Am. J. Hum. Genet. 50:852–858, 1992

Heteroplasmic mtDNA Mutation (T→G) at 8993 Can Cause Leigh Disease When the Percentage of Abnormal mtDNA Is High

Y. Tatuch,* J. Christodoulou,* A. Feigenbaum,* J. T. R. Clarke,* J. Wherret,* C. Smith,† N. Rudd,† R. Petrova-Benedict,* and B. H. Robinson*

*Departments of Biochemistry, Pediatrics, Pathology, and Medicine, University of Toronto, and the Research Institute, Hospital for Sick Children, Toronto; and †Department of Genetics, University of Calgary, Alberta

Summary

A female infant showing lacticacidemia, hypotonia, and neurodegenerative disease died at 7 mo of age. Autopsy revealed lesions typical of Leigh disease, both in the basal ganglia and in the brain stem. A maternal aunt and uncle died 1 year and 5 mo, respectively, after following a similar clinical course, while another uncle, presently 33 years of age, has retinitis pigmentosa and ataxia and is mentally retarded. PCR restriction-digest analysis of mtDNA isolated from the proband revealed a T-to-G change at position 8993, creating a new *AvaI* restriction site. The mutation present in the ATP 6 gene results in the substitution of an arginine residue for a leucine. The indexed patient had >95% abnormal mtDNA in her skin fibroblasts, brain, kidney, and liver tissues, as measured by laser densitometry. The maternal aunt who died at age 1 year had >95% abnormal mtDNA in her lymphoblasts. The uncle with retinitis pigmentosa had 78% and 79% abnormal mtDNA in his skin fibroblasts and lymphoblasts, respectively, while an asymptomatic maternal aunt and her son had no trace of this mutation. The mother of the index case had 71% and 39% abnormal mtDNA in her skin fibroblasts and lymphoblasts, respectively, showing that the heteroplasmy can be variable, on a tissue-specific basis, within one individual. This shows that mtDNA mutations at 8993 can produce the clinical phenotype of Leigh disease in addition to the phenotype of ataxia and retinitis pigmentosa described by Holt et al. The insertion of an arginine in the hydrophobic sequence at ATPase 6 probably interferes with the H^+ channel formed by subunits 6 and 9 of the ATPase, thus causing failure of ATP synthesis.

Introduction

Point mutations in mtDNA have now been shown to be responsible for Leber hereditary optic neuropathy (LHON) (Wallace et al. 1988), myoclonus epilepsy with ragged red fibers (MERFF) (Shoffner et al. 1990), and mitochondrial encephalomyelopathy with lactic acidosis and stroke-like episodes (MELAS) (Goto et al. 1990). In the case of LHON due to the 11778 mtDNA mutation, the mutation may be either homoplasmic or heteroplasmic, affecting the product of the ND4 gene, a component of complex I (Wallace et al. 1988; Lott et al. 1990). In the context of MELAS and MERFF in which the tRNA species for leucine and lysine are the predominant respective gene products affected, the defects are invariably heteroplasmic (Shoffner et al. 1990).

A further point mutation was reported by Holt et al. (1990) to cause retinitis pigmentosa and ataxia in one family. This mutation, which was heteroplasmic and affected the gene product of the ATPase 6 gene, is a T-to-G transition at 8993 and leads to a substitution of an arginine for a leucine residue near the c-terminal end of the protein. Another family was reported by Poulton to have pigmentary retinopathy due to a

Received August 22, 1991; final revision received December 18, 1991.

Address for correspondence and reprints: Dr. Brian H. Robinson, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1XB.

0002-9297/92/5004-0022$02.00

defect in the same gene, probably due to a mutation at 8994 or 8995 of mtDNA, though the sequence was not reported as such (Poulton et al. 1988). Here we report on another family in which the 8993 mutation is present in several family members and show that it can produce a severe disease phenotype, that of infantile Leigh disease with lactic acidosis.

