

X linked fatal infantile cardiomyopathy maps to Xq28 and is possibly allelic to Barth syndrome

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Abstract

A number of families with X linked dilated cardiomyopathy with onset in infancy or childhood have now been described, with varying clinical and biochemical features. Of these, one condition, Barth syndrome (BTHS), can be diagnosed clinically by the characteristic associated features of skeletal myopathy, short stature, and neutropenia, but not all of these features are always present. Molecular genetic studies have delineated the gene for BTHS, which maps to distal Xq28, from the gene for so called X linked dilated cardiomyopathy (XLCM), a teenage onset dilated cardiomyopathy, recently mapped to the 5' portion of the dystrophin locus at Xp21.

We report a large family in which male infants have died with congenital dilated cardiomyopathy, and there is a strong family history of unexplained death in infant males over at least four generations. Death always occurred in early infancy, without development of the characteristic features associated with Barth syndrome. Molecular analysis localised the gene in this family to Xq28 with lod scores of 2.3 at $\theta = 0.0$ with dinucleotide repeat markers, p26 and p39, near DXS15 and at F8C. The proximal limit to the localisation of the gene in this family is defined by a recombinant at DXS296, while the distal limit could not be differentiated from the telomere. This localisation is consistent with a hypothesis of allelic and clinical heterogeneity at the BTHS locus in Xq28.

(J Med Genet 1995;32:383-388)

The aetiology of familial cardiomyopathy, particularly those forms that are autosomally inherited, is heterogeneous, and cannot always be determined from the clinical or biochemical phenotype. Gene mapping of autosomal dominant hypertrophic cardiomyopathy (HCM) has implicated chromosomal regions 1q3, 11p13-q13, 14q11, and 15q2, and a number of missense point mutations in the β cardiac myosin heavy chain gene on chromosome 14 have been described.¹ HCM can, however, be sporadic. Maternally inherited cardiomyopathies associated with mtDNA mutations can present with marked clinical intrafamilial variation, from fatal infantile cardiomyopathy with lactic acid-

osis to adult onset skeletal and cardiac myopathy. Several mtDNA and tRNA mutations have been described.² The distinct X linked inheritance of dilated cardiomyopathy in some families has led to regional localisation of these genes by linkage mapping to the X chromosome.

The familial X linked cardiomyopathies so far reported tend to have infantile or childhood onset of symptoms.³⁻⁸ The best delineated clinically is Barth syndrome (BTHS) which features X linked cardiomyopathy with variable skeletal myopathy, short stature, and neutropenia.⁴ The clinical picture is quite variable both within and between families.^{8,9} Most affected people present with congestive cardiac failure and on echocardiogram have ventricular dilatation and decreased left ventricular ejection fraction (LVEF); thus they can be characterised as having a dilated cardiomyopathy. Reported laboratory findings have included mitochondrial ultrastructural abnormalities, decreased levels of respiratory chain enzymes, decreased plasma or muscle carnitine, and increased urinary excretion of 3-methylglutaconic and 2-ethylhydracrylic acids, but these are inconsistent. Two independent studies have recently localised the gene for BTHS to distal Xq28.^{10,11}

Berko and Swift¹² reported a large kindred with X linked dilated cardiomyopathy (now designated XLCM) in which affected males presented with rapidly progressive dilated cardiomyopathy in their teens to early twenties, with much later onset and slower course in manifesting female carriers. This disorder is associated with raised muscle creatine kinase and abnormalities of cardiac muscle dystrophin, but with no skeletal muscle involvement. XLCM has recently been linked to the 5' portion of the dystrophin locus at Xp21.¹³ These two major loci of X linked cardiomyopathy, BTHS and XLCM, have only been clearly delineated by linkage analysis although they do show some clinical differences.

We report a large family with apparent X linked inheritance of a fatal infantile cardiomyopathy, in which we have regionally localised the gene responsible to Xq28. The clinical features in this family are insufficient to permit a definite diagnosis of Barth syndrome, and the cardiomyopathy is consistently of congenital onset and fatal in infancy. The possibility that this represents allelic heterogeneity within the BTHS locus is discussed with respect to these findings.

