Deficiency of Complex II of the Mitochondrial Respiratory Chain in Late-Onset Optic Atrophy and Ataxia

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Defects of the mitochondrial respiratory chain are increasingly being recognized as an important cause of neurological disease in humans. In many of these patients, the biochemical defect results from an abnormality of the mitochondrial genome. Respiratory chain defects involving complex II, which is entirely encoded by the nuclear genome, are comparatively rare. We report the clinical and biochemical findings in 2 elderly sisters who presented with late-onset neurodegenerative disease. In both patients, a partial deficiency of complex II (approximately 50% of control values) was shown to be present in mitochondria from muscle and platelets. The enzyme defect was not expressed in cultured skin fibroblasts or immortalized lymphocytes. There was an overexpression of the 70-kd flavoprotein subunit in muscle mitochondria from both patients, although we showed that this subunit is present in normal amounts in mitochondrial membranes. Our studies highlight the diversity of the clinical presentation of respiratory chain disease and that complex II deficiency should enter the differential diagnosis of certain patients with late-onset neurodegenerative disease.


Defects of the mitochondrial respiratory chain are increasingly being recognized as an important cause of neurological disease in humans, with the clinical symptoms ranging from isolated organ involvement, such as muscle disease or blindness, to multisystem disease [1]. In many of these patients, defects of the mitochondrial genome with abnormalities of complexes I, III, IV, and V have been identified. Far less common are abnormalities of complex II of the respiratory chain.

Complex II (succinate:ubiquinone oxidoreductase, EC 1.3.5.1) is the only respiratory chain complex that does not have subunits encoded by mitochondrial DNA. It catalyzes the oxidation, in the mitochondrial matrix, of succinate to fumarate and transfers the electrons directly to the ubiquinone (UQ) pool [2]. Purified complex II can be resolved into two fractions, a soluble succinate dehydrogenase (SDH) and a membrane-anchoring fraction. SDH consists of a flavoprotein (Fp) subunit (70 kd) containing the succinate-binding site and the covalently bound flavin adenine dinucleotide (FAD) moiety of the enzyme, and an iron-sulfur (Ip) subunit (30 kd) carrying three dissimilar iron-sulfur clusters—[2Fe-2S], [4Fe-4S], and [3Fe-4S]. SDH is anchored to the mitochondrial inner membrane by two polypeptides, generally known as QPs1 and QPs2 (15 and 13.5 kd, respectively), which contain a single heme group (cytochrome bSS); these two small proteins are required for UQ binding.

We report the clinical and biochemical findings in 2 elderly sisters who had a partial complex II deficiency and who presented with encephalopathy and optic atrophy. Progressive neurodegenerative disease in later life has not been described in patients with complex II deficiency. In contrast to the low activities of complex II and SDH (50% of control values) in mitochondria of muscle and platelets, the activities of the other respiratory enzymes were normal. In both patients, the complex II deficiency was associated with an increase specifically in the level of the Fp subunit. The defect is not expressed in cultured skin fibroblasts or immortalized lymphocytes.

These patients highlight the clinical diversity seen in patients with mitochondrial respiratory chain disease. While the role of abnormalities of this pathway in the...
more common neurodegenerative diseases remains controversial [3], it is clear from our studies that late-onset neurodegenerative disease may be caused by defects of the respiratory chain, and that the genetic abnormality is not limited to defects of the mitochondrial genome.

**Patient Histories**

**Patient 1 (II-5)**
A 56-year-old woman came to neurological attention at the age of 46 with imbalance, vertigo, headaches, double vision with some visual impairment, recurrent aphony, and limb weakness. Her symptoms evolved over several months and were very gradually progressive. She gave a medical history of chronic backache for the preceding 15 years. Since onset, 10 years ago, symptoms included unsteady gait, episodic double vision, and periods of unrousable unresponsiveness without convulsions lasting several hours at a time. These episodes were not witnessed or investigated by either of our neurological units, and no helpful diagnostic information from her admissions was available. She also complained of weakness of all limbs with myalgia. Hearing never was impaired and there was no other history information of note. The patient has two healthy children; a third child (II-4) who had been a “floppy infant,” died at the age of 7 months. No other clinical details about this child were available.

