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RESEARCH ARTICLE

A novel splice site mutation in *OTC* gene of a female with ornithine transcarbamylase deficiency and her asymptomatic mosaic father

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Abstract. Ornithine transcarbamylase deficiency is an X-linked disease with a wide range of clinical severity and manifestation age both in males and females. Here, we describe a case which is caused by a novel c.78-1G>A splice site mutation, which on mRNA level leads to a 1-bp deletion and a frameshift (c.78delG (p.C27Vfs*11)) in *OTC* exon 2 in a young girl. The same mutation has been detected in a mosaic state in her asymptomatic father.

Keywords. ornithine transcarbamylase deficiency; splice site; minigene assay; mosaic.

Introduction

Ornithine transcarbamylase deficiency is an X-linked disease with a wide range of clinical severity and manifestation age both in males and females. It comprises half of all urea cycle defects. Disease prevalence is on an average about one per 14,000 of population (Brusilow and Maestri 1996). In most of the countries prevalence of OTC deficiency based on newborn screening and national health records is low and are ∼1:62000–77000. (Testai and Gorelick 2010; Caldovic *et al.* 2015; Laemmle *et al.* 2016; Shao *et al.* 2017). Due to the fact that the *OTC* gene is localized on the X chromosome, most of the patients are hemizygous males. Usually, the disease is manifested in males after the neonatal period. The symptoms are caused by toxic effects of ammonia on the central nervous system: repetative vomiting, neurobehavioural changes, seizures possibly resulting in hyperammonaemic coma. However, multiple cases of the disease manifestation in adult males are described. Approximately 20% of female carriers show signs of ornithine transcarbamylase deficiency such as protein intolerance, persistent vomiting or hyperammonaemic coma when increasing protein catabolism (consuming large amounts of meat, chemotherapy, postpartum period). It has been shown in different studies that the manifestation age in male patients and symptom presence/absence in heterozygous females depend on residual hepatic ornithine transcarbamylase activity and the type of *OTC* gene mutation. Missense mutations usually lead to various degrees of enzyme activity decrease and rarely show symptoms in female carriers (Caldovic *et al.* 2015), whereas loss-of-function (LoF) mutations lead to disease manifestation in heterozygous females as well (Gyato *et al.* 2004). Males with somatic *OTC* mutation mosaicism show later disease manifestation than hemizygotes (Lee *et al.* 2018). One study describes a case of 21% c.1046T>C (p.L349P) missense variant mosaicism in blood cells of a healthy father of a heterozygous female with OTCD (Qin *et al.* 2016).

In this study, we describe a case of ornithine transcarbamylase deficiency caused by a novel c.78-1G>A splice site mutation, which on mRNA level leads to a 1-bp deletion and a frameshift (c.78delG (p.C27Vfs*11)) in *OTC* exon 2 in a young female. The same mutation has been detected in a mosaic state in her asymptomatic father.

Materials and methods

The DNA analysis of the proband was carried out using paired-end reading (2x75 bp) on an IlluminaNextSeq 500 sequencer. The libraries were constructed using the IlluminaTruSeq Exome kit. The detected variants were annotated according to the standard nomenclature: http://varnomen.

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hgvs.org/recommendations/DNA v2.15.11. The sequencing data was analysed using the standard Illumina pipeline https://basespace.illumina.com. Mean coverage for this sample was ×102.4, with 3% of fragments, less than × 10 coverage.

Splicing alteration prediction was carried out using NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) and Human Splicing Finder (http://www.umd.be/HSF3/).

Polymerase chain reaction (PCR) was performed at an annealing temperature of 64°C and 6 mM $MgCl_2$ in the reaction mix. The length of the PCR product was 302 bp. The sequencing was carried out using the ABI Dye Terminator, v3.1 (Applied Biosystems), on a 3130xl ABI genetic analyser (Applied Biosystems) using the following oligonucleotide primers F: 5′-CAATGAATCACCATAGTACATGGGTC-3′ and R: 5′-GGGGACTGGTAGTAATGGAAC-3′. The reference cDNA sequence was taken from the GeneBank database (NM_000531.5).