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Received 5 October 1994
Revised version accepted for publication
9 December 1994

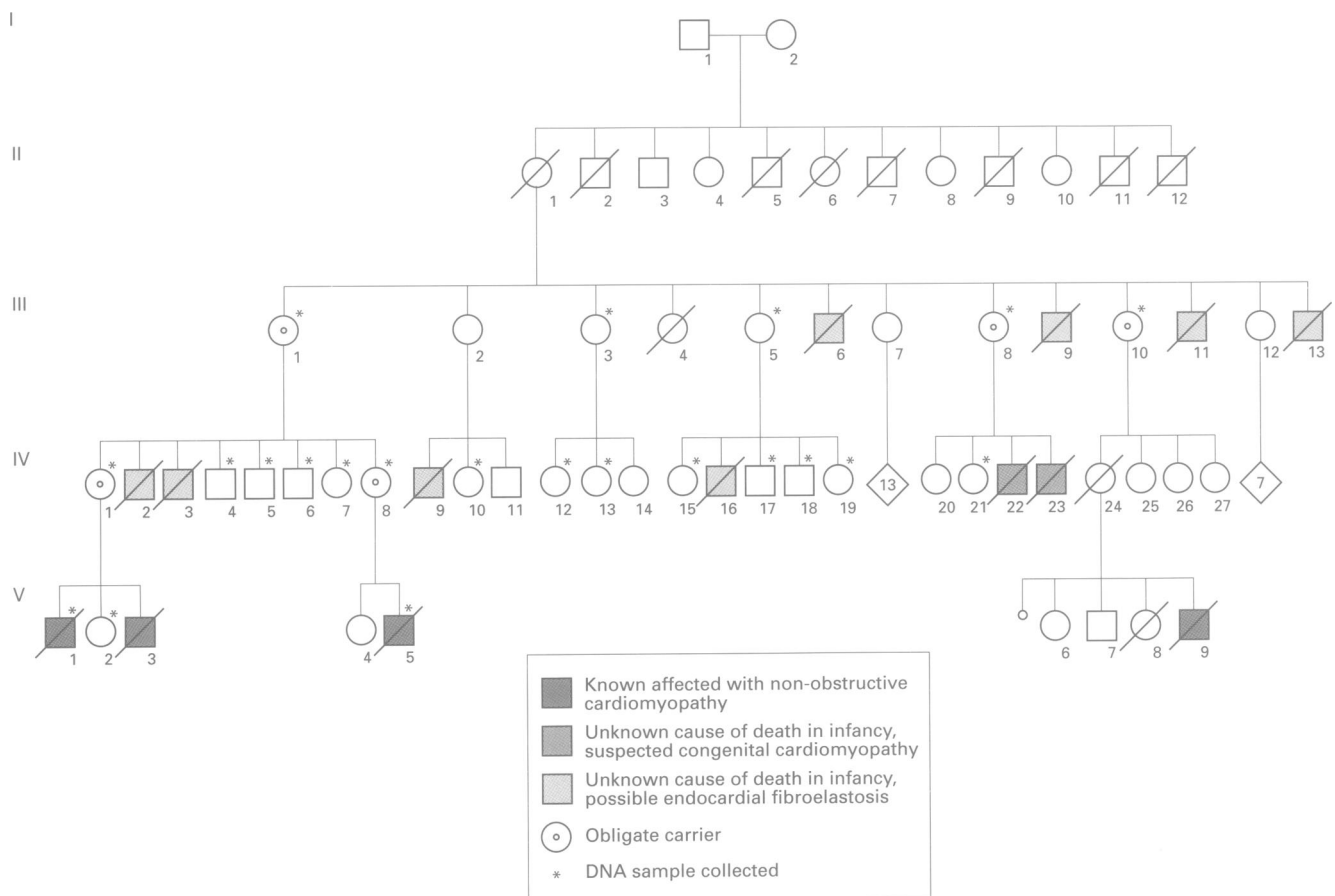


Figure 1 Pedigree of the family with fatal infantile cardiomyopathy.

Materials and methods

CLINICAL REPORT

The pedigree (fig 1) comprises several male infants (IV·22, IV·23, V·1, V·3, V·5, and V·9, known or believed to have died with hypertrophic cardiomyopathy. These affected males, with clinical findings given below, are related through apparently healthy women (III·1, III·8, III·10, IV·1 and IV·8) in a pattern consistent with X linked inheritance. Mitochondrial inheritance seems unlikely in the absence of any affected female offspring of carrier mothers and is also supported by the lack of clinical symptoms of cardiomyopathy in obligate carriers.