Examination revealed bilateral optic atrophy with cupped optic disks. There was no pigmentary retinopathy and ocular pressures were normal. Visual acuity was reduced to 6/12 in the right eye and 6/18 in the left eye and visual fields were severely constricted in both eyes although reliability indices were poor (Humphrey 24:2). Pursuit ocular movements were limited in all directions, but there was no abnormality of reflex eye movements. The patient had a mild proximal weakness (4+), tendon reflexes were generally brisk, but no pathological reflexes could be elicited. There was no objective sensory deficit. While limb coordination was normal, gait was ataxic with a tendency to fall to the right. Cognitive function was normal. Results of routine hematological and biochemical studies including measures of creatine kinase and thyroid function were normal; plasma lactate level was 0.64 mM (normal range <1.8 mM) and pyruvate level was 0.063 mM (normal range <0.18 mM). The cerebrospinal fluid lactate level was 2.23 mM (normal range <2.0 mM) and pyruvate level, 0.12 mM (normal range <0.2 mM). Urine organic acid analysis following a fast was normal. Electromyography findings and nerve conduction velocities were unremarkable and electroencephalography suggested a mild encephalopathic picture, with occasional sharp waves seen in addition in both temporal regions. Magnetic resonance images (MRIs) showed atrophic changes in the cerebellum and brainstem.

**Patient 2 (II-4)**
A 62-year-old woman presented with symptoms similar to those of her sister (Patient 1), the prominent features again being slowly progressive unsteadiness due to truncal ataxia, headaches, dizziness, blackouts, and a sensory disturbance with dysesthesia in the fingers and toes. Like her sister, she had noticed gradual visual impairment and episodes of diplopia. The onset of symptoms for both sisters occurred in their mid-40s with a slowly progressive course over the next 10 to 20 years. This patient did not experience any episodes of recurring stupor. Apart from a longstanding controlled arterial hypertension, there was no other medical history information of note.

On clinical examination she had bilateral optic atrophy with some disk cupping but no retinopathy (visual acuity of 6/9 in the right eye and 6/6 in the left eye). Visual field testing (Humphrey 24:2) showed significant loss of the peripheral visual field, but less marked than that in Patient 1. She had a full range of active and passive ocular movements with phasic nystagmus on lateral gaze and upgaze. All other cranial nerves, muscle power, and tone and sensory test results were normal and her reflexes were brisk, but with flexor plantar responses. She had mild limb ataxia and her gait was broad based and ataxic. Cognitive function was normal. Results of routine biochemical and hematological investigations were within normal limits. The resting plasma lactate level was 0.82 mM and pyruvate level was 0.086 mM; the cerebrospinal fluid lactate level was 1.49 mM and pyruvate level was 0.11 mM. Pattern-reversal visual evoked potentials, somatosensory evoked potentials, brainstem auditory evoked potentials, and nerve conduction were all normal. Electroencephalography only showed mild nonspecific diffuse changes. A cranial MRI appeared normal for her age.

Parkinson’s disease was diagnosed in a younger brother (II-2, see Fig 1) who had died 4 years previously; he also had visual disturbance and unsteadiness. A postmortem examination was not performed. One older sister (II-3) was well at the time of writing; another sister (I-1) died, but no clinical details were available. The parents were not consanguineous; their father died during the Second World War, while their mother lived until 77 years old and died of cardiac disease. Symptoms similar to the patients were not reported.
Materials and Methods

Muscle Biopsy
Skeletal muscle samples were obtained from the vastus lateralis by open biopsy under local anesthesia. Histochemistry was performed as previously described [4].

