Hypertrophic Cardiomyopathy with Mitochondrial Myopathy

A New Phenotype of Complex II Defect

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SUMMARY

Two brothers, 25 and 19 years old, were affected by asymmetrical hypertrophic cardiomyopathy. The older brother had waddling gait and weakness of the proximal girdle muscles, while the younger had a broad-based gait and weakness of selected limb girdle muscles. EMG exam was myopathic. Serum enzyme, CPK and aldolase were elevated. Histochemical reactions in muscle revealed " core-like " areas, subsarcolemmal rims of mitochondria and lipid accumulation. Succinate-dehydrogenase stain showed a lack of activity in both biopsies, with the exception of intrafusal fibers. Microphotometric quantitative measurements confirmed the defect in both biopsies. Biochemical measurements of several mitochondrial enzymes in muscle showed a reduced activity of succinate-dehydrogenase (33%) and succinate-cytochrome C reductase (36–47%) which are both components of complex II. On myocardial biopsy lipid and mitochondrial abnormalities were found. This mitochondriopathy represents a new phenotype of partial complex II defect. (Jpn Heart J **34**: 63–77, 1993.)

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Hypertrophic cardiomyopathy Mitochondria Complex II

THE term " mitochondrial myopathy " is used when a respiratory chain defect is expressed in skeletal muscle, although defects in respiratory chain complexes may also involve various organs and tissues (brain, heart,

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kidney, liver) leading to different clinical presentations.[1)-5)]

The mitochondrial oxidative phosphorylation system is composed of five protein complexes: NADH-CoQ oxidoreductase (complex I), succinate-CoQ oxidoreductase (complex II), CoQ-cytochrome C oxidoreductase (complex III), cytochrome C oxidase (complex IV), and ATP-synthetase (complex V). Defects of complexes I, III, IV, and V have been documented in a number of cases affected either by encephalomyopathy, ocular myopathy or cardiomyopathy,[6)-9)] while only rarely have patients with deficiency of complex II been described.[10)-13)]

Complex II is an important enzyme complex both for the tricarboxylic acid cycle and the respiratory chain of mitochondria. It catalyzes the oxidation of succinate to fumarate (succinate dehydrogenase: SDH) and transfers its reducing equivalent to ubiquinone in aerobic respiration. Complex II consists of four polypeptides, all encoded by nuclear DNA: two large polypeptides (SDH) and two small polypeptides (b-type cytochrome). SDH is a structural protein (M.W. 98,500) located on the matrix side of the mitochondrial inner membrane.[14)] The two proteic subunits of SDH (73,000 and 25,500 M.W.) contain three nonheme iron centers; the first subunit contains the binding site for succinic acid and a FAD molecule. The two small hydrophobic polypeptides contain heme b and supply the binding sites for the two large subunits to the membrane. These subunits seem essential for converting succinate dehydrogenase into succinate ubiquinone oxidoreductase.[15)] A complete complex II deficiency may be almost lethal since the impairment of the citric acid cycle[15)] severely decreases the efficiency of energy transduction.

Clinical phenotypes of partial complex II defect include: progressive external ophthalmoplegia,[10)] encephalomyopathy, ataxia and myoclonic jerks,[11)] myopathy and/or encephalopathy.[12),13)]

In this study we describe 2 brothers affected by a mitochondrial myopathy with hypertrophic cardiomyopathy due to a partial complex II defect. Their phenotype was peculiar since it was a limb girdle myopathy associated with hypertrophic cardiomyopathy and has not yet been reported.

Materials and Methods

Familial analysis

The family pedigree (Fig. 1) of the 2 brothers studied (III-20, III-21), showed the occurrence of ischemic cardiac disease in the father (II-7) and in 3 maternal uncles (II-21, II-22, II-23), and hypertension in the maternal grandmother (I-4), in 2 maternal aunts (II-18, II-20), in 1 uncle (II-23)

