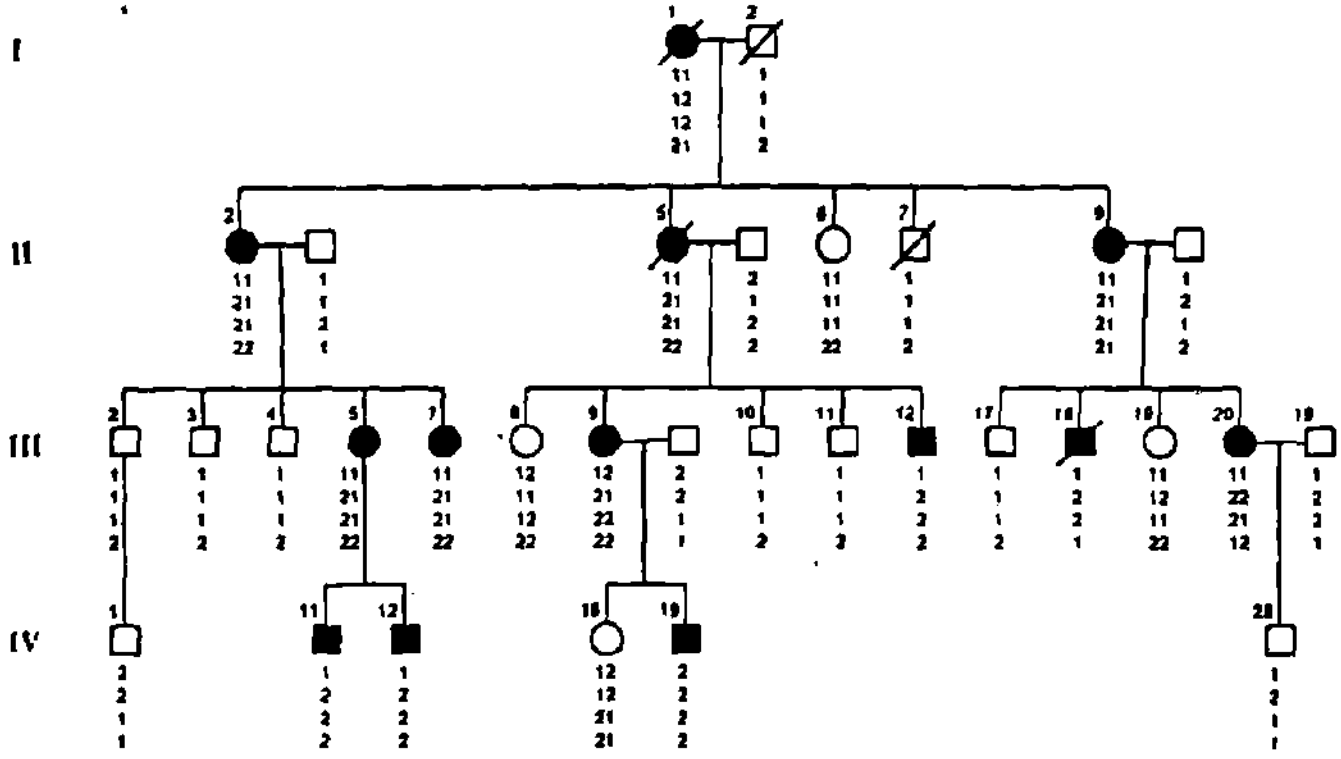
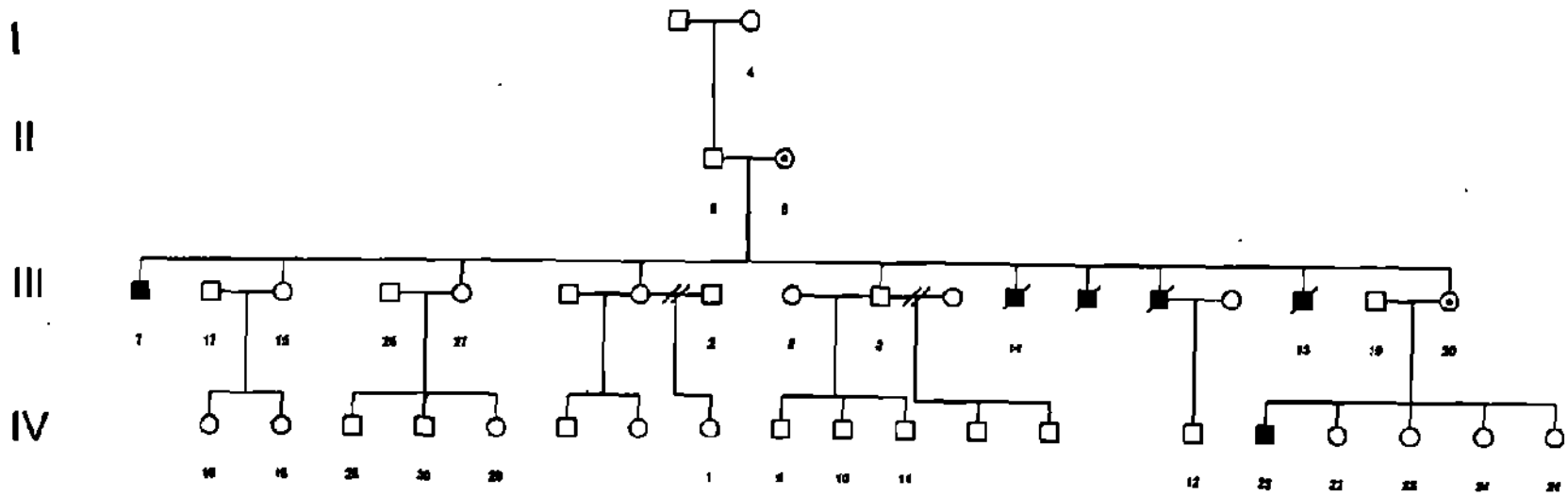
# ANEXO 1

