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RESEARCH NOTE

Exome sequencing identified a *de novo* frameshift pathogenic variant of *CTBP1* in an extremely rare case of HADDTS

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Abstract. Hypotonia, ataxia, developmental delay, and tooth enamel defect syndrome (HADDTS) is an extremely rare autosomal dominant genetic disease caused by disruptive pathogenic variants in *CTBP1*. There are merely 12 cases reported to have pathogenic variants in the *CTBP1* gene. Here, we report the first case with HADDTS in the Middle-Eastern population. In the present study, whole-exome sequencing was deployed to identify the variant(s) causing this condition. Subsequently, Sanger sequencing was performed to confirm the variant. The clinical evaluation of the patient is written according to the thoroughly carried out examinations and clinical investigations. A novel single frameshift pathogenic variant in *CTBP1* (NM_001328.3:c.1315_1316delCA, p.Gln439ValfsTer84) was identified as the cause for HADDTS in the proband. Our findings enhance the knowledge of poorly studied *CTBP1*. The newly reported patient is phenotypically different in comparison to the previously reported cases. He has no sign of hypotonia, difficulty in walking or standing.

Keywords. *CTBP1* gene; HADDTS; whole-exome sequencing; speech impairment; tooth enamel defects.

Introduction

C-terminal binding protein 1 (CTBP1) was initially discovered as a protein that could bind the human adenovirus E1A protein to its C-terminus (Schaeper *et al.* 1995). CTBP1 and its paralogue CTBP2 are highly conserved proteins (Kuppuswamy *et al.* 2008). *CTBP1* is ubiquitously expressed and located on chromosome 4p16. It is a transcriptional corepressor essential for gene regulation during human development and cell cycle. Additionally, a specific role is played by CTBP1 in cancer. Inhibition of CTBP1 causes apoptosis through activation of Bik, PUMA and Noxa in several types of cancer cells (Ding *et al.* 2020).

Mice studies show that both CTBP1 and CTBP2 play specific transcriptional roles during foetal development. Homozygous deletion of *CTBP2* gene is lethal to embryo beyond E10.5. However, homozygous deletion of CTBP1 leads to decreased lifespan and size (Beck *et al.* 2019). A study by Hu *et al.* (2019) indicates that CTBP1 plays a protective role in the hippocampal and cortical neurons against degeneration. Higher expression of *CTBP1* attenuated apoptosis of hippocampal and cortical neurons and enhanced neuronal activity.

Previously, it was reported that a pathogenic recurrent *de novo* missense pathogenic variant in *CTBP1* (NM_001328.3:c.1024C>T p.Arg342Trp) can lead to intellectual disability, hypotonia, ataxia, developmental delay, failure to thrive, and tooth enamel defects (Beck *et al.* 2016). In another study, it was claimed that the same recurrent *de*

This study was designed by ASS and HJK. Sanger and whole-exome sequencing was carried out by SM, MD, SMBT and SAD. Physical examination was performed by SZ and SAD. SMBT reviewed the literature. The final manuscript was written and edited by ASS, SZ, and HJK. All authors approved the final manuscript.

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Table 1. Phenotypic spectrum of HADDTS.

