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Prenatal Diagnosis of Mitochondrial DNA$^{8993\ T\rightarrow G}$ Disease

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Summary

We have previously described a family with a neurological syndrome comprising neurogenic muscle weakness, ataxia, retinitis pigmentosa, and variable sensory neuropathy, seizures, and mental retardation or dementia. This is associated with a heteroplasmic point mutation of mtDNA at bp 8993. The mother of a severely affected child underwent prenatal diagnosis in two further pregnancies. Analysis of chorionic villus samples showed a higher proportion of mutant mtDNA on both occasions, and this was reflected in the majority of fetal tissues, including brain and muscle. Prenatal diagnosis is a rational approach to the prevention of severe diseases caused by point mutations of mtDNA but is currently hampered by incomplete knowledge concerning the proportion of mutant mtDNA: its relationship to disease severity, how it may change during fetal and postnatal development, and its tissue distribution.

Introduction

An increasing number of maternally inherited diseases have been shown to be associated with point mutations of mtDNA. These include Leber hereditary optic neuropathy (Wallace et al. 1988), the syndrome of myoclonic epilepsy with ragged-red fibers (MERRF) (Shoffner et al. 1990; Zeviani et al. 1991*a*), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) (Goto et al. 1990; Hammans et al. 1991), mitochondrial myopathy with cardiomyopathy (Zeviani et al. 1991*b*), and a syndrome of neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP; Holt et al. 1990). In many reported families with these disorders, maternally related individuals have a mixture of mutant and wild-type mtDNA in investigated tissues (heteroplasmy), and the proportion of mutant mtDNA determines disease development or severity to some extent (Holt et al. 1989*a*, 1990; Shoffner et al. 1990). Quantified mtDNA studies in different tissues from patients with these disorders have so far been limited, and it is not known whether the proportions of mutant mtDNA in, for example, blood leukocytes, reflects that found in clinically affected organs such as the brain or optic nerve.

Prenatal diagnosis of diseases caused by point mutations of mtDNA is theoretically possible but has not as yet been reported. Here we describe both prenatal detection of the mtDNA^{8993} mutation causing NARP and the results of analysis of the proportion of mutant mtDNA in a variety of fetal tissues.

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Patients and Methods

Patients

The clinical and genetic features of the family studied were described by Holt et al. (1990). In brief, four members presented with a neurological syndrome comprising neurogenic muscle weakness, ataxia, and retinitis pigmentosa, with sensory neuropathy, seizures, and mental retardation or dementia in some of the patients. This was shown to be associated with a heteroplasmic point mutation of mtDNA (T→G) at bp 8993 in both blood and muscle, creating a new restriction site for *Ava*I and resulting in an amino acid change from a highly conserved leucine to arginine in subunit 6 of mitochondrial H^+-ATPase. There was some correlation between clinical severity and the amount of mutant mtDNA in the patients; the latter was present as only a small proportion of total mtDNA

in the blood of healthy elderly relatives in the same maternal line. The most severely affected individual (case 4 of Holt et al. 1990) is now a 5-year-old girl who is mentally retarded and ataxic with a pigmentary retinopathy and had only 3% normal mtDNA in leukocytes or muscle. Two other symptomatic family members were less severely affected, with 82% and 88% mutant mtDNA, respectively; the latter is case 3, the mother of case 4 (Holt et al. 1990).

Case 3 became pregnant again in 1988 and 1990. She and her husband were anxious to avoid having another severely handicapped child and had been advised that there was at least a 50% chance of further children being significantly affected. After much discussion, and with informed consent, they decided to opt for mtDNA analysis of a chorionic villus sample (CVS) to determine whether the proportion of mutant mtDNA was as high as in case 4. They were aware that it was not certain (*a*) that the percentage of mutant mtDNA in the CVS reflected that in other fetal tissues or (*b*) that this percentage would remain constant during fetal (or later) development. They also appreciated that it would be particularly difficult to make any predictions about disease status if there was between approximately 30%–80% mutant mtDNA in the CVS. Both pregnancies were eventually terminated.