## ARBOLES GENEALOGICOS

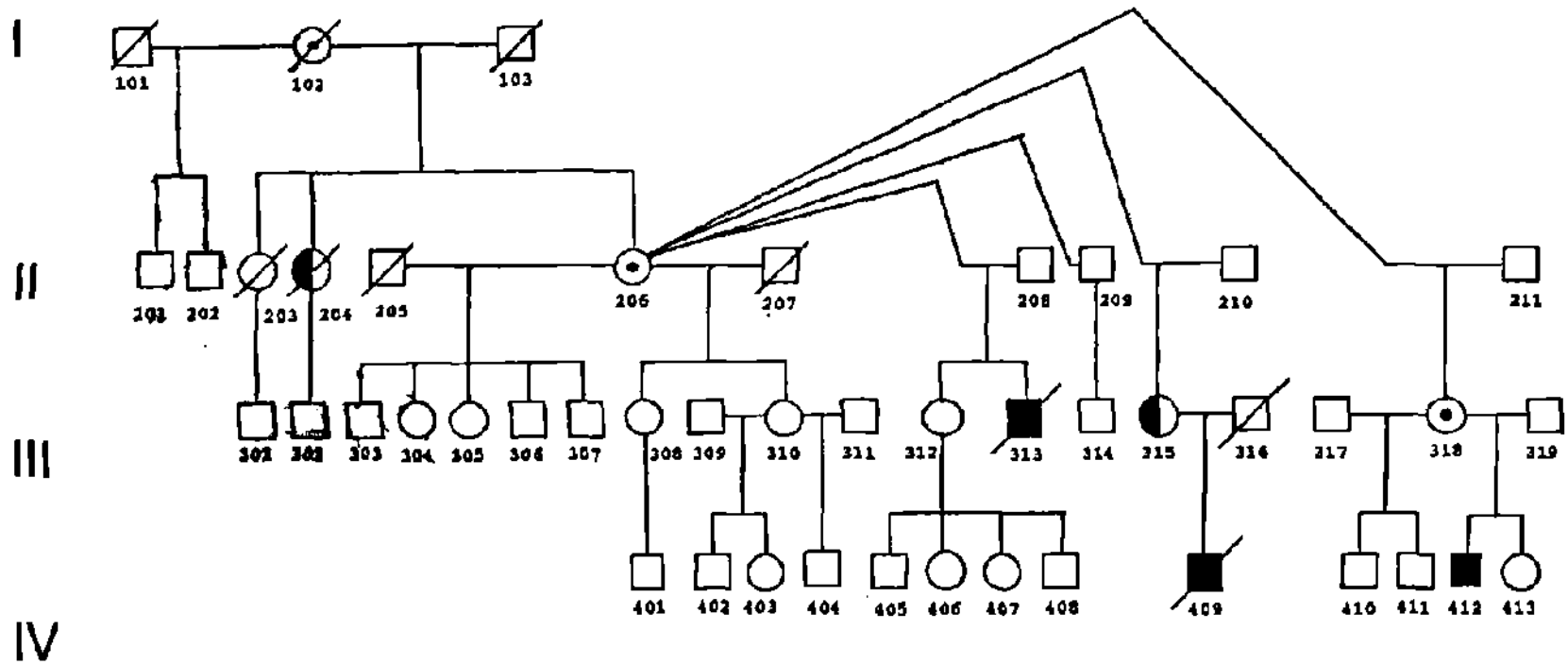
# Familia CDLX-1



# Familia CDLX-2



# Familia CDLX-3



# ANEXO 2

## PUBLICACIONES DERIVADAS DE ESTA TESIS

# The New England Journal of Medicine

Established in 1812 as The NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY

VOLUME 330 FEBRUARY 3, 1994 NUMBER 5


## Original Articles



## Images in Clinical Medicine



## Special Article



## Review Articles



## Clinical Problem-Solving



## Case Records of the Massachusetts General Hospital



## Editorial



## Sounding Board



## Correspondence



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of cardiac symptoms. Furthermore, the hypothesis put forth by Muntoni et al. that "the brain promoter is driving relatively high levels of transcription in skeletal muscle but not in the heart" is not supported by our report,[4] which was cited in their article. We demonstrated that the brain promoter is capable of driving dystrophin transcription in the human heart, perhaps more so than in skeletal muscle, a finding that contradicts their statement. The lack of molecular data from cardiac tissue (i.e., endomyocardial-biopsy specimens from an affected family member) makes it very difficult to speculate how the muscle-promoter deletion in the family Muntoni et al. studied might selectively affect dystrophin function in the heart, or even whether it has such an effect. Further investigation is required to uncover the molecular mechanisms that distinguish cardiac-muscle from skeletal-muscle dysfunction in these families.

ROGER D. BIES, M.D.
University of Colorado
Denver, CO 80262 Health Sciences Center

1. Muntoni F, Cau M, Ganau A, et al. Deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy. N Engl J Med 1993;329:921-5.
2. Towbin JA, Hejtmancik JF, Brink P, et al. X-linked dilated cardiomyopathy: molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation 1993;87:1854-65.
3. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet 1991;49:54-67.
4. Bies RD, Phelps SF, Cortez MD, Roberts R, Caskey CT, Chamberlain JS. Human and murine dystrophin mRNA transcripts are differentially expressed during skeletal muscle, heart, and brain development. Nucleic Acids Res 1992;20:1725-31.

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*To the Editor:* I was disturbed by the incorrect usage of genetic terms in the text and Table 1 of the article by Muntoni et al. The affected males (Subjects II-1, II-2, II-5, and II-6) are labeled "homozygous" for the deletion despite the fact that they are obviously hemizygous.* On the other hand, the carrier females (Subjects I-1, I-5, II-3, and II-4) are labeled "homozygous," although they are clearly heterozygous for the defect. The noncarrier females (Subjects I-4 and II-7) are described as "hemizygous" when they are in fact homozygous for the normal allele.

Another term used in a questionable manner is "obligate carriers," which is used for all four carrier females in the pedigree. This term can correctly be used to apply only to subjects whose carrier status is obvious on the basis of pedigree data; in this family, only Subject I-1 should be called an obligate carrier. The other three (Subjects I-5, II-3, and II-4) are not obligate carriers, since their carrier status was based on the molecular studies performed and not on pedigree information.

Since reports of genetic studies are increasingly read by nongeneticists, it is important to use correct terminology in order to avoid confusion.

MARTIN ROUBICEK, M.D.
Universidad Nacional
7600 Mar del Plata, Argentina de Mar del Plata

*King RC, Stansfield WD. A dictionary of genetics. 4th ed. New York: Oxford University Press, 1990:142.

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*To the Editor:* Muntoni et al. recently described a family in which a deletion in the muscle-promoter and exon 1 regions of dystrophin was thought to be the cause of X-linked dilated cardiomyopathy. The authors evaluated the muscle promoter and exon 1 by polymerase-chain-reaction (PCR) analysis of DNA extracted from blood and supported the results with immunohistochemical studies of skeletal muscle. No cardiac studies were performed. Unfortunately, the authors failed to refer to our report[1] in which two families with X-linked dilated cardiomyopathy were clinically evaluated, linkage to the 5′ portion of dystrophin was demonstrated, and Western blot analysis with *N*-terminal dystrophin antiserum showed low levels of cardiac dystrophin protein and normal levels of skeletal-muscle protein. In addition, multiplex PCR including the muscle-promoter region revealed no deletions.

We have now studied three families with X-linked dilated cardiomyopathy, and in all subjects the muscle-promoter and exon 1 regions are intact. Using the same primers as Muntoni et al., including DYSMSB (which was deleted in the family described), we could not find any deletions in the probands of our families (Fig. 1). Furthermore, extensive sequencing of this region revealed no abnormalities. Also unsupported is their hypothesis that selective deletion of the muscle promoter leads to cardiomyopathy without skeletal disease and that the brain promoter is selectively "driving . . . high levels of transcription in skeletal muscle but not in the heart." A similar deletion, but without selective cardiac disease (in fact, with no cardiac disease), has been described.[2,3] In addition, Bies et al.[4] demonstrated that the

