INTRODUCTION

Cyclic vomiting syndrome (CVS) is a chronic functional disorder of unknown etiology that is characterized by paroxysmal, recurrent episodes of vomiting separated by symptom-free intervals. Each individual is characterized by a specific stereotypic pattern concerning the time of onset, intensity, duration, frequency, and associated symptoms and signs (16). Although first described in 1882 by Samuel Gee in children, CVS has been recognized with increasing frequency in adults as well (8). The etiology and pathogenesis of CVS is still unknown. It is assumed that various factors can affect the occurrence of vomiting episodes in CVS. Multiple etiologic hypotheses have been suggested: migraine variant, mitochondrial diseases including mitochondrial fatty acid oxidation disorders, gastrointestinal motility disorder, corticotropin-releasing factor in response to stress, disorder of the brain–gut axis, autonomic dysfunction, abdominal epilepsy, ion channel dysfunction, and altered psychodynamics, etc. (16). Recent concepts also suggest a strong genetic component, with evidence of mitochondrial heteroplasmies that predispose to CVS and other related disorders, such as migraine and chronic fatigue syndrome. As pointed out by Boles et al. (5), one common mtDNA polymorphism, 16519T, is six-fold more common in pediatric CVS than in control populations (17). Another common mtDNA polymorphism, 3010A, was noted to increase the odds ratio for developing CVS in subjects with 16519T by as much as 17-fold (1). In children, most cases of CVS are associated with co-morbid and/or a close family history of migraine (12) and other “functional disorders” (e.g. depression, irritable bowel syndrome, etc.) (3), suggesting that at least some of the predisposing genetic factors are localized on the maternally inherited mtDNA (1).

Case report

An 18-year-old male patient was admitted to the Department of Gastroenterology and Hepatology of the St. George’s University Hospital in Plovdiv, with symptoms of intense vomiting accompanying with drowsiness and communication difficulties. He had no diarrhea, abdominal pain or fever. There was a long history of episodic intensive vomiting since the age of three, repeated visits to the emergency departments and multiple hospitalizations, and a reduced quality of life. According to his parents, these episodes of vomiting have the following invariable features: they occur 3–6 times per year; commence without any triggers, at any time of day (usually in the early morning, upon awakening); last usually for 1–3 days; and are self-limited. The vomiting has always been intense, sometimes bilious and several times there was hematemesis. The accompanying symptoms during the attacks include pallor, altered consciousness: the patient has always been lethargic and withdrawn, with muscle hypotonicity, mild elevation of blood pressure and hypersalivation. After the attacks, he remembers everything, denies lack of consciousness and is orientated and able to respond appropriately to commands, but restrains from speaking and moving because of incapacitating nausea. There had been no developmental and growth delay, no personal and family history of migraine, no co-morbid conditions such as anxiety, depression and irritable bowel syndrome during all these years.

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Materials and Methods

Participants
The patient was recruited at the Medical University Hospital in Plovdiv. Blood samples were obtained from him and his mother in EDTA-containing vacuum tubes. Written informed consent forms approved by the Medical University Ethics Committee in Plovdiv were signed by the participants.

Methods
Thorough personal and family history and physical examination were taken. The patient was consulted by an ophthalmologist, a neurologist and a psychiatrist during his hospital stay. Laboratory tests (complete blood count, serum glucose, serum transaminases, amylose, albumin, bilirubin, coagulation parameters, electrolytes and urinalysis) were performed on a Konelab 60i analyzer (Thermo Fisher, USA) at the Laboratory Center of the Medical University Hospital. Abdominal sonographies were made before and after the vomiting episodes on a Fukuda FF Sonic UF-750 XT analyzer, with a 2.5–3.5 MHz transducer in the Department of Gastroenterology and Hepatology. Contrast enhanced computed tomography was also performed in the symptom-free interval. Electroencephalographic examinations were done during the episode of vomiting and during the symptom-free interval, using a Mitzar EEG machine and bridge electrodes mounted according to the 10–20 system.

Genotyping methods
Genomic DNA was isolated from 1 mL whole-blood samples, using a QIAamp Blood DNA Midi kit (Qiagen). The DNA was eluted in 40 µL elution buffer. The presence or absence of the 7028C polymorphism that defines haplogroup H was determined by PCR/restriction fragment length polymorphism (RFLP) analysis following FastDigestAlul endonuclease digestion (Fermentas), with the following primer sequences: forward, TTT CGG TCA CCT AAG TTT A; reverse, AGC GAA GGC TTC TCA AAT CAT.

The participants with 7028C (haplogroup H) were tested for the 16519C_T polymorphisms by PCR/RFLP, applying FastDigestHaeIII endonuclease (Fermentas) and using the following primer sets (16519: HaeIII forward GGA TGA CCC CCA TCA GAT A and reverse CTT ATT TAA GGG GAA CGT G). Ffu DNA polymerase-based PCR was used to ensure the optimal balance between specificity and fidelity of specific PCR products. The PCR products were purified using the QIAquick PCR Purification Kit, following the manufacturer’s instructions, and eluted in 30 µL of elution buffer. Direct sequencing of the PCR products was used for confirmation of the presence of mtDNA polymorphisms.