Subjects and Methods

Case Report

The family pedigree is shown in figure 1. The index case was born at 38 wk by elective cesarean section; birth weight was 7 lbs 12 oz, and APGAR scores were 9 and 10 at 1 and 5 min, respectively. In the first 3 mo of life she was noted to feed poorly and slowly, her weight fell from the 70th to the 25th percentile, and she developed hypotonia and head lag. Blood lactate was elevated at 3–5 mM over a prolonged period. An magnetic resonance imaging scan of the head showed bilateral symmetrical basal ganglia abnormalities consistent with Leigh disease. Visual and auditory evoked potentials and electroencephalogram were normal at this time. At 6 mo she was readmitted because of increasing lethargy, an episode of apnea, and seizure activity thought to be caused by otitis media. Further apneic episodes occurred in the following weeks, and after discussion with the parents resuscitative support was withdrawn and the patient died of respiratory failure. Autopsy revealed bilateral cystic lesions of the basal ganglia, thalamus, periventricular region, substantia nigra, and tectal and tegmental brain stem. There was a proliferation of astrocytes and blood vessels in cystic areas, the gross and microscopic findings being consistent with Leigh disease. Examination of muscle showed that there were no ragged-red fibers and no mitochondrial proliferation present, and the mitochondria had normal ultrastructure on examination by electron microscopy.

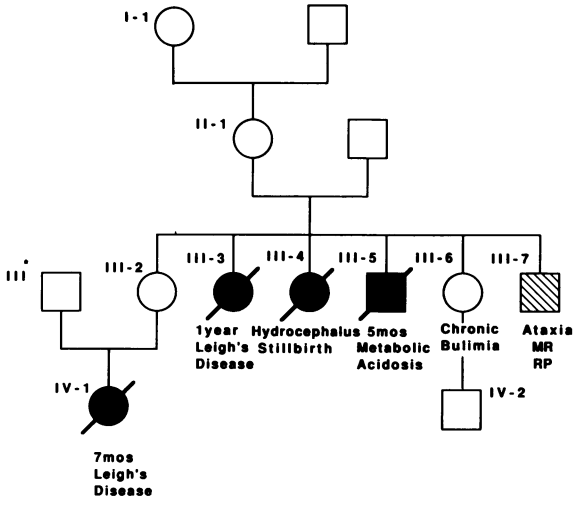

Figure 1 Family pedigree. This numbering system can be used to interpret table 1. The asterisk denotes that individual is nonconsanguinous.

The mother and the grandmother of this child were in good health, but the child had a maternal aunt and uncle who died of Leigh disease and an aunt who was a stillbirth. The aunt was first seen at 4½ mo of age in 1972 after an apneic spell. She had further apneic episodes and a chronic metabolic acidosis and continued to show little progression. She died of respiratory failure at 1 year of age, and the autopsy showed spongiform degeneration, capillary proliferation, and gliosis in the thalamus, medulla, basal ganglia, and brain stem. Urine sent for assay of the inhibitor of thiamine pyrophosphate phosphoryl transferase (Pincus et al. 1976) was positive, and small amounts of methylmalonic acid were present in the urine. The maternal uncle died at 5 mo of age from respiratory failure, the autopsy showing "lesions compatible with Leigh's disease." Another aunt and uncle, age 30 and 33 years, respectively, are still alive. The aunt suffers from anorexia nervosa and is a chronic bulimic. The uncle was normal until 12 years of age, when he suffered from a bout of weakness and ataxia from which he recovered after several months. After further bouts it was found that he had retinal degeneration and loss of IQ. He presently resides in an institution for the handicapped, is legally blind, has a somewhat ataxic gait, and is mentally retarded.

Methods

Skin fibroblast cultures were established on all living family members. Lymphoblast cultures were available from the maternal aunt who died in 1973 and were established on family members. Leukocyte pellets were prepared in some individuals. Liver, brain, and muscle samples from the index case that were taken within 2 h of death were stored at −70°C after immersion in liquid N_2.

DNA was extracted by standard techniques (Old 1986) and was used as a template for PCR or was digested with restriction enzymes. For amplification of mtDNA, 500 ng total DNA was amplified by PCR (Saiki et al. 1988) using the primers CCGACTAAT-

CACCACCCAAC and TGTCGTGCAGGTAGAGGCTT as used by Holt et al. (1990). After being cut with the restriction enzyme *Ava*I, the product was purified and run out on a 2% agarose gel. The gel was photographed, and the negative was used for densitometry using a PDI laser densitometer model 35, equipped with version 3.2 Discovery Series software. By preparation of gels with 0.1–10 μg DNA PCR product and by use of photographic negatives of these gels, the densitometer was calibrated to the gel system we were using.

