

Varying Loads of the Mitochondrial DNA A3243G Mutation in Different Tissues: Implications for Diagnosis

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Testing for common mutations in mitochondrial DNA (mtDNA), including the A3243G MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) mutation, is routinely done in DNA isolated from blood. Since the blood level of the A3243G mutation may be low in probands and even lower in asymptomatic or oligosymptomatic maternal relatives, we assessed the proportion of A3243G mutant genomes in other accessible tissues. We studied five tissues (leukocytes, skin fibroblasts, hair roots, urinary sediment, and cheek mucosa) in 61 individuals from 22 families harboring the A3243G mutation. Although mutational loads varied widely among these tissues, as a rule DNA from urinary sediment had the highest and blood the lowest proportion of mutant genomes; individual hair roots differed markedly from one another; fibroblasts and cheek mucosa tended to have higher mutation loads than blood but lower than urinary sediment. In all individuals in whom the mutation was detectable in blood, it was also detected in other tissues. Conversely, in some individuals the mutation was undetectable in blood but clearly present in other tissues. We conclude that urinary sediment and cheek mucosa are tissues of choice for the diagnosis of mtDNA mutations, as they are easy to obtain and the mutation load is almost always greater than in blood. © 2004 Wiley-Liss, Inc.

KEY WORDS: mtDNA; MELAS; A3243G; mutation load; accessible tissues; diagnosis

INTRODUCTION

MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) is the most common maternally inherited encephalomyopathy, and approximately 80% of patients harbor the A3243G mutation in the *tRNA^{Leu}(UUR)* gene of mitochondrial DNA (mtDNA). Although the mutation was initially identified in DNA isolated from muscle, it is usually present in all tissues from patients and, in lower

abundance, in tissues from oligosymptomatic or asymptomatic maternal relatives. Therefore, it became common practice to look for the mutation in blood, although it was noted that mutant loads were consistently lower in blood than in muscle. It was also observed that some maternal relatives expected to be carriers by pedigree analysis harbored no detectable A3243G mutation in blood. Thus, though usually informative, blood may not be the tissue of choice for the diagnosis of MELAS or for the detection of carriers. We, therefore, compared the mutation load in five accessible tissues (leukocytes, skin fibroblasts, hair roots, urinary sediment, and cheek mucosa) of 61 individuals from 22 families harboring the A3243G mutation.

MATERIALS AND METHODS

Subjects

Of the 61 individuals belonging to 22 families known to harbor the A2343G mutation, 22 were considered probands because they had either strokes or focal seizures; the remaining 39 individuals were either asymptomatic or oligosymptomatic maternal relatives of the probands.

Tissues

We obtained samples of blood, urine, hair roots, cheek mucosa, and skin fibroblasts. Urine (about 10 ml, preferably from first morning void) was centrifuged at 500 g for 10 min to obtain a pellet. Cells from cheek mucosa were obtained by scraping the inside of the mouth with a cotton swab. After dipping the swab in 3–5 ml of saline at room temperature, the saline was centrifuged at 500 g for 10 min and the pellet used. Roots from individual hairs were cut and placed separately in Cell Lysis Solution (Gentra systems). Fibroblasts were obtained from skin biopsies, and a cell pellet obtained from one T150 culture flask was used for DNA isolation.

Total DNA was extracted by standard protocols using Gentra Systems kits and following the manufacturer's instructions.

Molecular Analysis

mtDNA was amplified by the polymerase chain reaction (PCR) with oligonucleotide primers corresponding to nt positions 3116–3134 (forward) and 3353–3333 (backward). After 25 cycles, 3 μ C of ³²P-labeled ATP was added for the final PCR cycle ("hot finish PCR" [Moraes et al., 1992]). The 238 base pair (bp) fragment generated by PCR was digested with the restriction enzyme *Hae*III, which cleaves the fragment containing the A3243G mutation differently from the normal fragment. The digested PCR product was electrophoresed on a 12% acrylamide gel and the proportion (percent) of mutant genomes was assessed in a phospho-imager (Molecular Analyst; BioRad, Hercules, CA) using Image-Quant software (Molecular Dynamics, Sunyvale, CA).

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TABLE I. Proportion (percent) of A3243G Mutation in Five Tissues From 12 Different Patient

	Patient number											
	1	2	3	4	5	6	7	8	9	10	11	12
Blood	5	30	ND	57	14	11	55	65	3	16	13	40
Urine	84	86	11	98	67	84	63	92	10	22	34	98
Mucosa	35	61	17	71	52	46	69	73	14	37	17	63
Fibroblasts	22	47	56	59	40	66	97	45	ND	18	24	73
Hair 1	ND	42	45	71	74	22	85	83	10	50	7	38
Hair 2	7	37	ND	9	22	59	69	63	3	30	24	85
Hair 3	12	60	9	26	33	27	71	36	9	18	11	51

RESULTS

The proportion of mutant genomes varied widely among the five tissues tested. This is illustrated in Table I, which shows representative data in 12 individuals from 12 different families. Blood DNA consistently had the lowest mutation load and urinary sediment the highest. This is illustrated in Table II, which shows the mean mutation loads in 32 subjects (22 patients and ten maternal relatives) in whom accurate quantitative values could be obtained in both tissues, and excluding samples that contained clearly detectable but unmeasurable “trace amounts” of the mutation. The load in urinary sediment was threefold higher than in blood and the difference was highly significant. Values from fibroblast and cheek mucosa fell in between those of blood and urinary sediment, as illustrated in Table I. Individual hair follicles from the same subject showed striking variations.

