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SHORT REPORT

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A mitochondrial DNA mutation (A3243G mtDNA) in a family with cyclic vomiting

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We report the incidence of mitochondrial DNA (mtDNA) mutations and/or deletions in a family from Southern Italy in which four members were affected by a cyclic vomiting syndrome (CVS). The A3243G mtDNA mutation was detected in a boy, his mother, maternal grandmother, and aunt but not in other relatives of the family.

Patients with a CVS experience a minimum of three distinct episodes of vomiting and nausea, usually involving more than four emeses in one hour at the peak. They feel quite well between episodes. There is no apparent underlying cause for the vomiting [4]. In the differential diagnosis of CVS, mtDNA mutations should be considered when there is a maternal history of CVS and migraine, clinical findings of seizures, neuromuscular and gastrointestinal symptoms and laboratory evidence of lactate increase [5].

A family was recruited with four members suffering from CVS: a 5-year-old boy, his mother, the maternal grandmother and aunt. The three adults were affected by CVS during childhood, whilst they suffered from migraine at an adult age (Fig. 1). Metabolic investigations revealed permanent hyperlactataemia (5–8 mM) with elevated lactate/pyruvate ratios (L/P) (25–30) in the boy's mother. In the maternal grandmother and aunt, lactate levels (2.6 and 3.0 mM respectively) were slightly elevated and L/P molar ratios remained in the normal range.

The young patient suffered from vomiting fits which lasted for many hours until spontaneous resolution and recurrence with the same characteristics after an interval of 15–20 days. Metabolic acidosis (pH 7.3, bicarbonate 16 mM) with hyperlactataemia (2.5–5.35 mM) and elevated L/P (19–32; normal < 16) and ketone body molar ratios were found (2–4.6, normal < 2). Hyperalaninaemia, lactic aciduria, and an abnormal excretion of suberic, adipic, and 3-hydroxybutyric acids was observed. Laboratory, clinical and instrumental findings of all four patients excluded organic diseases.

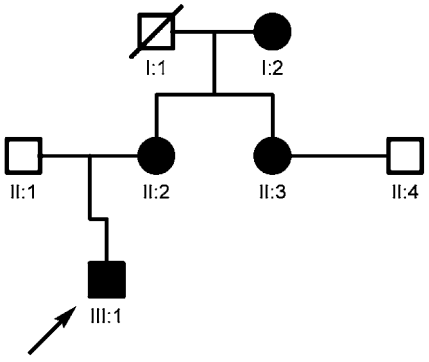

Fig. 1 Pedigree of the family with CVS; the *arrow* indicates the index case. The percentage of mutated mtDNA in blood and muscle of the patients is I:2 = 25% and 30%; II:2 = 35% and 38%; II:3 = 30% and 32%; III:1 = 70% and 75% respectively. Affected individuals are indicated by *solid* symbols

To check for point mutations and for a previously described 8.1 kb deletion [3], the region between nucleotides 6687 and 15123 was amplified by the polymerase chain reaction (PCR) in ten overlapping segments and completely sequenced. Screening for the 3243 np mutation in mtDNA was carried out by PCR-RFLP. The relevant region of mtDNA was amplified using the following primers according to the Cambridge sequence [1]: 5'CCCACAGGTCCTAAACTACC-3' (np 2770–2789) and 5'AGCGAAGGGTTGTAGTAGCC-3' (np 3456–3437). The PCR products (10 µl) were then purified and digested with 15 U of the restriction endonuclease ApaI for 1 h at 32°C. The A-to-G mutation at 3243 np created an ApaI restriction site (GAGCCC to GGGCCC) and was easily detected by ethidium bromide staining in a 2.5% agarose gel. In order to detect small amounts of mutated mtDNA in the presence of an excess of wild-

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type mtDNA, an allele specific mismatch priming was carried out using an upstream primer (np 3224–3243) in which there was the mutation (G instead of A) at np 3243 and a mismatch (G instead of C) at np 3240 in one site and the downstream primer 3551–3531 in the other site. We failed to detect the 8.1 kb deletion or unknown mutations in our patients.

The A3243G bp mutation was detected in the boy and in his above-mentioned relatives. In the boy's blood, the 3243 bp mutation with 70% mutant mtDNA was detected. We found 35%, 25% and 30% mutated mtDNA in the blood of the mother, maternal grandmother and aunt, respectively. The mtDNA in the muscle was heteroplasmic for the 3243 bp mutation in all cases. The percentage of mutant mtDNA ranged as follows: 75% in the boy, 38% in his mother, 30% in the maternal grandmother and 32% in the maternal aunt (Fig. 1).

To our knowledge, this is the first report describing the A3243G mtDNA transition in a family with CVS. This mutation has only been found in two unrelated females who suffered from gastrointestinal symptoms [2]. In our patients, the 3243 mtDNA mutation was heteroplasmic with a correlation between the amount of mutant mtDNA and the clinical severity in the family in both blood and muscle. Before such an association between a mitochondrial base-pair change and a clinical phenotype can be made, however, several criteria must be satisfied: (1) the mutation must be present in a high proportion in affected family members, typically in a heteroplasmic state and (2) it must be evolutionarily conserved throughout related species. In our patients, the heteroplasmic A > G 3243 in tRNA leucine fulfils these criteria. The degree to which various family members are clinically affected probably reflects the degree of mutant mtDNA. Similar to other mitochondrial tRNA point mutations, a threshold level of mutant DNA is required before the disease is manifest and symptoms usually appear in those tissues with a high metabolic demand where a reduction in ATP synthesis will result in impaired cellular function.

In cases where the CVS appears to show maternal segregation, particularly when it is associated with increased lactate, mtDNA may be a suitable subject for further investigation.

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