Deletions of mitochondrial DNA in Kearns-Sayre syndrome

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Article abstract—We have identified large-scale deletions in muscle mitochondrial DNA (mtDNA) in seven of seven patients with Kearns-Sayre syndrome (KSS). We found no detectable deletions in the mtDNA of ten non-KSS patients with other mitochondrial myopathies or encephalomyopathies, or three normal controls. The deletions ranged in size from 2.0 to 7.0 kb, and did not localize to any single region of the mitochondrial genome. The proportion of mutated genomes in each KSS patient ranged from 45% to 75% of total mtDNA. There was no correlation between the size or site of the deletion, biochemical abnormality of mitochondrial enzymes, or clinical severity. The data bolster arguments that KSS is a unique disorder and genetic in origin.

NEUROLOGY 1988;38:1339-1346

Holt, Harding, and Morgan-Hughes¹ opened a new field of investigation when they found deletions of mitochondrial DNA (mtDNA) in nine of 25 patients with "mitochondrial myopathies," defined by the appearance of morphologically abnormal organelles in muscle biopsy. However, they did not describe the clinical syndromes of the patients. As part of a continuing project in the molecular biology of mitochondrial diseases,^{2,3} we have used similar methods to study muscle mtDNA in patients with Kearns-Sayre syndrome (KSS) and related disorders, to determine whether there is any correlation between clinical phenotype, biochemical abnormality, and alteration in mtDNA.

Materials and methods. *Materials.* Restriction enzymes were from Boehringer Mannheim and New England Biolabs; Klenow fragment of *Escherichia coli* DNA polymerase I was from New England Biolabs; RNase A and hybridization chemicals were from Sigma; [α -³²P]dNTPs (800 Ci/mmol) were from New England Nuclear.

Patients. Patients were classified as follows: Seven patients had the Kearns-Sayre syndrome. Five were studied by us; all had ophthalmoplegia, pigmentary retinopathy, onset before age 20, and at least one of the following: high CSF protein content, heart block, or ataxia. The other two patients (nos. 4 and 6) were studied at other centers and were said to be characteristic of KSS cases. None had a relative with any of these manifestations (table 1).

Two patients had MELAS (myopathy, encephalopathy, lactic acidosis, and stroke-like episodes). Both had all of the following five abnormalities: proximal limb weakness, ragged red fibers, intermittent vomiting, lactic acidosis, and stroke-like episodes. Neither had pigmentary retinopathy.

Neither had affected relatives.

Two patients had MERRF (myoclonic epilepsy with ragged red fibers), defined by the following: clinical evidence of spinocerebellar degeneration, myoclonus, myoclonic epilepsy, and ragged red fibers in muscle. Neither had pigmentary retinopathy. One had an affected mother and two affected sisters. The other patient had affected siblings and half-siblings with the same mother. Both pedigrees were compatible with maternal inheritance. In the second family, an asymptomatic brother and the asymptomatic father of the patient were also studied.

Three patients had no clinical abnormality other than ophthalmoplegia and limb weakness; all had ragged red fibers. None had pigmentary retinopathy.

One patient had no ophthalmoplegia or retinopathy but had a myopathy of facioscapulohumeral distribution and cardiomyopathy; he also had ragged red fibers.

Two patients had Leigh's syndrome with cytochrome c oxidase deficiency.⁴ The diagnosis was confirmed on the basis of clinical features, brain CT, biochemistry and, in one patient, findings at autopsy.

We used three controls: the father in one of the MERRF families, and two autopsy cases of patients with no neurologic disease.

