Am. J. Hum. Genet. 62:262–268, 1998

Demonstration of a New Pathogenic Mutation in Human Complex I Deficiency: A 5-bp Duplication in the Nuclear Gene Encoding the 18-kD (AQDQ) Subunit

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Summary

We report the cDNA cloning, chromosomal localization, and a mutation in the human nuclear gene encoding the 18-kD (AQDQ) subunit of the mitochondrial respiratory chain complex I. The cDNA has an open reading frame of 175 amino acids and codes for a protein with a molecular mass of 23.2 kD. Its gene was mapped to chromosome 5. A homozygous 5-bp duplication, destroying a consensus phosphorylation site, in the 18-kD cDNA was found in a complex I–deficient patient. The patient showed normal muscle morphology and a remarkably nonspecific fatal progressive phenotype without increased lactate concentrations in body fluids. The child's parents were heterozygous for the mutation. In 19 other complex I–deficient patients, no mutations were found in the 18-kD gene.

Introduction

Among the inborn errors of metabolism, disturbances in the mitochondrial energy metabolism form a quantitatively major group, occurring in 1/10,000 live births (Bourgeron et al. 1995). In case of a defective pyruvate oxidation, skeletal muscle and brain tissue are often involved. In these mitochondrial encephalomyopathies (OMIM 251900[http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?251900]), the most frequently observed enzyme deficiencies are located in the pyruvate dehydrogenase complex and complexes I (OMIM 252010 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?252010]) and/or IV of the respiratory chain (Wallace 1992; Ruitenbeek et al. 1996). Numerous patients with isolated complex I deficiency have been reported (Korenke et al. 1990; DiMauro 1993; Shoffner et al. 1994). Their clinical presentation in general is that of a very severe, often fatal, multisystemic disorder frequently dominated by brain and skeletal muscle abnormalities. Less severe phenotypes have also been described (Wallace 1992; Trijbels et al. 1996). Until now, only one mutation in a nuclear gene has been described in a patient with a defective respiratory chain enzyme complex: a point mutation in the flavoprotein (FP) subunit of complex II (Bourgeron et al. 1995). No mutations in the nuclear genome of one of the other respiratory chain complexes have been reported. The relatively high frequency of complex I–deficient patients prompted us to investigate the molecular cause of this enzyme deficiency.

Complex I or NADH:ubiquinone oxidoreductase, the first multisubunit enzyme complex of the mitochondrial respiratory chain, plays a vital role in cellular ATP production, the primary source of energy for many crucial processes in living cells (Walker 1992; Pilkington et al. 1993; Trijbels et al. 1996). It removes electrons from NADH and passes them via a series of different protein-coupled redox centers (FMN and FeS clusters) to the electron acceptor ubiquinone (Yamaguchi and Hatefi 1993). In well-coupled mitochondria, the electron flux leads to ATP generation via the building of a proton gradient over the inner membrane.

Complex I is composed of $\geqslant 41$ subunits, of which 7 are encoded by the mitochondrial genome (ND1-6, ND4L) and the remaining by nuclear genes (Fearnley and Walker 1992). Limited information is available concerning the precise location and function of the individual subunits. Very recently, a low-resolution three-dimensional structure of complex I from *Neurospora crassa* has been elucidated (Guenebaut et al. 1997). A number of different subunits contribute to binding sites for FMN, NADH, and 4Fe-4S and 2Fe-2S centers (Walker 1992; Pilkington et al. 1993). It is hypothesized that

Received August 4, 1997; accepted for publication December 5, 1997; electronically published February 13, 1998.

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0002-9297/98/6202-0009$02.00

subunits of the FP fraction play a role in FMN and NADH binding. Proteins in the IP (iron-sulfur protein) fraction likely function in reduction-oxidation reactions via different iron-sulfur clusters. Very recently, it has been shown that the nuclear-encoded 18-kD (AQDQ) subunit of bovine heart complex I can be phosphorylated by a mitochondrial cyclic adenosine 5^3-monophosphate (cAMP)–dependent protein kinase (Papa et al. 1996). It is well established that the 18-kD (AQDQ) subunit is in the extrinsic domain of complex I (Walker 1992).

