A FAMILIAL MITOCHONDRIAL MYOPATHY WITH POSSIBLE MULTIPLE DELETIONS OF MITOCHONDRIAL DNA

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ABSTRACT

We have investigated the muscle mitochondrial DNA abnormalities in brothers suffered from mitochondrial myopathy *i.e.* Kearns-Sayre syndrome, together with their normal mother and a control patient, using Southern hybridization after digestion with restriction enzymes; *Bam*HI, *Eco*RI, *Hind*III, *Pst*I and *Sst*I. In two brothers, besides the normal size of DNA fragments, we found extra fragments suggesting the presence of approximately 4.1 kb shorter mitochondrial DNA in their muscle tissues. In addition, since these extra bands were extremely broad, multiple populations of deleted mitochondrial DNA were postulated. It is also noted that the Southern hybridization patterns of two patients were almost identical. On the other hand, muscle mitochondrial DNAs of their mother who is clinically normal showed exactly the same cleavage patterns as those of a normal control. This is the first report to investigate the state of the muscle mitochondrial DNA of the normal mother of the affected individuals. Based on these findings, possibilities of maternal inheritance, mutation in oocytes and autosomal recessive inheritance for underlying mechanism(s) of deletions in mitochondrial DNA in patients were discussed.

Several myopathies or encephalomyopathies are described to be caused by defect(s) of mitochondrial respiratory enzymes (2). Since important members of proteins in mitochondrial respiratory chain are encoded in mitochondrial DNA (mtDNA), much attention has recently been focussed on morphological and biochemical abnormalities of muscle mitochondria. Especially, in Kearns-Sayre syndrome (KSS) which is characterized by progressive external ophthalmoplegia, ragged-red fibers and retinopathy, deletions of muscle mtDNA were actually demonstrated by Holt *et al.* in 1988 (5). Subsequently, several confirmative reports were accumulated on this issue (6, 8, 9, 12-14). Although mitochondrial myopathy in general is considered to be trans-

mitted by maternal inheritance (3), most cases examined so far were sporadic. Familial cases with deletions in mtDNA have rarely been reported (9, 14). In order to make the mechanism(s) for deletion of muscle mtDNA clear, we investigated muscle mtDNAs not only of two patients (both are brothers) diagnosed as KSS but also of their asymptomatic mother by Southern analysis, and found deletions of mtDNA in two patients but not in their mother. Moreover, we found the size and the location of deletions of two patients to be identical. In addition, judging from the broadness of extra mtDNA bands on Southern hybridization we considered the deletions of mtDNA in our patients to be multiple.

Fig. 1 Family pedigree. Solid symbols indicate clinically affected individuals. Open symbols indicate clinically normal individuals. Barred symbols indicate deceased individuals. Figures shown at the lower right corners are ages of individuals when examined.

MATERIALS AND METHODS

Patients

Case 1 (51-year-old man) and case 2 (49-year-old man) are brothers, and their parents are first cousins (Fig. 1). Their clinical features were already reported under the title of 'Familial mitochondrial myopathy associated with peripheral neuropathy' by Mizusawa *et al.* (7). In short, both patients suffered from optic atrophy and blepharoptosis since childhood, and later developed muscle wasting and weakness of extremities, and glove and stocking type sensory impairment. Morphological investigations of biopsied muscles and sural nerves revealed mitochondrial myopathy with ragged-red fibers and peripheral neuropathy. Enzyme chemistry of muscles disclosed partial deficiencies of complexes I and IV of the mitochondrial respiratory chain in both cases. Although their mother is clinically normal, a muscle specimen was obtained by open biopsy after signed permission. Morphologically, muscle of the mother contained no ragged-red fibers and no defective fibers for cytochrome *c* oxidase activity.

Preparation of Total DNA

Muscle biopsy specimens from case 1, case 2, the mother and a control patient (mild polymyositis) were used for sources of DNA. Total DNA was isolated from approximately 150 mg of frozen muscles using the method of Davis *et al.* (1).

Southern Blot Analysis

Five micrograms of total DNA was digested with the following restriction enzymes; *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, and *Sst*I under the conditions recommended by the manufacture (Takara Shuzo, Kyoto). Digested DNAs were electrophoresed on a 0.7% agarose gel, and digestion patterns of mtDNA were detected by a hybridization technique using mtDNA purified from human placentas as a probe. Autoradiograms were developed for 1–2 h on Kodak X-Omat RP films at room temperature.

RESULTS

Fig. 2 shows the results of Southern hybridization using 5 different restriction enzymes. Since *Bam*HI cuts the normal mtDNA at only one site (Fig. 3), it is reasonable to find 16.6 kb fragment in all four cases examined. However, in cases 1 and 2, additional abnormal broad bands corresponding to approximately 12.5 kb in average were clearly recognized. Digestion with *Eco*RI which normally cleaves mtDNA at 3 sites (Fig. 3) producing 8.1 kb, 7.4 kb and 1.2 kb bands, revealed broad extra bands (11.5 kb) only in cases 1 and 2. Digestion with *Hind*III, which reveals 3 bands (10.2 kb, 5.5 kb and 0.9 kb) in the normal mtDNA, produced additive 12.5 kb band in cases 1 and 2. In addition, *Pst*I digestion which normally cleaves mtDNA at 2 sites into 14.5 kb and 2.1 kb fragments, revealed abnormal very broad bands corresponding to 10.4 kb only in cases 1 and 2. Finally, digestion with *Sst*I which reveals two fragments (9.6 kb and 7.0 kb) in the normal mtDNA, produced abnormal bands at 12.5 kb in cases 1 and 2.

