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Congenital Encephalomyopathy and Adult-Onset Myopathy and Diabetes Mellitus: Different Phenotypic Associations of a New Heteroplasmic mtDNA tRNA Glutamic Acid Mutation

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Summary

We report the clinical, biochemical, and molecular genetic findings in a family with an unusual mitochondrial disease phenotype harboring a novel mtDNA tRNA glutamic acid mutation at position 14709. The proband and his sister presented with congenital myopathy and mental retardation and subsequently developed cerebellar ataxia. Other family members had either adult-onset diabetes mellitus with muscle weakness or adult-onset diabetes mellitus alone. Ragged-red and cytochrome c oxidase (COX)–negative fibers were present in muscle biopsies. Biochemical studies of muscle mitochondria showed reduced complex I and IV activities. The mtDNA mutation was heteroplasmic in blood and muscle in all matrilineal relatives analyzed. Primary myoblast, but not fibroblast, cultures containing high proportions of mutant mtDNA exhibited impaired mitochondrial translation. These observations indicate that mtDNA tRNA point mutations should be considered in the differential diagnosis of congenital myopathy. In addition they illustrate the diversity of phenotypes associated with this mutation in the same family and further highlight the association between mtDNA mutations and diabetes mellitus.

Introduction

In recent years a large number of primary defects of mtDNA have been described in association with human disease (DiMauro and Moraes 1993). Although most of these defects are associated with a group of neurological diseases known as the mitochondrial myopathies and encephalomyopathies (Petty et al. 1986), there is increasing evidence that they may play a role in the pathogenesis of more common disorders, such as diabetes mellitus (Reardon et al. 1992; van den Ouweland et al. 1992; Kadowaki et al. 1994). The two main types of primary mtDNA defect are large-scale rearrangements and point mutations. Large-scale rearrangements include deletions and duplications of mtDNA and are commonly observed in patients with progressive external ophthalmoplegia (PEO) and the Kearns-Sayre syndrome (Holt et al. 1988; Moraes et al. 1989; Poulton et al. 1989). Point mutations in coding genes occur in Leber hereditary optic neuropathy (LHON) (Wallace et al. 1988), and those in noncoding regions, particularly tRNA genes, occur in encephalomyopathies such as MELAS (mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes) and MERRF (myoclonus epilepsy with ragged red fibers) (Goto et al. 1990; Shoffner et al. 1990).

There are now at least 20 point mutations described in mitochondrial tRNA genes that have been implicated in the pathogenesis of a variety of mitochondrial encephalomyopathy phenotypes (DiMauro and Moraes 1993; Morten et al. 1993; Yoon et al. 1993; Manfredi et al. 1994; Merante et al. 1994; Nelson et al. 1994; Sahashi et al. 1994). The precise molecular pathogenesis of disease associated with these point mutations is unclear, but available evidence suggests that mitochondrial translation and, possibly, transcription are impaired (Chomyn et al. 1991; Boulet et al. 1992; King et al. 1992). Although the phenotypes described are very varied, they usually have in common the presence of ragged red fibers on muscle biopsy and a progressive disorder with onset in childhood or later. Disorders of oxidative phosphorylation are well recognized to occur in the neonatal period (DiMauro 1992), but the majority of these have not been shown to be associated with tRNA point mutations (Houshmand et al. 1994; authors' unpublished observations). The few patients with neonatal onset in whom a tRNA point mutation has been identified have had a rapidly fatal course (Tanaka et al. 1990, 1992; Yoon et al. 1993).

In our series of 174 mitochondrial encephalomyopa-

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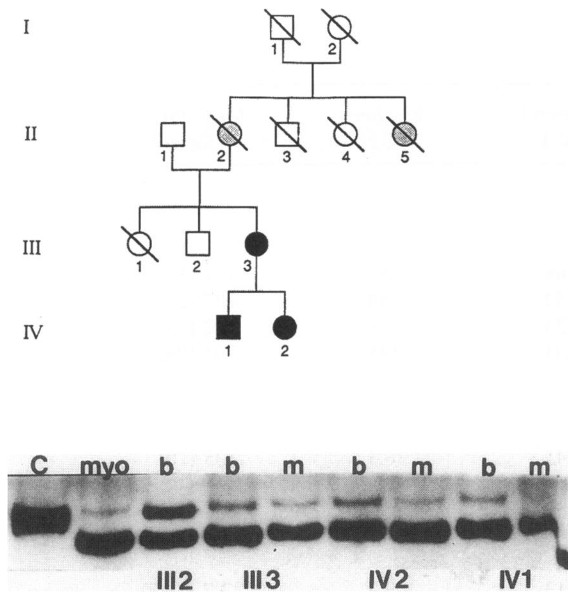