In some patients (i.e., in Leber hereditary optic neuropathy) a mutation in the ND4 subunit of the mitochondrial genome has been established (Brown et al. 1997). So far, the human cDNA sequences of seven nuclear-encoded subunits (i.e., 75 kD, 51 kD, 24 kD, B22, B13, PSST, and NDUFA1) have been published (Chow et al. 1991; Spencer et al. 1992; De Coo et al. 1995; Hattori et al. 1995; Gu et al. 1996; Hyslop et al. 1996; Zhuchenko et al. 1996; Pata et al. 1997). The availability of the bovine cDNA sequence for the 18-kD (AQDQ) protein (Walker et al. 1992) enabled us to apply a PCR-based strategy for generating the cDNA for the human 18-kD protein from human heart.

In this article, we present the human cDNA sequence of the nuclear-encoded 18-kD subunit, the chromosomal localization of the gene, and the results of mutation detection in 20 complex I–deficient patients all exhibiting a multisystem disorder. Detailed information is presented about the clinical symptoms of one patient in whom we found a 5-bp duplication in the 18-kD gene. This is the first report of a pathological mutation in a nuclear complex I gene of the mitochondrial respiratory chain.

Subjects and Methods

Case History

A male patient, born after an uncomplicated pregnancy as the second child of healthy nonconsanguineous Caucasian parents, was first admitted at the age of 8 mo with severe vomiting, failure to thrive, and hypotonia. Despite supportive care, his clinical condition deteriorated in the following months. At the age of 13 mo, he showed severe psychomotor retardation, convulsions, bradypnea, cyanosis, hypotonia, and depressed tendon reflexes. Results of routine clinical chemical blood and urine investigations, as well as amino-acid and organic-acid analyses in blood and urine, were normal. Lactic acid concentrations in blood and cerebrospinal fluid were within the range of controls. Electromyographic parameters were normal. Cerebral magnetic resonance imaging revealed generalized brain atrophy and symmetrical basal ganglia abnormalities. When the patient was 14 mo of age, a skeletal muscle biopsy (musculus quadriceps femori) was performed to investigate the mitochondrial energy-generating system (see Biochemical Methods). Light and electron microscopy and histochemistry did not reveal abnormalities. No ragged-red fibers were present. The child's condition deteriorated severely, and he died of cardiorespiratory failure at the age of 16 mo. The family history is normal for neuromuscular disorders. A younger brother and older sister of the proband are healthy.

Biochemical Methods

A biopsy of the patient's quadricep muscles was performed when he was 14 mo of age. Mitochondrial enzyme activities were determined in mitochondria isolated from frozen tissue, as described previously by Elpeleg et al. (1997). Respiratory chain enzyme activities in fibroblasts, cultured in M199 medium until confluency, were determined in mitochondria-enriched fractions as described by Bentlage et al. (1996). Enzyme assays were essentially identical to those described by Fischer et al. (1986) for skeletal muscle.

Cloning of the Human 18-kD cDNA

Human 18-kD cDNA was obtained by reverse transcription using oligo(dT) and random hexamer primers on poly A^+ RNA isolated from human heart (Clonetech). PCR amplification was performed with cDNA as template according to the protocol for *Taq* polymerase (Life Technologies) by use of oligonucleotides 5′-ATG GCG GCG GTC TCA ATG TC-3′ and 5′-CTA TTT TGT GGA TAC TCT TCT TC-3′. Oligonucleotide sequences were based on homology with bovine 18-kD cDNA sequence (Walker et al. 1992) and known human expressed sequence tags (ESTs). The PCR product was purified for direct DNA sequencing. DNA sequence analysis of both DNA strands was performed using the dyedeoxy-terminators on an automated ABI 377 sequencer according to a protocol provided by the manufacturer (Applied Biosystems).

Chromosomal Localization of the 18-kD Gene

Genomic DNA was isolated from human-rodent somatic cell hybrids containing all 24 human chromosomes (Coriell Cell Depositories) digested with *Eco*RI, fractionated, and blotted to Hybond nylon membrane. The blot was hybridized with α^{32}P-dCTP–labeled human 18-kD cDNA probe (of 528 bp). Binding of the probe was visualized by autoradiography.