Biochemical analysis of patient tissues. Analyses of mitochondrial enzyme activities were as described.⁴

Preparation of total patient DNA. Because we had only small specimens of frozen muscle biopsies available to us, it was difficult to isolate intact mitochondria in sufficiently high yield. Therefore, total DNA was isolated from 10 to 150 mg of frozen muscle taken at biopsy, using the method of Davis et al⁵ scaled down by a factor of 20 to accommodate the smaller sample size used here. Two steps of the method were modified as follows: the incubation with SDS/proteinase K was per-

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Supported by Center Grants from NINCDS (NS11766); RR-00645; and the Muscular Dystrophy Association. H.N. is supported by a grant from the Uehara Scientific Foundation, M.Z. by a grant from the Associazione Italiana per le Ricerche Neurologiche, C.T.M. by the Brazilian Research Council (CNPq), and E.A.S. by a grant from the Aaron Diamond Foundation.

Received May 5, 1988. Accepted for publication in final form June 30, 1988.

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Table 1. Clinical and laboratory features of seven patients with KSS

Clinical & laboratory features	Patients						
	1	2	3	4	5	6	7
Sex	M	M	F	F	M	F	M
Age at biopsy	15	7	28	33	27	26	12
Age at onset	8	4	14	24	17	14	3
Ophthalmoplegia	+	+	+	+	+	+	+
Pigmentary retinopathy	+	+	+	+	+	+	+
Heart block	+	+	+	—	—	—	—
CSF protein (mg/dl)	121	112	114	ND	65	ND	85
Cerebellar syndrome	—	+	+	+	—	—	+
Mental retardation or dementia	+	—	+	—	—	+	+
Ragged red fibers in muscle	+	+	+	+	+	+	+

ND Not determined.

formed for 2 hours at 50 °C, and the DNA was precipitated with isopropanol and then spooled, followed by washing in 70% ethanol. We routinely recovered about 1 to 2 µg total DNA per mg muscle.

Preparation of mtDNA probes. Mitochondria were isolated as described⁶ from normal frozen human liver. Mitochondrial DNA was then isolated using the method of Palva and Palva.⁷ For probes, 25 ng of mtDNA was linearized with *PvuII*, and then labeled in the presence of [α -³²P-dCTP] or [α -³²P-dTTP] by the random-primer method.⁸

Southern analysis. Five µg of mtDNA was digested with restriction enzyme, electrophoresed through a 0.8% agarose gel, and transferred to nitrocellulose (Schleicher and Schuell) as described.³ The filters were hybridized with about 5×10^6 cpm/ml of labelled mtDNA (specific activity about 5×10^8 cpm/µg) overnight at 65 °C, and washed and exposed for 2 to 4 hours to Kodak XAR-5 film with an intensifying screen (DuPont Cronex) at -70 °C as described.³ The possibility that hybridizing sequences present in the nuclear genome⁹ might interfere with the Southern analysis of authentic mitochondrial DNA was obviated by the short exposure times of autoradiography. Only high copy-number sequences (ie, mtDNA) are observable after short exposure times.

Detection of deletions and quantitation of mtDNA populations. Unlike nuclear DNA (which is linear), human mtDNA is circular. The two strands of the mtDNA duplex are called "heavy" and "light," based on their densities in CsCl gradients.¹⁰ It is possible to produce a full-length, 16.5-kb linear molecule by cutting the mtDNA with a restriction enzyme that has only one recognition site in the mitochondrial genome, such as *PvuII* (which cleaves at map position 2,650¹¹). In Southern blots, therefore, *PvuII*-digested normal mtDNA appears as a single band 16.5 kb in length. However, if a portion of the mtDNA has been deleted, digestion with *PvuII* will result in a linear molecule that migrates more rapidly in the gel (assuming that the *PvuII* site itself has not been deleted).

Thus, if a tissue contains mitochondria with both normal and deleted mtDNAs, cleavage with *PvuII* will produce two populations of molecules, one migrating in the gel at the normal position and another migrating more rapidly (because it is smaller). The appearance of two bands on a Southern blot is diagnostic of a subpopulation of deleted mitochondrial genomes (figure 1). If the autoradiograph is exposed for a short enough time for the bands to be in the linear range on the x-ray film, densitometry can be used to assess the relative proportions of the two bands, assuming that the total hybridization

in both bands accounts for all of the mtDNA (figure 2). On an equimolar basis, smaller fragments give a less intense signal than larger fragments; the densitometric data are therefore corrected for size, and the total signal is given a value of 100%. Each Southern analysis represents hybridization of mtDNA from the entire biopsy sample; we were unable to compare the proportion of deleted genomes in different muscle samples from the same patient.