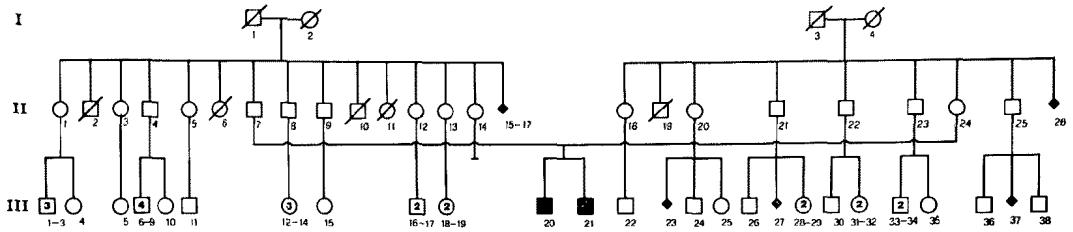


Fig. 1. Family pedigree.

and 1 nephew (III-30). In 1 paternal aunt muscle cramps were present (II-1); cramps and muscle weakness were present also in 1 paternal uncle (II-8). The mother (II-24) had hypertension, a myopathic EMG and lipid droplets in her muscle biopsy.

The diagnosis of coronary heart disease and hypertension was made in several family members by clinical history, EKG, M-mode, and bidimensional echocardiography. However the first degree relatives refused further cardiac analysis and biopsy.

Clinical studies

The following exams were carried out in patient 1 (III-20), a 25-year-old man, and in patient 2 (III-21), a 19-year-old man: neurological and cardiological examination, EKG, two-dimensional and Doppler echocardiography, heart catheterization, cineangiography, right ventricular biopsy, EMG, muscle serum enzymes and plasma and urine carnitine quantitation. The patients' mother (II-24) was also hospitalized for clinical and laboratory evaluation.

Echocardiography

Complete M-mode, two-dimensional, pulsed, continuous-wave, and color Doppler echocardiography were performed by means of standard techniques with a phased array ultrasound system (Hewlett-Packard 77020 AC and Sonos 1000 77030 A) using Duplex 2.5 and 3.5 MHz transducers and an independent Pedoff for imaging, spectral Doppler and color flow mapping. The color flow Doppler system was set to maximum packed size, and minimum reject with gain below the level of ambiguous color flooding.

Left ventricular (LV) volumes were calculated using an ellipsoidal biplane area-length model derived from LV images in the apical four-chamber view. Ejection fractions (EF) were calculated as:

$$EF = (EDV - ESV)/EDV$$

where EDV and ESV are the end-diastolic and end-systolic volumes.[16] Dop-

pler assessment of left ventricular systolic and diastolic flow velocities was obtained with the duplex transducer located at the apex. By means of conventional pulsed Doppler techniques, the sample volume was positioned to interrogate blood flow in various portions of the left ventricle inflow and outflow tracts and near the apex. When the velocity limit for pulsed Doppler echocardiography was exceeded at the site of an obstruction, continuous-wave technique was used to quantitate the flow velocity and pressure drop across the obstruction.

Skeletal muscle biopsy

Open muscle biopsy (biceps and quadriceps muscle) was obtained from both patients and their mother, after informed consent. Muscle specimens were processed for routine histochemistry and electron microscopy (Araldite embedded), frozen in liquid nitrogen and stored at −80°C for biochemistry. Microphotometric studies for analysing SDH activity in single fibers were performed as previously described.[17),18)] This method allows quantitative enzyme analyses in single fibers from a cross section.

Biochemical analyses

Mitochondrial enzyme activities: NADH dehydrogenase, succinate dehydrogenase, NADH-cytochrome C reductase, succinate-cytochrome C reductase, cytochrome C oxidase, and citrate synthase were measured by a spectrophotometric method on muscle specimens from patients and controls, according to previously published methods.[19)] Muscle, plasma and urine carnitine were assayed by a radiochemical method.[20)]

Results

Patients

Patient 1: A 25-year-old man, was hospitalized for acute pulmonary edema. An EKG tracing demonstrated atrial fibrillation with rapid ventricular rate, which was converted to sinus rhythm with i.v. verapamil. Cardiological examination revealed mild cardiomegaly. Echocardiography showed a nonobstructive hypertrophic cardiomyopathy and confirmed mesoventricular and apical hypertrophy of the left ventricle (Fig. 2). The interventricular septum measured 24 mm (normal up to 12 mm), and the posterior left ventricular wall 12 mm. The left ventricular end-diastolic volume (LVEDV) was 34 ml/m^2, and the ejection fraction (EF) was 71% (normal values: $\leq$70 ml/m^2 and $\geq$50%, respectively). No significant intraventricular or subaortic gradients were found by Doppler echocardiography.

