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Lack of Transmission of Deleted mtDNA from a Woman with Kearns-Sayre Syndrome to Her Child

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Summary

We have investigated the daughter of a woman with Kearns-Sayre syndrome. The woman had a high percentage of deleted mtDNA in muscle, but no deleted mtDNA was detected in fibroblasts, bone marrow, and peripheral blood cells by Southern blot analysis. With PCR, analytical sensitivity was significantly increased, and deleted mtDNA was detected in all examined tissues from this patient. The patient had healthy parents and nine healthy siblings. No deleted mtDNA was detected in blood from the mother of the patient. The patient had an uneventful pregnancy and delivered at term. Deleted mtDNA could not be detected in placenta by Southern blot analysis. With PCR, deleted mtDNA was detected in the majority of placental specimens. This finding may, however, be due to contamination with maternal DNA. The patient's daughter was healthy at age 5 mo, and morphologic examination of muscle was normal. No transmission of deleted mtDNA to the daughter could be detected by Southern blot and PCR analysis of peripheral blood cells, bone marrow, fibroblasts, and muscle. The presence of deleted mtDNA was excluded at a fractional level of less than 1:100,000 in all examined tissues from the daughter.

Introduction

Kearns-Sayre syndrome (MIM 165100) is a progressive multisystem disease characterized by onset before age 20 years, ophthalmoplegia, retinal pigmentary degeneration, and at least one of the following manifestations: heart block, elevated cerebrospinal fluid protein level, or ataxia (Rowland et al. 1988). Most cases are sporadic (Rowland et al. 1988) and show heteroplasmy with large deletions of mtDNA in muscle (Moraes et al. 1989). Deletions of mtDNA are also found in Pearson marrow-pancreas syndrome, which is characterized by severe sideroblastic anemia (Rötig et al. 1990). Patients who survive this disorder of infancy may later develop Kearns-Sayre syndrome (Larsson et al. 1990; McShane et al. 1991). There is often a widespread tissue distribution of deleted mtDNA in both Kearns-Sayre syndrome and Pearson syndrome, and the fraction of deleted mtDNA is high in organs manifesting disease, e.g., muscle, brain or bone marrow (Moraes et al. 1989; Larsson et al. 1990; Rötig et al. 1990; Shanske et al. 1990). It is currently unclear whether women with Kearns-Sayre syndrome can transmit deleted mtDNA to their offspring. We have investigated the daughter of a woman with deleted mtDNA and Kearns-Sayre syndrome.

Case Reports

The patient with Kearns-Sayre syndrome is the third child in a sibship of 10. Her parents and siblings are healthy. The patient was healthy until age 16 years, when her vision began to deteriorate. Ptosis and progressive ophthalmoplegia were noted from age 20 years. A pacemaker was implanted at age 25 years because of atrioventricular block with syncope. She gave birth to a daughter at age 26 years. The patient was investigated 5 mo after delivery. She had ptosis, ophthalmoplegia, retinal pigmentary degeneration,

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and muscular weakness. A muscle specimen was taken from the quadriceps muscle by open biopsy. Morphologic examination of muscle tissue showed mitochondrial myopathy. Polarographic and spectrophotometric measurement of the respiratory-chain function in isolated muscle mitochondria revealed a deficiency of complexes I and IV.

The daughter was investigated at age 5 mo. She was apparently healthy and had developed normally. A muscle specimen was taken from the quadriceps muscle by needle biopsy.

Routine laboratory investigations revealed no liver, kidney, or hematologic involvement and bone-marrow morphology was normal in both the patient and her daughter. Blood specimens were obtained from the healthy parents of the patient, for PCR analysis. The patient and her daughter were investigated after informed consent was obtained. The present study is part of an ongoing study on mitochondrial disorders that has been approved by the Ethical Committee of the Medical Faculty, Gothenburg University.

Material and Methods

Morphologic and biochemical analyses of muscle tissue (Tulinius et al. 1991), Southern blot analysis of mtDNA (Larsson et al. 1990), and PCR and direct sequencing (Larsson et al. 1991) were done according to methods described elsewhere.