Figure 1 *Top,* Pedigree of family (blackened symbols denote affected individuals, and gray-shaded symbols denote individuals with diabetes only). *Bottom,* Acrylamide gel of PCR products of 133 bp digested by *Sna*BI (mutant mtDNA digested into two fragments, of 113 and 20 bp). C = control; b = blood; m = muscle; and myo = myoblasts. The proportions (expressed as percentage total mtDNA) of mutant mtDNA were as follows: III-2 b, 65%; III-3 myo, 85%; III-3 b, 75%; III-3 m, 85%; IV-1 b, 93%, IV-1 m, 99%; IV-2 b, 93%; and IV-2 m, 98%.

thy patients, generally defined by the presence of ragged red fibers on muscle biopsy, ~30% do not have one of the known mtDNA defects described above. Although the majority had onset of disease during or after childhood, one family was notable in that two sibs had congenital onset of myopathy with mental retardation, followed by slowly progressive cerebellar ataxia. Other maternal relatives exhibited adult-onset muscle weakness and diabetes mellitus or diabetes mellitus alone. We report the clinical, biochemical, and molecular genetic findings in this family who have a novel mtDNA tRNA mutation.

Patients and Methods

Patients

The pedigree of the family is shown in figure 1.

Case IV-1.—This 28-year-old man was the product of a full-term delivery, but decreased fetal movements had been noted near term. He was a hypotonic infant who fed poorly. Motor development was markedly delayed, and he did not walk until age 4 years. Intellectual development was also delayed, with poor academic performance, although he attended a normal school. He was never able to run or play sports, because of muscle weakness and poor coordination, and he was always noted to have an unsteady gait with frequent falls. There was no history of deafness, seizures, or visual impairment. The course of his symptoms was very slowly progressive, apart from a prolonged episode in his midteens during which he was unable to walk for ~1 year. Clinical examination at age 28 years showed a thin man of low intellect. He had a mild lumbar scoliosis, pectus carinatum, and bilateral pes cavus. There was no ophthalmoplegia, retinopathy, or deafness. He had mild facial weakness and a cerebellar dysarthria. Muscle bulk was globally reduced, and he had moderate proximal weakness and ataxia affecting all limbs, as well as a waddling unsteady gait. Tendon reflexes were decreased and plantar responses flexor. Sensation and general physical examination were normal.

Resting plasma lactate was elevated, at 1.94 mmol/liter (normal <1.8 mmol/liter), but pyruvate was normal. Creatine kinase was 900 IU/liter (normal <195). Blood glucose, electroencephalogram, echocardiogram, nerve conduction studies, and visual and brain stem–evoked responses were normal. Electromyography showed mild myopathic changes. Brain magnetic resonance imaging demonstrated mild cerebellar atrophy. Formal psychometry indicated that he was functioning at a low average level. Muscle histochemistry was abnormal, showing a marked increase in fiber-diameter spectrum (10–100 microns). Ten percent ragged red fibers were noted on Gomori modified trichrome staining, and there were scattered COX-negative fibers. Abnormal mitochondrial morphology with type I curvilinear and rectangular paracrystalline inclusions were seen on electron microscopy. Polarographic and enzymatic analyses of freshly isolated mitochondria demonstrated a complex I and IV defect of the respiratory chain; cytochrome b and aa_3 concentrations were slightly reduced (table 1).

Case IV-2.—This 25-year-old female is the sister of IV-1. Her history is virtually identical to that of her brother; she was a hypotonic infant with subsequent motor and intellectual developmental delay. In addition, when 1 mo old she developed cardiac failure, and a diagnosis of primary pulmonary hypertension was made following cardiac catheterization. This resolved fully by the age of 1 year. Her gait had always been unsteady, with poor exercise tolerance because of muscle fatigue. She had never been able to run and never had played sports at school. Examination at age 25 years showed a thin female of low intellect. She had bilateral pes cavus but no spinal abnormality. The neurological findings were similar to those of her brother, with slightly less weakness but more ataxia. There were no signs of cardiac failure.