To determine this variant's functional significance, we applied the minigene assay as previously described (Filatova *et al*. 2019). In brief, the exon of interest with about 300 bp of flanking intronic sequences was cloned into a pSpl3-Flu2 vector. Wild type (WT) and c.78-1G>A reporter plasmids were transfected into HEK293T cells with the CaPO4 method. After 48 h, the RNA was extracted and reverse transcribed. The following primer sequences were used for functional analysis: 5′-TAACTCGAGACTTTGCAGAGACACATGAGGT-3′.

5′-AATGGATCCTGATTTCTTAGGTTCGATCCAGT- 3′ were used for minigene cloning, fragment size 842 bp and 5′-ACAAAGAGACCTACGTCGAGCA-3′; 5′-AGCTCGATCAGCACGGGCACGAT-3′ were used for minigene cDNA amplification and sequencing, fragment size of WT product 390 bp.

We used the FISH with DNA probes for X and Y chromosomes (SE X (DXZ1)/SE Y (DYZ3), KREATECH) and interphase nucleus analysis on peripheral blood lymphocytes to determine the number of X chromosomes in the proband's father. FISH results were scored using a fluorescent microscope AxioImager M.1 (Zeiss) with an oil immersed objective 100× and a proper filter set (FITC/Texas Red/DAPI). Images were acquired with a CCD camera and analysed using ISIS software (MetaSystems, Germany).

This variant was not detected in whole-exome sequencing (IlluminaNextSeq 500, IlluminaTruSeq ExomeKit) results of 877 nonrelated Russian patients with various hereditary pathologies (425 females and 453 males), 1303 chromosomes in total.

Written informed consent for genetic examination was obtained from all patients or their legal representatives. This work was performed within the framework approved by the ethics Committee of Research Centre for Medical Genetics (RF). All procedures performed involving human participants were in accordance with the ethical standards of the Wma Declaration of Helsinki 'Ethical Principles For Medical Research Involving Human Subjects' (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/).

Results

The proband, a 4-year-old girl, was born from a second pregnancy (mass 3890 g, length 58 cm) with an uneventful perinatal period. She refused to eat meat at the age of 1, when introduced to solid foods. At the age of 3, a biochemical blood analysis revealed a 10-fold increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. During the observation period, the level of transaminases increased. In addition, the following neurological manifestations of the disease were registered: mental confusion, speech retardation and body arching. A low-protein diet gave a significant positive effect, disappearance of neurological symptoms and transaminase levels decrease to 2× normal. Abdominal cavity and retroperitoneal space CT showed no pathology. Liver biopsy revealed signs of periportal hepatitis. The proband's parents and sister were healthy. Biochemical analysis for all asymptomatic family members showed normal hepatic enzyme (ALT, AST, ALP and CPK) levels.

Whole-exome sequencing for the proband revealed a novel heterozygous c.78-1G>A variant in the canonic splice site of *OTC* exon 2. Earlier, a different mutation (c.78-1G>C) was described in the same position in a patient with neonatal OTCD (Yamaguchi *et al*. 2006). Functional analysis was carried out to evaluate the c.78-1G>A variant's effect. cDNA amplification with minigene-specific primers (figure 1a) and further analysis (figure 1b) showed that the mutation disrupting the canonical AG dinucleotide created a new one 1-bp downstream, leading to deletion of first nucleotide of *OTC* exon 2 and a frameshift. Bioinformatic analysis with MaxEntScan (Yeo and Burge 2004) showed that the strength of the new splice site is significantly lower and this causes a minor event of exon skipping which is also a frameshift (figure 1c). Thus, the functional analysis showed that the major outcome is a frameshift deletion corresponding to c.78delG (p.C27Vfs*11) of *OTC* cDNA.

