

Possible X linked congenital mitochondrial cardiomyopathy in three families

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Abstract
Familial cases of childhood congestive cardiomyopathy with X linked recessive inheritance and abnormalities of heart muscle mitochondria have been previously reported. We report here three families with possible X linked congestive cardiomyopathy and specific mitochondrial abnormalities. The heart disorder presented as endocardial fibroelastosis with neonatal death in two brothers in one family, and as heart failure and death in infancy in two brothers in the other two families. In one family a maternal uncle may also have been affected. Pyodermia and neutropenia was reported in one of the boys. Electron microscopy of heart muscle after necropsy showed increased numbers of mitochondria and abnormal mitochondrial cristal condensations and paracrystalline inclusions in all sibships. Barth's syndrome has been mapped to Xq28 and includes cardiomyopathy, skeletal muscle myopathy, neutropenia, and mitochondrial abnormalities similar to those found in the three families reported here. Since the clinical picture differed in the three families, they may represent more than one entity (*J Med Genet* 1993;30:269-72)

Familial cases of childhood cardiomyopathy with probable X linked recessive inheritance and abnormal mitochondria have been

reported. Both the clinical and pathological-anatomical findings seem to vary. One family had only heart muscle affected,¹ whereas two families had abnormalities of both heart and skeletal muscle.^{2,3} We report here three families with cardiomyopathy and identical heart muscle mitochondrial abnormalities on electron microscopy. The cardiac abnormality presented as heart failure and death in infancy in two families and as endocardial fibroelastosis with neonatal death in one family.

Case reports

FAMILY 1

Patient 1 (II-1, fig 1) was born one week before term after induced birth because of delayed growth. Birth weight was 2145 g and length was not measured. He immediately developed respiratory distress syndrome and pyodermia, but was discharged from hospital after three weeks. However, he had feeding difficulties and poor weight gain and he sweated a lot. At 3 months of age he was admitted to hospital because of heart failure and a cardiomyopathy was found. Neutropenia was noted on several occasions. Leucocyte counts varied from 4 to $10 \times 10^9/l$, with the following differential counts at 6 months of age: lymphocytes 89%, monocytes 8%, segmented neutrophils 1%, eosinophils 2%. He had no obvious hypotonia or other signs of skeletal muscle disorder. Psychomotor development was normal. At 8 months of age carnitine in serum was normal (33.5 nmol/ml) but low in skeletal muscle (0.965 $\mu\text{mol/g}$). However, histochemical examination of the muscle biopsy showed normal muscle tissue. He was treated with L-carnitine without any effect. Cultured fibroblasts showed normal levels of α -glucosidase. He died at 9 months from heart failure. Necropsy showed an enlarged heart weighing 60 g. The hypertrophy mainly affected the left ventricular wall. The liver showed congestion and diffuse steatosis.

Patient 2 (II-2, fig 1) is the younger brother of patient 1. He was born at term after a normal pregnancy, weight 4750 g, length 56.5 cm. Echocardiography at 6 weeks showed a possibly enlarged and thick walled left ventricle. Six weeks later he was admitted to hospital with heart failure and he died the next day. A white blood cell count was not performed. Psychomotor development appeared normal. Necropsy showed a markedly enlarged heart weighing 90 g. Again, the hypertrophy mainly affected the left ventricular wall. The

