Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Genetics and Metabolism 88 (2006) 364–371
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Molecular–clinical correlations in a family with variable tissue mitochondrial DNA T8993G mutant load

Gregory M. Enns [a,*], Ren-Kui Bai [b,1], Anita E. Beck [a], Lee-Jun Wong [b]

[a] Department of Pediatrics, Division of Medical Genetics, Stanford University, 300 Pasteur Drive H-315, Stanford, CA 94305-5208, USA
[b] Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, NAB 2015, Houston, TX 77030, USA

Received 27 January 2006; accepted 1 February 2006
Available online 20 March 2006

Abstract

Unlike many pathogenic mitochondrial DNA mutations, the T8993G mutation associated with Leigh syndrome (LS) and neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) typically shows little variation in mutant load between different tissue types. We describe the molecular and clinical findings in a family with variable disease severity and tissue T8993G mutant loads. Real-time ARMS qPCR testing showed that two brothers with features of NARP and LS had high mutant loads (>90%) in all tissues tested, similar to previously reported cases. Their sister, who has mild speech delay but attends normal school, was found to have a relatively high mutant load (mean 93%) in tissues derived from endoderm (buccal mucosa) and mesoderm (blood and skin fibroblasts). However, in tissue derived from ectoderm (hair bulbs), she carried a considerably lower proportion of mutant mtDNA. Because both surface ectoderm, which gives rise to outer epithelia and hair, and neuroectoderm, which gives rise to the central nervous system, are derived from ectoderm, it is tempting to speculate that the mutant load detected in the oligosymptomatic sister's hair bulbs is a reflection of the brain mutant load. We conclude that significant variation in tissue mutant load may occur in at least some individuals that harbor the T8993G mutation. This adds additional complexity to genetic counseling and prenatal diagnosis in such instances. Given the shared embryonic origin of hair bulbs and brain, we recommend performing hair bulb mtDNA analysis in asymptomatic or oligosymptomatic individuals that have high blood mutant loads in order to understand better the genotype–phenotype correlations related to the T8993G mutation.

Keywords: Leigh syndrome; NARP; Mitochondrial DNA mutation T8993G; Mitochondrial DNA segregation; Prenatal diagnosis

Introduction

Point mutations in mitochondrial DNA (mtDNA) have been associated with a number of clinical syndromes, including mitochondrial encephalomyopathy with lactic acidemia and stroke-like episodes (MELAS), myoclonus epilepsy with ragged-red fibers (MERRF), Leber hereditary optic neuroretinopathy (LHON), and neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) [1]. Mutant mtDNA typically coexists with wild-type mtDNA in affected individuals, a situation termed heteroplasmy, although exceptions exist (e.g., homoplasmy is common in LHON) [1]. In general, the expression of the clinical phenotype is related to the percentage of abnormal mtDNA present (termed "mutant load") and the energy demands of a given tissue.

Variable tissue mtDNA mutant load has been documented in MELAS, MERRF and other mtDNA mutations [2–4]. However, the T8993G (and T8993C) mutation(s) associated with NARP and Leigh syndrome (LS) appear to behave differently than most other mtDNA point mutations. In particular, there appears to be a lack of tissue variability in pedigrees harboring 8993 mtDNA mutations [2,4–7]. Other properties of 8993 mtDNA mutations include rapid segregation toward homoplasmy, often within a single generation, and a relatively high frequency of de

* Corresponding author. Fax: +1 650 498 4555.
E-mail address: greg.enns@stanford.edu (G.M. Enns).
[1] Co-first author, contributed equally to first author.

1096-7192/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2006.02.001

novo mutations [5,8–12]. Such rapid segregation is consistent with the presence of a mitochondrial genetic "bottleneck," in which only a small subpopulation of mtDNA molecules are amplified during maturation of the primary oocyte [2,5].

In the present report, we correlate the molecular and clinical findings in a family with variable disease severity and tissue mutant loads. Two brothers with features of NARP and LS had high mutant loads in all tissues tested, similar to previously reported cases [13–16]. Their oligosymptomatic sister was found to have a relatively high mutant load in tissues derived from endoderm (buccal mucosa) and mesoderm (blood and skin fibroblasts). However, in tissue derived from ectoderm (hair bulbs), she carried a considerably lower proportion of mutant mtDNA when compared to her symptomatic siblings. These findings are discussed in light of current theories of mtDNA segregation during oogenesis.