Resting plasma lactate, pyruvate, creatine kinase, and glucose were normal. Electrocardiogram showed first-degree heart block, and mild mitral regurgitation was noted on echocardiography. Results of computed-to-

Table 1

Biochemical Analyses

	Proband (IV-1)	Mother (III-2)	Mean (SD) in Controls
Mitochondrial state 3 respiration rates for substrates (ng at 0/min/mg mitochondrial protein):			
Pyruvate + malate	64	51	101 (18)
Glutamate + malate	52	54	107 (25)
Succinate + rotenone	123	72	142 (43)
Ascorbate + TMPD	291	145	301 (99)
Enzyme activities in isolated muscle mitochondria:			
NADH CoQ_1 reductase (nmol/min/mg mitochondrial protein)	45	36.5	135 (70)
Succinate cytochrome c reductase (nmol/min/mg mitochondrial protein)	459	195	221 (102)
Cytochrome oxidase (first-order rate constant/min/mg mitochondrial protein)	13	9.2	33.7 (16.7)
Citrate synthase (nmol/min/mg mitochondrial protein)	828	796	1,655 (783)
Cytochrome concentrations (nmol/mg mitochondrial protein):			
b	.254	.353	.29–.48
c	.752	.513	.51–.83
aa_3	.268	.252	.30–.59

mography brain scan were normal. Psychometric testing suggested that she was performing at a borderline defective level. Muscle biopsy showed ragged red and COX-negative fibers.

Case III-3.—This 50-year-old female, the mother of IV-1 and IV-2, was well until the age of 45 years, when she developed diabetes mellitus. Initially this was treated with oral hypoglycemic agents, but she subsequently required insulin. She began to experience mild limb weakness and exercise intolerance at age 48 years, which progressed slowly to the point that she had difficulty walking up stairs and doing housework. Examination showed mild proximal muscle weakness of all four limbs and a mild background diabetic retinopathy, but there was no other abnormality. Her muscle weakness improved on insulin therapy. Muscle biopsy showed many ragged red fibers with succinate dehydrogenase staining, as well as a few COX-negative fibers. Biochemical analysis of freshly isolated muscle mitochondria demonstrated a complex I and IV defect, with slightly low cytochrome aa_3 concentrations (table 1). Nerve conduction studies showed evidence of a mild axonal neuropathy. Plasma insulin and glucose concentrations were 5 mU/liter and 22 mmol/liter, respectively; HOMA analysis (Matthews et al. 1985) suggested reduced β cell function (5% of normal) and increased (twice normal; 49% insulin sensitivity) peripheral insulin resistance. Results of autoantibody screening including anti-islet cell, anti-GAD, and anti-vagal antibodies were negative.

Case III-1.—This female died at age 3 mo and was said to have been a floppy infant who fed poorly. No further information was available.

Case III-2.—This male, age 57 years, is asymptomatic.

Cases II-2 and II-4.—These two females died at ages 60 years and 70 years, respectively, from myocardial infarction. Both developed diabetes mellitus in their late 40s and required insulin. Neither was known to have myopathy or other neurological disease.

Biochemical Analyses

Mitochondria were prepared from skeletal muscle removed from the quadriceps under general anesthesia, with informed consent and ethical committee approval, by standard procedures (Holt et al. 1989). Polarographic determination of mitochondrial function and low-temperature analysis of cytochrome content were performed as described elsewhere (Morgan-Hughes et al. 1977), as was spectrophotometric analysis of respiratory-chain enzyme activities (Mann et al. 1992).

mtDNA Analysis

Total DNA was extracted from blood and muscle by standard techniques. Southern blotting was performed on total muscle DNA (Holt et al. 1988). Direct sequenc-