Southern blots.—For Southern blot analysis 0.7 mg lymphoblast total DNA was cut with *Ava*I. The digested fragments were separated on 0.8% agarose gels and then were transferred to nylon membranes (Hybond). A blot was probed with a PCR fragment (^{32}P-labeled) from normal mtDNA in the region 8646–9199. Percentages of mutant mtDNA were determined by scanning the autoradiographs by laser densitometry.

Two standard curves were prepared using mixtures of cloned mutant mtDNA and normal mtDNA. PCR products generated from independent mutant and wild-type amplifications of mtDNA were mixed in known ratios to yield 500 ng. Mixtures were then digested with *Ava*I and were electrophoresed on agarose gels, photographed, and analyzed by laser densitometry (fig. 2, line A). mtDNA PCR fragments were mixed in known ratios again, but this time they were amplified by PCR before analysis as above (fig. 2, line B).

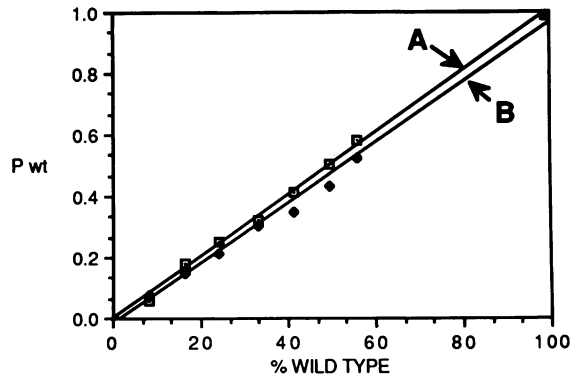

Figure 2 Standard plot for DNA amplification. Two 551-bp DNA samples (one with the *Ava*I site and one without it) corresponding to the amplified segment of mtDNA were prepared. These were mixed in differing proportions and were coamplified, run out on agarose gels, and photographed by ethidium bromide fluorescence. These were ready by laser densitometers, and the results (line B) were compared with gels run with unamplified mixtures (line A). The relative intensities of the cut vs. the uncut bands (Pwt) were plotted against the percentage of wild-type DNA in the original sample.

Enzyme analysis.—Determination of the activities of the pyruvate dehydrogenase complex, pyruvate carboxylase, succinate cytochrome *c* reductase, and cytochrome oxidase were performed on cultured skin fibroblasts according to a method described by Robinson et al. (1990).

Results

Initial results from the cultured skin fibroblasts showed that the activities of cytochrome oxidase and the pyruvate dehydrogenase complex were within normal limits. There was no elevation of the lactate/pyruvate ratio in cultured fibroblasts incubated with glucose, and the oligomycin-sensitive ATPase was normal both in fibroblast sonicates and in muscle mitochondrial sonicates (table 1).

When DNA extracts of the tissues of the index case were amplified using the primers as described in the Methods subsection, a 551-bp product was obtained from PCR and was found to be cleaved >95% by *Ava*I. The extent of cleavage was the same in all tissues. Product of the amplification of the patient DNA was subcloned and amplified, and serial dilutions were made with the 551-bp product obtained from a control subject. These heterogenous DNA samples were then amplified, run out on agarose gels after being cut with *Ava*I, and then subjected to densitometry. When a plot was constructed of product against percentage dilution of the precursor, a linear relationship was found (fig. 2). This is quite different from the skewing curve reported by Shoffner et al. (1990) for amplification of heterogenous DNA samples with the MERFF mutation sites and may reflect the different effects caused by a secondary-structure phenomena imposed by the symmetric *Ava*I site which would perhaps favor stability of nonheteroduplex DNA strands.

When the PCR products of fibroblast DNA amplification were obtained (fig. 3), it was obvious that the index case had a very high percentage of abnormal DNA. The mother and the uncle also had a higher percentage—71% and 78%, respectively—and the grandmother had a moderate 52% (table 2). The father, the asymptomatic aunt and her asymptomatic son had no trace of abnormal mtDNA. Amplification of the lymphoblast culture DNA showed that the aunt who died of Leigh disease had a profile similar to that of the index case, with no detectable normal mtDNA

