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Multiple Symmetric Lipomas with High Levels of mtDNA with the $tRNA^{Lys}$ A → $G^{(8344)}$ Mutation as the Only Manifestation of Disease in a Carrier of Myoclonus Epilepsy and Ragged-Red Fibers (MERRF) Syndrome

Elisabeth Holme,* Nils-Göran Larsson,* Anders Oldfors,† Már Tulinius,‡ Pelle Sahlin,§ and Göran Stenman§

Departments of *Clinical Chemistry, †Pathology, ‡Pediatrics, and §Oral Pathology, Gothenburg University, Gothenburg, Sweden

Summary

We have investigated the morphology, cytogenetics, and the fraction of mtDNA with the $tRNA^{Lys}$ A → $G^{(8344)}$ mutation in three lipomas in a carrier of this mutation. The son of the patient had myoclonus epilepsy and ragged-red fibers syndrome. The fraction of mtDNA with the $tRNA^{Lys}$ mutation varied between 62% and 80% in cultured skin fibroblasts, lymphocytes, normal adipose tissue, and muscle. In the three lipomas the mean fraction of mutated mtDNA was 90%, 94%, and 94%. Ultrastructural examination of the lipomas revealed numerous mitochondria with changes such as electron-dense inclusions in some adipocytes. When considered cytogenetically, the lipomas were characterized by a mixture of karyotypically abnormal and normal cells. An identical del(6)(q24) was found in two tumors. The fraction of mutated mtDNA in cultured lipoma cells was the same as in the lipoma in situ, indicating that the cultured cells were representative of the primary tumor. These findings indicate that the lipomas have originated with a grossly normal stem line and subsequently have developed the 6q deletion. We conclude that the lipomas represent clonal growth of adipocytes with a high content of mtDNA with the $tRNA^{Lys}$ mutation. The $tRNA^{Lys}$ mutation may be either the direct or the indirect cause of pertubation of the maturation process of the adipocytes, leading to an increased risk of lipoma formation.

Introduction

Myoclonus epilepsy and ragged-red fibers (MERRF) syndrome is a progressive mitochondrial encephalomyopathy characterized by myoclonic and tonic-clonic seizures, ataxia, dementia, muscle weakness, and mitochondrial abnormalities in skeletal muscle (Fukuhara et al. 1980). Genetic studies of families with MERRF syndrome revealed a maternal mode of inheritance of the disease (Rosing et al. 1985; Wallace et al. 1988). A mtDNA point mutation that changes a highly conserved adenine to a guanine at nucleotide 8344 in the $tRNA^{Lys}$ gene was subsequently found in patients with MERRF syndrome (Shoffner et al. 1990; Yoneda et al. 1990).

There is always heteroplasmy with a mixture of normal and mutated mtDNA in MERRF syndrome, but the fraction of mutated mtDNA varies widely between different individuals in the same family, and there are also differences between tissues in the same individual (Larsson et al. 1992). A common feature is that patients with MERRF syndrome have a high fraction of mutated mtDNA in muscle (Shoffner et al. 1990; Larsson et al. 1992). In vitro experiments with transmitochondrial cell lines have demonstrated that the $tRNA^{Lys}$ mutation impairs mitochondrial protein synthesis by inhibiting mitochondrial translation (Chomyn et al. 1991). The deficient translation seems to result in a deficiency of the respiratory chain complexes I and IV, which is found in most patients with MERRF syndrome (Wallace et al. 1988; Larsson et al. 1992).

Maternal relatives of patients with MERRF syn-

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Address for correspondence and reprints: Elisabeth Holme, Department of Clinical Chemistry, Gothenburg University, Sahlgren's Hospital, Gothenburg S-413 45 Sweden.

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drome are often clinically unaffected or have incomplete expression of the disease. Berkovic et al. (1989, 1991*a*, 1991*b*) described axial multiple large lipomas both in patients with MERRF syndrome and in otherwise unaffected carriers of the $tRNA^{Lys}$ mutation. Familial multiple lipomas symmetrically located around the neck have been described in several reports (MIM 151800 and 151900). In some of these families there have been associated neurological signs, and at least one description is fully compatible with MERRF syndrome (Ekbom 1975).

Chromosomal abnormalities are found in 50%–70% of solitary lipomas (Sandberg 1990, pp. 925–931; Sreekantaiah et al. 1991). At least six cytogenetic subgroups of lipomas may be distinguished: (1) tumors with a normal stem-line karyotype; (2) tumors with translocations of 12q13-14; (3) tumors with translocations involving 6p22-23; (4) tumors with rearrangements of 13q12; (5) tumors with ring chromosomes; and (6) tumors with sporadic rearrangements. There are only a few reports on cytogenetic investigations of lipomas from patients with multiple lipomas. Heim et al. (1988) found only normal karyotypes in seven cases, whereas Dal Cin et al. (1988) reported abnormalities involving 12q in one case.