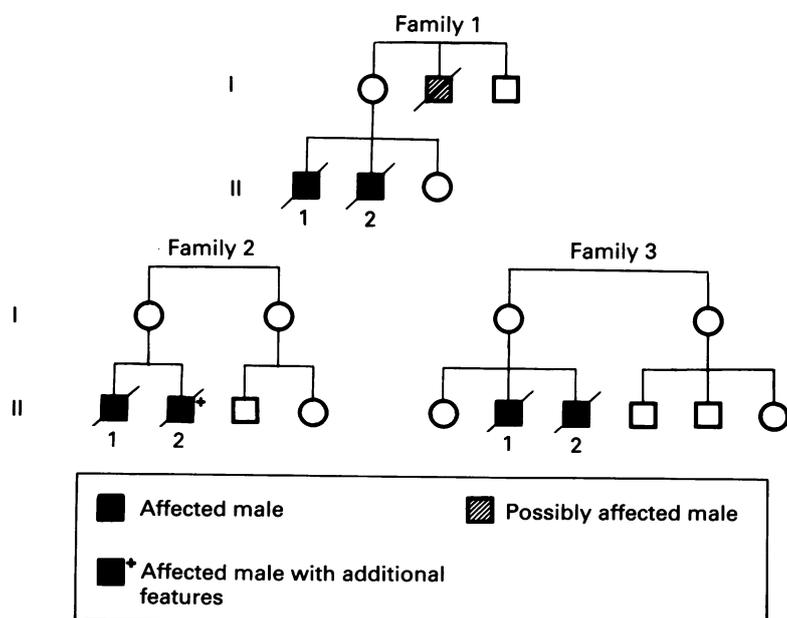


Figure 1 Family pedigrees.

liver showed congestion and moderate centrilobular steatosis.

Patient 3 (I-1, fig 1) is the maternal uncle of patients 1 and 2. He was born one week before term, weight 2050 g, length 46 cm. Development was normal until he was 6 weeks old, when he had several attacks of cyanosis. On admission to hospital he was cyanotic with cold extremities and shallow respiration and he died four days later. Necropsy showed a possibly enlarged heart weighing 45 g. Microscopic examination of heart muscle was not performed. The liver and kidneys showed moderate congestion.

FAMILY HISTORY

The parents of patients 1 and 2 are healthy and unrelated. 2D echocardiography showed no abnormality in either parent. A younger sister of the patients is healthy. Chorionic biopsy in a later pregnancy showed a female fetus with trisomy 22 and the pregnancy ended in a spontaneous abortion.

FAMILY 2

Patient 1 (II-1, fig 1) was the first child of healthy parents. At 32 weeks' gestation routine ultrasonography showed a cystic process in the left thorax and birth was induced two weeks before term. Birth weight was 3810 g and length was not measured. He was cyanotic and was treated on a respirator. An extremely dilated left ventricle was found and he died at 1 month of age. Serum carnitine was normal and he had no neutropenia. Necropsy showed an enlarged, markedly dilated heart, weighing 110 g. Microscopic examination showed thickened endocardium, variable interstitial fibrosis, and muscle fibres with increased diameter. The lungs and liver showed marked congestion.

Patient 2 (II-2, fig 1) was the younger brother of patient 1. From 28 weeks' gestation there was polyhydramnios. At 33 weeks ultrasonography showed neck oedema. Fetal blood karyotype was normal. He was born six weeks before term, weight 2900 g, length 45 cm, head circumference 37 cm. A dysmorphic appearance was noted with hypertelorism, antimongoloid eye slant, and thick and elaborate auricles. The arms were short and the thorax was small. Both testes were undescended. He was bradycardic and cyanotic and died 20 minutes after birth. At necropsy the heart weighed 25 g. There was possible left ventricular hypertrophy and preductal coarctation of the aorta with a patent ductus arteriosus. The liver was congested but the other organs were grossly normal. On microscopy the myocardium showed scattered hypertrophic fibres and some interstitial fibrosis.

FAMILY HISTORY

There were no other cases of cardiac anomalies in the family. Echocardiography of the parents was not performed.

FAMILY 3

Patient 1 (II-2, fig 1) was a boy born after a normal pregnancy, weight 4520 g, length 54 cm. He was healthy until he started coughing when he was 9 months old. One month later he was admitted to hospital because of cardiac failure and an enlarged left ventricle was found. Psychomotor development was normal and he had no hypotonia or neutropenia. He died within two days of cardiac failure. Necropsy showed a markedly enlarged heart, mostly affecting the left ventricle. The heart weighed 220 g. Microscopically the left ventricular endocardium was greyish and moderately thickened. The other organs were normal.

Patient 2 (II-3, fig 1) was the younger brother of patient 1. The pregnancy was normal, but weight and length is not known. Because of the brother's illness he was followed up regularly. From the age of 4 months he had a hypochromic microcytic anaemia and an increasing heart size. However, he remained healthy until he died suddenly at 20 months of age with a clinical picture of encephalitis. Psychomotor development was normal. He had no neutropenia. Necropsy showed an enlarged heart weighing 200 g, but no particular enlargement of the left ventricle. The brain was oedematous and the liver was fatty, otherwise no abnormalities were found.