Thus, we show that the c.78-1G>A variant in the *OTC* gene found in the proband is a LoF mutation, and therefore, may lead to disease manifestation even in a heterozygous state. Of the 877 nonrelated patients without hyperammonaemia (425 females and 453 males, 1303 chromosomes in total), none had this particular variant. The c.78-1G>A variant is also missing in the Genome Aggregation Database (gnomAD).

Sanger sequencing analysis of the DNA of proband's father, extracted from nucleus-containing peripheral blood cells, revealed a c.78-1G>A heterozygous variant. However, normal and mutant peak height ratio was different from his daughter. By scaling the father's peak height to his heterozygous daughter's peak height, we evaluated the mosaicism level in his white blood cells to be 30%. An analysis of 500 interphase nuclei of the proband's father revealed single

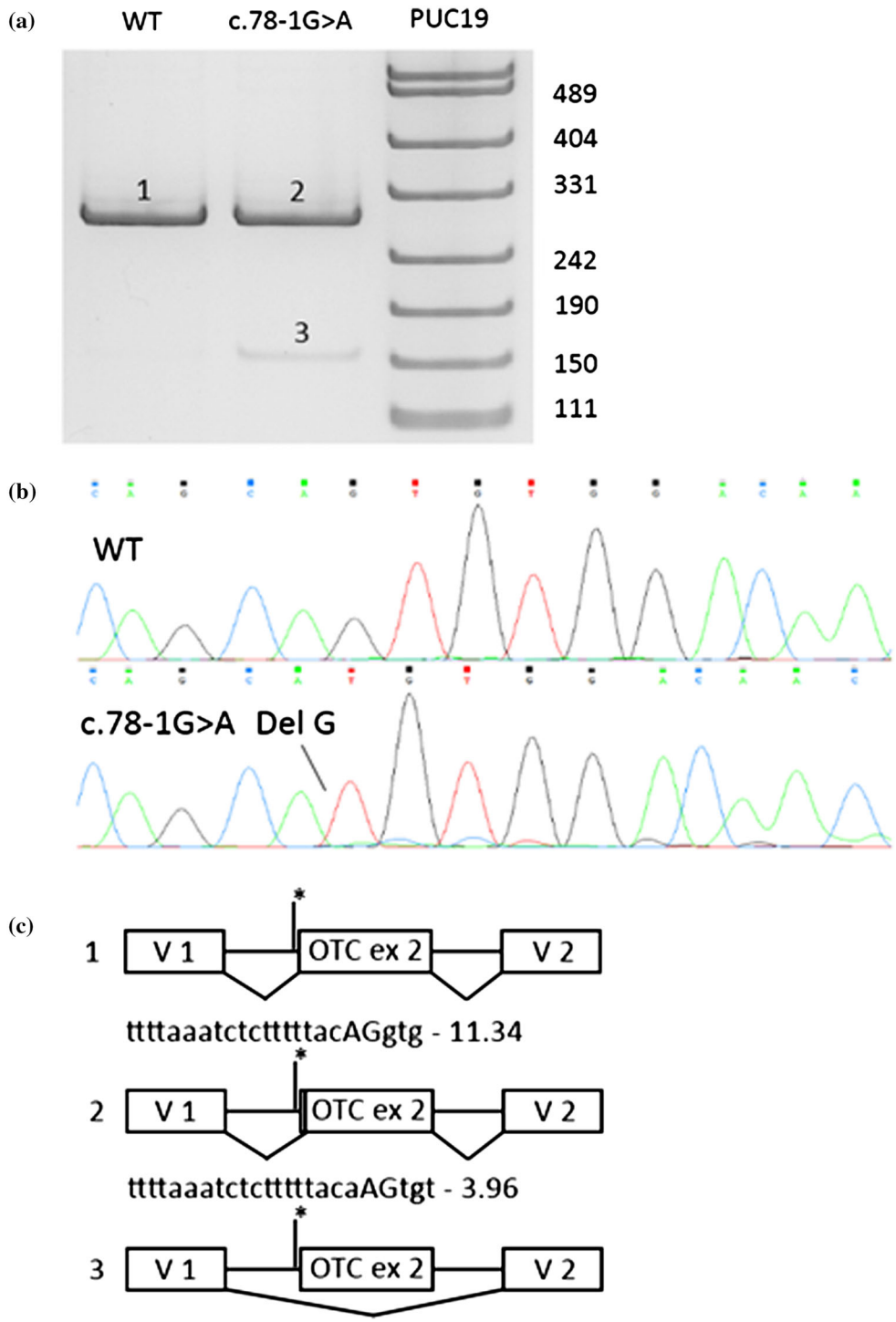

Figure 1. Results of minigene assay. (a) Visualization of PCR products on PAGE: 1, WT product; 2, c.78-1G>A product with 1-bp deletion; 3, c.78-1G>A product with exon skipping. (b) Sanger sequencing chromatograms. (c) Schematic representation of splicing events and scores for splice sites (*location of the mutation). WT product (1), c.78-1G>A disrupts canonical AG dinucleotide and creates new one 1 bp downstream (2), leading to deletion of OTC exon 2 first nucleotide. As the predicted strength of new acceptor site is much weaker (3.96 compared to 11.34), the minor event of exon skipping occurs (3).

copies of DXZ1 and DYZ3 loci that may correspond to the 46, XY karyotype. Seeing that the daughter inherited the c.78-1G>A variant from the father, his sperm should contain mutant cells, which was confirmed by Sanger sequencing of his semen DNA. The mosaicism level in this tissue was evaluated to be 13%. In addition, we analysed DNA samples from the father's buccal epithelium and urinary sediment cells and also found the c.78-1G>A variant. From the peak height on these chromatograms, the mosaicism levels were estimated to be 7% and 14%, respectively (figure 2).