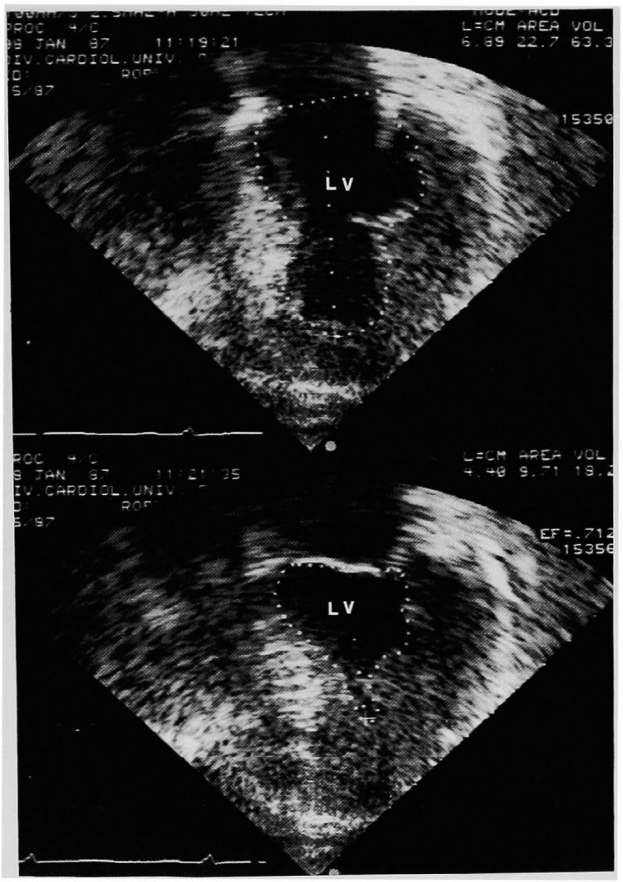


Fig. 2. Two-dimensional echocardiograms in patient 1. The apical four-chamber view shows a non-obstructive cardiomyopathy. Upper panel: diastolic frame. Lower panel: systolic frame. LV=left ventricle.

During heart catheterization, normal pressures were recorded. Cineangiographic study confirmed the normality of left ventricular size and systolic function and showed systolic obliteration of the apical portion of the left ventricle and hypertrophic papillary muscles (Fig. 3a, b).

Slit lamp examination revealed no cataracts. General neurological examination disclosed scoliosis, flat feet, proximal lordosis and weakness in the upper girdle (deltoid and triceps) muscles, Gowers' sign and waddling gait. EMG showed a myogenic pattern. CPK (201 U/l) and aldolase (8 U/l) levels were mildly elevated. Serum basal lactate was normal (8.8 mg/100 ml, n.v. 3.5–18.1). No cerebellar abnormalities were present, EKG was normal and mild mental retardation was evident.

Patient 2: A 19-year-old man, was admitted in 1986 for clinical evaluation of retrosternal pain after effort. He had a history of epileptic fits