V·1

This infant was born at term, birth weight 2440 g, Apgar scores 3 and 6. He was lethargic and mildly cyanosed from birth with periodic deeper cyanosis. He had mild talipes equinovarus but no dysmorphic features and no obvious skeletal myopathy. Echocardiography on day 11 showed a dilated, poorly contracting heart, with left ventricular hypertrophy (LVH) and LV dilatation; the interventricular septum was not hypertrophied. He was treated with digoxin, and showed some initial improvement but died at the age of 6 weeks. Necropsy showed a globular heart with biventricular hypertrophy and left ventricular dilatation. The endocardium was pale and thickened. Microscopy showed myocardial hyperplasia and endocardial fibroelastosis in the LV and right

ventricle and atrium. Skeletal muscle was reported as normal on light microscopy.

V·3

The brother of V·1 was born at term, Apgar scores 6 and 8. He had occasional cyanosis from birth and fed poorly at times. Echocardiogram at 1 week and 3 weeks showed minimal left ventricular hypertrophy, but normal LV function. He died suddenly at 3·5 weeks; no necropsy was performed.

V·5

He was born at term, birth weight 3000 g, Apgar scores 3 and 9. He was lethargic and cyanosed from birth and had mild talipes equinovarus. Echocardiography showed marked biventricular hypertrophy with tricuspid regurgitation and a right to left shunt at the foramen ovale. He required assisted ventilation for several days but was able to be extubated by day 4. Investigations included electrolytes, urea, creatinine, calcium, urine metabolic screen, lactate, pyruvate, full blood count and differential, carnitines, plasma amino acids, and very long chain fatty acids. Total, free, and acylcarnitines were normal as was his neutrophil count. There was no evidence of methylglutaconic aciduria. He was maintained on antifailure treatment and stabilised for several months, but had suboptimal weight gain and gradually deteriorated and died at the age of 5 months.

IV·9

Birth weight was 2700 g and Apgar scores 8 and 10. He fed poorly from birth but was first investigated at 6 weeks when he presented cyanosed and in cardiac failure; chest x ray showed cardiomegaly. He was clinically much improved after starting antifailure therapy, but the cardiomegaly persisted. He died at 5 months; no necropsy was performed, and no record of echocardiographic findings was available.

The available history for the following two infants was taken from medical records kept at the small country hospital where they were born.

IV·22

A birth weight of 2990 g was recorded at term. Dusky cyanotic episodes and some difficulty in feeding were documented in the nursing notes from birth and over the first 3 days of life, but he improved and was discharged home apparently well at 6 days of age; no investigations were documented. He died at the age of 2 to 3 weeks, cause unknown, and no necropsy was performed.

IV·23

Birth weight was 2710 g at term. He had frequent episodes of cyanosis from birth, with lethargy. He deteriorated rapidly and died at the age of 2 days; no investigations or necropsy were performed.

This clinical presentation is consistent with both babies (IV·22 and IV·23) having had congenital cardiomyopathy. No investigations or recognisable cause for the cyanotic episodes nor necropsy confirmation of these diagnoses is available.

There is also a strong family history of unexplained death of infant males, all less than 6 months old, occurring over at least four generations, including III·6, III·9, III·11, III·13, IV·2, IV·3, IV·9, and IV·16. Medical records for these and other possibly affected infants were either uninformative or not found.

DNA ANALYSIS

Genotyping of several dinucleotide repeat and RFLP markers in Xq27–28 was undertaken (table 1). The order of markers used was: cen-DXS548-DXS296(VK21A)-DXS1113-GAB-

RA3-DXS52(St-14)-p26, p39, DXS15(DX13)-DXS707(2-55)-DXS605(2-19)-F8C-DXS1108(SDF-2)-DXYS154(SDF-1)-tel.^{17,18,20,21} The markers p26 and p39 are dinucleotide repeat markers subcloned from a 500 kb YAC XY845, containing loci DXS52 and DXS15, and lie 220 kb and 10 kb proximal to DXS15 respectively.¹⁷ They are considered to be at the same genetic location as DXS15 for the purposes of multipoint linkage analysis. Genotypes at DXS296 (*TaqI*), DXS52 (*TaqI*), and DXS15 (*BglIII*) were determined by Southern blot hybridisation of the relevant restriction digest required to detect the RFLP. The markers 2-19 at DXS605 and 2-55 at DXS707 were described as RFLPs¹⁸; however, oligonucleotide sequences flanking each restriction site (supplied by D Toniolo) permitted amplification by PCR before digestion. Remaining markers were analysed by PCR amplification in the presence of [α -³²P]dCTP as previously described.²² The most distal polymorphic markers in Xq, DXS1108 within 500 kb of the telomere and DXYS154,²⁰ were genotyped in the family in an attempt to identify recombinant meioses that would define the localisation in Xq28 as distinct from the telomere. The STR44 and STR50 dinucleotide repeats, recently assigned D numbers DXS1238 and DXS1235 respectively,²³ lie within introns of the dystrophin locus and were analysed to show exclusion of this cardiomyopathy locus from Xp21.

