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BRIEF REPORT — MUTATION REPORT

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Combination of mtDNA mutations in a patient with a mitochondrial multisystem syndrome

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Abstract We report a patient who manifested a heterogeneous clinical presentation, including hypertrophic cardiomyopathy and hypothyroidism, with initially limited central nervous system involvement, and who harbored the mitochondrial (mt)DNA A3243G mutation. MtDNA analyses also revealed deleted genomes in muscle and blood. This atypical molecular combination may have influenced the clinical phenotype.

Key words mtDNA · MELAS genotype-phenotype correlation

Introduction

Ten years after the first pathogenic mutations in mitochondrial DNA (mtDNA) were discovered, and the concept of "mitochondrial genetics" was introduced into clinical medicine, we count over 50 pathogenic point mutations and rearrangements associated with an array of clinical presentations. However, the issue of genotype-phenotype relationships has become more complex than imagined (De Mauro and Bonilla 1997). We describe a patient with hypertrophic cardiomyopathy and hypothyroidism, but with limited central nervous system involvement, in whom we detected an unexpected combination of mtDNA defects.

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Clinical report and methods

A 51-year-old woman had suffered from severe hypertrophic cardiomyopathy with arrhythmia and bilateral hearing impairment since her thirties. At age 41 years, hypothyroidism became manifest. Serum free thyroxine (FT)3 and thyroid stimulating hormone (TSH) levels were reduced. Thyroid autoantibodies were negative. Hyperglycemia was also found, and non-insulin-dependent diabetes mellitus (NIDDM) was confirmed by an oral glucose tolerance test. After informed consent was obtained, muscle biopsy showed decreased cytochrome *c* oxidase (COX) staining in 10% of the ragged-red fibers (RRF) found. Biochemically, COX was also reduced (65% residual activity). Her condition worsened, with generalized muscle weakness and moderate ptosis, but there was no extraocular muscle involvement. Her mental state deteriorated progressively. A brain computed tomography (CT) scan showed bilateral calcifications in the pallidus and thalamus. When she was back at home, the patient suddenly suffered seizures and died of cardiopulmonary arrest.

Her 28-year-old daughter has non-specific ovary and thyroid gland dysfunction, but is otherwise asymptomatic. Neurophysiologic evaluation showed reduced nerve conduction velocities and abnormal visual evoked potentials. The patient's brother is healthy. No other relatives were tested.

Results and discussion

Clinically, in the patient, a disorder of the type seen in multisystem mitochondrial disorders or in mitochondrial encephalomyopathy, stroke-like episodes, and lactic acidosis (MELAS) was sustained by the morphological and biochemical findings in muscle, and indicated that an mtDNA disorder was likely. Southern blot analysis showed a relatively low proportion (17%) of the so-called "common" mtDNA deletion in muscle, but not in blood, from the

