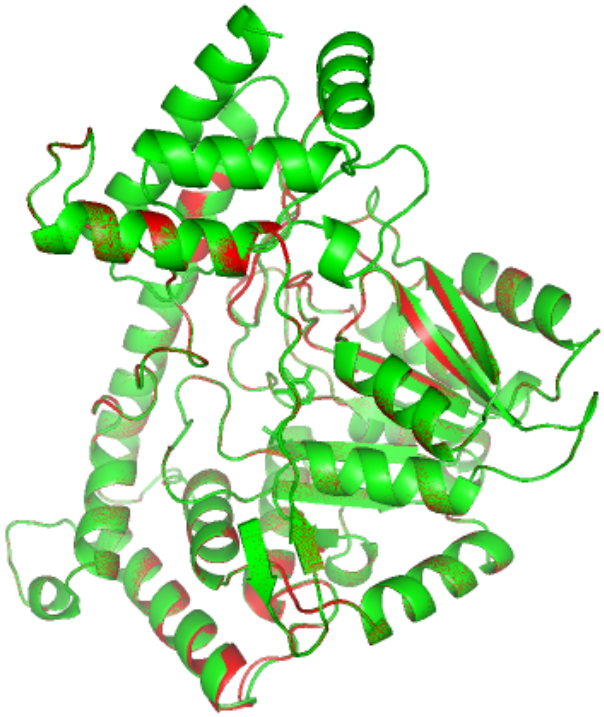

Figure 2: Superimposition of two models (wild-type and mutant) was done by PyMOL showing a truncation in mutant structure with the lack of three alpha-helix in the C-terminal.

6. Discussion

In the current report, for the first time in the literature, we presented a novel deletion leading to a frameshift in the 420th position of GPT2 gene through WES test in a ???-year-old woman with mental retardation and history of seizures. Mutation conformation was performed on her sister and brother by Sanger sequencing technique. Genotype of the case showed homozygosity in mutant allele (del/del), however, her sister was heterozygote (G/del) and her brother was completely normal (G/G). The pattern of this pathogenic mutation is autosomal recessive. Our findings broaden the phenotypic symptoms of GPT2 mutations including memory impairments in navigation and remembering the addresses, and also, aggressive behaviors. Interestingly, in silico analyses (modeling and superimposition) represented certain deletion resulting a serious frameshif in the mutant structure in comparison with wild-type model.

There are not enough reports about the disease-causing effects of GPT2 gene mutations; however, valuable documentaries are reported from Jewish, Pakistani, Turkish, and Palestinians cohorts [2-5]. Celis et al. found a homozygous missense mutation in the GPT2 gene (S153R) through WES in 3 sibs, born of consanguineous from Jewish parents. They had neurodevelopmental disorder with spastic paraplegia and microcephaly and functional in vitro analyses confirmed loss of function (Lof) [4]. In another study, Ouyang et al. reported two different homozygous mutations in the GPT2 gene (R404X, and P272L) in a large Pakistani family. By WES, linkage analysis and sequencing confirmation for the mutations, and HeLa cell transfection, they showed undetectable enzyme activity, consistent with a Lof [2]. Kaymakcalan et al. documented heterozygous missense mutations in the GPT2 gene including R134C and V479M which were found by WES and confirmed by Sanger sequencing [5]. In two Palestinian families (consanguineous marriage) Hengel et al. found Q24X in the GPT2 gene which was identified by WES and confirmed by Sanger sequencing; Notably, haplotype analysis showed a common ancestry. They predicted a Lof for the mutation effect. The patients had development delay with severely impaired intellectual development, no speech, microcephaly, and finally spastic paraplegia [3].

7. Conclusion

In conclusion, based on the previous and present reports about the missense mutations in GPT2 gene, the disease-causing impacts of these mutations lead to L of and neurodevelopmental disorders and hypotonic muscles with speech problems, seizures, and other neurological problems. Our study found a novel mutation (p.T420fs) in GPT2 gene by WES and sanger sequencing technique, introduced new symptoms of the disease, and suggested alterations in mutant structure and confirmed by in silico analyses through modeling and superimposition.

8. Acknowledgments

The authors would like to thank to the Dr. Shaveisi-zadeh lab experts and colleagues.

9. Disclosure Statement

No potential conflict of interest was reported by the author(s).

10. Funding

The author(s) reported there is no funding associated with the work featured in this article.

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