Biochemistry
Mitochondrial fractions were prepared from skeletal muscle [5]. Platelets were isolated from 60 ml of heparinized whole blood by centrifugation and a mitochondrial fraction was isolated by homogenization and differential centrifugation [6]. Human skin fibroblasts were cultured under standard conditions [7] and a mitochondrial fraction was isolated as described by Lowerson and coauthors [8]. Lymphocytes were obtained from blood samples of both patients and control subjects and transformed in vitro with Epstein-Barr virus (EBV) [9]. For growth in riboflavin-depleted medium, lymphoblasts were cultured for 1 week in RPMI 1640 medium without added riboflavin, and supplemented with 10% dialyzed fetal calf serum and 2 mM L-glutamine. After 1 week, cells were split and grown for another 2 weeks in either riboflavin-depleted medium or medium supplemented with riboflavin (0.6 mg·liter−1), respectively. Mitochondrial fractions were isolated essentially as described for cultured skin fibroblasts [8]. The activities of the individual respiratory chain complexes [10], mitochondrial substrate oxidation [10], and citrate synthase [11] were measured at 30°C. SDH activity was measured according to the method of Ackrell and associates [12] except that phenazine ethosulfate was substituted for phenazine methosulfate. The activity of aconitase (aconitate hydratase, EC 4.2.1.3) was assayed in skeletal muscle mitochondrial fractions according to the method of Rose and O’Connell [13], using 1.5 mM NADP and 0.8 units·ml−1 of isocitrate dehydrogenase (type IV, Sigma). Mitochondrial fractions were freeze-thawed three times in hypotonic media (25 mM potassium phosphate, 5 mM magnesium chloride, pH 7.2) to ensure optimal rates of enzyme activity. The protein concentration of mitochondrial fractions was determined by a modification of the Lowry method [14].

Membrane fractions were prepared from muscle mitochondrial fractions as follows: Mitochondrial fractions (1 mg of protein) were suspended in 1.0 ml of 25 mM potassium phosphate and 0.2 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, and sonicated (30 W) on ice for 3 × 10 seconds with a 1-minute cooling interval between bursts. Samples were subsequently centrifuged (100,000 g, for 1 hour at 4°C), the pellets resuspended in phosphate buffer, and respun. This second pellet contains less than 10% of the total citrate synthase activity found in the initial mitochondrial fraction. Membrane pellets were resuspended in 1.0 ml of phosphate buffer, incubated with Triton X-100 (1:1, detergent-protein) for 30 minutes on ice, and immediately snap-frozen in liquid nitrogen.

Immunoblot Analysis
The proteins in the skeletal muscle mitochondrial fractions and membrane fractions were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) [15] using 15% polyacrylamide gels, transferred to Immobilon PVDF membranes [16] in a buffer comprising 10 mM CAPS and 10% methanol (vol/vol), pH 11, and probed with antisera raised in rabbits to the individual subunits of complex II. Immunoreactive peptides were visualized using the enhanced chemiluminescence (ECL) system (Amersham) and quantitated using densitometry (BioImage, Millipore).

Results

Histochemistry
For both patients, the muscle biopsy specimens showed no histopathological abnormalities and a normal complement of the major metabolic fiber types. Cytochrome c oxidase and NADH tetrazolium reductase activities were unremarkable in both patients (data not shown). There was mild to moderate lipid storage observed in some fibers (predominantly type 2A) in Patient 2, but no excess lipid was seen in the muscle from Patient 1. Sections stained for SDH showed an overall decrease in activity in both patients, well outside the normal staining variations for this enzyme, with little differentiation between oxidative type 1 and glycolytic type 2 muscle fibers (Fig 2).

Biochemistry

MUSCLE. The specific succinate:ubiquinone oxidoreductase activity of complex II in disrupted skeletal muscle mitochondria of both patients was less than 50% of control values (Table 1). To avoid potential errors due to protein contamination, the activities of complex II and citrate synthase were compared in each of the skeletal muscle postnuclear supernatants. The ratios of activities, that is, 0.101 (Patient 1), 0.079 (Patient 2), and 0.234 ± 0.035 (control subjects [mean ± standard deviation], n = 7), confirm the data in Table 1. SDH activity in muscle mitochondrial fractions was also low (see Table 1). Furthermore, comparatively slow rates of succinate oxidation by intact mitochondria of Patient 1 (41%) and Patient 2 (58%) were demonstrated spectrophotometrically (data not shown). In contrast, the activities of complexes I, III, and IV of the patients’ mitochondria were all within the normal range, as was that of the matrix aconitase following full activation of the enzyme (see Table 1).

