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A New De Novo Mutation in HTRA1 Gene Associated With Painful Ataxia, Developmental Delay, and Autistic Behaviors Symptoms in An Iranian Boy Through Whole Exome Sequencing Followed by Homology Modeling

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1. Abstract

1.1. Introduction: Due to the insufficiency of understanding about Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) in general clinical studies, the process of diagnosis for most CADASIL patients is complex and often prolonged. The disease's symptomatic heterogeneity, which happens frequently even among family members, increases the complexity of diagnosis.

1.2. Methods: In vitro analysis was carried out by Whole Exome Sequencing (WES) for a 2-year-old boy. He had ataxia, developmental delay, delayed speech and language development, and autistic behaviors. Mutational confirmations were also done on both of his parents to find the genotypes. Also, bioinformatics predictions were performed by SWISS-MODEL, ProSA, Molprobity, and superimposition through MatchMaker in Chimera ver. 1.16.

1.3. Results: WES analysis uncovered a novel de novo missense mutation in the HTRA1 gene (exon1:c.320C>T:p.A107V) in the case. Mutation conformations documented the homozygosity of the normal allele in both of the case's parents. Superimposition predictions suggested two beta-sheet unfolded in the mutant model (T allele or Val107).

1.4. Conclusion: Consequently, in an autosomal dominant pattern of genetic inheritance, the current study described a novel de novo mutation in the HTRA1 gene (A107V) associated with neurological features such as painful ataxia, and developmental and speech delays. Pain management is necessary in this case and future cases with the same symptoms.

2. Introduction

Subcortical Infarcts and Leukoencephalopathy (CADASIL), a monogenic hereditary small vessel disease (SVD). It results from the most frequently heterozygous missense mutations (95%) in the NOTCH3 gene with an autosomal dominant inheritance pattern.

CADASIL has a delayed onset, with clinical symptoms appearing in the third and fourth decades of life. The most common clinical characteristics of CADASIL include migraines with aura, recurrent and transient ischemic attacks, progressive white matter degeneration, memory injury, painful dementia and disability, and diverse psychotic conditions [1-3]. Because of the insufficiency of understanding about the disease in the general clinical field, the diagnosis for most CADASIL patients is complicated and frequently prolonged. The disease's symptomatic heterogeneity, which occurs even within family members who share the same mutation but acquire distinct clinical characteristics, increases the difficulty [4].

Heterozygous HTRA1 mutations at specific loci can also cause a rare autosomal dominant cerebral artery disease (CADASIL-like disease) (5). The HTRA1 gene encodes the HTRA serine protease [5]. By now, 45 HTRA1 gene mutations have been related to Cerebral Small Vessel Disease (CSVD), including 23 various types of CADASIL-like disease caused by heterozygous HTRA1 mutations. CARASIL and CADASIL-like diseases have been connected to five more types of heterozygous HTRA1 mutations [6]. There are few reports of CADASIL-like disease caused by a c.497G>T heterozygous mutation in exon 2 of the HTRA1 gene in French and Chinese populations [7-8].

A novel de novo missense in HTRA1 (c. 320 C>T:p.A107V) was discovered based on the neurological manifestation of a boy with no indications in his parents. To understand more, the normal and mutant structures were processed to homology modeling and superimposition investigations.

3. Case Presentation

A 2-year-old boy with abnormal manifestations passed genetic counseling. Nonetheless, genetic counseling revealed severe signs of painful ataxia, developmental and speech delays; however, their parents' marriage was not consanguineous, and none of the parents in this case revealed any features of their children. As a result, the geneticist first requested that the boy undergo a WES test. WES analysis revealed a heterozygous missense mutation in the HTRA1 gene's first exon (HTRA1:NM_002775:exon1:c.320C>T:p.A107V). Confirmation of mutations in the boy and both of his parents was conducted for further examination. According to the results, both of his parents had normal genotypes (C/C); however, he had a heterozygote genotype (C/T). Thus, this mutation was de novo and occurred in the gametogenesis of one of his parents. This missense allele in the HTRA1 gene is associated with the symptoms of cerebral arteriopathy, autosomal dominant, with subcortical infarcts and leukoencephalopathy, type 2; CADASIL2 with OMIM number 616779. Notably, some of this case's manifestations were new such as developmental delay and autistic behaviors.

3.1. In Vitro Diagnosis

WES test utilized to detect the following possible pathogenic variants in the case: To extract and purify genomic deoxyribonucleic acid (gDNA) from the case's blood sample, a filter-based technology was applied, which was then measured. A total of 1.0g of gDNA utilized for DNA preparation. To generate sequencing datasets, the Agilent SureSelect Human All ExonV7 Kit (Agilent Technologies, CA, USA) was used, and x-index codes appended to the sample attribute sequences. The DNA was fragmented into 180-280 bp pieces using a hydrodynamic shear technique (Covaris, Massachusetts, USA). Exonuclease/polymerase activities blunted the residual overhangs, and enzymes were eliminated in the nest. After adenylation of the 3' ends of the DNA fragments, adapter oligonucleotides ligated; DNA fragments with adaptor molecules attached at both ends preferentially selected in a PCR reaction. To prepare for hybridization, captured libraries were enriched in a PCR reaction with index tags. The products were purified using the AMPure XP system (Beckman Coulter, Beverly, USA) and quantified utilizing Agilent Bioanalyzer 2100 System and the Agilent High Sensitivity DNA Assay. Qualified libraries fed into the Illumina NovaSeq 6000 sequencer. Data quality control, analysis, and interpretation were then performed on a Unix-based operating system running on a Generation G9 from an HP server.