LINKAGE ANALYSIS

Linkage analysis was performed using the computer programs MLINK for two point lod scores and LINKMAP for multipoint analysis. The analyses included all people from whom DNA samples were collected. The recombination fractions between loci included in the multipoint analysis were: DXS296-0·001-DXS1113-0·10-DXS52-0·008-p26, p39, DXS15-0·001-DXS707-0·001-DXS605-0·001-F8C-0·005-DXS1108. The disease gene frequency was set at 1/10 000 and analysis assumed X linked recessive inheritance. For diseases with an X linked mode of inheritance the critical value of the lod score is $Z_0 > 2.0$ for demonstration of linkage.²⁴

Results

Pairwise lod scores at 10 marker loci in Xq28 and at STR44 in Xp21 are given in table 2. The maximum lod score of 2·30 at a recombination frequency of 0·0 was generated at the p26 and p39 markers near DXS15 and at F8C. The STR50, DXS548, GABRA3, and DXYS154 loci were uninformative in this family. A recombination event was observed with DXS296 (VK21A) in subject III·8. Her status as a carrier is based on clinical records showing that her sons had a clinical presentation consistent with the suspected cause of death, congenital cardiomyopathy.

All of the people from whom DNA samples were collected were included in the analysis. The genotypes of III·3 and III·5, for example,

Table 1 Markers used for the localisation of fatal infantile cardiomyopathy

Locus	Marker/symbol	Polymorphism	Heterozygosity	Reference
DXS296	VK21A	<i>TaqI</i>	0·22	14
DXS1113	—	(TC) ₁₀ (AC) ₂₁	0·75	15
GABRA3	MGD34	(CA) ₁₅	0·29	16
DXS52	St14-1	<i>TaqI</i>	0·77	14
	p26	(CA) ₂₁	0·81	17
	p39	(CA) ₁₆	0·84	17
DXS15	DX13	<i>BglIII</i>	0·5	14
DXS707	2-55	<i>MspI</i>	0·42	18
DXS605	2-19	<i>EcoRI</i>	0·48	18
F8C	intron 13	(CA) ₂₀	0·69	19
DXS1108	sDF-2	(GT) ₂ GC(GT) ₁₃	0·75	20
DXYS154	sDF-1	(CS) ₁₈	0·70	20

Table 2 Two point lod scores between the fatal infantile cardiomyopathy gene and markers within Xp21 and Xq28

Locus	θ						Z_{max}	θ_{max}
	0.01	0.05	0.1	0.2	0.3	0.4		
Xp21								
STR44	-3.42	-1.44	-0.69	-0.09	0.12	0.13	0.15	0.36
Xq28								
DXS296 (VK21A)	0.67	1.23	1.34	1.23	0.95	0.54	1.35	0.11
DXS1113	1.66	1.52	1.33	0.96	0.58	0.21	1.70	0.0
DXS52 (St-14)	2.17	2.00	1.78	1.33	0.86	0.38	2.21	0.0
p26	2.26	2.07	1.84	1.36	0.87	0.37	2.30	0.0
p39	2.26	2.07	1.84	1.36	0.87	0.37	2.30	0.0
DXS15 (DX13)	1.43	1.31	1.16	0.85	0.51	0.18	1.45	0.0
DXS707 (2-55)	1.92	1.75	1.54	1.10	0.63	0.20	1.96	0.0
DXS605 (2-19)	1.39	1.26	1.10	0.77	0.43	0.11	1.42	0.0
F8C	2.26	2.07	1.84	1.36	0.87	0.37	2.30	0.0
DXS1108 (SDF-2)	1.65	1.51	1.32	0.95	0.57	0.21	1.69	0.0

could contribute to the inference of parental genotypes in generation II. Sufficient clinical records could not be found to confirm cause of death and include the probably affected males IV.2, IV.3, IV.9, and IV.16 into the analysis. Their inclusion under the assumption that they died of cardiomyopathy, increases the peak two point lod score to 2.84 at $\theta=0.0$ for the markers p26 and p39 and at F8C. Several males in generation III have also died of untraceable causes and descendants of the grandmaternal branch are said to have had boys with endocardial fibroelastosis: however, none of these was included in the analyses.