Mutation Detection

Total RNA was extracted (Chomczynski and Sacchi 1987) from cultured skin fibroblasts (Bentlage et al. 1996) and stored as ethanol precipitate at −80°C. A 5-

μg sample of RNA was reverse transcribed to cDNA in 1 h at 42°C with 200 U of Superscript II reverse transcriptase (RT) (Life Technologies), using oligo(dT) and random hexamer primers. One microliter of cDNA was subjected to PCR amplification. By PCR we generated one fragment covering the entire coding region, using the synthetic oligonucleotide primers described before. The PCR program consisted of 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. The cycles were preceded by an initial denaturation step of 3 min at 95°C and were followed by a final extension of 10 min at 72°C. The PCR reaction was analyzed on a 1.0% agarose gel with 0.5 μg/ml ethidium bromide in 1 × Tris-borate EDTA. The nucleotide sequences of the PCR products were analyzed by direct sequencing using the *Taq* Dye Deoxy terminator cycle sequencing kit according to the manufacturer's recommendations (Applied Biosystems).

To confirm the mutation at the genomic DNA level, a fragment consisting of 96 bp was amplified in the same PCR buffer as described above, using forward primer 5′-TAT GAC ATT GAA GAG AGG AAG G-3′ and reverse primer as decribed above. PCR fragments were resolved in a 20% polyacrylamide gel. Ethidium bromide was used to visualize the PCR fragments, and the presence of the duplication could be checked by size.

Results

Biochemical Results

From the decreased activity of NADH:ferricyanide oxidoreductase as well as NADH:cytochrome c reductase, a deficiency in complex I in skeletal muscle mitochondria can be concluded (see table 1). The NADH:Q_1 oxidoreductase assay revealed a complex I deficiency in cultured skin fibroblasts also. The activities of the other respiratory chain enzyme complexes and Mg^{2+}-ATPase were all normal.

Cloning of the Human 18-kD cDNA

We obtained a 528-bp cDNA fragment of the human nuclear-encoded 18-kD gene by RT-PCR using human heart RNA and oligonucleotide primers based on the 18-kD cDNA sequence from cow (Walker et al. 1992) and known human ESTs (Adams et al. 1995). Sequence analysis of the human 18-kD cDNA clone revealed an open reading frame of 175 amino acids (fig. 1). The amino acid sequence showed 91% homology with the bovine 18-kD amino acid sequence (data not shown). The calculated molecular weight of the protein encoded by the human 18-kD cDNA is 23.2 kD. As in the cow, the protein contains a leader sequence (amino acids 1–42) for import into the mitochondria. In the cow, this signal peptide is removed after import. There is a phosphorylation consensus site (RVS) at amino acid positions 171–173 (Pearson and Kemp 1991; Kemp et al. 1994). The human protein has no cysteine residues.

Table 1

Enzyme Activities in Skeletal Muscle and Fibroblasts

	Enzyme Activity	
	Patient	Control Range
Skeletal muscle:		
Cytochrome c oxidase	7.2[a]	2.8–11.8[a]
NADH:ferricyanide oxidoreductase	1.16[b]	1.7–4.1[b]
NADH:cytochrome c reductase	27.0[c]	63–529[c]
Succinate:cyt c oxidoreductase	100.0[c]	39–249[c]
Citrate synthase	1.28[b]	1.0–2.1[b]
Mg^{2+}-ATPase	634.0[d]	213–553[d]
Pyruvate dehydrogenase complex	18.3[c]	27–61[c]
Fibroblasts:		
NADH:Q_1 oxidoreductase	.075[e]	.10–.26[e]
Succinate:cyt c oxidoreductase	.23[e]	.21–.44[e]
Decylubiquinol:cyt c oxidoreductase	1.22[e]	1.25–2.62[e]
Cytochrome c oxidase	239.0[c]	147–252[c]
Citrate synthase	311.0[c]	144–257[c]

[a] In k/mg protein.
[b] In U/mg protein.
[c] In mU/mg protein.
[d] In mU/g protein.
[e] In mU/mU cytochrome c oxidase.

Chromosomal Localization of the Human 18-kD Gene

A Southern blot with genomic DNA from human-rodent somatic cell hybrids containing all human chromosomes has been used for hybridization with human 18-kD cDNA as probe. A specific hybridization signal was detected only for chromosome 5, indicating that the nuclear-encoded 18-kD (AQDQ) gene maps to this chromosome (fig. 2).