Identification of the site of the deletion in mtDNA.

Cleavage of mtDNA with the "single-cutter" *PvuII* gives information only about the size but not about the location of the deletion. To identify the site of the deletion, we cut the patient DNA with several restriction enzymes, each of which has multiple recognition sites in normal mtDNA—*EcoRI*, *PstI*, *HindIII*, *KpnI*, *StuI*, or *XbaI* (figure 3A).¹¹ (Not all patient DNA was cut with all six enzymes; some were cut with only four.) If a certain deletion included a particular restriction site, that enzyme would not cut at that site. As a result, the Southern blot would show a hybridizing band different in size than bands hybridizing to control DNA. The size of the anomalous band would indicate the distance to the nearest retained restriction site(s), and would also give an estimate of the size of the deletion. However, deletion of an entire restriction fragment (ie, two adjacent restriction sites) would not result in the absence of the appropriately sized band on the blot. Rather, the blot would display a normal-sized band with reduced intensity, corresponding to hybridization of the probe to the subpopulation of normal mtDNAs, which, of course, retained the restriction sites missing in the deleted genomes. Identification of these "reduced-intensity" bands provided further information about the location of missing sites in the deleted mtDNA. The data on "new" and "reduced-intensity" bands made it possible to estimate the location of each deletion (figure 3B) and the size of the deleted fragments in each patient (figure 4B). Each breakpoint of a deleted fragment was contained within a region of uncertainty (hatched areas in figure 4B) defined by the interval between pairs of retained and deleted restriction sites. As an example of this analysis, the deletion in the mtDNA of patient 3 (figure 4) extended from at least position 9,020 (*PstI* site loss) to position 12,978 (*StuI* site loss), but not beyond position 8,286 (*XbaI* site preserved) or 13,701 (*StuI* site preserved), a range of 3.9 to 5.4 kb. However, based on the *PvuII* mapping data, the size of the deletion in patient 3 was estimated to be 4.5 kb. Thus, the region of uncertainty was not 1.5 kb (ie, 5.4 - 3.9), but a smaller area of either 0.6 kb (ie, 4.5 - 3.9) on the "*XbaI* side" or 0.9 kb (ie, 5.4 - 4.5) on

Figure 1. Southern analysis of mtDNA in KSS. Five μ g of total DNA was digested with *PvuII*, electrophoresed through a 0.8% agarose gel, transferred to nitrocellulose, and probed with labeled mtDNA. Two controls (lanes C1 and C2) and the seven KSS patients (lanes 1 through 7) are shown. Marker sizes (lambda digested with *HindIII*) are shown at the left, in kb.

the "StuI side"; we chose the region of uncertainty to be the smaller of these two values.

Results. We performed genomic Southern analysis of total DNA isolated from seven patients with KSS, ten disease controls, and three normal controls, using mtDNA as the hybridization probe. Because mtDNA genomes are present in multiple copies per cell, the hybridization signal resulting from the mtDNA probe was easily detectable after exposure of the film for a few hours.