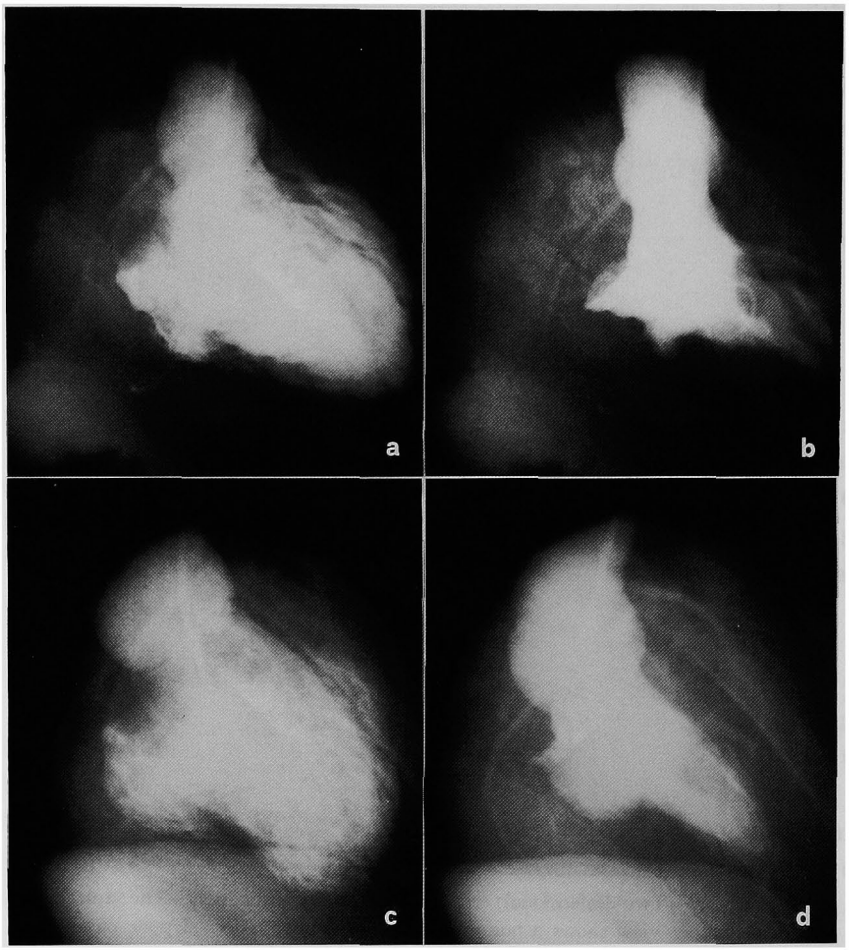
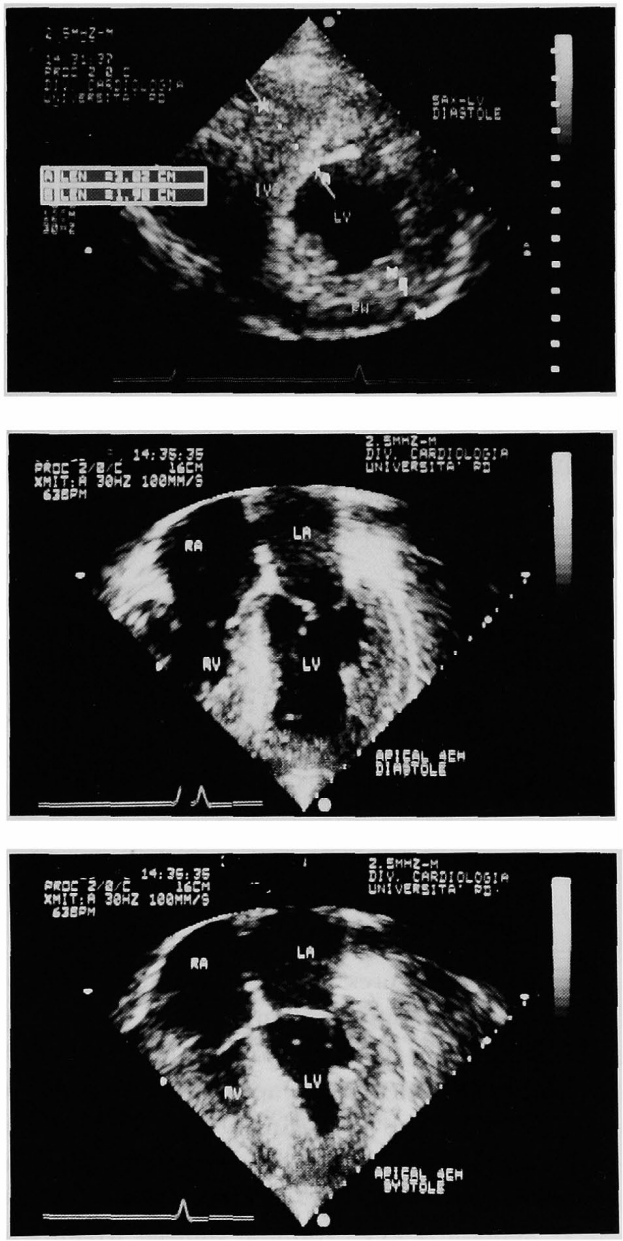