Mutation Detection in the Human 18-kD Subunit

A template of human cDNA was produced by first-strand synthesis of human fibroblast RNA originating from 20 patients with a complex I deficiency enzymatically established in fibroblasts and, when available, also in skeletal muscle and other tissues. One set of oligonucleotide primers enabled us to amplify the entire coding region of the 18-kD cDNA. Direct sequencing of this region revealed a novel homozygous 5-bp duplication of the cDNA sequence at positions 466–470 (AAGTC) in one patient (fig. 3*A*). No other sequence aberrations could be detected in the 18-kD cDNA of all other pa-

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                                                                    GC AGC AAG   -1

  1  ATG GCG GCG GTC TCA ATG TCA GTG GTA CTG AGG CAG ACG TTG TGG CGG AGA AGG GCA GTG   60
  1   M   A   A   V   S   M   S   V   V   L   R   Q   T   L   W   R   R   R   A   V   20

 61  GCT GTA GCT GCC CTT TCC GTT TCC AGG GTT CCG ACC AGG TCG TTG AGG ACT TCC ACA TGG  120
 21   A   V   A   A   L   S   V   S   R   V   P   T   R   S   L   R   T   S   T   W   40

121  AGA TTG GCA CAG GAC CAG ACT CAA GAC ACA CAA CTC ATA ACA GTT GAT GAA AAA TTG GAT  180
 41   R   L   A   Q   D   Q   T   Q   D   T   Q   L   I   T   V   D   E   K   L   D   60

181  ATC ACT ACT TTA ACT GGC GTT CCA GAA GAG CAT ATA AAA ACT AGA AAA GTC AGG ATC TTT  240
 61   I   T   T   L   T   G   V   P   E   E   H   I   K   T   R   K   V   R   I   F   80

241  GTT CCT GCT CGC AAT AAC ATG CAG TCT GGA GTA AAC AAC ACA AAG AAA TGG AAG ATG GAG  300
 81   V   P   A   R   N   N   M   Q   S   G   V   N   N   T   K   K   W   K   M   E  100

301  TTT GAT ACC AGG GAG CGA TGG GAA AAT CCT TTG ATG GGT TGG GCA TCA ACG GCT GAT CCC  360
101   F   D   T   R   E   R   W   E   N   P   L   M   G   W   A   S   T   A   D   P  120

361  TTA TCC AAC ATG GTT CTA ACC TTC AGT ACT AAA GAA GAT GCA GTT TCC TTT GCA GAA AAA  420
121   L   S   N   M   V   L   T   F   S   T   K   E   D   A   V   S   F   A   E   K  140

421  AAT GGA TGG AGC TAT GAC ATT GAA GAG AGG AAG GTT CCA AAA CCC AAG TCC AAG TCT TAT  480
141   N   G   W   S   Y   D   I   E   E   R   K   V   P   K   P   K   S   K   S   Y  160

481  GGT GCA AAC TTT TCT TGG AAC AAA AGA ACA AGA GTA TCC ACA AAA TAG GTT GGC ACT GAC  540
161   G   A   N   F   S   W   N   K   R   T   R   V   S   T   K   *                  175

541  TAT ATC TCT GCT TGA CTG TGA ATA AAG TCA GCT ATG CAG TAT TTA TAG TCC ATG TAT AAT  600

601  AAA TAC ATC TCT TAA TCT CCT AAT AAA TTG GAC CTT TAA ACT ACA AAA AAA AAA AAA AAA  660
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Figure 1 Human cDNA sequence for the nuclear-encoded 18-kD (AQDQ) subunit of mitochondrial NADH:ubiquinone oxidoreductase. Nucleotide sequence of the human 18-kD cDNA (*upper line*). Deduced amino acid sequence of the open reading frame of 525 bp (175 codons) is shown on the second line. The cDNA sequence and deduced amino acid sequence have been submitted to GenBank.

tients. The 5-bp duplication results in a frameshift at codon K158 and destroys a phosphorylation site present at the carboxy terminus of the 18-kD protein. Furthermore, the duplication changes the amino acid sequence of the 18-kD protein from amino acid 158 to the carboxy terminus and expands the length of the protein by 14 amino acids (fig. 3*B*). The mutation was confirmed on the genomic DNA level by PCR combined with PAGE. Both parents appeared to be heterozygous for the mutation (fig. 4). The segregation of this mutation in the family is consistent with an autosomal recessive mode of inheritance. The duplication was not present in 100 control alleles and the 19 other complex I–deficient patients.