The pathogenesis of multiple lipoma formation is unknown. To address this problem we have investigated the morphology, the fraction of mutated mtDNA with $tRNA^{Lys}$ mutation, and the cytogenetics of lipomas in a carrier of the $tRNA^{Lys}$ mutation.

Subject, Material, and Methods

Case Report

The patient is a 37-year-old woman. She is the mother of four boys. The oldest boy, 17 years of age, has MERRF syndrome. The second son, 14 years of age, has exercise-induced muscle pain but no neurological signs. The youngest sons, 3 and 5 years of age, are healthy. The mother of the patient has large confluent lipomas around the neck and shoulder region. The carrier state of the patient was diagnosed after the $tRNA^{Lys}$ mutation had been identified in her oldest son. She was subjected to a thorough investigation including biochemical and morphological examination of a muscle biopsy specimen and an extensive clinical investigation (Larsson et al. 1992). The respiratory rate and the activity of the different parts of the respiratory chain in isolated muscle mitochondria were normal. There were no ragged-red fibers or pathological accumulation of mitochondria, but a few (<1%) muscle fibers had deficient cytochrome *c* oxidase activity. She had lipomas in the neck and shoulder area, but no other clinical abnormalities were found.

At 22 years of age the patient noted a small lipoma in the nuchal region. This lipoma has gradually increased in size during the past 15 years, and it measured 6 cm × 3 cm × 3 cm (lipoma 1) when removed. During the past 5 years additional lipomas have appeared bilaterally in the clavicular areas and on the left shoulder. The lipomas in the clavicular areas, measuring 3 cm × 4 cm × 1.5 cm (lipoma 2) and 1.5 cm × 1.5 cm × 0.8 cm (lipoma 3), were removed together with the nuchal lipoma and a specimen of normal adipose tissue. The lipoma on the left shoulder was removed earlier and has not been investigated.

Morphological Examination of Lipomas

Samples for light-microscopic analysis were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin. Samples for ultrastructural analysis were fixed in 4% glutaraldehyde in sodium phosphate buffer, postfixed in 1% OsO_4, and embedded in Epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Cell Culture

Fresh tumor tissue from the three lipomas was minced into small pieces and was digested in a collagenase solution (1,000 U collagenase/ml, 146 mM NaCl, 4 mM KCl, 5 mM $CaCl_2$, 20 mM Hepes pH 7.3, and 0.1% glucose) diluted 1:1 in RPMI 1640 for 30 min at 37°C (Stenman et al. 1991). The tumor cell suspension was thereafter washed twice in fresh medium and explanted in 25-cm^2 tissue-culture flasks in RPMI 1640 supplemented with 17% FCS, 5 μg streptomycin/ml, 200 U benzylpenicillin-K/ml, and 200 mM L-glutamine. Fibroblasts from a skin biopsy were cultured in Eagle's minimum essential medium containing 9% FCS. The cultures were kept at 37°C in a water-saturated atmosphere containing 5% CO_2.

mtDNA Analysis

PCR amplification of DNA from different tissues was done with a ^{32}P end-labeled primer and a primer which created a *BglI* restriction site (Zeviani et al. 1991) in the mutant mtDNA during the reaction. Quantitation was done by densitometric scanning of autoradiograms and was corrected for heteroduplex formation, as described by Larsson et al. (1992). The fraction of mutated mtDNA was measured in three different PCR products from each tissue sample.

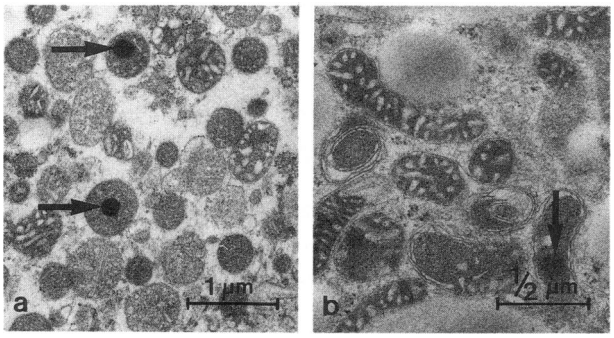

Figure 1 Pleomorphic mitochondria showing electron-dense inclusions (arrow) in univacuolar (*a*) and multivacuolar (*b*) adipocytes.