Using the restriction enzyme *PvuII*, which cuts only once in the mtDNA genome (and hence produces a single, 16.5 kb, linear molecule in normal mtDNA), the hybridization analysis in all seven patients with KSS showed a pattern consistent with the presence of two populations of mtDNA, one corresponding to full-length mtDNA molecules, and the other, migrating more rapidly, corresponding to smaller mtDNAs (minus the deleted fragment) (figure 1). The size of the deletions ranged from 2.0 kb in patient 4 to 7.0 kb in patient 1; patients 3 and 6 had identically sized deletions of 4.5 kb (figure 1; table 2). None of the other (non-KSS) patients or normal controls showed any evidence of a deletion, although formally we could not exclude a microdeletion that was not detectable in these blots.^{12,13}

The relative number of normal and deleted mitochondrial genomes in each KSS patient's muscle was estimated by densitometry (figure 2). The degree of hybridization was corrected for the length of the hybridizing fragment. The relative number of mutated

Figure 2. Quantitation of normal and deleted mtDNAs in KSS patients. The relative proportion of hybridizing signal from the full-length and deleted molecules is presented as a histogram, with 100% representing the total mtDNA population. Shaded boxes denote the proportion of normal full-length mtDNAs; hatched boxes denote the proportion of deleted mtDNAs.

genomes varied widely among patients, ranging from 45% to 75%.

To map the site of the deletions, we performed Southern analysis with other restriction enzymes whose map locations on the mitochondrial genome are known (figure 3A).¹¹ The presence of two populations of sizes of mtDNA genomes could be attributed to extra bands or reduced intensity (ie, nonstoichiometric relationships) among digested fragments. As an example of a mapping experiment (figure 3B), DNA from patient 2 was digested with *EcoRI*, *HindIII*, *PstI*, or *XbaI*. With each enzyme, the control DNA gave the Southern hybridization pattern predicted by the normal mtDNA map, while the patient's mtDNA showed an extra band (eg, the 9.3-kb *EcoRI*, 10.4-kb *HindIII*, 8.3-kb *PstI*, and 2.7-kb *XbaI* fragments).

In addition, the two populations of mtDNA were revealed by differences in the intensity of hybridization of one or more restriction fragments. Fragments that were conserved in normal and deleted genomes were more intense than those found only in the normal genome but missing in the deleted one. Based on the size of the extra bands and of those in reduced amount, we calculated the size and location of each deletion (figure 4).

The location of the deletion was different in each patient, with one exception. Not only did patients 3 (low cytochrome *c* oxidase activity) and 6 (normal

Figure 3. Mapping the deletion of mtDNA in KSS patient 2. (A) Map of the human mitochondrial genome,¹¹ showing the restriction enzyme sites used to map the deletions in all seven KSS patients. E = EcoRI, H = HindIII, K = KpnI, P = PvuII, Pt = PstI, S = StuI, X = XbaI. (B) Autoradiograph of a Southern analysis of total DNA (5 µg in each lane) from a control subject (C) and KSS patient 2 (P), digested with the denoted enzymes. Size markers (lambda digested with HindIII) are shown at left, in kb. The mtDNA deletions from the other six KSS patients were mapped in a similar manner.

cytochrome *c* oxidase activity) have identically sized deletions, but both deletions mapped to the same region of mtDNA (figure 4) and, within the level of resolution of our analysis, deleted the same genes (table 2).

Biochemical analysis of mitochondrial enzymes in muscle biopsies of six KSS patients showed a partial defect of cytochrome *c* oxidase activity in patients 3, 5, and 7 (table 3). Other enzymatic activities of the respiratory chain were normal, as was citrate synthase, a matrix enzyme.

Discussion. We found deletions of mitochondrial DNA in all seven cases of Kearns-Sayre syndrome studied, but there were no detectable deletions in cases of MELAS, MERRF, or ophthalmoplegic syndromes with ragged red fibers. We know of three other cases of KSS with deletions of mtDNA (reference 14 and A.E. Harding, personal communication), so that deletions have been found in all ten cases of KSS so studied.