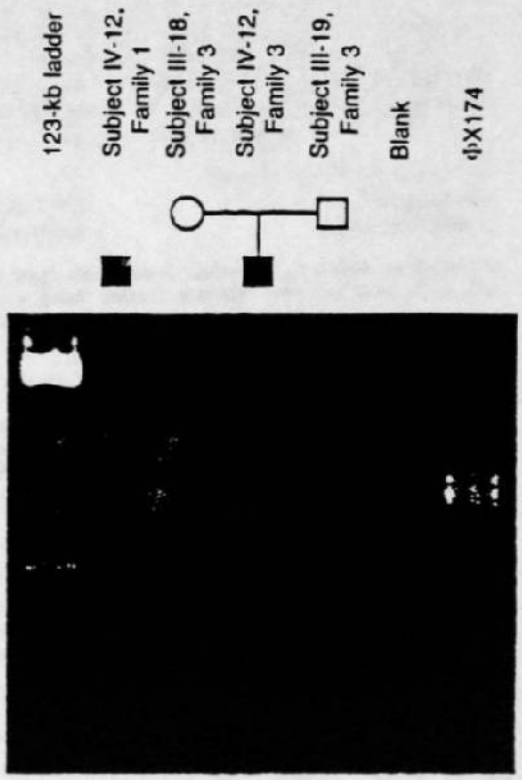


Figure 1. PCR Analysis of the Dystrophin Muscle-Promoter Microsatellite Locus DYSMSB in the Probands from Two Families with X-Linked Dilated Cardiomyopathy (Subjects IV-12 and IV-12 in Families 1 and 3), a Probable Carrier Female (Subject III-18 in Family 3), and a Normal Male (Subject III-19 in Family 3).

Note that in all subjects, the expected amplification product is seen (i.e., no deletions occurred). The amplification products were resolved on 1 percent agarose gel. The 123-kb ladder and ϕX174 are size markers.

brain promoter is capable of driving dystrophin transcription in the human heart at a level even greater than that seen in skeletal muscle, directly contradicting, rather than supporting, the results of Muntoni et al.

We believe that the report of Muntoni et al. describes a defect inconsistent with a cardiospecific abnormality and that this defect has been prematurely accepted as the cause of X-linked dilated cardiomyopathy. Further careful clinical and molecular study of cardiac tissue from patients with X-linked dilated cardiomyopathy is needed to clarify the molecular mechanisms that distinguish cardiospecific dysfunction from the typical skeletal-muscle disease expected with dystrophin defects.

JEFFREY A. TOWBIN, M.D.
ROCIO ORTIZ-LOPEZ, M.S.
Houston, TX 77030 Baylor College of Medicine

1. Towbin JA, Hejtmancik JF, Brink P, et al. X-linked dilated cardiomyopathy: molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation 1993;87:1854-65.
2. Boyce FM, Beggs AH, Feener C, Kunkel LM. Dystrophin is transcribed in brain from a distant upstream promoter. Proc Natl Acad Sci U S A 1991;88:1276-80.
3. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet 1991;49:54-67.
4. Bies RD, Phelps SF, Cortez MD, Roberts R, Caskey CT, Chamberlain JS. Human and murine dystrophin mRNA transcripts are differentially expressed during skeletal muscle, heart, and brain development. Nucleic Acids Res 1992;20:1725-31.

Dr. Muntoni replies:

*To the Editor.* I am grateful to Dr. Roubicek for his comment on the use of the term "obligate carrier." With respect to his comment on Table 1, an error was introduced during the preparation and editing of column 11. A revised version is shown.

The letters of both Dr. Bies and Dr. Towbin and Mr. Ortiz-Lopez raise the issue of the genotypic or phenotypic specificity in our family (the report by Towbin et al. appeared only after the final version of our manuscript was accepted). As we have already stated, the two previously described patients with a muscle-promoter deletion but no cardiac symptoms are young (10 and 12 years old) and may well be clinically asymptomatic.[1,2] Alternatively, the deletion found in our family might encompass regulatory sequences not affected in those boys.

We initially hypothesized that the brain promoter was driving dystrophin transcription in the skeletal muscle of our patients. We have now found, after detailed quantitation of the reverse-transcribed messenger RNA, that this is the case (unpublished data). Because of the unavailability of cardiac tissue, we have not studied transcription in the heart of the proband.

The work of Bies et al. showed that the brain promoter is not capable of driving dystrophin transcription in human Purkinje fibers or in the heart of the mouse.[3] The importance of the faint signal obtained from the heart in humans is difficult to interpret because no quantitation of transcription was attempted[4] and the contributing role of contaminating smooth muscle was not established.

Regardless of the mechanism responsible for the dissociation between the skeletal-muscle and cardiac-muscle involvement in our patients, their phenotype is undoubtedly due to a dystrophin mutation, and this is what we emphasized. We proposed that biochemical and genetic screening for a dystrophinopathy should be performed in all families with X-linked dilated cardiomyopathy. The observations of Dr. Towbin and Mr. Ortiz-Lopez and those of Dr. Bies confirm that this recommendation is valid.