For asymmetric PCR, 50 pmol of a primer corresponding to nucleotides 12801–12820 (NG42) of the light chain of mtDNA and 4 pmol of a primer corresponding to nucleotides 7377–7396 (NG21) of the heavy chain were used. For sequencing, 0.5 pmol of a primer corresponding to nucleotides 7631–7650 (NG45) of the heavy chain was used. For PCR analysis of mtDNA in different tissues, the primer NG45 and a primer corresponding to the nucleotides 12401–12420 (NG46) of the light chain were used. This pair of primers gave a fragment of 124 bp only when deleted mtDNA was present.

DNA was prepared from 26 scattered specimens from the placenta and from one specimen from the umbilical cord. The specimens were extensively washed in saline to remove gross maternal blood contamination. For deleted mtDNA, the detection limit for Southern blot and PCR analyses was determined by analyzing the patient's muscle DNA in 10-fold serial dilutions in DNA from normal muscle tissue.

Results

Southern blot analysis of mtDNA in muscle from the patient showed heteroplasmy with deletion of mtDNA. The fraction of deleted mtDNA in muscle was 70%. No deleted mtDNA was detected in peripheral blood cells, bone marrow, and fibroblasts. With PCR, analytical sensitivity was significantly increased, and deleted mtDNA was detected in all examined tissues from the patient (fig. 1). Sequencing of the breakpoint region showed that the mtDNA sequence between nucleotides 7697 and 12364 was deleted. No repeats were detected at the junction site.

Morphologic examination of muscle from the pa-

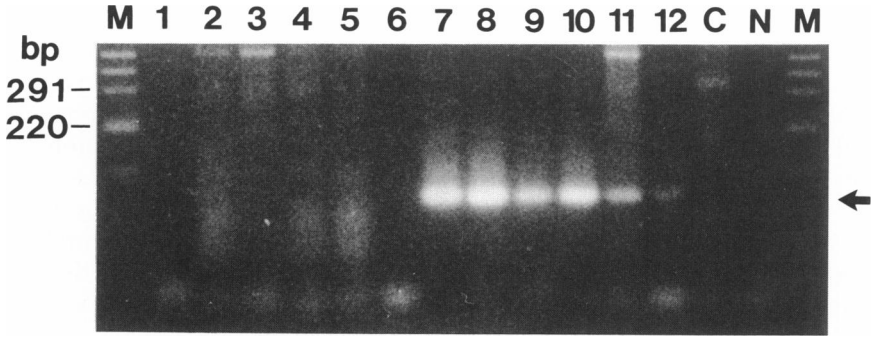

Figure 1 PCR analysis of deleted mtDNA in various tissues from a woman with Kearns-Sayre syndrome and her daughter. PCR was used to amplify a 124-bp fragment (arrow) corresponding to the breakpoint region of the deleted mtDNA molecule, by using DNA from different tissues from the daughter (lanes 1–6) and her mother (lanes 7–12) and a normal individual (lane C). A negative control without DNA (lane N) and size markers (lane M) are also shown. The analysis of DNA from muscle (lanes 1 and 7), fibroblasts (lanes 2 and 8), bone marrow (lanes 3 and 9), lymphocytes (lanes 4, 10, and C), granulocytes (lanes 5 and 11), and thrombocytes (lanes 6 and 12) are shown. PCR products were separated by electrophoresis in an agarose gel with ethidium bromide.

tient's daughter revealed no abnormalities. The respiratory-chain function was not analyzed. No deleted mtDNA was detected in muscle, peripheral blood cells, bone marrow, and fibroblasts, either by Southern blot or PCR analysis (fig. 1).

With Southern blot analysis, no deleted mtDNA was detected in the placenta. With PCR, deleted mtDNA was detected in 20 of the 26 placental specimens and in the umbilical cord specimen. With PCR, no deleted mtDNA was detected in blood from the mother (and father) of the patient.

The fraction of deleted mtDNA detectable by Southern blot analysis was about 1:100 (results not shown); the detection limit for PCR was 1:100,000 (fig. 2).