Cases	Variant	Age	Gender	Global development delay	Enamel defects	Hypotonia	Neurologic exam	Oculomotor apraxia	MRI	EMG/muscle biopsy	Developmental regression	References
I-1	c.991C>T	12	Male	+	Enamel defect	+	Dysarthria, ataxia, weakness	NA	Mild volume loss of cerebellum, residual white matter changes	Evidence of myopathy, fibre splitting, necrosis, no clear evidence of neuropathy	None	Beck *et al.* (2016)
II-1	c.991C>T	25	Male	+	Soft enamel with discolouration	+	Ataxia, dysarthria, decreased muscle strength in upper and lower extremities	+	Cerebellar atrophy with progression on MRI	NA	None	Beck *et al.* (2016)
III-1	c.991C>T	11	Female	+	Wide spaced incisors, brown discolouration of primary incisors	+	Ataxia, dysarthria, muscle weakness, areflexia, hyperflexibility, tremors	+	Superior cerebellar vermis is small, uncertain if this represents volume loss or hypoplasia	NA	None	Beck *et al.* (2016)
IV-1	c.991C>T	14	Female	+	Enamel hypoplasia	+	Ataxia	+	Normal	NA	None	Beck *et al.* (2016)
I-2	c.991C>T	18	Female	+	NA	+	Hypotonia, increased tone at elbows, wrists, knees, ankles	NA	Mild cerebellar and brainstem atrophy	NA	Motor, language	Beck *et al.* (2019)
I-3	c.991C>T	22	Male	+	NA	+	Ataxia	NA	NA	Fibrillation activity and a recruitment pattern of motor unit potentials c/w myopathic process	NA	Ding *et al.* (2020)
II-3	c.991C>T	8	Male	+	Dental enamel defect	+	Dysarthria, ataxia, muscle weakness	NA	Normal brain MRI at age 3 years	NA	NA	Ding *et al.* (2020)
III-3	c.991C>T	8	Male	+	Enamel dysplasia	+	Low tone, absent to areflexia in arms and legs	NA	Cerebellar atrophy primarily involving the superior aspect of the cerebellum	A diffuse mild to moderate chronic non-irritable myopathy	NA	Ding *et al.* (2020)
IV-3	c.991C>T	12	Male	+	Unspecified, multiple cavities	+	Axial hypotonia, increased tone at ankles, ataxia, wide based gait, difficulty with balance	NA	Mild enlargement of the cisterna magna and hypoplasia of the inferior cerebellar vermis at age 7	NA	Motor, cognitive	Ding *et al.* (2020)
V-3	c.991C>T	7	Male	+	NA	+	Low tone, ataxia, dysarthria	NA	Cerebellum was underdevel-oped and has not changed	NA	Motor	Ding *et al.* (2020)
VI-3	c.991C>T	13	Male	+	Discoloured dental enamel, crowded dentition	+	Low tone, ataxia, dysarthria, marked tuncal ataxia when standing	+	Significant vermian atrophy, cerebellar volume loss	NA	NA	Ding *et al.* (2020)
VII-3	c.991C>T	24	Female	+	Protuberant malpositioned teeth, widely spaced incisors, brown discolouration of roots	+	Limb-girdle and bulbar weakness, significantly decreased muscle bulk and tone throughout	Horizontal nystagmus on lateral gaze	Significant cerebellar volume loss	EMG bilateral unlar monnoneurop-athies	Slow regression of motor and language function	Ding *et al.* (2020)

Table 1. *(contd)*

Cases	Variant	Age	Gender	Global development delay	Enamel defects	Hypotonia	Neurologic exam	Oculomotor apraxia	MRI	EMG/muscle biopsy	Developmental regression	References
I-4 (our study)	c.1315-1316del	26	Male	+	Dental enamel defects, teeth deformation, brown discolouration of roots	-	Dysarthria,	Normal	NA	NA	Normal	

novo mutation in *CTBP1* (c.991C > T, p.Arg342Trp) is associated with progressive neurodegenerative disorder with decreased activity in mitochondrial complex I and IV in skeletal muscles, which results in reduced respiratory chain activity (Sommerville *et al.* 2017).

In the current paper, we report a 25 year-old man with a novel pathogenic variant (NM_001328.3:c.1315_1316delCA, p.Gln439ValfsTer84), a 2-bp deletion, that causes a phenotype similar to that previously reported. Intellectual disability (ID), seizure, speech disorder, and tooth enamel defects have been observed in our case. The patient is heterozygous for the variant.

Materials and methods

Subject

The proband is a 25 year-old male born to consanguineous parents. The proband was physically examined thoroughly and further laboratory and diagnostic tests were carried out as required. Peripheral blood sample was obtained for further studies. The sibling of the proband were examined for the issues related to this study.

DNA extraction

Genomic DNA was extracted from 5 mL of patient's peripheral white blood cells using a QIAamp DNA Blood Mini kit according to the manufacturer's protocol.

Exome sequencing

Exome sequencing was performed on the extracted genomic DNA using a HiSeq 3000/4000 SBS kit.