Methods

CVS was obtained transcervically after ultrasonography to confirm gestation of 11 and 9 wk, respectively. Both pregnancies were terminated, one at 12 wk and one at 11 wk, by the suction method and the fetal tissue was dissected. DNA was extracted from each CVS and from samples of different fetal tissues using standard methods (Old 1986; Holt et al. 1989*b*). Five micrograms of these samples and of those previously studied from other family members were digested with 10 u of *Ava*I under conditions recommended by the manufacturers (Northumbria Biologicals Limited), with the addition of BSA and spermidine. The digested DNA fragments were separated in horizontal agarose gels (0.8%) by electrophoresis for 16 h at 50 V and then were transferred to nylon membrane (Hybond-N; Amersham, U.K.) by Southern blotting. Purified human placental mtDNA was oligolabeled (Feinberg and Vogelstein 1983) with ^{32}P to a specific activity of $>1 \times 10^8$ cpm/μg. Prehybridization and hybridization were as recommended for Hybond-N. mtDNA fragments were visualized by autoradiography for 24–72 h at −70°C, and those from each CVS were compared with cases 3 and 4 in adjacent lanes. Densitometry was performed using an LKB Ultroscan densitometer.

Results

There was a higher proportion of mutant mtDNA in chorionic villus tissue taken from both pregnancies than there was in case 4 (fig. 1). The parents opted for termination of pregnancy on both occasions. Figure 1 also shows that this high percentage of mutant mtDNA was reflected in placenta, brain, muscle, lung, and limb in the first fetus but that there was relatively more wild-type mtDNA in liver and kidney. The fetus from the second pregnancy was more macerated than the first, and fewer tissues were identifiable. However, there was very little normal mtDNA in muscle, and findings in the liver were similar to those in the first fetus.

Discussion

The present study confirms the potential application of prenatal diagnosis of severe neurological disease caused by mtDNA mutations. It was undertaken with caution for a number of reasons. We had no reasonable doubt that the bp 8993 mutation caused the maternally inherited disease in this family, as discussed elsewhere (Holt et al. 1990). There was also evidence that the proportion of mutant mtDNA was correlated with disease severity. However, these observations were made in only eight individuals in what was, to

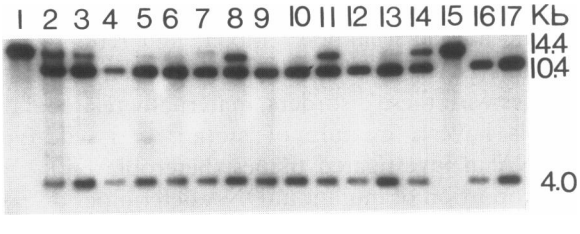

Figure 1 mtDNA fragments after digestion with *Ava*I and hybridization to whole mtDNA. Lanes (with percentage mutant mtDNA from densitometry) are as follows: 1, Control (0%); 2, case 3 (69%); 3, case 4 (87%); 4–11, first fetus (4, CVS [100%]; 5, placenta [97%]; 6, brain [98%]; 7, muscle [97%]; 8, liver [79%]; 9, lung [89%]; 10, limb [94%]; and 11, kidney [82%]); 12–14, second fetus (12, CVS [100%]; 13, muscle [96%]; 14, liver [68%]); 15, control (0%); 16, first CVS (91%); and 17, second CVS (96%) (last two have increased loading). In both control subjects (lanes 1 and 15) there were fragments 14.4, 1.3, and 0.8 kb in length (last two are not visible in figure). In the patients, both CVS samples, and fetal tissues, a variable portion of the 14.4-kb fragments was cleaved to two: one each of 10.4 and 4.0 kb.

our knowledge, a unique kindred; two further families with this mutation and with similar clinical features have been observed subsequently (B. Robinson, personal communication). Although the proportion of mutant mtDNA was similar in blood and muscle in three affected members of our kindred, we were concerned that observations made in CVS DNA may not reflect the mtDNA status of possibly seriously affected tissues, particularly brain. The potential problem of maternal contamination of CVS tissue was avoided by careful dissection and, in any case, would have influenced our results in favor of a lower percentage of mutant mtDNA, which seems unlikely to have occurred.