In ten asymptomatic or oligosymptomatic relatives (16% of all subjects), the mutation was undetectable in both blood and urinary sediment. However, in all individuals in whom the mutation was detectable in blood, it was also detected in other tissues. Also, there were eight individuals (13% of the total) in whom the mutation was not detected in blood while it was clearly present in other tissues. For example, in patient #3 (Table I) the mutation was undetectable in blood DNA but was clearly present in DNA from the urinary sediment. This particular case also illustrates how hair follicles can be informative (with mutation loads as high as 45%), but unreliable for diagnosis because of marked individual variability: notably, another hair bulb from the same patient had no detectable mutation.

The most striking differences were between mutation loads in urinary sediment, an easily accessible tissue, and blood, which, although less easily accessible, is almost always used for diagnosis (Table II and Fig. 1). The percent mutation could be as high as 92% in urine from a patient whose blood had a mutant load of only 7%.

This variation of mutant loads is illustrated by PCR-restriction fragment length polymorphism (RFLP) analysis of DNA from all five tissues in a single patient (Fig. 2).

The variation of mutation loads in different members of a single family is shown in Figure 3. We detected the A3243G mutation in blood and fibroblasts (the only tissues available) from a child with MELAS (II-1), who had died at 10 years of age. The mutation was also present in all five tissues from the

TABLE II. Mean Mutation Loads in Blood and Urine

Tissue	Mean \pm SD*
Blood	23.47 \pm 19.16
Urine	63.03 \pm 29.10

*N = 32; P < 0.001.

mother (I-1) and from an oligosymptomatic brother (II-2). Notably, however, none of the tissues from the asymptomatic 10-year-old sister (II-3), who is expected to be a carrier, showed the mutation.

DISCUSSION

The acronym MELAS was first proposed in 1984 [Pavlikis et al., 1984] to highlight the features of a clinical entity defined by encephalopathy, frequently with seizures and migraine-like headache, stroke-like episodes occurring before age 40 and often in childhood, lactic acidosis, and morphological evidence of mitochondrial dysfunction, such as ragged-red fibers (RRF) in the muscle biopsy. Early development is usually normal, and other frequent features include short stature, seizures, recurrent vomiting, neurosensory hearing loss, and dementia [Hirano and Pavlikis, 1994]. In 1990, Goto et al. identified a mutation in the *tRNA^{Leu}(^{UR})* gene of mtDNA, at nucleotide (nt) position 3243 (A3243G) in patients with MELAS [Goto et al., 1990].

Over 150 pathogenic point mutations in mtDNA have been reported, but fewer than ten are encountered with any frequency. The A3243G is the most common: in one large screening it was identified in approximately 20% of patients with myopathies and encephalomyopathies [Moraes et al., 1993], and in another study the population frequency was estimated at 1:15,000 [Majamaa et al., 1998]. While originally associated with typical MELAS [Goto et al., 1990], this same mutation can cause other clinical phenotypes, including progressive external ophthalmoplegia (PEO), diabetes mellitus, or hypertrophic cardiomyopathy [Ciafaloni et al., 1992; Moraes et al., 1993; Kadowaki et al., 1994; van den Ouweland et al., 1994]. In one study, the most frequently observed syndrome was one consisting of hearing impairment, cognitive

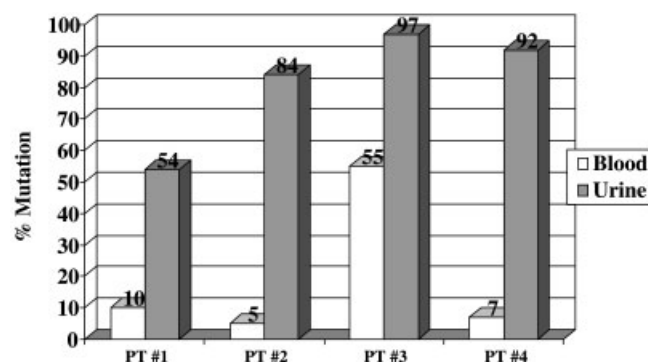


Fig. 1. Representative patients harboring the A3243G mutation, in whom DNA from urine had higher proportion of mutation than blood.

