3.1-kb deletion of mitochondrial DNA in a patient with Kearns-Sayre syndrome

Abstract  Mitochondrial DNA (mtDNA) deletions have been found in the majority of patients with chronic progressive external ophthalmoplegia and Kearns-Sayre syndrome. A large number of different mtDNA deletions have been identified. They generally spare the two origins of replication and are frequently flanked by direct or indirect repeats. We have found a 3.1-kb deletion of mtDNA in a patient with Kearns-Sayre syndrome that has some unusual features. First, it encompasses nucleotides 11259 to 14368, a localization that was not described before. Second, the deletion is not flanked by direct or indirect repeats, supporting the view that homologous recombination and slip-replication do not account for all mtDNA deletions.

Key words  Chronic progressive external ophthalmoplegia  •  Kearns-Sayre syndrome  •  Mitochondrial DNA deletion

Introduction

Chronic progressive external ophthalmoplegia (CPEO) and Kearns-Sayre syndrome (KSS) may be regarded as the extreme manifestations of a clinical continuum. While CPEO is characterized by ocular myopathy, KSS is commonly defined as a multisystem disorder with progressive external ophthalmoplegia, pigmentary retinopathy, onset before age 20 and at least one of the following symptoms: heart block, cerebellar dysfunction, or high cerebrospinal fluid protein content [14]. Muscle morphology is characterized by “ragged red fibers” that are a hallmark of mitochondrial proliferation. Biochemically, both syndromes often show reduced respiratory chain enzyme activity, particularly of cytochrome c oxidase [7, 13, 23]. Almost all patients with KSS and about half with CPEO were found to harbor deletions of mitochondrial DNA (mtDNA) [5, 12, 16, 23] that differ in size, location and relative amount among patients. Since these deletions are frequently flanked by direct or indirect repeats, it has been proposed that their emergence might involve homologous recombination [6, 10, 16] or slip-replication events [19]. We report here an unusual mtDNA deletion in a patient with KSS.

Case report

The 51-year-old male patient has been suffering from slowly progressive ptosis and double vision for more than 20 years. In addition, he noticed weakness of facial, neck and limb muscles with an abnormal fatiguability during the day. There was no past medical history and no family history of neuromuscular disease. Physical examination showed severe external ophthalmoplegia with almost completely lost ocular motility and severe ptosis bilaterally. There was pigmentary retinopathy and mild hypacusis of the left ear. Muscle weakness was most marked in neck and proximal limb muscles. There were no signs of cardiac dysfunction. Routine laboratory analysis of venous blood revealed mildly elevated sedimentation rate, hyperlipoproteinemia and moderate elevation of creatin kinase to 169 U/l (normal, < U/l). CSF total protein was elevated to 900 mg/l (normal, < 450 mg/l), while cell count was normal. Electromyography of proximal and distal leg muscles showed a myopathic pattern. Nerve conduction velocities were normal. ECG and echocardiography showed no abnormalities.

Methods

Morphology

A muscle biopsy specimen was taken from the left quadriceps muscle and frozen in a slightly stretched position in melting isopen-
tine. Ten-micrometer serial sections were stained with hematoxy-
lin-eosin, modified Gomori trichome and oil red O and by histo-
chemical methods, including NADH-tetrazolium reductase, succi-
nate dehydrogenase and ATPase, as outlined in [3]. A second part
of the muscle biopsy specimen and a sural nerve biopsy specimen
were immediately fixed in 3% glutaraldehyde, washed in buffer,
postfixed in 1% buffered osmium tetroxide, dehydrated in alcohol
and embedded in Epon according to routine procedures [4]. Sec-
tions, 1 μm thick, were stained with methylene blue azure, tolui-
dine blue and p-phenylenediamine for light microscopy. Ultrathin
sections were stained with uranyl acetate and lead citrate and ex-
amined in a Zeiss EM 900 electron microscope.

Muscle biochemistry

About 50 mg of muscle that had been stored at -80°C was ho-
mogenized and assayed for the enzyme activities of respiratory
chain complexes as previously described [15].

Molecular genetic studies

Total DNA was isolated from 50 mg muscle tissue as described
[17] by homogenization and digestion overnight at 55°C with 100
μg/ml proteinase K and 0.5% SDS. DNA was extracted with phen-
ol and chloroform and precipitated with 2 volumes of ethanol and 0.3
M sodium acetate. The precipitate was dissolved in 50 μl of
H2O and stored at -20°C. For Southern blot analysis [20], DNA
was digested with the restriction endonucleasesPvuII and BamHI
(Boehringer Mannheim, Germany), respectively, subjected to elec-
trophoresis on a 0.5% agarose gel and blotted onto a nylon mem-
brane. mtDNA was visualized by hybridization to radioactively la-
beled probes [nucleotide position (nt) 4831–5917 and nt 11673–
12576 of mtDNA, respectively, corresponding to the Cambridge
Polymerase chain reaction (PCR) amplification was performed for
30 cycles at a denaturation temperature of 93°C (1 min), the re-
spective hybridization temperature of the primers used (1 min) and
an elongation temperature of 72°C (1 min). The 100-μl PCR reac-
tions contained 200 μM of each dNTP, 50 μM KCl, 10 mM Tris-
HCl (pH 8.3), 1.5 mM MgCl2, 0.01% gelatin, 30 μM of each primer
and 2.5 U of AmpliTaq polymerase (Perkin Elmer/Cetus). Ten
microliters of the respective PCR reactions were loaded on analyt-
cal agarose gels and the amplified fragments were visualized by
ethidium bromide staining. A wild-type fragment was amplified
with primer A: nt 10714–10737 and primer B: nt 12576–12557.
Deleted DNA was amplified with primer A: nt10712–10728 and
primer B: nt 15360–15343. Sequencing was done using asymmet-
rical amplified (primer nt 11141–11158) single-stranded mtDNA
as described [9].

Results

Morphology

Muscle biopsy revealed mild myopathic changes with nu-
merous ragged red fibers. Those fibers stained also hyper-
intensely in NADH-tetrazolium reductase and succinate
dehydrogenase stains (Fig. 1a) and showed an increased
lipid deposition in the oil red O stain. Electron micros-
copy revealed many fibers with an increase in the number
of mitochondria, enlarged mitochondria and structural al-
terations such as distorted cristae or paracrystalline inclusions
(Fig. 1b). The sural nerve appeared normal by light
and electron microscopy.

Biochemistry

Analysis of the respiratory chain enzymes in muscle
showed normal activities for rotenone-sensitive NADH
dehydrogenase (complex I), NADH cytochrome c reduc-
tase (complex I + III), succinate dehydrogenase (complex
II), succinate cytochrome c reductase (complex II + III)
and cytochrome c oxidase (complex IV).

Molecular genetics

Southern blot analysis with hybridization to a radioac-
tively labeled probe ranging from nt 4831 to 5917 showed
a second, faster migrating form of mtDNA at 13.4 kb, in-
dicating a 3.1-kb deletion (Fig. 2, lane 1, PvuII restric-
tion). The absence of this faster migrating band and the
appearance of two slower migrating bands with BamHI