It is likely that more than one mutation will be found in this group of patients, as is the case for both Duchenne's and Becker's muscular dystrophies. However, most reports of the families with this disease (including one Italian family [Angelini C: personal communication] and seven Japanese families [Takeda S: personal communication]) in which a proper biochemical evaluation was performed revealed skeletal-muscle dystrophin of normal molecular weight although in lower than normal levels, strongly reinforcing the view that mutations in regulatory regions of the gene are likely to be implicated in this phenotype.

FRANCESCO MUNTONI, M.D.
London, W12 0NN, Royal Postgraduate
United Kingdom Medical School

1. Boyce FM, Beggs AH, Feener C, Kunkel LM. Dystrophin is transcribed in brain from a distant upstream promoter. Proc Natl Acad Sci U S A 1991;88:1276-80.
2. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet 1991;49:54-67.
3. Bies RD, Phelps SF, Cortez MD, Roberts R, Caskey CT, Chamberlain JS. Human and murine dystrophin mRNA transcripts are differentially expressed during skeletal muscle, heart, and brain development. Nucleic Acids Res 1992;20:1725-31.
4. Chelly J, Concordet JP, Kaplan JC, Kahn A. Illegitimate transcription: transcription of any gene in any cell type. Proc Natl Acad Sci U S A 1989;86:2617-21.

Table 1. Selected Laboratory Findings in Various Family Members.*

| Subject No. | Sex | Age | Serum CK | Serum CK-MB | Echocardiography | | | NYHA Class | ECG Results† | Zygosity for the Deletion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | FS | EDD | Events | | | |
| | | yr | U/liter | | % | mm | | | | |
| I-1 | F | 51 | 106 | 4 | 31 | 48 | Normal | — | Normal | +/− |
| I-4 | F | 44 | 98 | 4 | 43 | 46 | Normal | — | Normal | +/− |
| I-5 | F | 39 | 71 | 5 | 28 | 50 | Mild hypokinesia | I | Normal | +/− |
| I-8 | M | 27 | 112 | 3 | 41 | 45 | Normal | — | Normal | −/− |
| II-1 | M | 36 | 1368 | 40 | 20 | 66 | Severe hypokinesia | II | Abnormal | − |
| II-2 | M | 30 | 1826 | 60 | 15 | 60 | Severe hypokinesia | II | Abnormal | − |
| II-3 | F | 28 | 171 | 7 | 33 | 48 | Normal | — | Normal | +/− |
| II-4 | F | 26 | 158 | 6 | 35 | 40 | Normal | — | Normal | +/− |
| II-5 | M | 27 | 3312 | 37 | 18 | 59 | Severe hypokinesia | II | Abnormal | + |
| II-6‡ | M | 23 | 3362 | 60 | 14 | 58 | Severe hypokinesia | IV | Abnormal | − |
| II-7 | F | 18 | 90 | 3 | 43 | 44 | Normal | — | Normal | −/− |

*CK denotes creatine kinase (normal level, <250 U per liter), CK-MB creatine kinase, MB isoform; FS fractional shortening; EDD left ventricular end-diastolic diameter, and NYHA New York Heart Association.
†ECG denotes electrocardiographic. A finding of deep Q waves from lead $V_4$ to lead $V_6$ was considered abnormal.
‡Proposita.

## CAT SCRATCH DISEASE

*To the Editor:* We wish to point out a potentially confusing result in the work of Zangwill and colleagues (July 1 issue).[1] In the Abstract and Results section they state that the positive predictive value of the indirect fluorescent-antibody test for *Rochalimaea henselae* was 91 percent. We suggest that it is not

ELSEVIER

Progress in Pediatric Cardiology 6 (1996) 71–82

Progress in Pediatric Cardiology

# Genetic aspects of dilated cardiomyopathy

Rocio Ortiz-Lopez[a], Karla R. Schultz[b], Jeffrey A. Towbin[c,*]

[a]Pre-doctoral Student, Department of Pediatrics (Cardiology), Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA

[b]Pre-doctoral Student, Department of Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA

[c]Associate Professor of Pediatrics (Cardiology) and Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, One Baylor Plaza, Room 333E, Houston, TX 77030, USA

**Abstract**

*Dilated cardiomyopathy*, which is characterized by ventricular dilatation and reduced systolic function, is the most common form of primary myocardial disease and the leading indication for cardiac transplantation. Although the majority of cases are described as idiopathic, a significant number are identified as being acquired due to an inflammatory response, and 20–30% are familial. Molecular biologic methods have been increasingly used during the past decade to attempt to better characterize the underlying etiology and pathogenetic mechanisms leading to both acquired and genetic forms of dilated cardiomyopathy. The aim of this chapter is to describe recent developments in the understanding of the inherited forms of this disease. This chapter will first describe the clinical aspects of dilated cardiomyopathy before outlining the progress currently being made in the genetic forms of dilated cardiomyopathy.