The proximal limit for the localisation of this gene is defined by the recombination in subject III.8 at DXS296. The DXYS154 locus was not informative so that the distal limit to this localisation is not differentiated from the telomere at Xqter, since recombination was not observed in any markers distal to DXS296. The regional localisation covers approximately 11 cM, virtually all of the Xq28 band to the

telomere. The peak location score is 12.27 (equivalent to a lod score of 2.67) at the p26, p39, and DXS15 cluster between the loci DXS52 to DXS707 (fig 2). This multipoint analysis did not include the probably affected males IV.2, IV.3, IV.9, and IV.16. The significant two point lod scores of 2.3 with p26, p39, and F8C, and 2.2 with DXS52 depend upon the assumption that IV.22 and IV.23 were affected with fatal infantile cardiomyopathy. The peak two point lod score obtained by exclusion of III.8 and her sons is reduced to 2.21 at $\theta=0.0$ at p26 and p39, but remains significant in support of linkage to Xq28.

Discussion

The large family described here has a severe familial form of cardiomyopathy with early, often congenital onset which is invariably fatal in infancy. The clinical diagnosis of Barth syndrome (BTHS) is based on the presence of the characteristic triad of cardiac and skeletal myopathy in association with short stature and neutropenia, but BTHS could not be diagnosed clinically in this family as none of the patients developed other features. The cardiomyopathy in BTHS is of congenital, infantile, or childhood onset and very variable progression, sometimes fatal in the neonatal period, but in others within the same family may stabilise or improve in early childhood. The cardiac findings are of a predominantly dilated cardiomyopathy often with ventricular hypertrophy and sometimes associated with echocardiographic or histological evidence of endocardial fibroelastosis. The cardiac findings in the patients described here were of this type, but although there was some response to antifailure medication in the two treated boys, death still ensued within months, and no known affected male has survived past infancy. As wider family investigation has been limited, it is not known whether there are surviving asymptomatic affected boys.

None of the affected infants in this family had definite skeletal myopathy, and creatine kinase, measured in V.1 and V.5, was not raised. It is interesting to note that two infants had mild talipes equinovarus, which has been reported in patients with BTHS,¹¹ and could be a consequence of either congenital myopathy or in utero hypotonia. Mild neutropenia was present on one occasion in V.1, but was not observed in other affected infants, nor was there a history of skin or other infections. Others^{9,11} have commented on the relatively low birth weight for gestational age, although this does not apply to all patients with BTHS; we also noted birth weights under the 10th centile in three of the six infants reported. Information regarding birth length and subsequent linear growth was lacking, but poor feeding and failure to thrive, consistent with symptomatic cardiomyopathy occurred in the two infants who survived the longest (V.5 and V.9 survived to 5 months).

Although the clinical features were inconclusive, linkage analysis in this family was

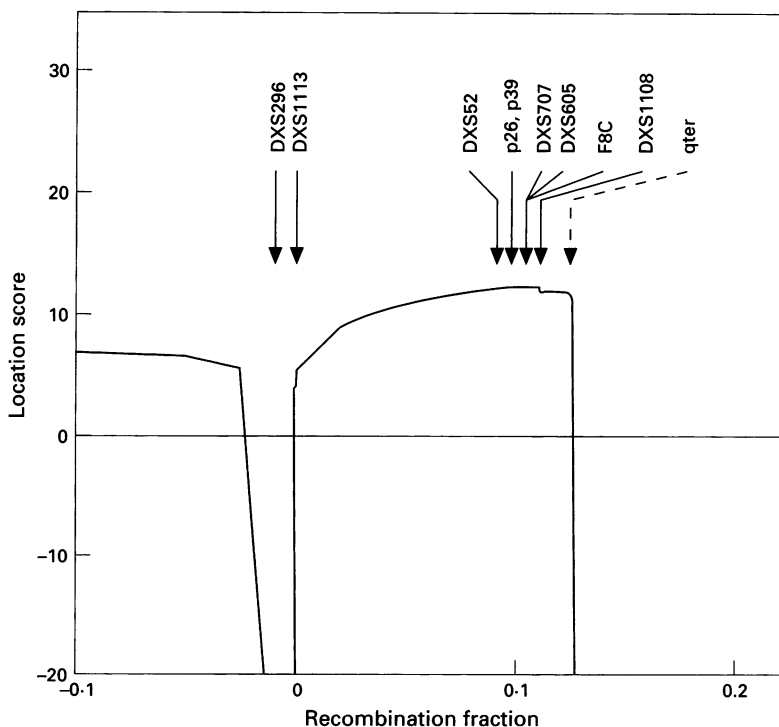


Figure 2 Location of the gene for fatal infantile cardiomyopathy on the background map of X chromosome markers. The location score reached a peak at 12.27, equivalent to a multipoint lod score of 2.67.