3.2. In Silico Analysis

In silico investigations were performed to have a better understanding of the impact of amino acid changes on the normal structure (Ala107) versus the mutant structure (Val107). As a result, homology modeling for HTRA1 protein structures in wild-type and mutant models was performed using the PDB IDs 3NZI, 3NWU, and 3TJQ structures.

SWISS-MODEL was used to model Serine protease HTRA1 (480 amino acids) (UniProt ID: Q92743), and both structures were energy minimized using SPDBV ver. 4.10.

Molprobity (http://molprobity.biochem.duke.edu/index.php)ProSA (https://prosa.services.came.sbg.ac.at/prosa.php), and ERRAT (http://services.mbi.ucla.edu/ERRAT/) were used to validate the final models. Surprisingly, superimposition was accomplished using Chimera's MatchMaker tool in version 1.16. MatchMaker used the Needleman-Wunsch algorithm with the BLOSUM-62 matrix.

4. Results

4.1. Molecular Reports

WES analysis revealed a novel de novo missense mutation in the first exon of the HTRA1 gene (exon1:c.320 C >T:p.A107V) associated with Autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy type 2. (OMIM: 616779). WES screening of the HTRA1 gene revealed heterozygosity for the position (C/T), and Sanger sequencing confirmed genotype C/C for both his mother and father (Figure 1). The parents were both normal homozygotes for the HTRA1 missense mutation, however, the case was heterozygotes.

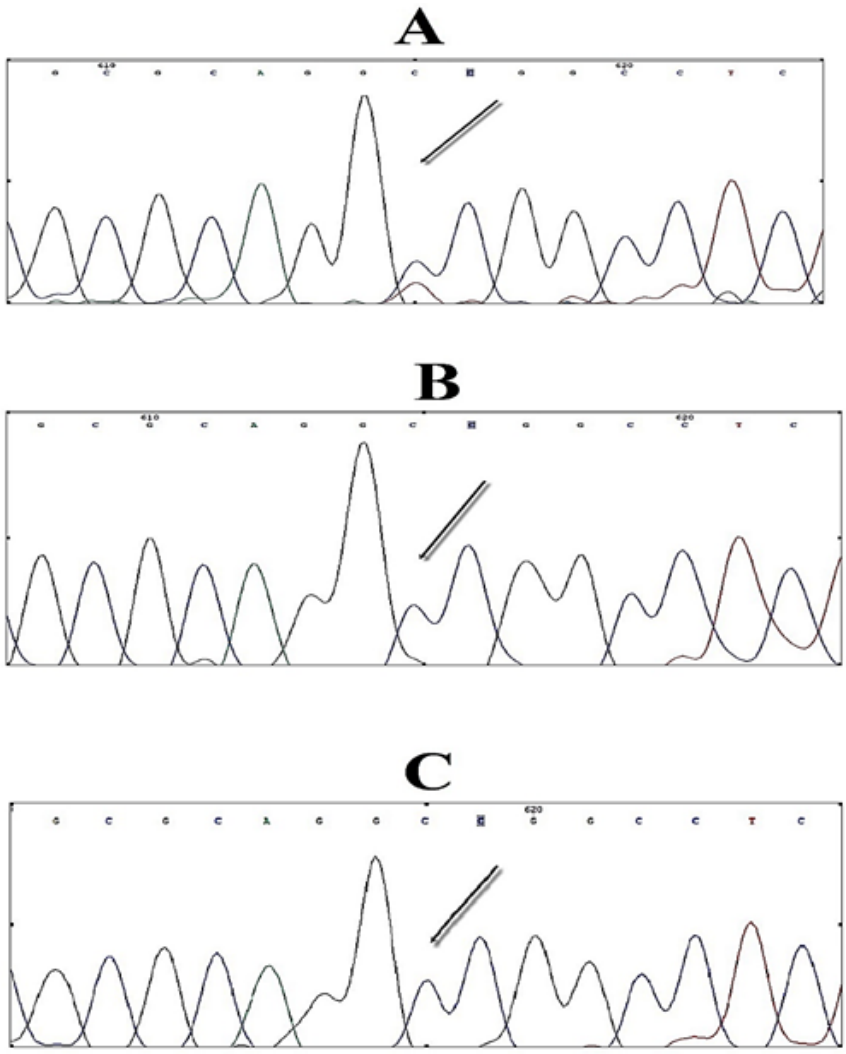

Figure 1: The mutational confirmations of HTRA1 missense mutation Ala107Val by Sanger sequencing technique for the case with heterozygote genotype CT (A), and his normal mother (B) and father (C) with genotypes CC

4.2. In Silico Results

Homology modeling results followed by validation of modeled structures including Ramachandran plots and scores, Z-scores, ERRAT scores, and Verify3D pass/failed mode. Ramachandran assay revealed that 96.2% and 97.3% of all residues were in allowed regions for Ala107 (Wild-type) and Val107 (Mutant) models, respectively. Also, additional confirmations by ProSA showed z-scores of -10.34 for Ala107 and -10.35 for Val107 models. Notably, both models passed the Verify3D with ERRAT values higher than 85%. MatchMaker displayed two significant unfolded beta-sheets in the mutant model (Val107) compared with the wild-type model (Ala107) (Figure 2).