*Keywords:* Dilated cardiomyopathy; Familial dilated cardiomyopathy; Molecular cardiology; Gene mapping; Inherited cardiomyopathy

## 1. Introduction

The purpose of this review is to describe the current clinical and molecular genetic understanding of dilated cardiomyopathy. Rapid progress is expected in the next 1–2 years and therefore, this review should provide background for future discoveries.

## 2. Clinical aspects of dilated cardiomyopathy

Dilated cardiomyopathy (DCM), a significant cause of morbidity and mortality, is characterized by impaired ventricular systolic function and abnormal diastolic function, usually associated with left ventricular or biventricular dilatation [1–3]. Children commonly present with signs and symptoms of congestive heart failure, including dyspnea, exercise intolerance, decreased appetite, and in some children, vomiting. Ventricular tachycardia, syncope, and sudden cardiac death are relatively frequent occurrences as well. The 5-year survival of patients with dilated cardiomyopathy is poor, commonly believed to be $< 50\%$ [1–3]. Physical examination findings usually include a displaced apical impulse consistent with cardiomegaly, a gallop rhythm, and evidence of mitral regurgitation. Occasionally, the heart sounds are muffled due to pericardial effusion. The lungs of older children and adolescents commonly have rales, although this finding is rare in neonates and infants unless pneumonitis or pneumonia co-exists. Hepatomegaly is usually identified at all ages. Peripheral edema is variable, but when seen, usually is notable in adolescents.

The diagnosis of DCM is usually confirmed on chest radiograph, which demonstrates cardiomegaly and increased pulmonary vascular markings, and by echocardiography [1–3]. Two-dimensional echocardiography clearly demonstrates reduced ventricular systolic function and ventricular dilatation (Fig. 1). In many patients, left atrial dilatation is notable. Pericar-

*Corresponding author. Tel.: +1 713 7987342; fax: +1 713 7988085.


PII S1058-9813(96)00173-7

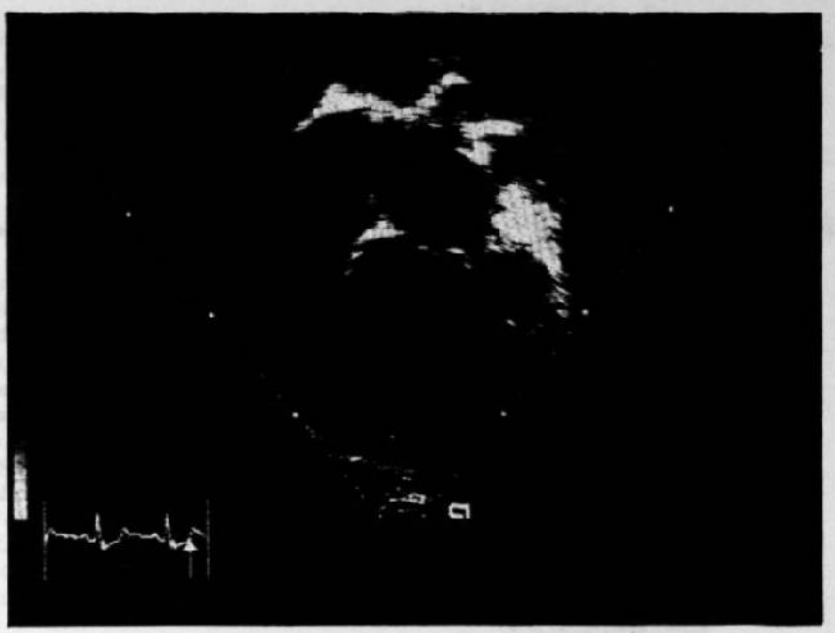

Fig. 1. Two-dimensional echocardiogram of a child with dilated cardiomyopathy. Note the large, dilated left ventricle with bulging of the interventricular septum into the right ventricular chamber. In real-time, the left ventricular function is severely depressed.

dial effusions may also be identified. Doppler and color Doppler interrogation demonstrates atrioventricular regurgitation when it is present and may allow for prediction of right ventricular pressure using tricuspid regurgitation and the Bernoulli equation.

A small minority of patients present with predominant right heart failure, ventricular tachycardia, and sudden cardiac death. This disease, most commonly termed arrhythmogenic right ventricular dysplasia (ARVD) [4], is usually associated with good exercise tolerance. On electrocardiogram, inverted T-waves in the right precordial leads, late potentials, ventricular tachycardia with left bundle branch block, and ventricular fibrillation may be seen. Endomyocardial biopsy or autopsy examination generally reveals cardiomegaly, thinning and fatty infiltration of the right ventricle. The left ventricle is usually spared.