Table 1

Data Obtained from Skin Fibroblasts from Index Patient

	MEAN ± SEM (n^a) (nmol/min/mg protein)	
	Patient	Control
Fibroblast:		
Pyruvate dehydrogenase complex:		
Native	.630 ± .031 (4)	1.06 ± .23 (3)
DCA activated	.80 ± .12 (4)	1.14 ± .18 (3)
Pyruvate carboxylase	1.54	.65
Succinate cytochrome *c* reductase	5.5 ± .5 (2)	7.1 ± .7 (2)
Cytochrome oxidase	9.7 ± 1.1 (2)	14.2 ± .2 (2)
Lactate/pyruvate ratio	30.5 ± 2.2 (14)	30.7 ± 3.3 (11)
Fibroblast mitochondria:		
Oligomycin-sensitive ATPase	29.2 (10)	37.6 ± 13 (3)
Muscle mitochondria:		
Oligomycin-sensitive ATPase	77.2 (1)	65.8 ± 15.2 (3)

[a] Number of determinations.

(fig. 4). The results for the asymptomatic aunt and child showed the same unaffected status as did the fibroblasts. The results for the mother (39%) and ataxic uncle (79%) were different and reflected better their clinical status. Leukocyte DNA showed results similar to those shown by lymphocyte DNA. An aliquot of PCR-amplified mtDNA from the index case was subcloned into a plasmid vector and was sequenced. This showed that the DNA sequence contained the T-to-G mutation at 8993 of mtDNA, confirming that the *Ava*I site was within the ATPase 6 sequence.

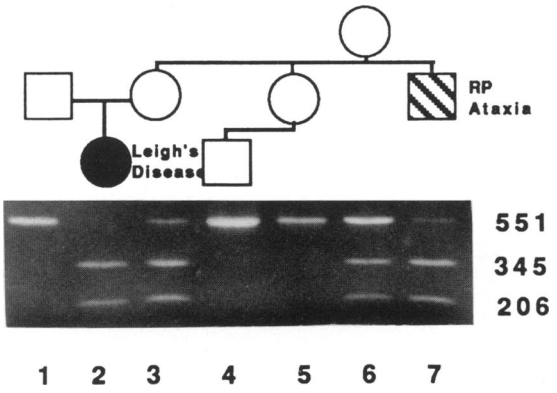

Figure 3 Detection of 8993 mutation in amplified fibroblast DNA. Agarose gel of the 551-bp amplified segments of fibroblast mtDNA after they were cut with *Ava*I is shown. Template DNA was prepared from fibroblasts of family members as indicated. See Methods subsection for details of amplification. Family members are as indicated in fig. 1. Lane 1, III. Lane 2, IV-1. Lane 3, III-2. Lane 4, IV-2. Lane 5, III-6. Lane 6, II-1. Lane 7, III-7.

A Southern blot performed both with DNA from the postmortem tissues and with DNA from the lymphoblasts was carried out to check that the PCR conclusions were valid. The blot shown in figure 5 shows that family members' DNA digested with *Ava*I gave a variable pattern when probed with ^{32}P-labeled ATPase 6 DNA. Brain, liver, and kidney from the index case and lymphoblasts from the aunt who died of Leigh disease showed two bands—one at 10.4 kb and one at 4 kb. Partially affected family members displayed all three bands—one uncut by *Ava*I at 14.4 kb and one cut band each at 10.4 and 4 kb. On prolonged exposure of the Southern blot to X-ray film, the brain and liver of the patient showed very faint bands at 14.4 kb. mtDNA from controls always shows only one band at 14.4 kb (Holt et al. 1990). This agreed with the original observations of Holt et al. (1990), who showed that the mtDNA cut with *Ava*I gave a 14.4-kb band on Southern blots in normal individuals but that, in carriers of the 8993 T-to-G mutation, there were, in addition to the 14.4-kb band, bands at 10.4 kb and 4 kb.

Discussion

We have shown in a family with a form of Leigh disease which appears to have a strong element of maternal inheritance that there is a segregating heteroplasmic T-to-G mutation of mtDNA at position 8993.

Table 2

Percentage of Abnormal mtDNA in Family Members

	% Abnormal mtDNA in								
	PCR Digest Analysis of					Southern Blot Analysis of			
Case	Fibro-blasts	Lympho-blasts	Brain	Kidney	Liver	Lympho-cytes	Brain	Kidney	Liver
IV-1	>95		>95	>95	>95		>95	>95	>95
IV-2	0	0				0			
III-2	71	39				43			
III-3		>95							
III-4									
III-5									
III-6	0	0				0			
III-7	78	79				78			
II-1	52	45				44			
I-1		0				0			
III[a]	0					0			

[a] Nonconsanguineous.