ing of all 22 tRNA genes was undertaken using a Sequenase DyeDeoxy Terminator sequencing kit and a 373A automated DNA sequencer (Applied Biosystems). Sequence data were analyzed using SeqEd software (ABI). PCR fragments encompassing all tRNA genes were purified with Magicprep (Promega) and were rendered single stranded by capture onto streptavidin-coated beads (Dynal). Internal primers were then used for the sequencing reaction. Primers for tRNA gene PCR and subsequent sequencing were as follows: phenylalanine 371–390 forward (F), 920–901 reverse (R), and 706–687 internal (I); valine 1412–1431 (F), 1856–1837 (R), and 1751–1732 (I); leucine (UUR) 3054–3073 (F), 3558–3539 (R), and 3116–3135 (I); isoleucine/glutamine/methionine cluster 4021–4040 (F), 5920–5901 (R), and 4211–4230 (I); tryptophan/alanine/asparagine/cysteine/tyrosine cluster 4021–4040 (F), 5920–5901 (R), and 5454–5473 (Ia) and 5651–5670 (Ib); serine/aspartic acid cluster 7377–7396 (F), 7865–7846 (R), and 7650–7631 (I); lysine 8196–8216 (F), 8726–8707 (R), and 8426–8445 (I); glycine/arginine cluster 9282–9301 (F), 10604–10584 (R), and 9911–9930 (Ia) and 10361–10380 (Ib); histidine/serine/leucine (CUN) cluster 11835–11854 (F), 12585–12566 (R), and 12072–12091 (I); glutamic acid 13926–13945 (F), 14883–14864 (R), and 14839–14820 (I); and threonine/proline cluster 15788–15807 (F), 16259–16240 (R), and 16150–16131 (I) (Anderson et al. 1981). Template strands were biotinylated.

A mismatch PCR (Zeviani et al. 1991*a*) was designed in order to screen for the 14709 mutation, creating a restriction site for *Sna*BI in mutant mtDNA. The forward primer was 14596–14615, and the reverse mismatch primer was 14729–14710 AAATACAACGATGGTTTTAC (the mismatch nucleotide is underlined). Conditions for mismatch PCR were as follows: one cycle of 94°C for 3 min, followed by 30 cycles of 92°C for 30 s, 50°C for 30 s, and 72°C for 30 s and, finally, one cycle of 72°C for 10 min. After digestion with *Sna*BI, fragments were separated in a 3.2% agarose gel and stained with ethidium bromide. The 133-bp product was digested into two fragments, of 113 and 20 bp, in mutant mtDNA but not in wild-type mtDNA.

To confirm the presence of heteroplasmy and to exclude the possibility of partial digestion, a second mismatch PCR was designed to detect the wild-type nucleotide at position 14709, creating a *Nde*I restriction site in wild-type mtDNA but not in mutant mtDNA. The forward mismatch primer was TACAACCACGACCAATCATA, and the reverse primer was 14839–14820. Conditions for this second mismatch PCR were identical to those described above, except for an annealing temperature of 52°C. After digestion with *Nde*I, the 150-bp fragment generated was cut into two fragments, of 131 and 19 bp, only in wild-type mtDNA. For quantification of the 14709 mutation, the first mismatch PCR was performed, with the addition of ^{35}S dATP prior to the final cycle. The products were then digested with *Sna*BI and separated on a 12% nondenaturing polyacrylamide gel, and the fragments were quantified by scanning the dried gel on a Phosphor Imager (Molecular Dynamics).

To screen for the change identified at position 5558, a third mismatch PCR was designed, creating a *Hinf*I restriction site in the presence of the A-to-G change at this position. The forward primer was 5454–5473, and the reverse mismatch primer was (5578–5569) AGAAATTAAGTATTGCAACT. Conditions for PCR were identical to those of the first PCR described above. In the presence of the 5558 change, the 124-bp fragment generated was digested into two, of 104 and 20 bp.

Myoblast and Fibroblast Culture

Mass myoblast cultures were established from the diagnostic muscle biopsy by standard techniques (Yasin et al. 1977; Blau and Webster 1981). Standard growth medium for myoblasts comprised DMEM (Dulbecco's modified Eagle's medium) with GLUTAMAX I, 4.5 g glucose/liter, sodium pyruvate (110 mg/liter), 20% FCS, 2% detoxified chick embryo extract, uridine (50 μg/ml), and penicillin and streptomycin. Cells were confirmed to be myoblasts by their ability to fuse and form myotubes in fusion medium (identical to growth medium but containing 2% horse serum instead of FCS). Fibroblast cultures were established from skin-punch biopsies by standard methods. Growth medium for fibroblasts was as described above but without chick embryo extract.

Total RNA was extracted from 60-mm plates of cells by standard methods (Chomzynski and Sacchi 1987). RNA was separated in 1.8% agarose gels and then was transferred to nylon membranes for hybridization with ^{32}P dATP–labeled oligonucleotide probes, prepared by PCR, for ND5 (13926–14217) and COX subunit I (5971–6968). Blots were analyzed by densitometry using an LKB 220 densitometer. The amount of RNA blotted for each sample was normalized to the nuclear 18S rRNA probe.