To determine if the decreased efficiency of complex II function might be attributable to structural changes at the active site of the enzyme, succinate:ubiquinone oxidoreductase activity was assayed in the presence of varying concentrations of the competitive inhibitor malonate [17]. Figure 3 shows that the inhibition exhibited by the muscle mitochondrial fractions of Patient 2 and a control subject were identical, inferring that it is unlikely that structural modifications at the substrate-binding site contribute to the lowered activity.

PLATELETS. The activities of complex II (51% and 64% of control values) and SDH (54% and 65% of control values) were low in platelet mitochondrial frac-
Table 1. Activities of Individual Respiratory Chain Complexes, Succinate Dehydrogenase (SDH), and Aconitase in Skeletal Muscle Mitochondrial Fractions

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Controls (mean ± SD)</th>
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<tbody>
<tr>
<td>Complex I</td>
<td>195</td>
<td>387</td>
<td>243 ± 59 (n = 20)</td>
</tr>
<tr>
<td>Complex II</td>
<td>149</td>
<td>127</td>
<td>340 ± 54 (n = 40)</td>
</tr>
<tr>
<td>Complex III</td>
<td>2.37</td>
<td>2.29</td>
<td>2.35 ± 0.40 (n = 18)</td>
</tr>
<tr>
<td>Complex IV</td>
<td>4.37</td>
<td>4.65</td>
<td>4.99 ± 1.66 (n = 13)</td>
</tr>
<tr>
<td>SDH</td>
<td>101</td>
<td>115</td>
<td>236 ± 18 (n = 5)</td>
</tr>
<tr>
<td>Aconitase</td>
<td>39.2</td>
<td>41.4</td>
<td>42.4 ± 8.1 (n = 5)</td>
</tr>
</tbody>
</table>

Respiratory chain enzyme activities are expressed as nmol NADH oxidized min⁻¹ mg protein⁻¹ for complex I; nmol DCPIP reduced min⁻¹ mg protein⁻¹ for complex II; and apparent first-order rate constants (s⁻¹ mg protein⁻¹) for complexes III and IV. SDH activity is expressed as nmol DCPIP reduced min⁻¹ mg protein⁻¹; aconitase activity is expressed as nmol NADP reduced min⁻¹ mg protein⁻¹.

DCPIP = 2,6-dichlorophenol-indophenol; SD = standard deviation.

Fig 2. Histochemical analysis of succinate dehydrogenase activity. Skeletal muscle sections (×250 before 43% reduction) are shown from a control (A) and the 2 patients (B, Patient 1; C, Patient 2). These sections were all stained at the same time.

Fig 3. Malonate titration of complex II activity in human skeletal muscle mitochondrial fractions. Inhibitor titrations were performed on mitochondrial fractions isolated from Patient 2 (closed circles) and a control subject (open circles). For each plot, complex II activities without inhibitor are designated 100%, and were calculated as 127 nmol min⁻¹ mg protein⁻¹ and 321 nmol min⁻¹ mg protein⁻¹ for the patient and control subject, respectively.

Immunoblot Analysis
There was no difference in the steady-state levels of the 30-kd (lP) and 15-kd (QPs1) subunits in the mitochondrial fractions from the patients compared with control subjects (Fig 4). However, the amount of the 70-kd (Fp) subunit in both patients was 6.5-fold higher than that observed in 2 control subjects (see Fig 4). These same relative proportions were obtained whether mitochondrial fractions were loaded on gels according to the citrate synthase activity of the samples, or on the basis of mitochondrial protein concentration.
Table 2. Respiratory Chain Enzyme Activities in Platelet Mitochondrial Fractions

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Complex I</th>
<th>Complex II</th>
<th>Complex IV</th>
<th>SDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>+6 wk riboflavin</td>
<td>125</td>
<td>153</td>
<td>0.97</td>
<td>150</td>
</tr>
<tr>
<td>+3 mo riboflavin</td>
<td>115</td>
<td>135</td>
<td>1.02</td>
<td>105</td>
</tr>
<tr>
<td>Patient 2</td>
<td>143</td>
<td>190</td>
<td>0.80</td>
<td>179</td>
</tr>
<tr>
<td>+3 mo riboflavin</td>
<td>130</td>
<td>176</td>
<td>1.34</td>
<td>175</td>
</tr>
<tr>
<td>Controls (mean ± SD)</td>
<td>98 ± 17 (n = 10)</td>
<td>298 ± 44 (n = 10)</td>
<td>1.09 ± 0.17 (n = 8)</td>
<td>276 ± 46 (n = 9)</td>
</tr>
</tbody>
</table>