This information addresses two questions about KSS. (1) It is uncertain whether KSS is a specific disorder, or merely the full expression of a pleiotropic disease in which ptosis or ophthalmoplegia alone might be isolated manifestations.¹⁵ (2) It is uncertain whether KSS is inherited or acquired, because almost all fully expressed cases have been "simplex," with no other cases in the family.¹⁵

Specificity of KSS. The finding of deletions in KSS

but not in the clinically different syndromes of MERRF or MELAS reinforces the view that KSS is a specific disorder. However, it is already evident that not all deletions of mtDNA are associated with KSS.¹ Moreover, it cannot yet be stated that deletions will be found in all cases of KSS. For these reasons, KSS could still be the full expression of a pleiotropic disorder, but in addition to clinical evidence,¹⁵ the mtDNA data make it more likely that KSS is a specific disorder. Analysis of mtDNA may even be diagnostically useful in cases that meet some but not all criteria for diagnosis.

Etiology and pathogenesis of KSS. The finding of deletions in mtDNA in all cases of KSS so studied suggests that the deletions are involved in pathogenesis. However, this is not proven, because there was no relationship between site or size of the deletion and biochemistry or severity of the disease. Of the six mitochondrial enzyme activities studied, only cytochrome *c* oxidase was decreased, and that was seen in three of six patients so studied (table 3). Although all the deletions of mtDNA were large enough to be obviously different from controls on agarose gels, they ranged in size from 2.0 kb in patient 4 to 7.0 kb in patient 1. Also, the ratio of normal to deleted mitochondrial genomes in muscle varied from 25:75 in patient 3 to 55:45 in patient 1. The fact that no patient had less than 45% deleted mtDNAs supports the hypothesis that phenotypic expression of a mitochondrial disease

Figure 4. Localization of deletions of mtDNA in KSS. (A) Map of the human mitochondrial genome. The structural genes for the mitochondrial-encoded subunits of NADH-coenzyme Q reductase (ND),³³ cytochrome c oxidase (CO), cytochrome b (Cyt. b), ATP synthase (ATPase),³⁴ the 12S and 16S ribosomal RNAs (rRNA), and each tRNA (1-letter amino acid nomenclature) are shown. The origins of heavy-strand (O_H) and light-strand (O_L) replication, and of the promoters for initiation of heavy-strand (P_{H1} and P_{H2}) and light-strand (P_L) transcription are shown by arrows. (B) Maps (same as in A) of the deletion in each of the seven KSS patients. Numbering of the patients is as in figure 1. The open pie-shape denotes the location and size of the deletion; the hatched pie-shapes denote the region of uncertainty (see text).

requires a minimal "threshold" number of mutated mtDNAs.¹⁶ Furthermore, there was no obvious correlation between severity of symptoms or biochemical abnormality and location of the mtDNA deletion or number of deleted mitochondrial genomes. The only clear-cut correlation was that all KSS patients had deletions in mtDNA and none of the other patients had any detectable deletion.

Although the deletions differed among patients, there were some common features. All deleted genomes preserved the promoters of heavy- and light-strand transcription, the 12S and 16S ribosomal RNA genes, and the origin of heavy-strand replication. Six of the seven deletions also spared the origin of light-strand replication; in patient 1, it was uncertain whether the deletion included the origin of light-strand replication, because the origin fell within the region of uncertainty. Since the deleted genomes were seen in large numbers in these patients (regardless of the age of the patient), all of the deleted mtDNAs must have been competent for replication.

The deleted mtDNAs could be transcriptionally active, but since they lack indispensable tRNAs, translation would be impossible in any organelle that contained only mutated mtDNAs. However, translation of mutant mtRNA could take place in organelles containing both normal and mutant genomes by intra-

organelle complementation; that is, the residual normal mtDNA could serve for both transcription and translation. Normal mitochondria contain multiple copies of the mitochondrial genome (from 2 to 10 mtDNAs in each mitochondrion^{17,18}) and complementation has been demonstrated in cultured cells.¹⁶ On the other hand, a mitochondrion bearing only mutant mtDNAs might still be viable, even in the absence of a functionally active, endogenous respiratory chain, because it might be able to import essential molecules, including ATP,¹⁹ from the cytoplasm.²⁰⁻²²