Mitochondrially encoded translation products were studied by using methods adapted from Attardi and Ching (1979). Sixty-milliliter plates of cells in 2 ml labeling medium (modified Eagle's medium without methionine or cysteine, with 5% dialyzed FCS) were exposed to ^{35}S methionine (Trans label ICN) (150 μCi/ml) in the presence of emetine (200 μg/ml), a specific inhibitor of cytosolic protein synthesis, for 2.5 h. Cells were preincubated in labeling medium without label for 20 min and were chased with normal growth medium for 30 min after the labeling period. After the labeling, cells were harvested, by cell scrapers, into ice-cold PBS saline pelleted and frozen at −70°C. Cell pellets were subse-

quently resuspended in 120 μl loading buffer (62.5 mM Tris pH 6.8, 4% SDS, 20% glycerol, and 5 mM aminobenzamidine) and were sonicated (three 10-s bursts at 10 microns). Equal amounts of total cellular protein (100 μg), determined by a DC protein assay kit (Biorad), were loaded onto 6 M urea/18% polyacrylamide gels (Kadenbach et al. 1983). After electrophoresis, gels were fixed in 50% methanol/10% acetic acid, were treated with Entensify (Dupont), and then were dried and exposed to Kodak X-Omat film. Mitochondrial translation products were assigned on the basis of chloramphenicol sensitivity and molecular weight. With this gel system, COX subunits II and III often migrate as a single band (Kadenbach et al. 1983). Translation products were analyzed by densitometry; the total area of the peaks detected in the patient sample was expressed as a percentage of control values.

Results

Southern blot analysis showed no evidence of a major rearrangement. Changes in four tRNA genes were identified in a comparison with the published mtDNA sequence (Anderson et al. 1981); these changes were tRNA arginine T→C 10463, tRNA glutamic acid T→C 14709 (fig. 2), tRNA threonine G→A 15928, and tRNA tryptophan A→G 5558. The changes at positions 15928 and 10463 have recently been reported in healthy controls (Houshmand et al. 1994). The 5558 change was not detected in 100 control subjects but was homoplasmic in all cases analyzed. The change at position 14709 was heteroplasmic in blood and muscle. It was not detected in a total of 203 additional individuals (71 mitochondrial myopathy patients and 132 healthy ethnically matched control subjects). The proportion of mutant mtDNA was highest in the muscle of the proband and his sister (99% and 98%, respectively) and was lowest in the blood of their unaffected uncle (65%) (fig. 1).

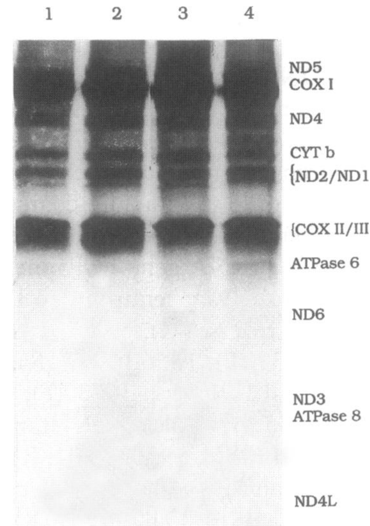

Figure 3 Analysis of mitochondrial protein synthesis in fibroblasts: autoradiography of mitochondrial translation products after electrophoresis in a 6 M urea/18% polyacrylamide gel. Cells were derived from skin biopsies of IV-1 (lane 1), III-3 (lane 2), and a control subject (lanes 3 and 4). COX I, COX II, and COX III = subunits of cytochrome c oxidase; ND = subunits of NADH-CoQ reductase; CYT b = cytochrome b; ATPase6 and ATPase8 = subunits of ATP synthase.