1Respiratory chain enzyme activities are expressed as ratios of activity (nmol NADH oxidized · min⁻¹· for complex I; nmol DCPIP reduced · min⁻¹· for complex II and SDH; and an apparent first-order rate constant (k · sec⁻¹·) for complex IV) compared with the activity of citrate synthase (μmol · min⁻¹·) rather than milligrams of protein (see text for explanation).

SDH = succinate dehydrogenase; SD = standard deviation; DCPIP = 2,6-dichlorophenol-indophenol.

To confirm similar loading, immunoblots were probed with antibodies specific to subunit IV of cytochrome c oxidase and no difference between patients and control subjects was observed (see Fig 4).

Immunoblot analysis of a skeletal muscle mitochondrial membrane fraction prepared from Patient 2 revealed no difference in the membrane content of the 70-kd (Fp) subunit compared to 3 control subjects (Fig 5). Similar loading of samples onto the gel was confirmed by probing immunoblots with antibodies specific to subunit IV of cytochrome c oxidase and ETF-dehydrogenase (see Fig 5).

Biochemical Studies in Cultured Cells
Respiratory chain enzyme activities were determined in mitochondrial fractions isolated from cultured skin fibroblasts from Patient 1 (Table 3) and transformed lymphoblasts from both patients (Table 4). No evidence of the defect in complex II could be found in the cells from either patient. This becomes more appar-
Fig 5. Immunoblot analysis of skeletal muscle mitochondrial membrane fractions. Lanes 1, 3, and 4, controls (30 μg each); lane 2, Patient 2 (30 μg). The separated proteins were reacted with antibodies raised against (A) the 70-kd (Fp) of complex II and (B) subunit IV of cytochrome c oxidase (COX IV) and ETF-dehydrogenase (ETF-dh). A comparable amount of the Fp subunit is seen in the patient and the 3 control subjects.

Table 3. Respiratory Chain Activities in Mitochondrial Fractions Isolated from Cultured Skin Fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>Complex I</th>
<th>Complex II</th>
<th>Citrate Synthase</th>
</tr>
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<tbody>
<tr>
<td>Patient 1</td>
<td>49</td>
<td>84</td>
<td>0.215</td>
</tr>
<tr>
<td>Controls (mean ± SD)</td>
<td>63 ± 15 (n = 10)</td>
<td>77 ± 14 (n = 10)</td>
<td>0.162 ± 0.037 (n = 10)</td>
</tr>
</tbody>
</table>

1Enzyme activities are expressed as nmol NADH oxidized · min⁻¹ · mg protein⁻¹ (complex I), nmol DCPIP reduced · min⁻¹ · mg protein⁻¹ (complex II), and μmol · min⁻¹ · mg protein⁻¹ (citrate synthase).

SD = standard deviation; DCPIP = 2,6-dichlorophenol-indophenol.

Table 4. Respiratory Chain Activities in Mitochondrial Fractions Isolated from B Lymphoblastoid Cell Lines

<table>
<thead>
<tr>
<th></th>
<th>Complex I</th>
<th>Complex II</th>
<th>Ratio of Complex I to Complex II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin-supplemented medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>0.88</td>
<td>1.72</td>
<td>0.51</td>
</tr>
<tr>
<td>Patient 2</td>
<td>0.66</td>
<td>1.07</td>
<td>0.62</td>
</tr>
<tr>
<td>Controls (n = 5) (mean ± SD)</td>
<td>0.99 ± 0.08</td>
<td>1.72 ± 0.32</td>
<td>0.58</td>
</tr>
<tr>
<td>Riboflavin-deficient medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>0.104</td>
<td>0.171</td>
<td>0.61</td>
</tr>
<tr>
<td>Patient 2</td>
<td>0.100</td>
<td>0.152</td>
<td>0.66</td>
</tr>
<tr>
<td>Controls (n = 3) (mean ± SD)</td>
<td>0.115 ± 0.037</td>
<td>0.177 ± 0.018</td>
<td>0.65</td>
</tr>
</tbody>
</table>

1Results are expressed as nmol NADH oxidized · min⁻¹ · ml · mitochondrial fraction⁻¹ (complex I) and nmol DCPIP reduced · min⁻¹ · ml · mitochondrial fraction⁻¹ (complex II).