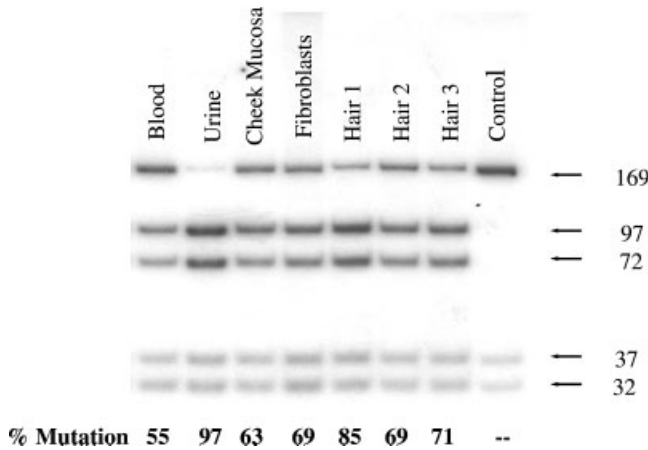


Fig. 2. A 238 base pair (bp) DNA fragment encompassing the site of the mutation is amplified by polymerase chain reaction (PCR). The fragment is then digested with the diagnostic restriction enzyme, *Hae*III, and subjected to restriction fragment length polymorphism (RFLP) analysis. The normal pattern includes three fragments, 169, 37, and 32 bp in length. In the presence of the mutation, the 169 bp fragment is further digested into 97 and 72 bp fragments.

decline, and short stature [Majamaa et al., 1998]. It is, therefore, important to have a simple and reliable screening assay to evaluate patients with diverse clinical phenotypes for the A3243G mutation. In addition, maternal relatives of typical patients may be oligosymptomatic or totally asymptomatic, but most of them want to know whether they carry the mutation.

Most pathogenic mtDNA mutations, including the A3243G transition, are heteroplasmic, with normal and mutant mtDNA coexisting in individual tissues. While initial studies of patients with MELAS used muscle, many subsequent analyses used blood DNA [Hammans et al., 1991; Poulton and Morten, 1993], and it was soon noted that mutant mtDNA levels were consistently lower in blood than in muscle [Ciafaloni et al., 1992; Sue et al., 1998]. One study suggested that mutant mtDNA levels decline with age due to preferential selection of cells containing high levels of normal DNA

[Rahman et al., 2001]. Although these findings raised the possibility of false negative diagnostic conclusions, the relatively noninvasive nature of blood DNA testing made this the routine procedure for the diagnosis of MELAS.

Our study indicates that other even more easily accessible tissues are at least as reliable as blood for diagnosis, and probably more so. In none of the 61 individuals studied by us, was the A3243G mutation detected in blood and not in either urine or cheek mucosa. Conversely, in eight cases (patient #3 in Table I is one example), the mutation could not be detected in blood whereas both urine and mucosa were positive.

In ten cases, however, the study of other accessible tissues was no more informative than the study of blood. For example, the sister of a typical MELAS patient (Fig. 3, II-3) was expected to carry the mutation, but this was undetectable in blood or in any one of the other four accessible tissues studied. It remains unclear whether this woman has the mutation in less accessible tissues (muscle? brain? ovary?) or whether the mother did not transmit any mutation at all with this particular ovum.

Our finding that individual hair follicles from the same individual had strikingly different mutation loads may be explained by the existence of a special epithelial stem cell population in the bulge of the hair bulb [Ghazizadeh and Taichman, 2001]. If each follicle derives from one or very few stem cells, clonal expansion could account for the uniform yet varied degrees of heteroplasmy. The cells in urinary sediment and cheek scrapes are sloughed off cells of predominantly epidermal origin. Perhaps, their higher mutation loads are due to the fact that they presumably turn over more slowly than blood cells (the life-span of granulocytes is a few hours and that of platelets about 1 week).

We conclude that urinary sediment and cheek mucosa are tissues of choice for the diagnosis of MELAS and, probably, most other mtDNA mutations, because they are easy to obtain and the mutation load is almost always greater than in blood.

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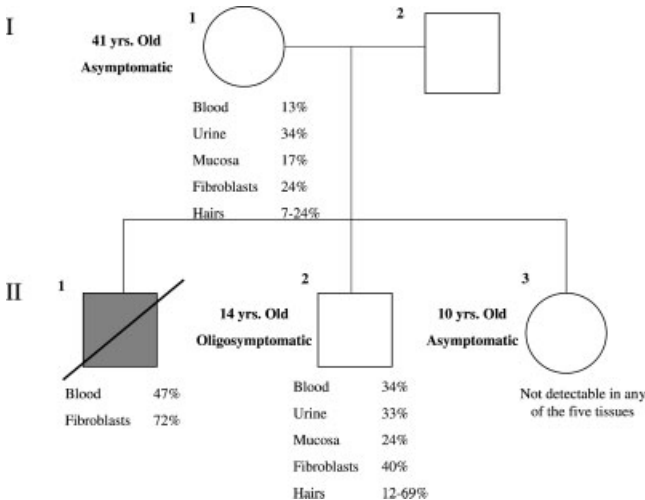


Fig. 3. Pedigree of a family with the MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) mutation. The proband died at the age of 10.

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