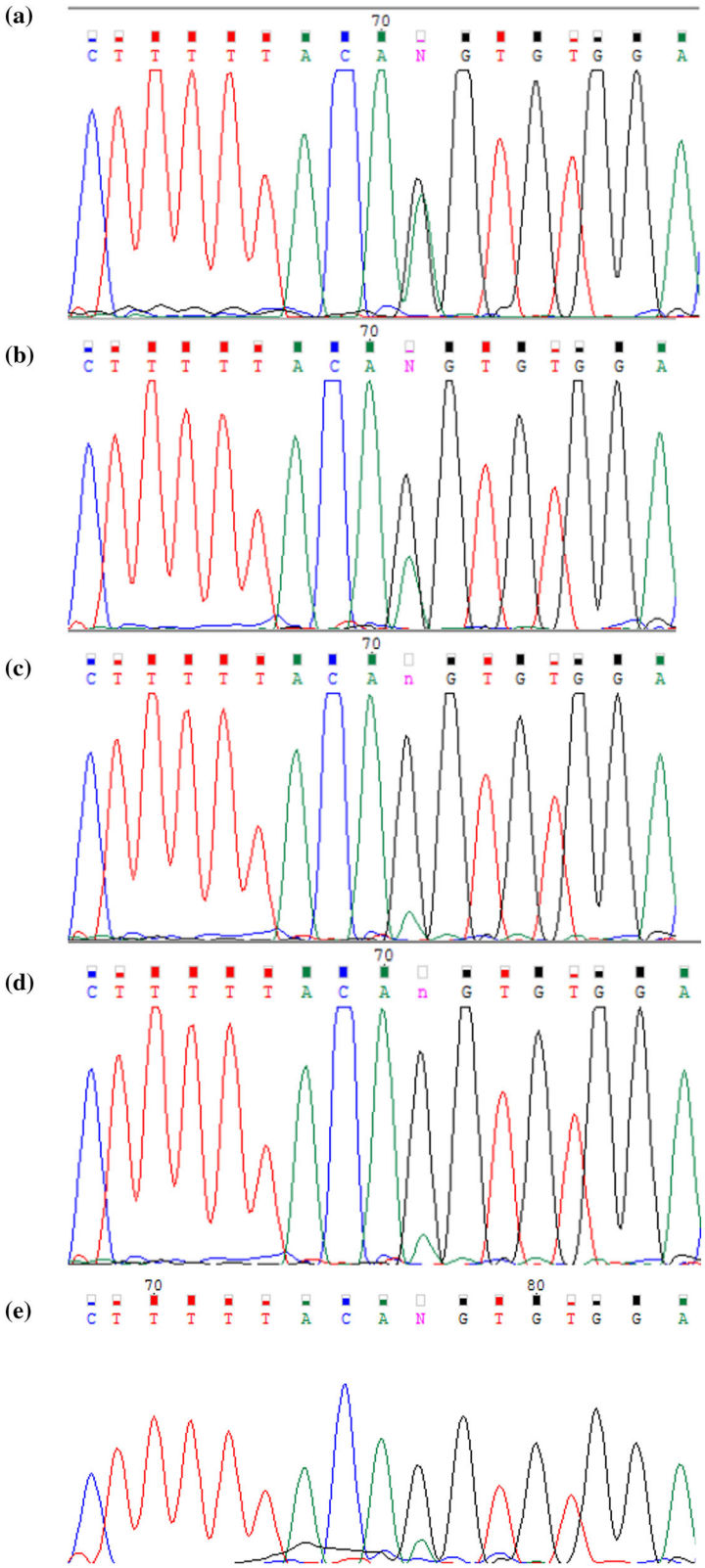

Figure 2. Sanger sequencing chromatograms: (a) proband (c.78-1G>A variant of the *OTC* gene in heterozygous state); (b) proband's father (30% c.78-1G>A mosaicism in white blood cells); (c) proband's father (7% c.78-1G>A mosaicism in buccal epithelium cells); (d) proband's father (13% c.78-1G>A mosaicism in sperm cells); (e) proband's father (14% c.78-1G>A mosaicism in urinary sediment cells).

Discussion

This study shows that a novel c.78-1G>A variant in the *OTC* gene (intron 1) on mRNA level leads to a 1-bp deletion (c.78delG) and a frameshift with premature stop codon formation (p.C27Vfs*11). It is known that LoF mutations in the *OTC* gene can lead to OTCD manifestation in heterozygous females. Besides that, carriers of such mutations often show high hepatic enzyme levels, as detected in this patient. It is worth noting that the proband's father (35 years old at the moment of examination) had no OTCD symptoms. His liver enzyme activity levels were also normal: ALT 10 u/L (normal 5–41), AST 13 u/L (normal 0,1–40). It is possibly a result of low mutant allele mosaicism levels in his liver cells. Unfortunately, it is impossible to determine the foetal development stage, on which the mutation occurred, no endodermal tissues or internal secretion glands are available. All tissues provided for research (mesodermal: blood cells, semen, urinary sediment; ectodermal: buccal epithelium) show c.78-1G>A variant mosaicism. This allows us to suggest that the mutation has probably occured on an early stage (before gastrulation), and therefore, mutant cells can be present in all organs, including his liver. The proband's father may possibly have late disease manifestation (hyperammonemic intoxication), which without knowledge of its nature could be fatal (Thurlow *et al*. 2010).

In conclusion, this study proves the pathogenicity of a novel c.78-1G>A variant in the *OTC* gene, which leads to loss of protein function. The uniqueness of this case is the proband's 35-year-old father with the pathogenic LoF variant mosaicism having no OTCD symptoms or biochemical hepatic malfunction markers.

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