FAMILY HISTORY

The parents were healthy and unrelated. Echocardiography showed no abnormalities in either parent.

CONTROL SUBJECTS

Heart muscle from two male infants aged 13 and 16 weeks was used as controls for electron microscopy. The cause of death in both cases was sudden infant death syndrome. All organs were normal at necropsy.

Electron microscopy

METHODS

Necropsies were performed 12 to 48 hours after death. Tissue samples taken at necropsy were diced, fixed in 2% buffered glutar aldehyde, postfixed in 1% osmium tetroxide for two hours, and embedded in epoxyresin. Areas to be studied by electron microscopy were selected from semithin, toluidin-blue sections. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 301 electron microscope. Micrographs were taken at $\times 3\text{-}4000$ to $25\text{-}000$ and enlarged photographically $\times 3$. Neutrophil granulocytes were prepared from buffy coat prepara-

tions and processed similarly for electron microscopy.

RESULTS

Myocardium

In all six patients there was a reduced number of contractile fibres, and a marked increase in the number and size of mitochondrial profiles. Between 8 and 50% of mitochondrial profiles showed cristal condensations, frequently with paracrystalline appearance (fig 2). Control subjects lacked such changes (table 1). Most mitochondria, including those of control subjects, contained flocculent densities.

Skeletal muscle

Skeletal muscle from patient II-2, family 1 was normal. No mitochondrial abnormalities were found.

Liver

Liver tissue from patient II-2, family 1 showed diffuse steatosis, but no mitochondrial abnormalities.

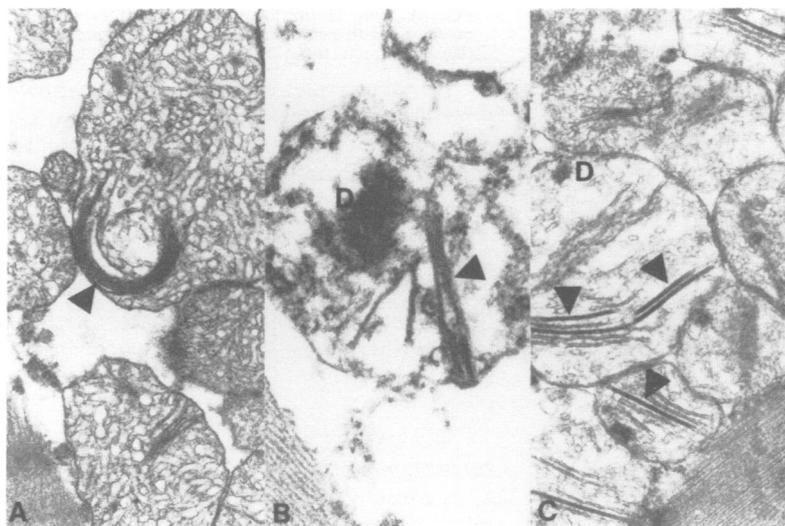


Figure 2 Myocardial mitochondria with cristal condensations (necropsy specimens). Arrowhead: cristal condensations. D: flocculent density (non-specific). (A) Family 1, II-1. Well preserved ultrastructure. Semicircular cristal condensations. (B) Family 2, II-1. Less well preserved, but mitochondrial condensation with parallel array of membranes visible at arrowhead. (C) Family 3, II-1. Multiple well preserved cristal condensations, frequently with faint cross striations.

Table 1 Percentage of mitochondrial cristal condensations in patients and controls.

	%
Family 1	
Patient II-1	8
Patient II-2	14
Family 2	
Patient II-1	ND
Patient II-2	13
Family 3	
Patient II-1	50
Patient II-2	9
Control 1	0
Control 2	0

ND = not done, material not adequate.

Neutrophil granulocytes

Barth's syndrome includes neutropenia and abnormal mitochondria in bone marrow cells.³ A white blood cell count of the mothers of the affected boys in families 1 and 2 was performed and no neutropenia was found. Electron microscopy of granulocytes showed no abnormal mitochondria in these females.