Discussion

Complex I deficiencies, whether or not as part of multiple enzyme deficiencies, are a major cause of mitochondrial encephalomyopathies. So far, no mutation in a nuclear gene of one of the numerous complex I subunits has been described. The multisystemic progressive

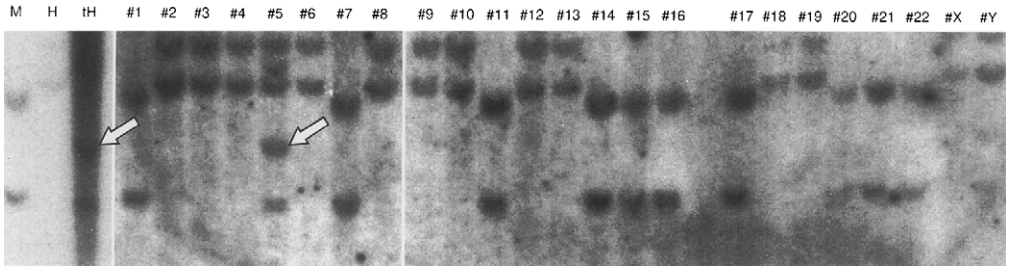

Figure 2 Chromosomal location of the human 18-kD (AQDQ) gene. The 18-kD gene was localized to chromosome 5 by chromosomal somatic cell hybrid analysis. Genomic DNA was isolated from a panel of mouse or hamster/human cells, each containing a single human chromosome. The DNA was digested with *Eco*RI, fractionated, transferred to Hybond nylon, and probed with a 528-bp 18-kD cDNA. A positive signal was observed in the sample of total DNA of human origin (*left arrow*). Mouse and hamster genomic DNA showed no 18-kD signal. A strong signal for 18 kD was seen only on hybrids containing chromosome 5 (*right arrow*).