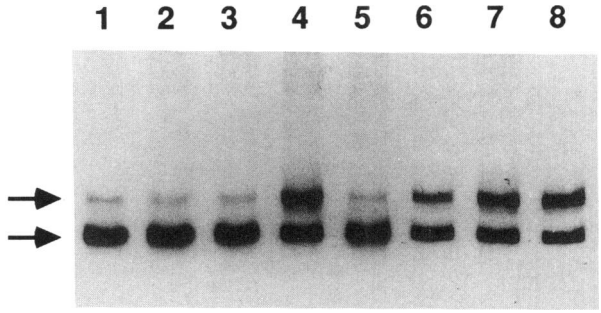

Figure 2 PCR analysis of the distribution of normal mtDNA and of mtDNA with the $tRNA^{Lys}$ mutation in different tissues from the patient. The upper band, 230 bp, is the fragment obtained from normal mtDNA. The lower band, 184 bp, is the fragment obtained from mtDNA with the $tRNA^{Lys}$ mutation. The samples are from the lipomas (lanes 1–3), normal adipose tissue (lane 4), cultured lipoma cells (lane 5), cultured skin fibroblasts (lane 6), lymphocytes (lane 7), and skeletal muscle (lane 8).

Cytogenetic Analysis

Chromosome preparations were made from exponentially growing primary cultures, after 6–8 d. Sixteen hours before harvest, colchicine was added, to a final concentration of 0.02 μg/ml. The methods for chromosome preparation and G-banding were essentially as described elsewhere (Stenman and Mark 1983). Magnified photographs were used for the karyotype analysis. The nomenclature follows that of the International System for Cytogenetic Nomenclature (Harnden and Klinger 1985).

Results

Morphological Analysis

Light microscopic examination.—The three lipomas were composed of several lobules and had the appearance of ordinary benign lipomas, with mature, univacuolar adipocytes and a sharp demarcation to the surrounding tissue by a thin fibrous capsule.

Ultrastructural examination.—The predominant cell type was mature univacuolar adipocytes with a distinct basal lamina and a peripheral rim of cytoplasm containing mitochondria and other cell organelles. Some of the adipocytes contained numerous mitochondria of variable appearance. A frequent finding was large electron-dense inclusions (fig. 1*a*). Occasional small, apparently immature adipocytes with multiple lipid droplets were also observed. These cells contained numerous mitochondria, some with large electron-dense inclusions (fig. 1*b*). Mitochondrial changes were not seen in subcutaneous adipose tissue from individuals without mitochondrial disease.

mtDNA Analysis

The PCR analysis for the occurrence of the $tRNA^{Lys}$ mutation in mtDNA is shown in figure 2. The proportion of mtDNA with the $tRNA^{Lys}$ mutation varied between 62% and 80% in various nontumorous tissues of the patient. The highest concentration was found in muscle. Normal adipose tissue from the same area in which the lipomas were found contained 75% mutated mtDNA. The fraction of mtDNA was significantly higher in the lipomas than in the surrounding adipose tissue or in other tissues from the patient. The fraction of mutated mtDNA was approximately the same in cultured lipoma cells as in the lipomas (table 1).

Cytogenetic Analysis

A total of 55 cells were analyzed from lipomas 1 and 3. The morphology of the metaphases from lipoma 2 was not of sufficient quality for detailed analysis. All cells had chromosome counts in the diploid-hypodip-

Table 1

Proportion of mtDNA with $tRNA^{Lys}$ Mutation in Different Tissues

Tissue	Mean (%)	Range (%)
Muscle	80	78–83
Lymphocytes	71	70–72
Cultured skin fibroblasts	62	62–62
Adipose tissue	75	72–79
Lipoma 1	90	89–93
Lipoma 2	94	92–95
Lipoma 3	94	93–96
Cultured lipoma cells	94	92–95

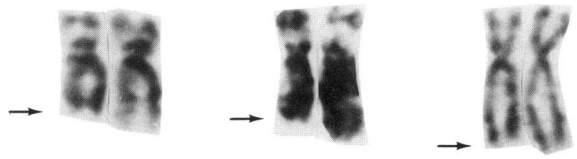

Figure 3 G-banded partial karyotypes showing del(6)(q24) (arrows) in lipomas 1 (first pair) and 3 (second and third pairs).

loid region. Of the eight cells from lipoma 1 that were karyotyped in detail, four had a terminal deletion of chromosome 6, with a breakpoint at band 6q24, del(6)(q24). In one cell this was the sole anomaly (fig. 3). The other cells showed, in addition to the 6q deletion, losses of single chromosomes (two cells) or a deletion of the X chromosome, del(X)(q23-24) (one cell). Of the remaining cells, one had +1,−3, and three had a normal female karyotype. Lipoma 3 showed a similar picture, with a mixture of karyotypically aberrant and normal cells. Five of the 15 cells karyotyped in detail had a del(6)(q24) indistinguishable from the 6q− marker in lipoma 1 (fig. 3). In four cells, the 6q deletion was the only abnormality, and in one cell it was found together with a rearranged chromosome 17, der(17)t(10;17)(q24-25;q25). There were also (*a*) one hypodiploid cell with a translocation and an inversion—t(5;17)(q12;q25),−10,inv(16)(p11.2q22-23)—and (*b*) nine cells with a normal female karyotype.