The indication that this was an mtDNA defect came first from the maternal inheritance of the disease and second from the presence of two distinct disease entities in the family, a fact strongly suggestive of heteroplasmy. The presence of a family member with retinitis pigmentosa and ataxia suggested that we were

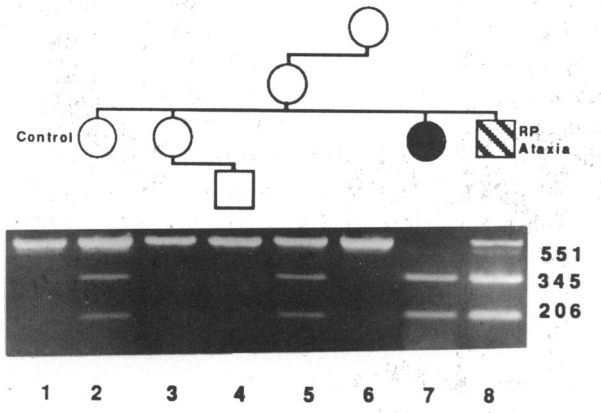

Figure 4 Detection of 8993 mutation in amplified lymphoblast DNA. Agarose gel of the 551-bp amplified segments of mtDNA after they were cut with *Ava*I is shown. Template DNA was prepared from lymphoblasts of family members as indicated. See Methods subsection for details of amplification. Family members are as indicated in fig. 1. Lane 1, Unrelated control. Lane 2, III-2; Lane 3, III-6; Lane 4, IV-2. Lane 5, II-1. Lane 6, I-1. Lane 7, III-3. Lane 8, III-7.

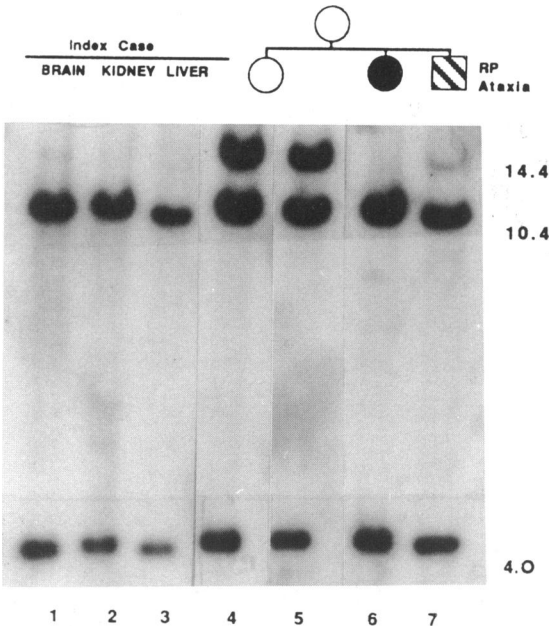

Figure 5 Southern blot of *Ava*I-digested DNA. DNA prepared from cultured skin fibroblasts, brain, kidney, liver, or cultured lymphoblasts was digested with *Ava*I and was separated on a 0.8% agarose gel. This was then blotted onto Hybond and was probed with ^{35}P-labeled amplified mtDNA, as described in the Methods subsection. Samples used for each lane are indicated in fig. 1. Lane 1, IV-1, brain. Lane 2, IV-1 kidney. Lane 3, IV-1 liver. Lane 4, III-2 fibroblasts. Lane 5, II-1 fibroblasts. Lane 6, III-3 fibroblasts. Lane 7, III-7 fibroblasts.