The prevalence of DCM has been estimated to be approximately 40 cases per 100000 population and most cases are believed to be sporadic [2,5,6]. There are numerous causes of DCM (Table 1), but in most cases the underlying etiology goes unrecognized and the cause is considered idiopathic [7].

## 3. Clinical genetic aspects of dilated cardiomyopathy

Familial dilated cardiomyopathy (FDCM) was initially considered to be a rare and distinct entity, causing an estimated 6–9% of cases of DCM based on retrospective studies [3,8,9]. However, Michels and colleagues [10] prospectively analyzed (physical examination and echocardiography) 315 relatives of 59 index patients with so-called idiopathic DCM, demonstrating that approximately 20% of the index patients had familial disease, an estimate much higher than previously reported. Interestingly, only 5% of individuals in these families had suspected hereditary disease based on family history alone. In addition, several clinically healthy individuals (9%) were found to have ventricular dilatation without dysfunction. This relatively high incidence of FDCM has been confirmed by several investigators including Keeling et al. [11]. Indeed, these authors suggested an even higher incidence, at least 25–30%.

### *3.1. Autosomal dominant inheritance*

The most common inherited form of FDCM is characterized by an autosomal dominant pattern of transmission with age-related penetrance [12,13]. Autosomal dominant inheritance is characterized by disease appearance in multiple generations, equal occurrence in both sexes, the presence of male-to-male transmission, and a 50% chance of expression in offspring of an affected parent (Fig. 2). It most commonly presents in the second or third decade of life; presentation in early childhood occurs less commonly and may be particularly severe. In the usual situation, patients with autosomal dominant DCM present with 'pure' DCM. However, a few families have been reported that present with conduction abnormalities initially, followed by DCM (typically one to two decades after the onset of conduction disturbance). The largest of these families, initially described by Graber et al. [14], was a six-generation family (at least 30 affected individuals). Sudden death occurred in 5% of individuals. A second family, described by Greenlee and colleagues [15], had remarkably similar symp-

Table 1
Causes of dilated cardiomyopathy

**A. Familial dilated cardiomyopathy**
- Familial dilated cardiomyopathy, autosomal dominant (AD)
- Familial dilated cardiomyopathy, autosomal recessive (AR)
- Cardioskeletal myopathy, X-linked Barth syndrome (XR)
- X-linked cardiomyopathy (XLCM)
- Endocardial fibroelastosis (EFE) (XR)

**B. Heredefamilial disorders**
- Muscular dystrophies/myopathies
  - Duchenne/Becker muscular dystrophy (XR)
  - Emery-Dreifuss muscular dystrophy (XR)
  - Steinert myotonic dystrophy (AD)
  - Erb limb-girdle (Scapulohumeral) muscular dystrophy (AR)
- Kugelberg-Welander spinal muscular atropy
- Nemaline myopathy (AD, AR)
- Myotublar myopathy (AD, AR, XR)
- Roussy-Levy polyneuropathy (AD)

**C. Mitochondrial**
- Kearns-Sayre syndrome
- MELAS
- NADH-Coenzyme Q reductase deficiency
- MERRF

**D. General system diseases**
- Connective tissue disorders
- Systemic lupus erythematosis
- Juvenile rheumatoid arthritis
- Polyarteritis nodosa
- Kawasaki disease
- Progressive systemic sclerosis
- Polymyositis

**E. Sensitivity/Toxic Reactions**
- Sulphonamides
- Penicillin
- Anthracyclines
- Chloramphenicol
- Alcoholic cardiomyopathy
- Arsenic
- Cocaine
- Lithium
- Cobalt
- Lead

**F. Tachyarrhthmias**
- Supraventricular tachycardia
- Atrial flutter
- Atrial ectopic tachycardia
- Ventricular tachycardia

**G. Fatty acid oxidation**
- Carnitine deficiency (AR)
- Long-chain-acyl-CoA dehydrogenase (LCAD) deficiency (AR)
- Medium-chain-acyl-CoA dehydrogenase (MCAD) deficiency (AR)

**H. Infectious Myocarditis**
- Viral
  - Adenovirus
  - Coxsackie B
  - Coxsackie A
  - Echo
  - Mumps
  - Rubella
  - Parvovirus B19
- Bacterial
  - Diphtheria
  - Meningococcal
  - Pneumococcocal
  - Gonococcal
- Protozoal
  - *American trypanosomiasis (chagas)*
  - Toxoplasmosis
- Rickettsial
  - Rocky mountain spotting fever
- Spirochetal
  - *Lyme disease*
- Fungal
  - Candidiasis
  - Aspergillosis