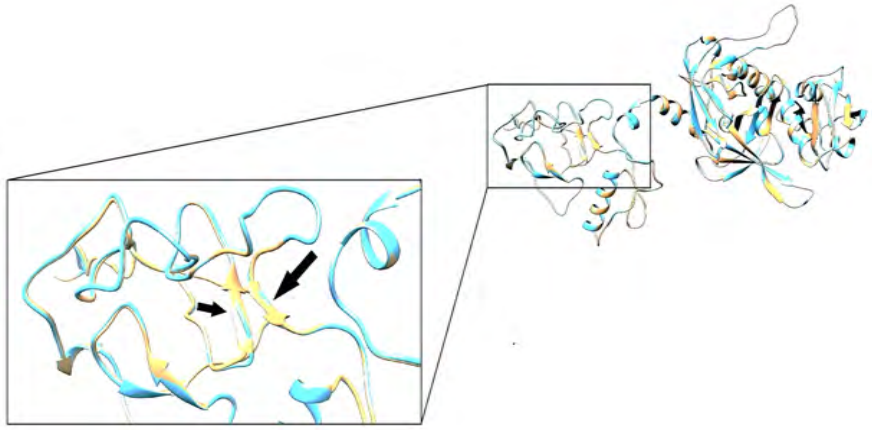

Figure 2: The superimposed state of wild-type in orange (Ala107) and mutant in blue (Val107) structures performed and visualized by MatchMaker (Chimera ver. 1.16.). The black arrows refer to the unfolded beta-sheets and the plausible differences of the two modeled structures.

5. Discussion

In the present case report by WES test, Sanger sequencing confirmation, and in silico analyses, we reported a new de novo mutation in the first exon of HTRA1 gene (Ala107Val) in an Iranian boy with painful ataxia, developmental and speech delays, and also autistic behaviors. Due to the lack of aforementioned phenotypes in either his mother or his father, mutational confirmation was performed for both of them. The results of sequencing demonstrated that this missense mutation was de novo and not previously reported.

Hara et al demonstrated that CARASIL was associated with mutations in the HTRA1 gene. Their data suggested a relationship between repressed TGF-family signaling inhibition and ischemic cerebral small-vessel disease, alopecia, and spondylosis [9]. Verdura et al proved that heterozygous HTRA1 mutations are a significant cause of familial small vessel disease and also that HTRA1 genotyping should be investigated in all individuals with a hereditary small vessel disease with an unknown etiology [7]. Wu and colleagues discovered that a heterozygous HTRA1 missense occurred in an arginine to glutamine substitution for the encoded protein positioned at 302nd amino acids. The mutation type causes a change in the structure of the protein, which may lead to changes in the spatial structure, and then to changes in the physical, chemical, and physiological features of the protein [10]. Noticeable investigations reported musculoskeletal pain in people with HTRA1 gene mutations. Tiaden et al discovered that the HTRA1 gene has a negative impact on the pathophysiology of joint and intervertebral disk degeneration, which causes joint and back pain. Ziaei et al. also reported a novel mutation in the HTRA1 gene in a family with widespread white matter lesions and inflammatory features, with lower back pain being the most common symptom in three of their three cases [12]. The current study depicted the painful ataxia observed in the case, which must be handled and managed.

The only indication of speech problems was in Wu et al's study, which detected as one of the symptoms of proband's sister. To the best of our knowledge, there is no report about the autistic behaviors associated with any mutations of the HTRA1 gene in the CADASIL disease. Based on the in silico analysis through PROSITE (https://prosite.expasy.org/), the 107th residue of HTRA1 is situated in a Kazal domain (98-157 amino acids). Kazal domains contain disulfide bonds which act in the regulating of serine proteases by inhibiting them [13]. Thus, this could be a noticeable clue for the disruptive impact of Val107 mutation on the beta-sheets and formation of Kazal domain disulfide bonds which might lead to the severe signs reported here.

In conclusion, HTRA1 mutations especially this new de novo mutation (exon1:c. 320C>T:p.A107V) with an autosomal dominant pattern of genetic inheritance have remarkable effects on the neurological system. By further molecular investigations in the future reports, the in silico findings of this study may pave the way for detecting the molecular disease-casing pathways of this de novo missense leading to the aforementioned phenotypes of such this case specifically developmental delay and autistic behaviors.

6. Acknowledgement

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7. Conflict of Interest

The authors have no conflict of interest to disclose.

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