consistent with localisation of the gene to Xq28, overlapping with the regional localisation for BTHS. The proximal limit to the gene localised in this family is defined by recombination at DXS296 in a single subject III-8, given the clinical evidence that her sons died of congenital cardiomyopathy. Since the parental genotypes in generation II are not available and could not be inferred at any of the markers used distal to DXS296, no further recombinations could be detected. The gene responsible for fatal infantile cardiomyopathy is located within an interval spanning 11 cM of Xq28 from DXS296 to qter. The BTHS locus has been mapped between DXS369 and qter¹⁰ and in the interval DXS374 to qter.¹¹ The marker DXS369 is proximal to DXS548 and DXS296, while DXS374 is an RFLP proximal and close to GABRA3 and distal to DXS1113. Co-localisation of both disorders could imply genetic homogeneity, with clinical heterogeneity explained by allelism at the same locus. Alternatively, it must be considered that there may be more than one gene for dilated cardiomyopathy in Xq28, which is a known gene rich area of the genome.

The two patients in family 2 of Orstavik *et al*⁸ died within four weeks of birth from heart failure presenting prenatally. These cases with congenital cardiomyopathy may represent examples of the same severe entity that we describe, which may be a congenital variant of BTHS.

Major features described in BTHS include mitochondrial structural changes suggestive of a disorder in mitochondrial energy metabolism. Nuclear encoded genes affecting mitochondrial function may be possible candidates for this disorder. Mitochondrial abnormalities are not always seen in Barth syndrome families^{10,11} and, if present, are non-specific so may be a secondary effect. Therefore genes for structural muscle proteins could equally well be candidates as genes involved in mitochondrial energy metabolism.

In the XLCM form, there is no weakness or histological evidence for skeletal muscle dystrophy although raised creatine kinase (CK) levels were reported.¹³ It is known that some Becker muscular dystrophy (BMD) patients, despite mild skeletal myopathy, may have severe cardiomyopathy. It is suggested that the cardiomyopathy in XLCM and in these BMD cases may arise through particular mutations in the dystrophin gene, or perhaps in dystrophin regulatory regions at Xp21.

Emery-Dreifuss muscular dystrophy (EDMD) mapped to distal Xq28 is a slowly progressive, later onset disorder of skeletal myopathy and contractures, and a cardiomyopathy usually presenting with prominent atrial involvement, heart block, and rhythm disturbances.²⁵ Candidate genes derived for EDMD should therefore also be examined in cardiomyopathy families linked to Xq28. The possibility exists that, in the same way that XLCM has been shown to be linked to the DMD/BMD region but without skeletal myopathy, Barth syndrome may be a specific mutation in the gene region causing cardiac

defect in EDMD. The actin binding protein 280 (ABP-280) encoded by the filamin gene (FLN1) is a potential candidate gene for a structural protein rearrangement in these cardiomyopathies, by virtue of its location in Xq28.²⁶ It is interesting that the actin binding protein shares some sequence with dystrophin. So far no gross rearrangements or mutations of this gene have been shown in patients with EDMD.²⁷

Linkage studies mapping X linked forms of cardiomyopathy^{9-11,13} are an important step towards their classification. The clinical distinction between the various forms of X linked cardiomyopathy including X linked endocardial fibroelastosis (EFE) is not clear. Since at least two loci (XLCM and BTHS) have already been identified, and given the variability of expression, delineation of the cardiomyopathy locus is essential before carrier or prenatal diagnoses in a family. The requirement for a large number of meioses within single families will be the rate limiting step to the refinement of these gene localisations by linkage and subsequent cloning of the gene. Once more families have been localised in this manner it may be possible to differentiate between these forms based on differences in clinical findings and age of onset. It has not been possible to delineate genetically the more severe congenital form of cardiomyopathy reported in this study from Barth syndrome. If the family described here represents a severe form of Barth syndrome, at least some of the variable expression between families might represent allelic rather than locus heterogeneity.

This work was supported by the National Health and Medical Research Council of Australia.

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