The data presented here pertaining to fetal tissue analysis show that there was a good correlation between the amount of mutant mtDNA in both brain and muscle and that in the CVS from the first pregnancy, confirming that, at least in NARP associated with mtDNA^{8993G}, prenatal diagnosis using CVS mtDNA analysis has a rational basis. A further practical difficulty may have arisen in this study and was discussed with the parents in advance; this was the possibility of the CVS DNA yielding a medium-range result, with a percentage of mutant mtDNA between about 30% and 80%. Lower or higher values than this in NARP should predict a reasonable chance of a good or bad prognosis; intermediate results would have had an even less certain predictive value. This problem is exacerbated by some inconsistency in our densitometric data, with generally lower proportions of mutant mtDNA observed in the present study as compared with previous studies (Holt et al. 1990). This is not particularly surprising, given the limitations of the technique, and was overcome by comparing proportions between samples rather than using absolute values.

The higher proportion of normal mtDNA seen in both fetus' liver and in kidney from the first fetus may be related either to random segregation of normal and mutant genomes, or to selection against the mutant population resulting from the high metabolic activity and cell division in these organs during early fetal life. Relatively low amounts of abnormal mtDNA in these tissues as compared with muscle have been described in patients with mtDNA deletions (Obermaier-Kusser et al. 1990; Ponzetto et al. 1990; Shanske et al. 1990).

The possibility of prenatal diagnosis may tentatively be considered in the prevention of other disorders caused by mtDNA mutations. It would not currently be reliable in the majority of families with Leber hereditary optic neuropathy, as correlation between the percentage of mutant mtDNA and disease development is not generally high. Some families seem to demonstrate homoplasmy for the mutation, including unaffected old males (Vilkki et al. 1989). Furthermore, there may be an X-linked gene which determines susceptibility to visual loss (Vilkki et al. 1991). In MERRF, Shoffner et al. (1990) showed that there is a correlation between the percentage of mutant mtDNA in muscle and development or severity of disease, if age is taken into account. To date, there has only been one study of the bp 8344 mtDNA mutation in different tissues from a patient with MERRF, and it showed heteroplasmy in brain, heart, liver, kidney, and muscle, but this was not quantified (Zeviani et al. 1991*a*). This mutation and that associated with MELAS are detectable in leukocyte mtDNA in the majority of patients (Hammans et al. 1991). Further work is required to determine the relationship between the phenotypic characteristics of these diseases and the amount of mutant mtDNA in different tissues.

Extrapolation of results of such studies to give prenatal or predictive genetic advice will require careful validation, as levels of mutant mtDNA may change both between fetal and adult life and also during adult life. In humans, mtDNA heteroplasmy has been observed only in individuals who have a population of disease-related mutant mtDNA, despite the high mutation rate of mtDNA which implies a need for heteroplasmy during the transition from one nondeleterious genotype to another (Holt et al. 1990). Ashley et al. (1989) suggested that bovine mtDNA could switch genotypes completely in two to three generations, on the basis that the number of mtDNAs is greatly reduced at some point in oogenesis and that there is uneven transmission to progeny. This switch appears not to occur so rapidly when mtDNA mutations are harmful, presumably because of selection in favor of wild-type mtDNA (Holt et al. 1990). In Leber hereditary optic neuropathy, fairly rapid switches in predominant mtDNA genotype have been described, but heteroplasmy is often maintained over several generations (Bolhuis et al. 1990; Vilkki et al. 1990).

It is not known whether an initially high proportion of mutant mtDNA, as may be detected in CVS, could fall as a result of selection at a cellular level during fetal development and later, with possible reduction of predicted disease severity. The beneficial effect of such a phenomenon on tissues containing cells which do not divide after very early development, such as brain and muscle, is likely to be slight. mtDNAs with

large deletions have been described in other mitochondrial diseases (Holt et al. 1989*b*; Moraes et al. 1989); there is indirect evidence that in dividing tissues the proportion of these falls with age, but it increases in muscle (Larsson et al. 1990; McShane et al. 1991). However, deleted mtDNAs are not strictly comparable to those with point mutations, as they are probably more selected against in dividing cells, and they increase in number as a result of the mitochondrial proliferation which occurs in response to oxidative deficiency.

As case 3 described here has borne three pregnancies containing a very high proportion of mutant mtDNA, it seems likely that this applies to many of her ova. For this reason, she has been offered in vitro fertilization of donated ova as an alternative to further natural pregnancies. This may be the only practical solution for women who are shown to have a high risk of transmitting severe mitochondrial genetic disease and who wish to avoid it.

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