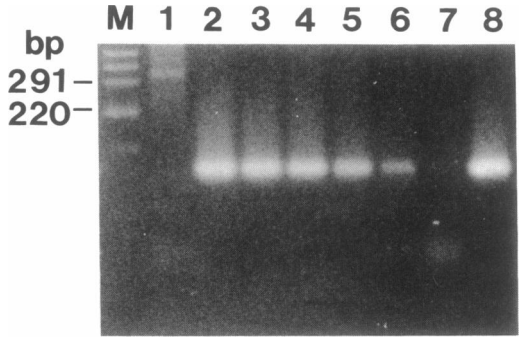

Figure 2 PCR analysis of serial dilutions of deleted mtDNA. PCR was used to amplify a 124-bp fragment corresponding to the breakpoint region of the deleted mtDNA molecule, by using 100 ng of muscle DNA from a normal individual that was mixed with 10 ng, 1 ng, 0.1 ng, 10 pg, 1 pg or 0.1 pg of the patient's muscle DNA with mtDNA deletion (lanes 2–7, respectively). Controls with 100 ng of either normal muscle DNA (lane 1) or muscle DNA with mtDNA deletion (lane 8) are also shown, as is a size marker (M). PCR products were separated by electrophoresis in an agarose gel with ethidium bromide.

Discussion

We have found no transmission of deleted mtDNA to the daughter of a woman with Kearns-Sayre syndrome. The presence of deleted mtDNA in all examined tissues from the daughter was excluded at a level of less than 1:100,000. This means that the majority of the daughter's cells probably contain exclusively normal mtDNA. The probability for future development of Kearns-Sayre syndrome in the daughter because of transmission of deleted mtDNA from her affected mother thus seems unlikely, although a progressive increase of deleted mtDNA with age has been found in Kearns-Sayre syndrome (Larsson et al. 1990).

Deleted mtDNA was detected in most, but not all, placental tissue specimens. The placenta contains both maternal and fetal tissue and is in close contact with maternal tissues during delivery. The PCR assay is extremely sensitive and detects a very low grade of contamination. The finding of deleted mtDNA in this placenta—and even in the umbilical cord—is therefore difficult to interpret. The failure to detect deleted mtDNA in some placental specimens indicates that some parts of the placenta contained very little, if any, deleted mtDNA.

There have been no previous reports of childbirth in women with Kearns-Sayre syndrome. Low levels of identical mtDNA deletions have been found in the healthy mother and sister of a patient with Kearns-Sayre syndrome (Poulton et al. 1991). This indicates that deleted mtDNA was present in more than one germ cell in the mother. A woman and her daughter who both had progressive external ophthalmoplegia but two different mtDNA deletions have also been described (Ozawa et al. 1988). The patient's mother in the family that we studied did not have any detectable amounts of deleted mtDNA in blood, and this is in accordance with the results from studies of other families (Moraes et al. 1989; Zeviani et al. 1990).

Most cases of Kearns-Sayre syndrome are sporadic and are probably due to de novo deletions of mtDNA. The widespread tissue distribution of identical deleted mtDNA in our and other cases is only possible if the deletion occurs either in the oocyte or at a very early stage of embryogenesis. However, once established, the mtDNA deletions should be maternally transmitted according to the principles of mitochondrial inheritance.

Deleted mtDNA was found in all examined tissues from our patient. Although this has not been proved, we find it likely that deleted mtDNA also was present in the oocytes, which is a prerequisite for maternal transmission. To assess the probability for such transmission, both random and selective factors have to be considered. Investigations in Holstein cows have shown that heteroplasmic mtDNA point mutations are maternally inherited and that the proportion of the different mtDNA species varies considerably between animals of the same sibship (Ashley et al. 1989). This rapid shift in the levels of point-mutated mtDNA has also been found in humans with mitochondrial diseases, e.g., MERRF (myoclonus epilepsy and ragged-red fibers) syndrome (Shoffner et al. 1990) and Leber hereditary optic neuropathy (Vilkki et al. 1990). It has

been suggested that this rapid shift is due to a founder effect in which only a fraction of the thousands of mtDNA copies in the oocyte are transmitted to the offspring (Ashley et al. 1989). The proportion of mutated mtDNA molecules in the oocyte, together with the absolute numbers of mtDNA molecules that belong to the founder population, then determine the risk of random transmission to the fetus. The findings of spontaneous recovery in patients with Pearson syndrome, together with progressive increase of deleted mtDNA in muscle in Kearns-Sayre syndrome patients, indicate that both negative and positive selection of deleted mtDNA may occur (Larsson et al. 1990). Selection of deleted mtDNA may also be of importance during the rapid cell division of embryonal development and may, together with random transmission, determine whether the fetus is affected.

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