Southern hybridization analysis of mtDNA using five restriction enzymes revealed that both patients have certain populations of muscle mtDNAs which are approximately 4.1 kb shorter in average than that of normal mitochondria. The location of the deletion in mtDNA may be determined by loss of restriction sites. Apparently, an *Sst*I site, an *Eco*RI site and two *Hind*III sites were lost by the deletion. Although a *Bam*HI site seemed to be maintained, a possibility still remained that some were lost, since the deletions including the *Bam*HI site brought about non-linear

Fig. 2 Autoradiogram of a genomic Southern blot hybridization of muscle mitochondrial DNA from affected brothers (elder: P1, younger: P2), their mother (M) and a control patient (C). Restriction enzymes used are shown at the top of each set. Sizes of each band are shown at left in kilobases (kb). Bands marked with white triangle are normal bands and those marked with black triangle are extra bands.

Fig. 3 Map of the human mitochondrial genome showing the locations of cleavage sites of each restriction enzyme; B: *Bam*HI, E: *Eco*RI, H: *Hind*III, P: *Pst*I and S: *Sst*I. The extent of the deleted regions in mutant mitochondrial DNA in patients is indicated with solid line. Interrupted lines at each end of deletion define its possible extension (see text).

mtDNA some of which were difficult to detect by the ordinary Southern hybridization analysis (Fig. 3). In addition, it is noteworthy that those additional digested bands in patients are broad. It is interesting to note that in Southern hybridization mtDNA of the phenotypically normal mother revealed absolutely normal patterns. This finding was further confirmed by another autoradiogram which was obtained with much longer exposure time (6 h).

DISCUSSION

Deletions of muscle mtDNA in mitochondrial myopathies were first described by Holt *et al.* in 1988 (5). They found various size and location of deletions in 9 out of 25 sporadic cases of mitochondrial myopathies. Since then, totally at least 35 cases of mitochondrial myopathies (especially of Kearns-Sayre syndrome or progressive external ophthalmoplegia) out of 65 cases examined reported to have a peculiar population of deleted muscle mtDNA in addition to a normal mtDNA population (6, 8, 9, 13). In this respect, our findings are largely confirmative to the previous reports. However, the present results contain several unusual and unique features when compared with previous reports.

Firstly, in two patients extra mtDNA bands which were observed after digestion with restriction enzymes were broad. This has not been reported in previous studies (6, 8, 13). Based on this unique pattern on Southern

analysis, it is possible to speculate that each patient in the present study may contain more than two populations of mtDNA in muscle. Indeed, according to the most recent report of Zeviani *et al.* (14), there are multiple populations of deleted mtDNA (7–8 different sizes of deletions were detected in a single person) in two patients whose *Pvu*II digested mtDNA pattern showed extremely broad extra bands. It is needed to determine the extra site of the deletion breakpoint by DNA sequencing for the final conclusion on this issue.

Secondly, it is interesting to know that the deletions of muscle mtDNA found in both elder (case 1) and younger (case 2) brothers are quite similar to each other judging from the patterns of Southern hybridization. Zeviani *et al.* (14) have also reported similar deletion patterns in two of one family, in which the mode of inheritance of the disorder was exceptionally an autosomal dominant. These findings contribute much to the better understanding of the mechanisms involved in the generation of partially deleted mtDNA (*vide infra*).

Thirdly, this is the first report to analyse the muscle mtDNA from a clinically normal mother. Since mitochondria of cells in general are believed to be brought in exclusively from a maternal germ cell (3), mitochondrial myopathy is postulated to be transmitted through maternal inheritance (11). In spite of this general concept, almost all cases examined so far were sporadic, and muscle mtDNA of their mother was rarely examined. Ozawa *et al.* (9) actually investigated a mother and her daughter (both were clinically affected), and found the size and the position of mtDNA deletions to be different each other. On the other hand, the present study revealed the muscle mtDNA of the phenotypically normal mother having affected children to be normal in terms of patterns of Southern hybridization. Of course, the present results do not deny the possibility for the existence of a trace amount of deleted mtDNA in the total mtDNA of the mother which can not be detected by the ordinary Southern analysis.

Based on these relevant findings, it is possible to consider the following mechanisms for deletion of mtDNA in this particular family. 1) If there exists an extremely small amount of deleted mtDNA in somatic cells (including muscle) of the mother, these delet-

ed mtDNA could be amplified during transmission to her two children through 'maternal inheritance'. However, there is no definite evidence to support it so far. It is really needed to sequence the mtDNA of mother in order to obtain the evidence for or against this hypothesis. 2) If mtDNA of the mother is normal in the strict sense, then it may be possible to consider the 'mutation' mechanism occurred in mtDNA of oocytes. It is relevant here to recall previous reports suggesting the mitochondria and mtDNA to be amplified during mammalian oocyte development (4, 10). For instance, Hauswirth *et al.* (4) found about a 100-fold increase of mtDNA in mature bovine oocytes when compared with somatic cells. If the same type of amplification of mtDNA occurs in human oocytes, a chance for the deletions in mtDNA could be high enough to produce mitochondrial myopathy. However, it may be difficult to interpret the fact that two brothers in our family seem to have the same deletions, unless very rigid hot spots on mtDNA are assumed to exist. 3) It may also be possible to consider the 'autosomal recessive inheritance' of the defective chromosomal regulation to prevent deletions in mtDNA or of the active chromosomal control to enhance deletions in mtDNA. It is difficult at the moment to draw a conclusion about which mechanism actually underlies in the present family.

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