Fig. 3. Left ventricular angiographic frames in right anterior oblique view of patient 1 (a: diastole, b: systole) and of patient 2 (c: diastole, d: systole).

at 8 months of age, which subsided with treatment. Echocardiographic study demonstrated nonobstructive hypertrophic cardiomyopathy. The maximum diastolic septum thickness was 30 mm and left ventricular posterior wall thickness was 19 mm (Fig. 4A) at the level of the papillary muscles. LVEDV was 32 ml/m^2 and EF 63%. The distribution of the hypertrophic process was similar to that observed in the older brother (Fig. 4B, C). Heart catheterization disclosed normal pressures. Cineangiographic findings were similar to those observed in case 1, although hypertrophy of papillary muscles was less evident (Fig. 3c, d).

Slit lamp examination revealed no cataracts. At 12 years of age the patient presented with right basal pneumonia and developed left ventricular



A

B

C

Fig. 4. Stop frames of two-dimensional echocardiograms from patient 2 with hypertrophic cardiomyopathy. A: parasternal short-axis view at level of papillary muscles, showing severe hypertrophy of the ventricular septum. B, C: apical four-chamber views during diastole (B) and systole (C) showing hypertrophy confined to mesoventricular and apical portions of the ventricular septum and lateral wall. IVS=intraventricular septum; LA=left atrium; LV=left ventricle; PW=posterior wall; RA=right atrium; RV=right ventricle.

insufficiency. During the following years he suffered from headache, mild fever, dyspnea and hypertension.

Neurological examination revealed an ataxic broad based gait with difficulty in tandem gait, Gowers' sign, slight weakness of the left deltoid, biceps and triceps muscles and moderate weakness of the ileopsoas muscles. Lower limb muscles were thin and slightly atrophic, particularly in the leg compartment. Deep tendon reflexes were normal. A reddish face and slight mental retardation were noted. EMG showed a myogenic pattern; CT scan and EKG were normal. CPK (576 U/l, n.v. 0–190), aldolase (33.8 U/l) and LDH (694 U/l) were elevated. Serum basal lactate was normal (13.5 mg/100 ml, n.v. 3.5–18.1).

The patients' mother presented with exertional muscle pain and, on neurological examination, slight weakness of pectoral and ileopsoas muscles and distal atrophy of the lower limbs. EKG was normal, but hypertension was present. EMG showed a myogenic pattern. CPK was normal.

Molecular genetic studies

The karyotype (GTG bands) did not reveal any abnormality in either patient. Likewise, PCR amplification of 18 different exons which are most frequently involved in mutations of the dystrophin gene, failed to detect any deletion. Dystrophin immunoblot was normal in both patients and in the mother. Analysis for mtDNA revealed no deletion in either patient.

Skeletal muscle biopsy

In both biopsies, NADH-TR reductase showed irregular staining of the intermyofibrillar mitochondria appearing as " core-like " areas in case 1 (Fig. 5A) and " multiple " cores in case 2. There was also a poor differentiation of fiber types and subsarcolemmal rims of mitochondria. PAS staining showed an increase in glycogen content in case 2. Oil red O and Sudan black B staining revealed lipid accumulation in muscle fibers, especially in case 2. There were no definite ragged-red fibers; only a few scattered fibers with rims, vacuoles and cytoplasmic bodies were seen in the younger brother.

Cytochrome C oxidase (COX) staining showed irregularity of reaction and subsarcolemmal rims in both muscle biopsies; in case 1 some fibers were almost COX negative. SDH stain showed a very faint reaction at the periphery of fibers in both cases, while the central fiber areas were clearly devoid of any reaction as compared to control. On the contrary, a clear SDH reaction was seen in the intrafusal fibers of a muscle spindle of case 1 (Fig. 5B).