Our data do not imply that all deletions of mtDNA must cause KSS, or that all patients with KSS must have detectable deletions of mtDNA, but the data suggest that most patients fulfilling the clinical and morphologic criteria of KSS will be found to have deletions in mtDNA. The uniformity of phenotype among KSS patients contrasts with the heterogeneity of the deletions. This might be explained if the entire mitochondrial genome functions as a single genetic entity rather than as a collection of individual genes. In fact, transcription of mtDNA is polycistronic²³; that is, all the genes encoded on the heavy and light strands are transcribed as two large precursor RNAs, which are cleaved into the individual tRNAs and mRNAs in subsequent steps.^{16,23-25} Thus, a major deletion anywhere in the genome may have serious consequences on transcrip-

Table 2. Size and location of deleted regions in mtDNA of KSS patients*

	Patients						
	1	2	3	4	5	6	7
Deletion (kb)	7.0	6.0	4.5	2.0	4.2	4.5	5.4
Map coordinates	5275 (E) 11680 (H)	9020 (Pt) 16048 (K)	8286 (X) 13701 (S)	7440 (X) 10256 (X)	8286 (X) 13701 (S)	8286 (X) 13701 (S)	10256 (X) 16048 (K)
Retained regions							
O _H , P _H , P _L	+	+	+	+	+	+	+
12S rRNA	+	+	+	+	+	+	+
16S rRNA	+	+	+	+	+	+	+
ND1	+	+	+	+	+	+	+
ND2	?	+	+	+	+	+	+
O _L	?	+	+	+	+	+	+
COI	—	+	+	+	+	+	+
COII	—	+	+	?	+	+	+
ATPase8	—	+	?	—	?	?	+
ATPase6	—	?	—	—	—	—	+
COIII	—	?	—	—	—	—	+
ND3	—	—	—	?	—	—	+
ND4L	—	—	—	+	—	—	?
ND4	—	—	—	+	—	—	—
ND5	?	—	—	+	—	—	—
ND6	+	—	+	+	+	+	—
Cytb	+	—	+	+	+	+	—
7S DNA	+	?	+	+	+	+	?

* The estimated sizes of the deletions are based on the mapping analyses. The distance between the map coordinates for each deletion differs from the actual measured size, because it denotes only the borders of retained restriction sites (notation of figure 3A). Retained (+) and deleted (—) genes in each deleted mtDNA are shown; genes present in the region of uncertainty are indicated with a (?). The gene regions are designated as in figure 4.

tion (and translation) even of those genes that are not encompassed by the deletion.^{26,27}

In support of this view is the observation that patient 7 had a 50% reduction of cytochrome *c* oxidase activity, but had a deletion of mtDNA that spared all three mitochondrial-encoded subunits of cytochrome *c* oxidase; conversely, patients 1, 4, and 6 all had normal levels of cytochrome *c* oxidase activity, but had at least one cytochrome *c* oxidase subunit gene deleted. In patient 1, all three subunits were deleted. The discrepancy between enzyme activity and presence or absence of genes encoding subunits for the enzymes of the respiratory chain may be due to intracellular or intraorganelle complementation.

Although the biochemical abnormality is not known, the deletions almost certainly have something to do with pathogenesis because the only mtDNA deletions described in any asymptomatic individuals who have been studied are tiny (about 10 bp), and are localized to noncoding regions.^{12,13,28,29}

We did not find deletions in ophthalmoplegic syndromes that lacked the other manifestations of KSS, but it would be important to study mtDNA in all cases of ophthalmoplegia with ragged red fibers and other "incomplete" cases of KSS to determine the full range of manifestations. In addition, there is evidence that deletions similar in size and location to those found in KSS may be associated with some other syndrome.¹