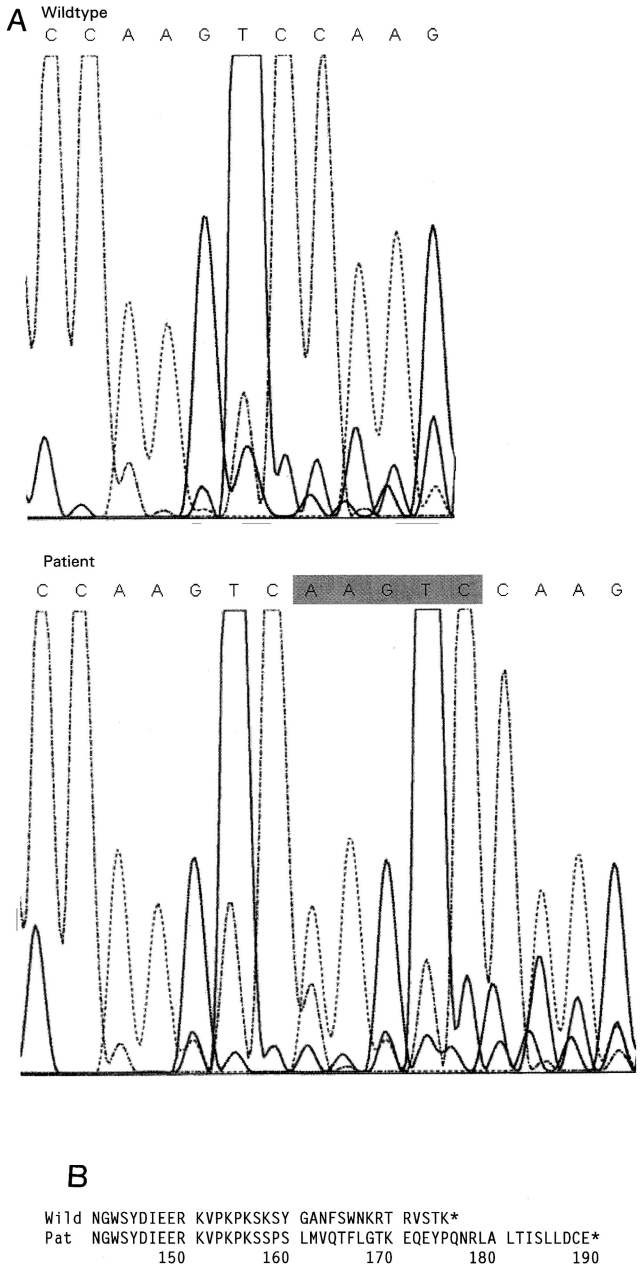

Figure 3 *A,* DNA sequence of 18-kD cDNA segments for wild type (*top*) and patient with the mutation (*bottom*). For the patient, a 5-bp duplication (AAGTC) was observed, resulting in a frameshift and extension of the C-terminus of the 18-kD protein. *B,* Primary C-terminal amino acid sequence of wild-type and patient's 18-kD protein.

clinical presentation of our patient was highly suggestive for a mitochondriocytopathy (Munnich et al. 1992; DiMauro 1993; Schoffner et al. 1994; Ruitenbeek et al. 1996). Despite normal clinical chemical features, biochemical analyses indeed revealed a reduced complex I activity both in skeletal muscle and cultured skin fibroblasts. In addition, a 5-bp duplication in the cDNA and

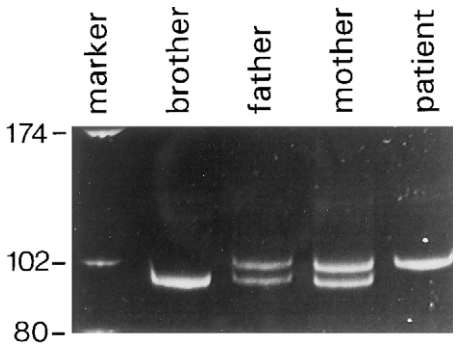

Figure 4 Molecular analysis of the 18-kD (AQDQ) gene. Segregation of the 5-bp duplication in the 18-kD gene in the family, detected by PCR on genomic DNA and by PAGE. Mutant and wild-type alleles are 101 bp and 96 bp, respectively. Lane 1, Molecular weight markers. Lane 2, Brother. Lane 3, Father. Lane 4, Mother. Lane 5, Index patient.

genomic DNA encoding the nuclear-encoded 18-kD (AQDQ) subunit of complex I of the respiratory chain could be demonstrated. Both parents were heterozygous for this mutation.

We started our mutation analysis in complex I–deficient patients with examination of the 18-kD (AQDQ) gene, because Papa et al. (1996) recently found that the bovine 18-kD subunit can be phosphorylated. A mitochondrial inner membrane–bound kinase acts via a cAMP-dependent mechanism. The presence of a phosphorylation/dephosphorylation site is frequently associated with regulation of biological processes. In the case of phosphorylation of the 18-kD subunit, one can hypothesize an influence on complex I activity or a role in posttranslational processes. The cDNA, encoding the 18-kD subunit of bovine complex I, had already been cloned. We cloned the human 18-kD subunit by RT-PCR, using human heart mRNA as template. The protein encoded by the human 18-kD gene is 175 amino acids long, and its calculated molecular mass is 23.2 kD. The amino acid sequences of the human and bovine 18-kD protein are highly conserved. There is ~91% identity between these two species. The phosphorylation consensus site (RVS) is also present in the human subunit. As in the cow, the human 18-kD (AQDQ) subunit has a leader sequence for import into the mitochondria. Although the human, like the bovine, 18-kD protein is supposed to be part of the IP fraction (Walker et al. 1992), it has no cysteine residues that might provide ligands for iron-sulfur centers. A Blast search for homologous sequences revealed two different EST clones (AA258924 and N29566) originating from different human tissues. The EST clones, whose functions are not known, have sequences homologous with different parts of the 18-kD protein.