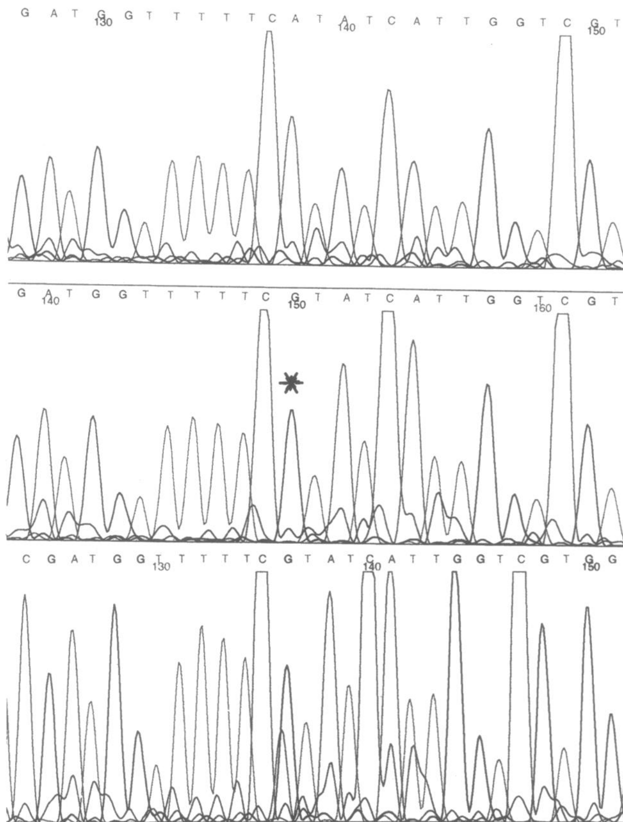

Figure 2 Chromatogram from the automatic sequencer, showing the transition A→G (heavy strand) at position 14709 of the $tRNA^{Glu}$. *Top,* Control. *Middle,* IV-1. The asterisk (*) indicates mutation at bp 14709. *Bottom,* III-2.

Analysis of mass cell cultures showed the following amounts of mutant mtDNA: myoblasts from III-3, 85%; fibroblasts from III-3, 85%; and fibroblasts from IV-1, 90%. Mitochondrial translation products in fibroblasts from III-3 and IV-1 did not differ qualitatively or quantitatively from those in control cultures (fig. 3). In myoblasts from case III-3, densitometry indicated a 35% reduction in the rate of total translation, compared with that in control cultures. There was no obvious specific subunit deficiency (fig. 4). Northern blots showed no abnormalities in the transcripts studied, compared with controls, or any extra bands (data not shown).

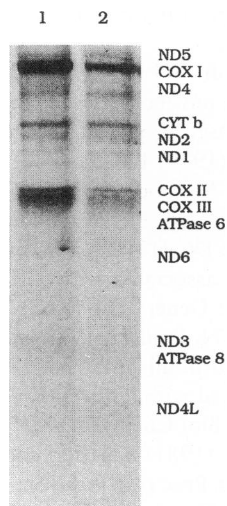

Figure 4 Myoblast mitochondrial translation products in a control subject (lane 1) and III-3 (lane 2) harboring 85% mutant mtDNA.

Discussion

We have described the clinical, biochemical, and molecular genetic findings in members of a family exhibiting a range of clinical phenotypes, including possible premature infantile death, congenital myopathy with cerebellar ataxia and mental retardation, and adult-onset diabetes mellitus with muscle weakness. Although the initial presentation of the proband, a picture suggestive of a congenital myopathy, is unusual for disease associated with primary defects of mtDNA, the histochemical and biochemical findings in biopsied muscle prompted us to undertake detailed mtDNA analysis.

Investigation for large-scale rearrangements and known mtDNA point mutations was negative. On sequencing all 22 mitochondrial tRNA genes in muscle mtDNA extracted from the proband, four changes from the published human mtDNA sequence were identified (Anderson et al. 1981). Those at positions 10463 and 15928 most likely represent neutral polymorphisms, since they occur in healthy control individuals (Houshmand et al. 1994). The other two changes have not been reported previously, and we investigated them further for evidence of pathogenicity. The following criteria were applied: heteroplasmy, absence from controls, and evolutionary conservation. Although the change at position 5558 was not present in 100 control subjects and occurs at a conserved position within the tRNA gene for tryptophan, it was homoplasmic in blood and muscle from all individuals investigated, both symptomatic and asymptomatic. In contrast, the change at position 14709 was heteroplasmic, with a correlation between amount of mutant mtDNA and clinical severity in the family, in both blood and muscle. Heteroplasmy, the coexistence of mutant and wild-type mtDNA, is a phenomenon generally associated with pathogenic mutations as opposed to harmless polymorphisms (Holt et al. 1990; Zeviani et al. 1991*b*). Furthermore, the mutation was absent in >200 controls and occurs at a highly conserved position, immediately adjacent to the anticodon of the tRNA gene for glutamic acid. The G-A base pair at 14709 is conserved in all mammalian and nearly all other species, extending back to yeasts and even chloroplasts, the only exception being *Ascaris suum* (Sprinzl et al. 1989). The change at position 14709 is therefore likely to be the pathogenic mutation in this family. This is supported by a brief report of a second family with this mutation (Moraes et al. 1994). We cannot exclude the possibility that the change at position 5558 is contributing to the disease phenotype, but it seems unlikely that it represents the primary pathogenic mutation.