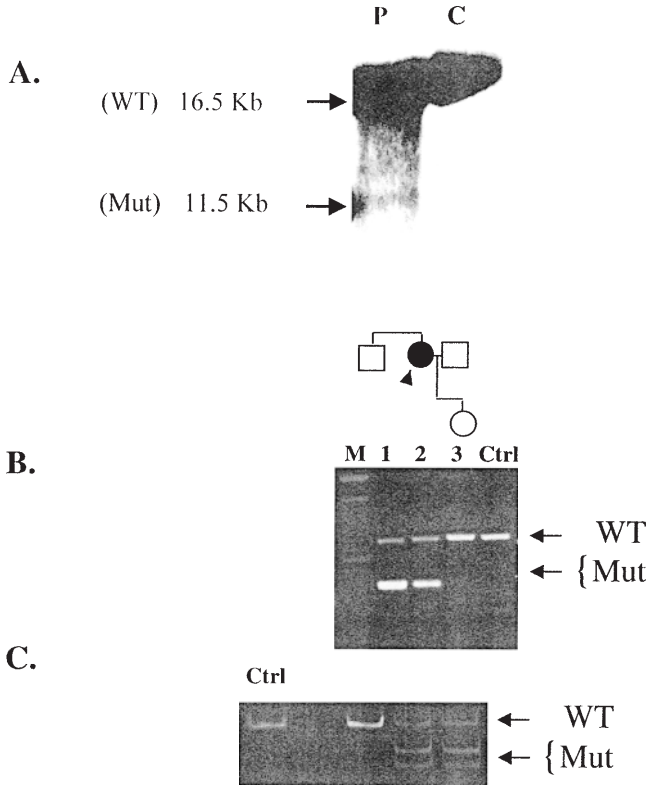

Fig. 1. **A** Southern blot analysis of muscle from our patient (*P*) and a control (*C*) used a standard methodology described in Santorelli et al. (1996). In addition to the wild-type 16.5-kb band, an additional band of approximatively 11.5 kb, corresponding to a deletion of about 5.0 kb, was seen. **B** The *pedigree* of this family is illustrated with proposita indicated by *filled circle*. The strategy to quantitate the "common deletion" of about 5.0 kb, employing oligonucleotide primers (5′-3′) 8200-8225F, 9030-9055B, and 13530-13500B, is also shown. A wild-type fragment of 850-bp was found in the patient's muscle and blood (*lanes 1 and 2*), her daughter's lymphocytes (*lane 3*), and in control (*Ctrl*) muscle and blood. An additional, mutated fragment was detected only in the patient. **C** Autoradiogram showing the mutant load of the A3243G mutation. The 238-base pair (bp) polymerase chain reaction (PCR) amplified fragment is normally cleaved by the endonuclease *Hae*III in three fragments of 169, 37, and 32 bp. The A3243G mutation creates an additional restriction site, producing 72-bp and 97-bp fragments. The levels of the A3243G mutation were determined using a reported radioactive methodology (Ciafaloni et al. 1992). WT, Wild-type; *Mut*, mutant mtDNA; *M*, molecular marker size

proposita (Fig. 1A). The "common" deletion of about 5 kb accounted for 47% of total genomes in blood and 67% of total genomes in muscle when assessed using a sensitive three-primer polymerase chain reaction (PCR) method, as described (Sciacco et al. 1994) (Fig. 1B). We also found the A3243G mutation in blood and muscle mtDNA in our proposita in similar percentages (55%), as well as detected this mutation in lymphocytes from her daughter (Fig. 1C), who did not harbor deletions. The unusual clinical and molecular combination suggested that additional factors may have determined the phenotype. We hypothesized that other alterations affecting genes important for protein translation or impairing energy production could account for the more severe clinical phenotype in the mother, and we sequenced 80% of mtDNA in the proposita and in two maternal relatives. We found no variations of pathogenic significance in the tRNAs and rRNA-coding sequences, whereas we identified several changes in the two adenosine triphosphatase (ATPase) subunits, but most had already been reported in the normal population (Mitomap 1999). We also found a new, homoplasmic T9137C mutation in the patient, but not in 50 control subjects: the mutation converts a moderately conserved isoleucine by tryptophan but its pathogenic role is still unclear.

The typical manifestations of the A3243G mutation include extraocular and skeletal myopathy, with RRF, lactic acidosis, encephalopathy with stroke-like episodes, NIDDM and deafness, and cardiomyopathy. However, in affected families, most carriers are either asymptomatic or present with different combinations of symptoms, such as hearing loss, cardiomyopathy, or NIDDM. An endocrine disorder such as hypothalamic growth hormone deficiency or delayed puberty may also occur (Manouvrier et al. 1995; Yang et al. 1995). Hypothyroidism, which was observed in our patient, could be an underestimated manifestation (Manouvrier et al. 1995). Interestingly, a Japanese patient with hypothyroidism and features of the Kearns-Sayre syndrome has been reported to harbor a similar "double" mtDNA mutation (Ohno et al. 1996).

Differences between levels of mutant mtDNA in organs and tissues within the same individual are often considered to be the principal factor responsible for the varied clinical expression of the mtDNA defect. It has been shown that most, but not all, A3243G individuals harbor more abundant mutant genomes in muscle than in blood, and that clinical involvement correlates well with mutant load in muscle but not in blood, with higher percentages of the mutated mtDNA resulting in severe involvement of the central nervous system (Chinery et al. 1997). In our patient, intermediate levels of mutant A3243G were detected, and this probably determined the relatively milder phenotype. We cannot exclude the possibility that different tissues, other than blood and muscle, harbored higher percentages of mutated mtDNAs. Also, we assessed levels of the mutations at a particular time point and it is uncertain whether the aging process influences the severity of presentation and the mutant load. For instance, this influence has been noted in some mtDNA tRNA mutations (Fu et al. 1996; Weber et al. 1997).

In conclusion, we suggest that, in patients with mitochondrial DNA defects, signs and symptoms in the patient be considered by the appropriate internist, as these manifestations may precede the development of a central nervous system disorder.

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