SD = standard deviation; DCPIP = 2,6-dichlorophenol-indophenol.
ent in the transformed lymphoblasts when the activities of complexes I and II are expressed as a ratio to each other (see Table 4). Lymphoblasts from both patients and control subjects were grown in riboflavin-deficient media to determine if the high concentration of riboflavin typically present in tissue culture media corrected the complex II defect. The activities of both complexes I (FMN requiring) and II (FAD requiring) were considerably compromised in all cell lines grown in riboflavin-deficient media (see Table 4), but the effect of riboflavin deficiency was no different in the patient and control cells.

Discussion
This article details the biochemical investigation of a family in which at least 2 individuals had a deficiency of complex II of the respiratory chain. The diagnosis specifically of low complex II (44% of control values in the mitochondrial fraction) and SDH (43% of control values) activities was established in Patient 1, which led to referral of her sister. She had similar clinical symptoms and signs, and also had low activities of complex II and SDH (37% and 49% of control values, respectively) in skeletal muscle mitochondria. Histochemical analysis of biopsy specimens revealed a generalized decrease of SDH activity in both patients. Biopsy was performed on a third clinically unaffected sister (H-3), but complex II and SDH activities were normal by biochemical and histochemical criteria.

While defects of complexes I, III, and IV, all of which have some subunits encoded by mitochondrial DNA, have been extensively reported, patients with defects involving complex II seem to be less common. The clinical presentation of complex II deficiency is very variable and there is a remarkable difference in the clinical phenotype of our patients compared to those reported previously. Firstly, the late onset of clinical symptoms is very different from the onset in previous patients with complex II deficiency, who presented in childhood or early adulthood [18-26]. Secondly, a clinical picture of progressive neurodegenerative disease with optic atrophy and ataxia is a novel presentation of complex II deficiency. In most, but not all published reports, a myopathic presentation associated with muscle weakness and exercise intolerance was a prominent feature [20, 21, 25, 26]. Patients with complex II deficiency have also presented with Kearns-Sayre syndrome and progressive ophthalmoplegia [19], encephalopathy [18], cardiomyopathy [23], and Leigh's disease [22, 24].

Of the patients with complex II deficiency reported to date, only 4 underwent comprehensive studies of the biochemistry of the respiratory chain and the levels of immunoreactive subunits of complex II in skeletal muscle [18-21]. In all these patients, the activities of complex II and SDH were very low (between 9% and 25% of control values). Apart from the patient studied by Rivner and coworkers [19], in whom an abnormally high activity of complex I was also demonstrated, the defect of complex II was found to be associated with a decrease in the activities of the other complexes, particularly complex I [18, 20] and complex III [18, 21]. This is very different from the biochemical defect in our patients, in which the abnormality was restricted to complex II. Furthermore, with the exception of the studies of Rivner and coworkers [19], who reported normal levels of cross-reacting material corresponding to the subunits of complex II, the low activities of complex II were associated with abnormally low amounts of immunoreactive complex II peptides. The patient described by Desnuelle and coauthors [18] had low amounts of the Fp, Ip, and QPs1 (15-kd) subunits. The patients described by Schapira and colleagues [20] and Haller and associates [21] had a deficiency of mitochondrial aconitase in association with low amounts of the Fe-S peptides of complexes I, II, and III. This observation is compatible with a disturbance of the transport or metabolism of iron, and an abnormality of heme metabolism was postulated as a cause of the biochemical findings (low complexes I, II, and III) in another patient [18]. The activity of aconitase, another iron-containing enzyme, was measured in both of our patients (see Table 1) and was normal.