Discussion

We have found a high proportion of mutated mtDNA in lipomas of an otherwise healthy carrier of the $tRNA^{Lys}$ mutation. A frequent finding was mitochondria with electron-dense inclusions, which is often seen in muscle mitochondria of patients with mitochondrial disorders (Oldfors et al. 1991). Similar morphological changes of mitochondria have not been described in lipomas (Fu et al. 1980; Kim and Reiner 1982) or in lipomatous tissue from patients with multiple symmetric lipomatosis (Zancanaro et al. 1990). Berkovic et al. (1989, 1991*a*, 1991*b*) reported multiple symmetric lipomas in three different families with MERRF syndrome and suggested that lipoma formation is a manifestation of the disease. The findings of lipomas in our patient and her mother, both of whom are carriers of the $tRNA^{Lys}$ mutation, further support this suggestion. The high proportion of mutated mtDNA and mitochondria with ultrastructural changes in the lipoma cells suggest that the appearance of lipomas is not a secondary phenomenon but is a primary manifestation of the disease in adipose tissue of patients carrying the $tRNA^{Lys}$ mutation.

The proportion of mutated mtDNA in muscle tissue of patients with MERRF syndrome is generally above 90%, which seems to be the threshold for development of respiratory-chain dysfunction (Larsson et al. 1992). In our patient the proportion of mutated mtDNA was between 62% and 80% in tissue samples other than the lipomas. The patient had no neuromuscular disease. There is always heteroplasmy in MERRF syndrome, and the proportion of mutated mtDNA varies between patients and between different tissues of the same patient (Shoffner et al. 1990; Zeviani et al. 1991). The proportion of mutated mtDNA may also vary between cells within the same tissue. Microheterogeneity has been shown by in situ hybridization in muscle-tissue samples from patients with Kearns-Sayre syndrome, who have heteroplasmy with large deletions of mtDNA (Mita et al. 1989; Shoubridge et al. 1990; Oldfors et al. 1992). In Kearns-Sayre syndrome a deficiency of normal mtDNA is found in cytochrome *c* oxidase–deficient fibers (Mita et al. 1989; Hammans et al. 1992; Oldfors et al. 1992). A characteristic finding in MERRF syndrome is cytochrome *c* oxidase–deficient fibers. The occasional cytochrome *c* oxidase–deficient fibers in our patient probably represent muscle-fiber segments with a deficiency of normal mtDNA. It is reasonable to assume that there are occasional cell clones with mainly mutated mtDNA also in tissues other than muscle. The lipomas of our patient may represent expansion of such clones in adipose tissue. The presence of both karyotypically normal and abnormal cells in the present lipomas raises the question whether the normal cells represent truly neoplastic cells or derive from the stromal component of the tumor. The fact that the proportion of mutated mtDNA was the same in the cultured lipoma cells as in the lipoma in situ strongly indicates that the cultured cells were representative of the primary tumor. It is thus likely that the karyotypically normal cells are tumor cells and that the lipomas have originated with a grossly normal stem line and that during progression they developed the 6q deletion. A similar karyotypic evolution has been observed in solitary lipomas (Sreekantaiah et al. 1991) and other benign tumors (Mark et al. 1990; Sandros et al. 1990).

Deletions, translocations, and inversions with breakpoints on 6q have previously been found in solitary lipomas (Sandberg et al. 1986; Mandahl et al. 1988; Sreekantaiah et al. 1991). In a series of 109 lipomas, all tumors located in the head and neck region were cyto-

genetically abnormal, in contrast to tumors located in the trunk, extremities, or at other sites (Sreekantaiah et al. 1991). The finding of a clonal del(6)(q24) in the lipomas in the neck region of our patient is thus in line with previous cytogenetic findings in solitary lipomas. Since this is the first report of cytogenetic changes in a patient carrying the $tRNA^{Lys}$ mutation, it remains to be shown whether the del(6)(q24) is characteristic of these tumors or whether the lipomas in MERRF syndrome show the same spectrum of cytogenetic abnormalities as do other lipomas.

The high levels of mutated mtDNA and the occurrence of abnormal mitochondria in the lipomas indicate that the oxidative phosphorylation was deficient. Normal adipocyte precursors contain numerous mitochondria (Zancanaro et al. 1990), which indicates that these cells are highly energy dependent. A deficient oxidative phosphorylation may thus interfere with the normal maturation process and may be either the direct or the indirect cause of lipoma formation. On the other hand, the occurrence of lipomas has hitherto only been reported in MERRF syndrome and not in other mitochondrial disorders, and the possibility that the $tRNA^{Lys}$ mutation plays a specific role in lipoma formation cannot be excluded.

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