The muscle biopsy of the patients' mother showed subsarcolemmal rims

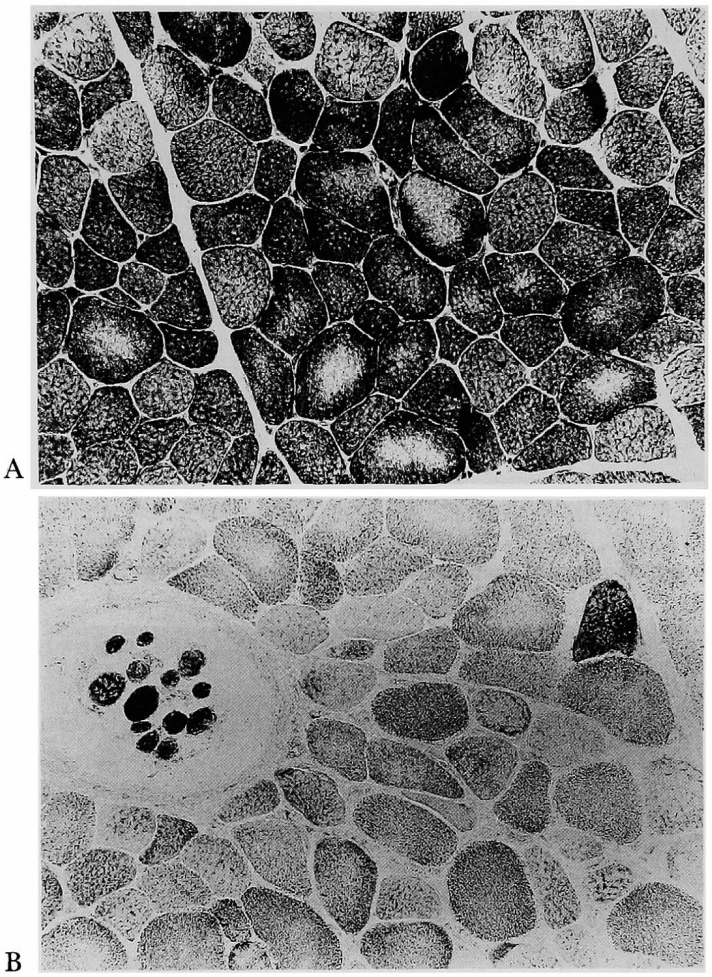


Fig. 5. A: NADH-TR reductase stain of skeletal muscle in patient 1; note irregular staining in the intermyofibrillar mitochondria similar to " core areas " (×200). B: SDH stain of skeletal muscle in patient 1, showing a very faint reaction at the periphery of fibers and no staining in central areas. Intrafusal fibers react for SDH (×200).

of mitochondria, rare hyporeactive areas in myofibers and mild lipid accumulations.

Quantitative single fiber analyses of SDH activity in 2 patients and 2 controls were performed in 2 composite blocks (one block consisted of patient 1 and control 1, the second of patient 2 and control 2). Significant differences were found between patients and control in type I and type IIA fibers ($p<0.001$). A significant decrease in SDH activity in type I (28.8% and 29.2%, respectively) and type IIA (42% and 32.5%, respectively) com-

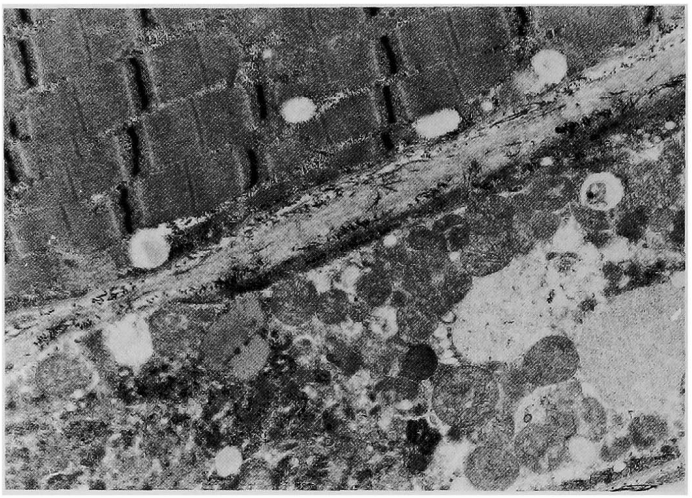


Fig. 6. Electron microscopic examination of skeletal muscle in patient 1: lipid droplets, granular β-glycogen and mitochondria in both intermyofibrillar and sarcolemmal positions are seen (original magnification $\times$16,250).

pared to normal control values occurred. Type IIB fibers were not detected in the 2 patients.