Etiology of the deletions. KSS seems to be a genetic disease, but not necessarily an inherited one. Although the deletions of mtDNA presumably cause KSS, it is

not clear how they arise or how they cause the disease. The deletions could arise from either of two possible mechanisms: (1) inheritance of a preexisting deletion in the mother, or (2) spontaneous mutation of mtDNA, either in the oocyte or in the zygote. Against the first possibility, and in support of the second, is the absence of affected mothers in KSS pedigrees and the paucity of affected siblings. However, we cannot rule out maternal inheritance completely, because the mother could carry a number of defective mitochondria below the threshold number required for phenotypic expression.¹⁶ We plan to analyze tissues from mothers of individuals affected with KSS for the presence of deleted mtDNAs.

The presence of two patients with deletions that are apparently identical in size and in location raises the intriguing possibility that the sites of the deletions are not random. There may be "hot spots" on the mitochondrial genome that are more prone to deletion than other regions of mtDNA. Only direct DNA sequencing of each of the deletion breakpoints will help resolve this issue.

We have previously considered the possibility that KSS is a lethal autosomal dominant mutation, not lethal for the patient but rather in the sense that affected individuals do not reproduce (because they are disabled or for other reasons). If so, the only clinical cases would be new mutations. Advanced paternal age in KSS is consistent with that pattern of inheritance.³⁰ Based on the results presented here, however, KSS is not an autosomal dominant mutation, but rather a genetically lethal mitochondrial mutation. If so, advanced mater-

Table 3. Mitochondrial enzymes in crude muscle preparations of six patients with KSS and in controls*

Patient	COX	Succinate-Cyt c red	NADH-Cyt c red	SDH	NADH-DH	CS
1	2.16	0.70	1.17	2.09	25.86	6.86
3	0.40	0.65	0.76	1.70	32.26	8.09
4	3.33	0.72	0.72	1.00	26.88	10.29
5	0.72	0.47	1.32	0.61	28.20	6.98
6	2.34	0.76	1.08	1.61	24.49	9.80
7	0.97	0.53	0.90	2.15	30.86	17.65
Controls						
Mean	2.80	0.70	1.02	1.00	35.48	9.88
± SD	0.52	0.23	0.38	0.53	7.07	2.55
n	71	69	78	78	76	73

*Activities are μ moles substrate utilized/min/g fresh tissue.

COX Cytochrome c oxidase.
SDH Succinate dehydrogenase.
NADH-DH NADH-coenzyme Q reductase.
CS Citrate synthase.

nal age may be important, but this remains to be documented (eg, mtDNA analyses of individual ova from mothers of KSS patients). This interpretation is consistent with the paucity of fully documented cases of KSS in parent and child, siblings, or twins; there have been only single pairs of each.³⁰

Although we do not know how these deletions arise, the presence of multiple copies of an identical deletion in muscle fibers implies that the deletion is a clonal amplification of a single initial mutational event.³¹ Since we have not yet analyzed mtDNA in tissues or organs other than muscle, it is not known if the deletion is confined to muscle or is widespread, as is suggested by the multisystem nature of KSS. If the deletion is found in several tissues or organs, the mutation must have occurred early in development, or even in the oocyte. It is known that mitotic segregation can result in different numbers of mitochondria in different tissues, or even between cellular subpopulations of the same tissue or organ.¹⁶ Thus, the pleiotropic nature of KSS could depend on the proportion of normal and deleted mtDNAs in different organs.

Finally, the present findings make it unlikely that a viral infection early in life alters mtDNA to cause the syndrome. There is no evidence that a persistent viral infection, as previously suggested for KSS,³⁰ could cause large deletions of mtDNA, and there is no evidence of any insertion of foreign (viral) DNA in these cases.³² However, until we know how the deletions arise and how deletions cause the clinical manifestations of KSS, it is necessary to consider the possibility that deletions of mtDNA in KSS may be an effect rather than a cause.

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Neurology 1988;38;1339

DOI 10.1212/WNL.38.8.1339

This information is current as of August 1, 1988

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