We have included in this study 20 patients with a complex I deficiency in fibroblasts and, in most patients, in at least one other tissue as well. Direct sequencing of the entire 18-kD cDNA of these patients revealed in one patient a homozygous 5-bp duplication. A mistake made by the RT or *Taq* DNA polymerase in the RT-PCR was excluded by repeating the RT-PCR and DNA sequencing of this patient and three controls in three independent experiments, all of which revealed the homozygous duplication in the patient's cDNA. The presence of the duplication in the patient was confirmed on the genomic DNA level. The 5-bp duplication causes a shift in the translational reading frame. The shift results in (1) an elongation of the mature protein by 14 amino acids and (2) destroys the phosphorylation consensus site. Although the gene product belongs to the IP fraction and therefore is probably involved in crucial redox reactions, its relation to the adjacent subunits and its exact function are still obscure. Our results, a mutation with an autosomal recessive mode of inheritance, associated with a fatal multisystemic complex I deficiency biochemically established in two different tissue types, stress the physiological importance of this subunit in humans. Because the phosphorylation site in the 18-kD subunit is disrupted in the patient, we speculate that the mutation in this vital site for regulation of protein function in eukaryotic cells is responsible for the observed complex I deficiency. Alternatively, the obviously changed C-terminus of the 18-kD subunit of the patient has caused the complex I deficiency. The protein has been elongated by 14 amino acids, whereas amino acids 158–175 have been modified. A proper transport and/or folding and orientation of the 18-kD protein in the enzyme complex or in the mitochondrial inner membrane may be hindered. Perhaps the alterations in the patients 18-kD subunit also lead to instability and premature breakdown of the protein.

In conclusion, we present the first mutation in a nuclear gene of one of the subunits of complex I of the human respiratory chain. Although the frequency of the described mutation must be further established, the finding improves the potentials for genetic counseling and prenatal diagnosis. These preventive tools are of utmost importance, because therapeutic intervention is unsuccessful in the majority of complex I patients. Mutation analysis of other nuclear genes in patients with a complex I deficiency are in progress.

Acknowledgments

We are very grateful to Antoon Janssen, Frans van den Brand, Jeanette Elsink, and Ronney de Abreu for technical assistance. Asher Barak and Ann Saada are gratefully acknowledged, for clinical and biochemical assistance, and Rob Sengers, for critical reading the manuscript and ongoing enthusiastic support. This study was financially supported by the Stichting Voor Kinderen Die Wel Willen Maar Niet Kunnen to L.H. and J.S.

References

Adams MD, Kerlavage AR, Fleischman RD, Fuldner RA, Bult CJ, Lee NH, Kirkness EF, et al (1995) Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. Nature 377: 3–174

Bentlage H, Wendel U, Schägger H, Ter Laak H, Janssen A, Trijbels F (1996) Lethal infantile mitochondrial disease with isolated complex I deficiency in fibroblasts but with combined complex I and IV deficiencies in muscle. Neurology 47:243–248

Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Péquignot E, Munnich A, et al (1995) Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat Genet 11: 144–149

Brown MD, Sun F, Wallace DC (1997) Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 1778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 60:381–387

Chow W, Ragan I, Robinson BH (1991) Determination of the cDNA sequence for the human mitochondrial 75 kDa Fe-S protein of NADH-coenzyme Q reductase. Eur J Biochem 201:547–550

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

De Coo R, Buddiger P, Smeets H, Geurts Van Kessel A, Morgan-Hughes J, Olde Weghuis D, Overhauser J, et al (1995) Molecular cloning and characterization of the active human mitochondrial NADH:ubiquinone oxidoreductase 24-kDa gene (NDUFV2) and its pseudogene. Genomics 26:461–466

DiMauro S (1993) Mitochondrial encephalomyopathies. In: Rosenberg RN, Prusiner SB, DiMauro S (eds) The molecular and genetic basis of neurological disease. Butterworth-Heinemann, Boston, pp 665–694

Elpeleg ON, Saada AB, Shaag A, Glustein JZ, Ruitenbeek W, Tein I, Halevy J (1997) Lipoamide dehydrogenase deficiency: a new cause for recurrent myoglobinuria. Muscle Nerve 20: 238–240

Fearnley I, Walker JE (1992) Conservation of sequences of subunits of mitochondrial complex I and their relationships with other proteins. Biochim Biophys Acta 1140:105–134

Fischer JC, Ruitenbeek W, Gabreëls FJ, Janssen AJM, Renier RO, Sengers RCA, Stadhouders AM, et al (1986) A mitochondrial encephalomyopathy: the first case with an established defect at the level of coenzyme Q. Eur J Pediatr 144: 441–444

Gu JZ, Lin X, Wells DE (1996) The human B22 subunit of the NADH-ubiquinone oxidoreductase maps to the region of chromosome 8 involved in branchio-oto-renal syndrome. Genomics 35:6–10