The ultrastructural study showed Z-line streaming in 1 brother (case 1); in both patients' muscles and in the mother, there were large collections of lipid vacuoles, β-glycogen granules and clusters of enlarged mitochondria with irregular cristae, in both intermyofibrillar and subsarcolemmal positions (Fig. 6).

Heart biopsy

In patient 1 electron microscopy revealed mitochondrial abnormalities associated with lipid droplets and various electron-dense figures (Fig. 7). Although abundant mitochondria may be seen in the normal heart, their association with lipid droplets was highly characteristic. In patient 2 there were areas of cardiac myofibers packed with mitochondria, that had irregularly arranged cristae. Myofibrillar disarray and β-glycogen particles were also seen (Fig. 8).

Biochemical data

Mitochondrial enzyme activities related to complex II in muscle homogenates were markedly reduced (Table I). SDH activity was 35% and 33% of control, respectively, in cases 1 and 2; succinate-cytochrome C reductase activity was 36% and 47% of controls. Three years later, a second muscle biopsy of patient 1 confirmed the above partial defect: SDH ac-

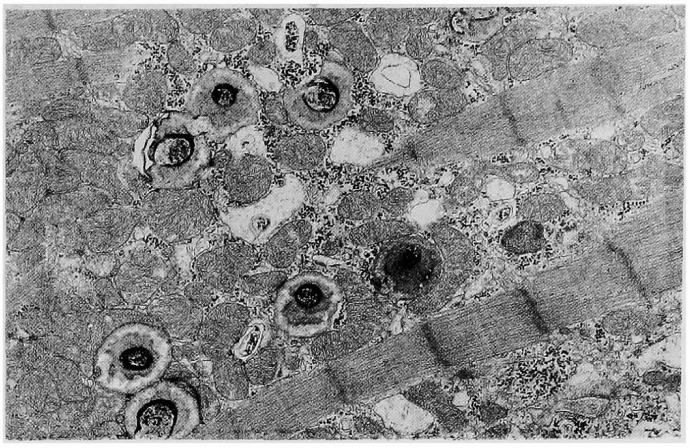


Fig. 7. Electromicroscopic examination of right endomyocardium in patient 1. Mitochondrial-associated lipid droplets of various electron densities are seen. The muscle shows abnormal mitochondria (original magnification ×19,000).

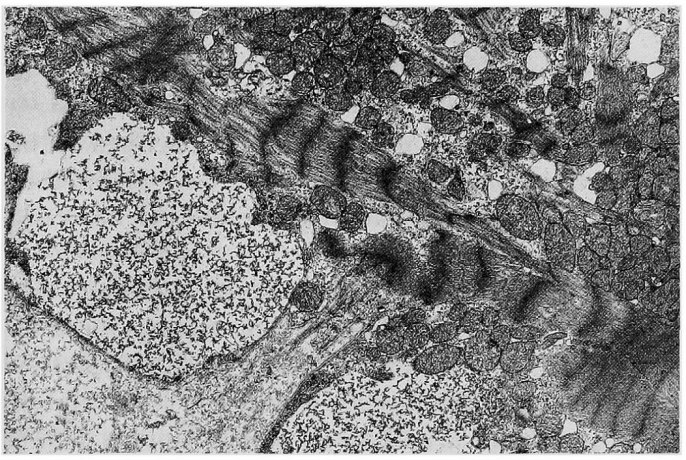


Fig. 8. Electromicroscopic examination of right endomyocardium in patient 2. Heart muscle areas are packed with mitochondria that have irregularly arranged cristae. Note also myofibrillar disarray (original magnification ×13,500).

tivity was 34%, succinate-cytochrome C reductase was 34% and cytochrome C oxidase was 40% of controls. Case 2 also showed a reduced activity in cytochrome C oxidase (51%).