dealing with the known heteroplasmic mtDNA mutation at 8993, because of the strong association between these symptoms and this defect (Holt et al. 1990). This was confirmed in the affected child's tissues, where there was a very high percentage of abnormal mtDNA in muscle, brain, kidney, and skin fibroblasts. The status of the mother was interesting in that both hers and the grandmother's abnormal DNA content was quite substantial. Differential segregation of abnormal DNA in the family correlates with the clinical phenotype, especially if the lymphoblast data are used. Leigh disease has been shown by ourselves and others to be caused by a number of inborn errors of energy metabolism, which include deficiency of the pyruvate dehydrogenase complex (Wexler et al. 1988; Robinson 1989) and isolated deficiency of complexes I (Fujii et al. 1990; Robinson et al. 1990) and IV (DiMauro et al. 1987; Glerum et al. 1988) of the respiratory chain. Initial investigations on the fibroblasts of the index case showed that the pyruvate dehydrogenase complex was normal (table 1). The measurements of cytochrome oxidase activity and the presence of a normal redox state in the skin fibroblasts as measured by the lactate pyruvate ratio indicated that the respiratory-chain assembly was not involved (Robinson et al. 1990). In addition, the skin fibroblast cultures of the index patient could grow quite well in galactose containing culture medium. We have shown that in severe respiratory-chain defects leading to Leigh disease the cells will not grow in a medium in which galactose is substituted for glucose as a carbon source (Sodeberg et al. 1988; Petrova-Benedict et al., in press). Further, when we measured oligomycin-sensitive ATPase in isolated mitochondria from muscle or fibroblasts, the activity was normal. Thus this mtDNA mutation, like that in the ND4 gene observed in patients with LHON, does not give rise to an easily distinguishable biochemical phenotype (Petrova-Benedict et al., in press). Neither does it produce the mitochondrial proliferation in muscle which is typical of the mtDNA mutations leading to MELAS and MERFF.

How, then, does the mutation in ATPase 6 at 8993 lead to energy deprivation and the neuronal death that precipitates the symptoms and pathology associated with Leigh disease? To understand this, we must look at how the product of the ATPase 6 gene functions in the ATP synthetase complex. The catalytic portion (F_1) of the complex that synthesizes ATP is made up of α, β, γ, δ, and ε subunits all nuclear encoded to form an $\alpha_3\beta_3\ \gamma\delta\varepsilon$ complex (Boyer 1989; Papa et al. 1990). It is connected to a membrane-spanning component (F_0) by a stalk which comprises a number of small portions including the gate protein γ, the oligomycin sensitivity–conferring protein (OSCP), an ATPase protein inhibitor (H), and part of ATPase 8 (Joshi and Pringle 1989; Papa et al. 1990). The F_0 is made up of ATPase 6, 8, and 9, of which 6 and 8 are encoded in mtDNA in higher animals (Boyer 1989; Papa et al. 1990). The basic structure and mechanism of the H^+-transporting ATPase has been conserved from prokaryotes through to higher mammals and is designed so that the energy derived from the respiratory chain–driven proton gradient is harnessed to drive ATP synthesis (Boyer 1989). The F_0 portion of the complex spans the mitochondrial membrane such that ATPase 6 with five membrane-spanning helices and ATPase 9 with two membrane spanning helices form a channel which admits an incoming proton (Cox et al. 1986; Boyer 1989) (fig. 6). The two helices which are crucial to the mechanism,—i.e., the ATPase 6 fourth helix, at amino acids 142–166 in humans, and the ATPase 9 second helix, at amino acids 42–56—are adposed within the membrane (Cox et al. 1986). In the proposed mechanism a negatively charged residue—aspartate in prokaryotes and yeast but glutamate in mammals—is intimately involved in absorbing the energy of the proton ascending the channel (Boyer 1989; Cox et al. 1986). This residue is positioned halfway up the second membrane-spanning helix of ATPase 9 (Farrell and Nagley 1987).

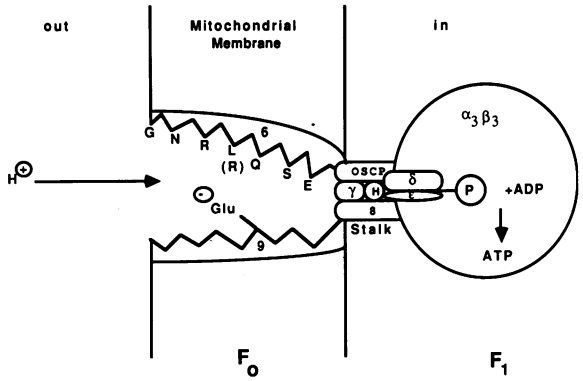

Figure 6 Schematic view of complex V membrane channel formed by ATPase 6 and ATPase 9 genes in mitochondrial membrane. The mutation at 8993 mtDNA changes a conserved leucine in the ATPase 6 channel segment to an arginine, thus changing the charge distribution in the channel and potentially interfering with both the entry of the proton and ATP formation. For further details, see text.