Case reports

Case 1

The proband (III-2, Fig. 1) was born at 40 weeks of gestation to a then 23-year-old G3 P2 SAb 1 mother via spontaneous vertex delivery following an uneventful pregnancy. His early history was unremarkable and developmental milestones in infancy were normal. At age one year, he was noted to have poor growth. Cystic fibrosis testing was negative. He was found to be allergic to egg whites and peanuts, so these were removed from his diet. A gluten-free diet was also attempted, but he continued to exhibit failure to thrive. A developmental assessment at age 17 months noted hypotonia and motor development at the 10–12 month level. An ophthalmology evaluation at 18 months was normal. Laboratory studies performed at age 21 months showed elevated lactate (4.1 mM, normal 0.7–2.1 mM) and moderately increased tricarboxylic acid cycle intermediates on urine organic acid analysis. He spoke his first words at age 21 months and, at age 22 months, he was starting to take steps independently. At 22 months, lactate was 4.5 mM, pyruvate was 0.093 mM (normal 0.03–0.08 mM), and the lactate to pyruvate ratio was elevated at 48 (normal <25). Carnitine levels showed low free carnitine (14.2 μM, normal 18–58 μM) and an elevated acyl to free carnitine ratio (1.7, normal <0.4), but the acylcarnitine profile was normal. Urine organic acids showed minimal elevation of adipic, suberic, 2-hydroxyglutaric, and 2-oxoglutaric acids. Ammonia level, plasma amino acids, and high resolution karyotype were normal. Mitochondrial DNA analysis was performed at age $2\frac{1}{2}$ years , after the presentation of the younger brother (Case 2), and was positive for the T8993G mutation (see Results). At age 2 years 8 months, he had mild hypotonia and an ataxic gait. He

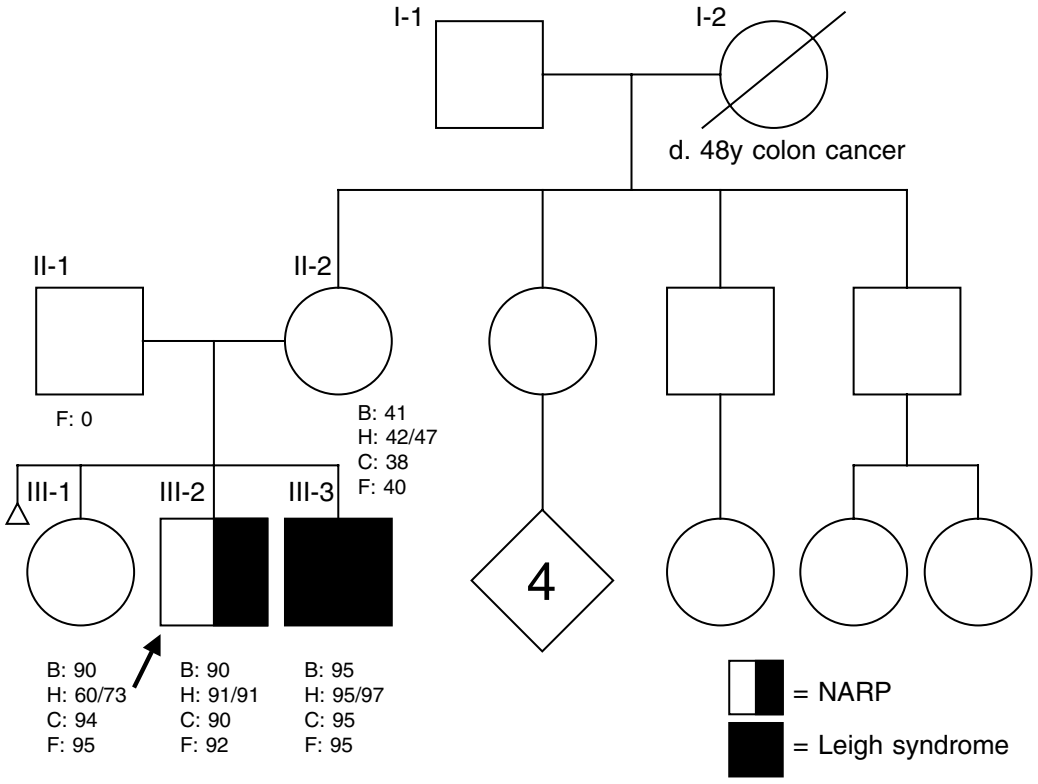

Fig. 1. Pedigree and T8993G mutant load in different tissue types analyzed by real-time ARMS qPCR. B, blood; H, hair bulbs (both mutant load from pooled samples and mean mutant load from individual hair bulbs are shown); C, cheek (buccal) cells; F, skin fibroblasts.