Data analysis

The raw data, converted by HiSeq X, were filtered and aligned against the human reference genome (hg19) by Burrows-Wheeler Aligner (Li and Durbin 2010). The single-nucleotide polymorphisms (SNPs) were called by the GATK software (Genome Analysis Toolkit) 2. Variants were annotated using ANNOVAR (Wang *et al.* 2010). All variants were classified as per the standards for the interpretation of sequence variations recommended by ACMG and categorized to be pathogenic, likely pathogenic, variants of unknown clinical significance (VUS), likely benign and benign. The associated phenotypic features of candidate genes were evaluated against the phenotype of the patient. Core phenotypes of the variants were obtained from OMIM

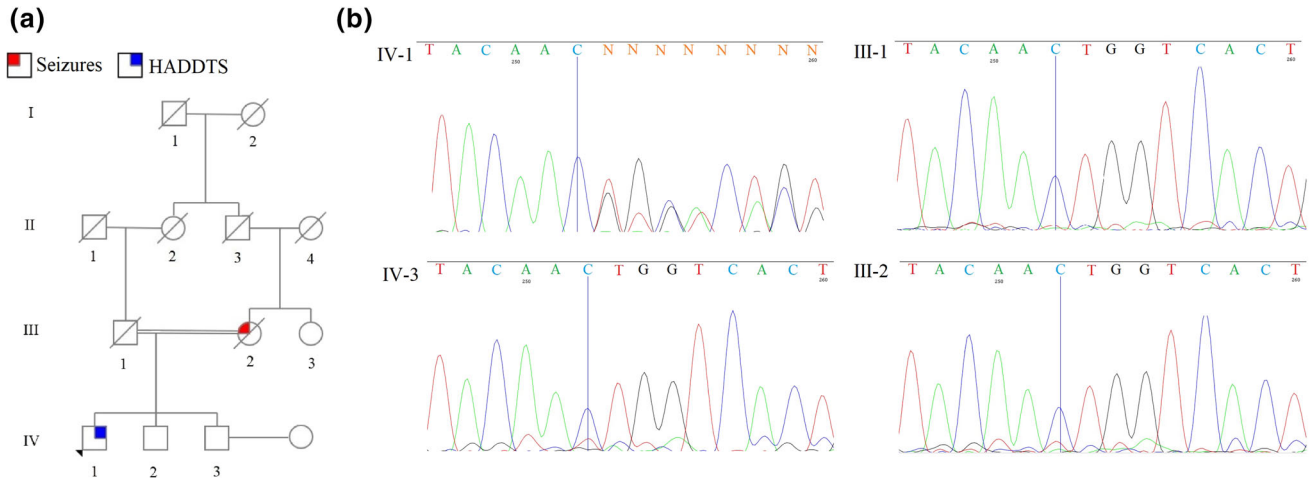

Figure 1. The pedigree and electropherogram of the proband. (a) the pedigree. (b) electropherogram of the proband (IV-1), his father (III-1), his mother (III-2), and his unaffected brother (IV-3).

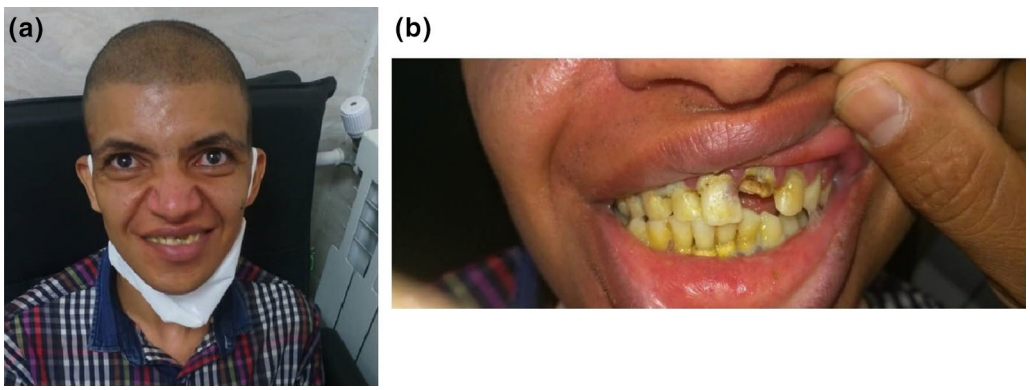

Figure 2. Facial features of the proband. (a) Face; (b) tooth enema defect.

database and utilized to acquire a gene list of the virtual panel by OMIM database (OMIM: 617915).