The mutation at 8993 of human mtDNA changes the leucine residue, which is adposed to the critical glutamate residues in ATPase 9, to a positively charged arginine. This would be expected to interfere with the energy-driven synthesis of ATP, since it puts a second positive charge in the proton channel of F_0. In ATP hydrolysis assay systems ATPase would still be able to function as we have observed but would be hindered in ATP synthesis.

Acknowledgments

We thank the National Centres of Excellence (Canada) and the Leigh's Disease Research Fund.

References

Boyer PD (1989) A perspective of the binding change mechanism for ATP synthesis. FASEB J 3:2164–2178

Cox GB, Fimmel AL, Gibson F, Hatch L (1986) The mechanism of ATP synthase: an assessment of the function of the b and a subunits. Biochim Biophys Acta 849:62–69

DiMauro S, Servidei S, Zeviani M, DiRocco M, DeVivo DC, DiDonato S, Uziel G, et al (1987) Cytochrome c oxidase deficiency in Leigh syndrome. Ann Neurol 22: 498–506

Farrell LB, Nagley P (1987) Human liver cDNA clones encoding proteolipid subunit 9 of the mitochondrial ATPase complex. Biochem Biophys Res Commun 144:1257–1264

Fujii T, Ito M, Okuno T, Mutoh K, Nishikomori R, Mikawa H (1990) Complex I deficiency in two patients with probable Leigh syndrome. J Pediatr 116:84–87

Glerum DM, Yanamura W, Capaldi R, Robinson BH (1988) Characterization of cytochrome c oxidase mutants in human fibroblasts. FEBS Lett 236:100–104

Goto Y, Noneka I, Horai S (1990) A mutation in $tRNA^{Leu}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–654

Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA (1990) A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 46:428–433

Joshi S, Pringle MJ (1989) ATP synthase complex from bovine heart mitochondria. J Biol Chem 264:15548–15551

Lott MT, Voljavec AS, Wallace DC (1990) Variable genotype of Lebers hereditary optic neuropathy patients. Am J Hum Ophthalmol 109:625–631

Old JM (1986) Fetal DNA analysis. In: Davies KE (ed) Human genetic diseases: a practical approach. IRL, Oxford, pp 1–17

Papa S, Guerrieri F, Zanotti F, Fiermonte M, Capozza G, Jirillo E (1990) The y subunit of F_1 and the PVP protein of F_0 ($F_0$1) are components of the gate of the mitochondrial F_0 F_1 H^+-ATP synthase. FEBS Lett 272:117–120

Petrova-Benedict R, Buncic JR, Wallace DC, Robinson BH. Selective killing of cells with oxidative defects in galactose medium: a screening test for affected patients. J Inherited Metab Dis (in press)

Pincus JH, Solitaire GB, Cooper JR (1976) Thiamine triphosphate levels and histopathology: correlation in Leigh disease. Arch Neurol 33:759–763

Poulton J, Turnbull DM, Mehta AB, Wilson J, Gardine RM (1988) Restriction enzyme analysis of the mitochondrial genome in mitochondrial myopathy. J Med Genet 25: 600–605

Robinson BH (1989) The metabolic basis of inherited disease. In: Scriver C, Baudet A, Sly W, Valle D (eds) McGraw-Hill, New York, pp 869–888

Robinson BH, Glerum DM, Chow W, Petrova-Benedict R, Lightowlers R, Capaldi R (1990) The use of skin fibroblast cultures in the detection of respiratory chain defect in patients with lacticacidemia. Pediatr Res 28:549–555

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Millis KB (1988) Primer dictated enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–494

Shoffner JM, Lott MT, Lezza AMJ, Seibel P, Bellinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red fibre disease (MERFF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. Cell 61:931–937

Sodeberg K, Nissinen E, Bakay B, Scheffler IE (1988) The energy charge in wild-type and respiration deficient hampster cell mutants. J Cell Physiol 103:169–172

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AMS, Elsas LJ, et al (1988) Mitochondrial DNA mutation associated with Lebers hereditary optic neuropathy. Science 242:1427–1430

Wexler ID, Kerr DS, Ho L, Lusk MM, Pepin RA, Javed AA, Mole JE, et al (1988) Heterogeneous expression of protein and mRNA in pyruvate dehydrogenase deficiency. Proc Natl Acad Sci USA 85:7336–7340