There are an increasing number of mtDNA tRNA mutations not involving the tRNA leucine gene, and it remains to be seen whether this is, in fact, a hotspot for mutations, as has been suggested by some authors (DiMauro and Moraes 1993). This is the third mutation to be described in the anticodon loop of a tRNA molecule (Moraes et al. 1993; Yoon et al. 1993), clearly a functionally important region; a base change in this position may well have structural and therefore functional consequences.

In order to investigate the molecular pathogenesis of disease induced by the 14709 mutation, we examined its effect on translation of mitochondrial proteins in skin fibroblasts from the proband and his mother and in mass myoblast cultures from the mother. Translation in fibroblast cultures did not differ from that in controls, which may suggest either that fibroblasts are not affected by this mutation, as has been suggested in relation to other point mutations (Bindoff et al. 1993), or that the amount of mutant mtDNA was not high enough in these cultures to result in impaired translation in fibroblasts. We plan to investigate this question further by isolating clonal cultures with higher amounts of mutant mtDNA. There was, however, evidence of impaired translation in the myoblasts from the mother harboring 85% mutant mtDNA, which was not associated with abnormal transcripts. Taken together, these data would be consistent with a posttranscriptional pathogenetic mechanism.

The phenotypic variation associated with this mutation is striking. Such diversity within families has been described in relation to other mtDNA point mutations and has been suggested to be related, at least in part, to differences in tissue distribution and amount of mutant mtDNA between individuals, although this is probably not the whole explanation (Shoffner et al. 1990; Sweeney et al. 1994). The brother and sister reported here are unusual in having congenital onset. It is also possible that the aunt who died at age 3 mo had a severe

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form of this mitochondrial disease. The majority of patients with pathogenic tRNA gene mutations have disease onset in childhood or after. There are two case reports of tRNA mutations with infantile onset. Yoon et al. (1993) described a girl who died at 2 ½ d, with multisystem failure, lactic acidosis, and hypoglycemia, who had a heteroplasmic mutation in the anticodon loop of tRNA threonine. Tanaka et al. (1990) identified a mutation at position 4317 in a boy who died of cardiomyopathy at age 1 year. However, these mutations have not subsequently been identified in further patients.

Biochemical analysis of skeletal muscle mitochondria indicated impaired activity of respiratory-chain complexes I and IV, consistent with a tRNA mutation impairing intramitochondrial protein synthesis. It is notable that the biochemical phenotype appeared to be quantitatively similar in the muscle of the proband and his mother, despite the latter having much milder muscle weakness that improved on insulin therapy.

There are an increasing number of reports suggesting that there is an association between diabetes mellitus and primary defects of mtDNA, both large-scale rearrangements and point mutations of tRNA genes (Ballinger et al. 1992; Reardon et al. 1992; van den Ouweland et al. 1992; Kadowaki et al. 1994). Normal insulin secretion by the pancreatic islet cells is known to be highly dependent on oxidative phosphorylation (Ashcroft and Ashcroft 1992), and therefore it is possible that this process might be impaired if oxidative phosphorylation is compromised. In keeping with this hypothesis, Reardon et al. (1992) and Kadowaki et al. (1994) found impaired insulin secretion in patients with the tRNA leucine (UUR) 3243 mutation. In the present study, three members in the maternal line developed diabetes that required insulin therapy. Only one patient, III-3, was still living, and measurements of plasma insulin and glucose were consistent with markedly impaired pancreatic β-cell function and a relatively mild degree of peripheral resistance. van den Ouweland et al. (1992) found normal plasma insulin concentrations in their diabetic patients with the 3243 mutation, suggesting that mechanisms of diabetogenesis in patients with mtDNA defects may be heterogeneous.

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