The 2 patients we describe here did not have depleted levels of complex I based on Western blotting. Indeed the amount of Fp in mitochondria from both patients exceeded some sixfold (see Fig 4) that of control subjects, and levels of Ip and the 15-kd (QPs1) subunit appeared normal. The status of the fourth subunit (QPs2) was not determined due to unavailability of an appropriate antibody. However, assembly of the complex in the inner mitochondrial membrane is contingent on both anchor polypeptides. The underlying genetic defect(s) in these patients has not yet been identified. That the Fp, Ip, and QPs1 subunits appear to have the correct molecular weights on gels and are not depleted argues against there being a major deletion in one of the alleles for any of these proteins. The great surfeit of Fp in these patients' mitochondria is most unusual, however, and together with the biochemical data would suggest that an abnormal form is being synthesized. Our studies showed (at least from the amounts of Fp, Ip, and QPs1) that normal levels of complex II are assembled in the membrane but with markedly reduced (>50%) catalytic efficiency. Stability apparently is not a problem, inasmuch as the aberrant complex behaved similarly to that in control membranes during washing and centrifugation; that is, there was no dissociation of subunits. As expected, the excess of Fp was in the soluble fraction of the mitochondria. It could well represent a compensatory response to the defective enzyme.
Further biochemical investigations revealed that the defect of complex II was present in the platelet mitochondrial fraction (see Table 2), where the activities of both complex II and SDH were also 50% of control values. The defect however was not expressed in either cultured skin fibroblasts (see Table 3) or lymphoblast cell lines (see Table 4). This is quite a common occurrence with respiratory chain defects involving mutations in mitochondrial DNA, where recovery is attributable to heteroplasmy. Such cannot be the case in our patients, however, since all four subunits of complex II are nuclear encoded.

One testable explanation for the normal complex II activity exhibited by cultured cells was that the growth medium contains factors able to effect “repair” of the defective enzyme. A most likely candidate was riboflavin, since synthesis and covalent binding of FAD are clearly essential for catalytic activity of complex II. “Riboflavin responsiveness” previously was demonstrated in some patients with multiple acyl coenzyme A dehydrogenase deficiency, a metabolic disorder of mitochondrial fatty acid oxidation [27] and in 1 patient with a combined defect of fatty acid oxidation and the respiratory chain [28]. This deficiency often only becomes apparent when cultured cells from these patients are grown in riboflavin-depleted media. Treatment of these patients with riboflavin is associated with a dramatic clinical improvement, with the resolution of both their symptoms and associated lipid storage myopathy [29, 30]. Overexpression of the FAD-binding FP subunit of complex II in our 2 patients may have been compensation for, or poor binding of, the FAD cofactor. We tested this possibility by growing lymphoblastoid cell lines from both patients and control subjects in riboflavin-deficient medium for 3 weeks. While the activities of both complexes I and II were found to be dramatically lower in both the patients and the control subjects (about a tenfold decrease) than those in cells grown in riboflavin-supplemented media, there was no difference in the ratio of the activity of complex I to complex II between either patient and the control cell lines (see Table 4). Furthermore, these activity ratios were identical to those measured in the lymphoblasts cultured in riboflavin-supplemented media (see Table 4). Concurrent with these experiments, both patients were started on riboflavin (150 mg/day). Blood samples (60 ml) were taken following 6 weeks and 3 months of riboflavin treatment, and platelet mitochondrial fractions were isolated. The measurement of both complex II and SDH activities revealed no improvement in the biochemical defect (see Table 2). Furthermore, there was no clinical improvement.

One of the most interesting clinical features of these patients is the late onset of symptoms relative to other patients with complex II deficiency. We believe this is compatible with the partial defect of this complex, and it is possible that other late-onset neurological syndromes may be due to similar deficiencies.

In conclusion, our findings in these 2 patients define a novel presentation of complex II deficiency in which progressive neurodegenerative disease is the salient clinical feature. While the precise molecular basis of the defect has not yet been elucidated, our studies do highlight the diversity of the clinical presentation of respiratory chain disease and that complex II deficiency should enter the differential diagnosis even in patients with late-onset neurodegenerative disease.

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References