A summary of the findings in the three families is presented in table 2.

Discussion

Neustein *et al*² reported the first family with X linked cardiomyopathy and abnormal mitochondria. The abnormalities were found in both heart and skeletal muscle, liver, and kidneys, and seem to be similar to the abnormalities found in heart muscle in the three families of the present study. In the family described by Barth *et al*,³ affected boys had structural mitochondrial abnormalities both in cardiac muscle and neutrophil bone marrow cells, but only occasionally in skeletal muscle. Respiratory chain abnormalities were found in isolated skeletal muscle mitochondria. In addition, the affected boys had neutropenia and repeated infections. Plasma carnitine was borderline and muscle carnitine low. Hodgson *et al*¹ reported a family with six affected boys where endomyocardial fibroelastosis was confirmed in two of the boys and abnormal mitochondria with cristae arranged in a circular fashion were found in the only patient studied by electron microscopy. Kelley *et al*⁴ reported dilated cardiomyopathy in boys from five families in which raised urinary levels of 3-methylglutamate, 3-methylglutarate, and 2-ethylhydracrylate were a consistent feature. The disease in these boys seemed to have a more benign course and abnormal mitochondria were not found. Ino *et al*⁵ described two unrelated boys with cardiomyopathy, neutropenia, and short stature. Both children had low free plasma carnitine values and showed clinical improvement in cardiovascular manifestation after L-carnitine supplementation. Skeletal muscle biopsy showed normal mitochondria, but heart muscle mitochondria were not examined. Kelley *et al*⁴ suggested that these reports all refer to the same entity, Barth's syndrome.

The features in common to the three families reported here are the early onset and fatal outcome of the disorder, the affectedness of boys only, and the mitochondrial abnormalities. The number of heart muscle mitochondria was increased and the cristal condensations and paracrystalline densities were identical in all affected subjects. Family 1 could well represent a case of Barth's syndrome, since both neutropenia and pyoderma were found in patient II-1 who lived for the longest period. Although heart muscle was not available for microscopy, the clinical picture of the maternal uncle is in agreement with a cardiomyopathy. Normal skeletal muscle mitochondria were found in the only patient examined (II-2, family 1). However, both Neustein *et al*² and Barth *et al*³ found abnormal skeletal muscle mitochondria only occasionally. It is note-

Table 2 Summary of clinical and morphological findings.

	Family 1			Family 2		Family 3	
	I-1	II-1	II-2	II-1	II-2	II-1	II-2
Age at onset of symptoms (mth)	1.5	3	1.5	0	0	9	4
Age at death (mth)	1.5	9	3	1	0	10	20
Abnormal heart mitochondria	?	+	+	+	+	+	+
Neutropenia	?	+	?	?	?	-	-

worthy that whereas II-1 and his maternal uncle (I-1) had a low birth weight, his younger affected brother (II-2) had a birth weight of 4750 g.

In family 2 the heart failure presented prenatally in both the affected brothers. These patients could therefore represent a variant of Barth's syndrome. However, patient 2 had neck oedema which was detected prenatally and dysmorphic features which had not been found in his brother. Some of the dysmorphic features could be explained by his hydrops. This patient may also have had another disorder in addition to a cardiomyopathy. One possible diagnosis is Noonan syndrome. The affected members of family 3 had a later onset of the symptoms and no neutropenia. However, neutropenia has been reported to be fluctuating.³

Idiopathic dilated cardiomyopathy is generally considered to be a sporadic, non-genetic disorder but familial cases, usually with autosomal dominant inheritance, have

been reported.^{6,7} However, the congenital or early childhood cardiomyopathies probably have a different aetiology. There are no empirical risk data for these cardiomyopathies. Recently a linkage study of the original family described by Barth *et al*³ showed mapping of this syndrome to Xq28.⁸ This was an important step towards classification of the cardiomyopathies and providing empirical risk data for genetic counselling. Further studies are needed to clarify whether the various cardiomyopathies with mitochondrial abnormalities represent different entities or genetic variants of Barth's syndrome.

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