Guenebaut V, Vincentelli R, Mills D, Weiss H, Leonard KR (1997) Three-dimensional structure of NADH-dehydroge-

nase from Neurospora crassa by electron microscopy and conical tilt reconstruction. J Mol Biol 265:409–418

Hattori N, Suzuki H, Wang Y, Minoshima S, Shimizu N, Yoshino H, Kurashima R, et al (1995) Structural organization and chromosomal localization of the human nuclear gene (NDUFV2) for 24-kDa iron-sulfur subunit of complex I in the mitochondrial respiratory chain. Biochem Biophys Res Commun 216:771–777

Hyslop SJ, Duncan AVM, Pitkänen S, Robinson BH (1996) Assignment of the PSST subunit gene of human mitochondrial complex I to chromosome 19p13. Genomics 37: 375–380

Kemp BE, Parker MW, Hu S, Tiganis T, House C (1994) Substrate and pseudosubstrate interactions with protein kinases: determinants of specificity. Trends Biochem Sci 19:440–444

Korenke GC, Bentlage HACM, Ruitenbeek W, Sengers RCA, Sperl W, Trijbels JMF, Gabreëls F, et al (1990) Isolated and combined deficiencies of NADH dehydrogenase (complex I) in muscle tissue of children with mitochondrial myopathies. Eur J Pediatr 150:104–108

Munnich A, Rustin P, Rötig A, Chretien D, Bonnefront JP, Nuttin C, Cornier V, et al (1992) Clinical aspects of mitochondrial disorders. J Inherit Metab Dis 15:448–455

Papa S, Sardanelli AM, Cocco T, Speranza F, Scacco SC, Technikova-Dobrova Z (1996) The nuclear encoded 18 kDa (IP) subunit of bovine heart complex I is phosphorylated by the mitochondrial cAMP-dependent protein kinase. FEBS Lett 379:299–301

Pata I, Tensing K, Metsspalu A (1997) A human cDNA encoding the homologue of NADH ubiquinone oxidoreductase subunit B13. Biochim Biophys Acta 1350:115–118

Pearson RB, Kemp BE (1991) Protein kinase phosphorylation site sequences and consensus specificity motifs: tabulations. Methods Enzymol 200:62–81

Pilkington SJ, Arizmendi JM, Fearnley JM, Runswick MY, Shehel JM, Walker JE (1993) Structural organization of complex I from bovine mitochondria. Biochem Soc Trans 21:23–26

Ruitenbeek W, Wendel U, Trijbels F, Sengers R (1996) Mitochondrial energy metabolism. In: Blau M, Duran M, Blaskovics ME (eds) Physician's guide to the laboratory diagnosis of metabolic diseases. Chapman & Hall Medical, London, pp 391–406

Shoffner JM, Wallace DC (1994) Oxidative phosphorylation diseases and mitochondrial DNA mutations: diagnosis and treatment. Annu Rev Nutr 14:535–568

Spencer SR, Taylor JB, Cowell IG, Xia CL, Pemble SE, Ketterer B (1992) The human mitochondrial NADH:ubiquinone oxidoreductase 51 kDa subunit maps adjacent to the glutathione S-transferase P1-1 on chromosome 11q13. Genomics 14:1116–1118

Trijbels JMF, Ruitenbeek W, Sengers RCA, Janssen AJM, Van Oost BA (1996) Benign mitochondrial encephalopathy in a patient with complex I deficiency. J Inherit Metab Dis 19: 149–152

Walker JE (1992) The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. Q Rev Biophys 25: 253–324

Walker JE, Arizmendi JM, Dupuis A, Fearnley IM, Finel M, Medd SM, Pilkington S, et al (1992) Sequences of 20 subunits of NADH:ubiquinone oxidoreductase from bovine heart mitochondria. J Mol Biol 226:1051–1072

Wallace DC (1992) Diseases of the mitochondrial DNA. Annu Rev Biochem 61:1175–1212

Yamaguchi M, Hatefi Y (1993) Mitochondrial NADH:ubiquinone oxidoreductase (complex I): proximity of the subunits of the flavoprotein and the iron-sulfur protein subcomplexes. Biochemistry 32:1935–1939

Zhuchenko O, Wehnert M, Bailey J, Sun ZS, Lee CC (1996) Isolation, mapping, and genomic structure of an X-linked gene for a subunit of mitochondrial complex I. Genomics 37:281–288