Muscle carnitine was normal in case 1, while in case 2 there was a decrease of short chain acyl-carnitine fraction (14% of controls). Plasma carnitine was normal in case 2, while in case 1 there was a decrease of free carnitine fraction (62%) and of total carnitine (67%). Urine carnitine

Table I. Mitochondrial Enzyme Activities in Muscle Biopsy

	Case I (%)	Case II (%)	Controls (12) mean±SD
NADH Dehydrogenase	569.3 (91)	559.4 (89)	627.7±185.1 (399.6-943.0)
Succinate Dehydrogenase	5.8 (35)	5.5 (33)	16.5±7.7 (6.4-30.6)
NADH Cyt-C Reductase	65.6 (95)	43.0 (62)	69.4±38.1 (21.3-138.2)
Succ. Cyt-C Reductase	6.0 (36)	7.8 (47)	16.6±5.6 (9.5-28.7)
Cytochrome-C Oxidase	33.8(106)	16.4 (51)	32.0±18.0 (14.6-67.5)
Citrate Synthase	108.1 (50)	138.1 (64)	217.1±64.8 (91.1-350.2)

Values are expressed in nanomoles/min/mg non-collagen protein. The range and number of controls are given in brackets.

was normal in both cases. Plasma carnitine and mitochondrial enzyme activities in muscle homogenate from the patients' mother were normal.

Discussion

Complex II deficiency has been previously described to be associated with Kearns-Sayre Syndrome (KSS)[10] and has been reported in 2 patients with mitochondrial encephalomyopathy on the basis of decreased succinate-cytochrome C reductase activity.[11]

Cardiac muscle involvement is frequent in respiratory chain defects, although rarely the predominant clinical features are those of heart failure.[21-23] Sengers et al[24] described 7 patients with hypertrophic cardiomyopathy associated with mitochondrial myopathy, congenital cataracts and lactic acidosis. Histochemical analysis of muscle biopsies showed lipid and glycogen storage. This disorder was designated as " Sengers' syndrome " (McKusick 21235). Since then several other cases of cardiomyopathies due to mitochondrial dysfunction have been reported.[21,22] In 1985 Sengers studied 2 additional cases: 2 brothers of 33 and 18 years presented with the same syndrome, but no clear-cut biochemical defect was found in their mitochondria.[25] The mitochondriopathy reported here differs from Sengers' syndrome by the absence of congenital cataracts and evidence of a partial complex II defect.

Two siblings with a mitochondrial encephalomyopathy characterized by ataxia, intellectual impairment, myoclonic jerks, seizures and small stature have been reported by Riggs.[11] Muscle biopsy showed abnormal accumula-

tion of mitochondria and lipid droplets and decreased succinate-cytochrome C reductase activity, as in our siblings; however evidence of complex II deficiency in the patients described by Riggs is not conclusive. In fact, in one of their patients succinate-cytochrome C reductase was low, but within the range of 2 SD, SDH activity was normal and also complex IV was significantly low. It is not surprising that complex II defect causes lipid accumulation because of its dual function in both the citric acid cycle and respiratory chain.[15] In our patients a defect of complex II was proved by low activity of both succinate-cytochrome C reductase and succinate dehydrogenase. This pattern suggests a defect of either a non heme iron sulfur protein or of the b-cytochrome of complex II. The most prominent morphological finding was a striking change of mitochondrial ultrastructure and its abnormal distribution in both myocardial and skeletal muscle biopsies.

In 1 case with KSS, complex II deficiency has also been found on biochemical analysis.[10] KSS and progressive ophthalmoplegia are well known mitochondrial myopathies. These clinical syndromes have been associated with cardiac involvement of the conduction system and in some cases left or right ventricular dilatation has been reported.[26),27] However, the usual changes in these patients are " ragged-red " fibers associated with mitochondrial DNA deletion,[28] that were not found in our cases.

Depressed enzyme activity in skeletal muscle in severe heart chronic failure has been observed by Ralston et al.[29),30] Biopsies of patients with heart failure, compared with normal controls, demonstrate both moderate reduction in citrate synthase and marked reduction in SDH and COX activity. It is unlikely that the observed complex II defect is a secondary biochemical change since in our patients citrate synthase was normal and complex II activity was much more severely affected than other respiratory chain enzymes. In addition, in a previous series of dilated and hypertrophic cardiomyopathies[31] we found no specific enzyme decrease in skeletal muscle.

Complex II subunits are coded by nuclear DNA. The pattern of inheritance of our cases could be either autosomal recessive, X-linked recessive, or dominant with incomplete penetrance since minimal signs were found in the mother.

The 2 brothers reported here differ from previously reported cardiomyopathies by the presence of overt muscle weakness, relevant bioptic changes, and a partial complex II enzyme defect.

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