Sanger sequencing

The primers were designed using Oligo Primer Designer (Rychlik 2007). PCR was carried out to amplify the fragments covering the mutated sites.

Case presentation

The proband is a 25 year-old male who was referred to our centre with tooth enamel defects, ID, speech disorder, and history of a myoclonic seizure attack.

In the current study, severe ID (IQ<50), morphological, and semantic speech disorders were recorded in proband. Developmental delay, hypotonia and nystagmus, although being one of the most widely reported symptoms, were not observed in the physical examination or the history. The patient had a normal physical development. During infancy, breast refusal was recorded, and breastfeeding was substituted by infant formula. His specking ability are underdeveloped; even relative to the previously reported cases of HADDTS. He is able to say sentences shorter than four or five words in length.

His weight and height were comparable to healthy siblings. In comparison to previously reported cases, he had no difficulty in walking or standing and, by extension, no sign of ataxia was detectable except that he had a slightly wide-based gait and difficulty with balance. Nevertheless, he does not require any assistance for ambulation. His eyesight is intact and no sign of visual impairment in the field or acuity were recorded. No sign of immune disorder was detectable. He did not take any drug in particular for his condition. Although he is very calm in general, in crowded places he can lose his control.

Neurological examination revealed normal deep tendon reflexes. Babinski signs were present bilaterally. Mild

hyperextensible of the major joints and finger-to-nose dysmetria were the only remarkable physical examinations. Although he is physically developed, he had difficulty and delay in learning to walk or talk, movement skills, learning new things and interacting with others socially and emotionally. He has history of an episode of myoclonic seizure when he was five-year-old. Bearing in mind that the seizure was not repeated no treatment was initiated for this particular issue.

The proband has two siblings; one healthy and one with a similar condition as his. His brother, who has a similar condition, lives in special case facility. He has sever behavioural issues and intellectual disability. Enrolling him in this study was exceedingly troublesome both for the special care facility and his family. The MRI imaging of the brain could have come up with valuable findings; yet, the proband was not able to go through MRI or any other type of medical imaging. Written informed consent was obtained from each subject individually or, in the case of minors, from their parents. This study was approved by the Ethical Committee, Shiraz University of Medical Science.

Result and discussion

HADDTS is an extremely rare and understudied disease. Thus far, only 12 cases of HADDTS caused by a single variant in *CTBP1* (NM_001328.3:c.1024C>T p.Arg342Trp) have been described in the literature (table 1). The patient described in this paper is the 13th patient caused by a novel pathogenic variant (NM_001328.3:c.1315_1316delCA, p.Gln439ValfsTer84) (figure 1). The true prevalence of HADDTS is not well-known because it has just been described and is only discoverable by advanced sequencing techniques. Phenotypic variability adds to the complexity of HADDTS. Thus, further studies on the patients having pathogenic variants in *CTBP1* can be highly beneficial for developing diagnosis and management strategies. Loss of function variants in *CTBP1* are present in the population. Moreover, Beck *et al.* (2019) suggested the pathogenic mechanism behind p.Arg342Trp to be a gain of function in PXDLS motif. By extension, we suggest that the novel variant reported here, p.Q439Vfs*84, also causes a gain of function in *CTBP1*. However, molecular and functional studies are needed to reinforce this assertion.

We have detected a new *de novo* variant as the cause of the distinct phenotype of the subject of this study. Compared to the previously reported cases, our patient has obvious discolouration and deformation of teeth, which is the distinct landmark of the disease (HADDTS). However, a study by Sommerville *et al.* (2017) showed that in a case with mitochondrial disorders, carrying a pathogenic variant, (p.Arg342Trp) teeth had no clinically detectable defects (Sommerville *et al.* 2017). The proband of this study has a considerable differences compared to the previously reported ones emphasizing the phenotypic variability of HADDTS patients. Tooth enamel defects, ID, and dysarthria (figure 2). However, he did not experience a physical developmental delay, oculomotor apraxia or severe gait abnormality. We suggest that we should actively look for the behavioural issues than the other disorders in these patients according to the findings of our study.

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