Results and Discussion
The history, clinical and laboratory examinations excluded triggers of vomiting such as physical and emotional distress, substance abuse, mild infection, fasting, high protein intake. Psychiatric co-morbidities like anxiety disorders, depressive symptoms and disturbed parent–child relationships were also excluded. After careful physical examination, we did not find any abdominal tenderness and/or severe abdominal pain, severe alteration of mental status, abnormal eye movements, motor asymmetry and/or ataxia, hallucination, seizures, photophobia and paresis during the vomiting. The laboratory screening showed no abnormalities in the complete blood count, no hypoglycemia, no hyperammonemia, no abnormal anion gap, no metabolic acidosis or respiratory alkalosis. There were transitory high levels of serum amylose accompanied by normal urine amylose. The urinalysis showed sterile urine, transitory acetonuria and absence of aminocidurid. Ultrasonography and computed tomography did not show any abnormalities of the hepato-biliary system and pancreas. Brain computed tomography (naive and contrast enhanced) showed completely normal results. There were no supra- and subtentorial brain tumors or lesions and no signs of oedema that could explain the altered consciousness and lethargy of the patient during the vomiting episode. The ventricular system was symmetric and the subarachnoid space was normal. The multiple upper gastrointestinal endoscopies that were made during the present and the previous hospitalizations revealed signs of chronic erythematous gastritis and reflux-oesophagitis. Electroencephalography during an episode of vomiting showed no ictal or interictal epileptiform activity. Verification of the mtDNA polymerism 16519T and mtDNA polymorphism 3010A in the CVS patient and his mother was performed by restriction endonuclease digestion and direct PCR product sequencing (Microsynth). Agarose gel electrophoretic analysis of restriction fragment length polymorphism (RFLP) is shown in Fig. 1. Direct sequencing of PCR-amplified products did not show any PCR mutations in the sequenced PCR products and confirmed the presence of mtDNA 7028C/T and 16519C/T polymorphisms in the tested patient and his mother (Fig. 2). Nucleotide changes were confirmed by replicate PCR amplification and sequence analysis from the same DNA samples.

Fig. 1. Agarose (2 %) gel electrophoresis of RFLP showing mtDNA polymorphisms 16519T and 7028. Identification of 7028C mtDNA polymorphism (haplogroup H) - (A). Identification of mtDNA 16519 polymorphism - (B). Lanes 1–3: mother, patient and control DNA samples, respectively; Lane M: molecular weight marker (O’Range Ruler Fermentas).
Fig. 2. Nucleotide alignment (Geneous 4.8.4) of PCR-amplified mtDNA sequences, showing the presence of 7028C SNP defining haplogroup H (A), 3010 SNP (B), and 16519 SNP (C), in the tested patient and his mother. Comparison was made with control DNA samples. The positions of a genetic variant (i.e. SNPs) are shown in boxes above the alignments.

The results of our study confirm the role of a predisposing mtDNA sequence polymorphisms in CVS pathogenesis. CVS is a functional vomiting disorder that affects individuals of all ages. Accurate diagnosis based on diagnostic criteria for CVS and the exclusion of organic diseases mimicking the clinical manifestations of cyclic vomiting is absolutely required (16). The first diagnostic criteria for CVS were defined at the 1st International Symposium on CVS held in 1994 (16). Later on, a series of revised criteria for CVS were proposed at the 2nd International Scientific Symposium in 1998, followed by the Rome II criteria in 1999, and the Rome III criteria (10, 13, 15). The most important point is that the earlier criteria of CVS were valid for children only, whereas the newly revised Rome III criteria for CVS include adults as well (10, 13, 15). In 2008, the latest diagnostic criteria for CVS in childhood were suggested as a part of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition Consensus Statement on the diagnosis and management of CVS (11). According to this consensus definition, all of the following criteria must be met: at least 5 attacks at any interval (or a minimum of 3 attacks during a 6-month period); episodic attacks of intense nausea and vomiting lasting 1 hour to 10 days and occurring at least one week apart; stereotypical pattern and symptoms in the individual patient; vomiting during attacks occurs at least 4 times/hour for at least 1 hour; return to baseline health between episodes and absence of another disorder (11).

In about 40 % to 80 % of CVS patients, vomiting episodes are evoked as a result of some specific triggering factors (7). Such factors, however, were not found in our case. Migraine and family history of migraine have been reported in 39 % to 81 % of pediatric CVS patients, and in 24 % to 70 % of adult patients (6, 12). However, in our patient there was no such history either. Although it is known that haplogroup H is defined by the mtDNA 7028C polymorphism, it still remains unclear which mtDNA SNP(s) actually provoke(s) the functional predisposition to CVS. This is due to the highly complex nature of haplogroup H, which includes multiple constituent subhaplogroupings. When a patient presents with acute vomiting, more severe disorders can usually be excluded based on history, physical examination, and basic laboratory tests. Most cases of CVS were described as sporadic (7), but family history may be an important factor. Our data point to maternal inheritance because of mitochondrial DNA mutations. There are several reported familial CVS cases with multiple members involved (2, 9, 14), with predominantly matrilineal mode of inheritance. However, the functional effects of the SNPs at position 3010 and 7028 have not been demonstrated in a model cell or reporter system.
Conclusions
The results from this study confirmed the presence of mtDNA 7028C/T and 16519C/T polymorphisms in the tested CVS patient and his mother. Mitochondrial mutation plus precipitating stress or excitement may predispose the onset of vomiting episodes in patients with CVS. The functional effects of the two SNPs at position 3010 and 16519 in a model cell or reporter system have not been demonstrated, and, therefore, even if there is an association, the mechanism whereby dysfunction or symptoms occur is unclear. Moreover, even though universal genetic associations of CVS have not yet been identified, some case studies of patients and/or families indicate that the A3243G mitochondrial DNA mutation, along with several other less well-described mitochondrial DNA mutations, are commonly associated with vomiting (4, 14). In this regard, genetic testing could be considered as an additional method that may support diagnosis of CVS.

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REFERENCES