**I. Metabolic**
- Endocrine
  - Thyrotoxicosis
  - Hypothyroidism
  - Diabetic Cardiomyopathy
  - Hypoglycemia
  - Pheochromocytoma
  - Neuroblastoma
  - Catecholamine cardiomyopathy
- Familial storage disease
  - Glycogen storage disease (AR)
    - GSDIV (Andersen's)
    - GSDV (McArdle's)
- Mucopolysaccharidoses
  - Sanfillipo syndrome
  - Morquio's syndrome
  - Maroteaux-lamy syndrome
- Sphingolipidoses
  - Niemann-pick diseases
  - Farber's disease
  - Caucher's disease (AR)
  - Tay-Sach's disease (AR)
  - Sandhoff's disease (AR)
  - GM1 Gangliosidosis (AR)
  - Refsum's disease (AR)
- Nutritional deficiency/metabolic
  - Kwashiorkor (protein)
  - *Beri-beri (thiamine)*
  - Selenium deficiency (kershan)
  - β-Ketolase deficiency
  - Hypertaurinuria
  - Hypocalcemia
  - Hypoposphatemia
  - Hypokalemia
  - Uremia

**J. Other**
- Histiocytosis X
- Hemolytic-Uremic syndrome
- Reye's syndrome
- Peripatum cardiomyopathy
- Osteogenesis imperfecta (AD, AR)
- Hereditary hemochromatosis (AR)

Legend: AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; XR, X-linked recessive inheritance

toms and natural history. Another form of autosomal dominant DCM, arrhythmogenic right ventricular dysplasia (ARVD), typically demonstrates incomplete penetrance [4].

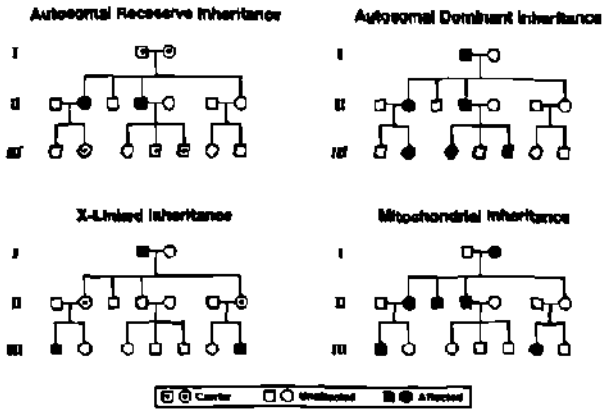


Fig. 2. Inheritance patterns as demonstrated by pedigrees with autosomal recessive, autosomal dominant, X-linked, and mitochondrial transmission of disease.

### 3.2. Autosomal recessive inheritance

Autosomal recessive forms of DCM have also been described [12,16]. In these families, one-fourth of offspring become affected, equal involvement of sexes is seen (Fig. 2), and presence of heterozygous carriers can be identified; most cases are seen in families where inbreeding has occurred (i.e. consanguinity) [13]. This type of inherited DCM is uncommon.

### 3.3. X-linked inheritance

X-linked forms of FDCM have also been recognized. Berko and Swift [17] reported a five-generation kindred with a sex-linked dilated cardiomyopathy (XLCM) in which no male-to-male transmission occurred, early-onset and rapid course in men was seen, and later and milder onset occurred in females, consistent with heterozygosity (Fig. 2). No skeletal muscle disease, normal mitochondria, and normal biochemistries except for an elevated muscle isoform of creatine kinase (CK-MM) was reported. The affected males presented in their teens or early 20s with clinical evidence of mitral regurgitation, heart failure, and echocardiographic evidence of DCM. Episodes of ventricular tachycardia were noted in several patients. The males progressed rapidly (within 1 or 2 years) to death or transplantation. Manifesting female carriers developed mild symptoms of heart failure and evidence of cardiomyopathy in the fourth or fifth decade and progressed slowly. Right ventricular endomyocardial biopsy in affected males revealed minimal interstitial fibrosis, while postmortem examination demonstrated marked ventricular dilatation, widespread patchy fibrosis worst in the posterior wall, and normal mitochondria on electron microscopy. Similar clinical and pathologic findings have been reported in other families.

A second X-linked form of DCM, called Barth syndrome or X-linked cardioskeletal myopathy [18], is characterized by dilated cardiomyopathy with endocardial fibroelastosis (EFE), neutropenia, skeletal myopathy, abnormal mitochondria, growth retardation, and increased levels of urinary 3-methylglutaconic acid and 2-ethyl-hydracrylic acid [13]. Lactic acidemia not provoked by prolonged fasting and decreased plasma and muscle carnitine concentrations are also seen. The affected males are usually severely affected, present in early infancy in severe heart failure, and succumb in infancy or early childhood from cardiac decompensation or septicemia. Ultrastructural abnormalities in mitochondria from heart and skeletal muscle, as well as in neutrophil bone marrow cells are also typically seen. Mitochondrial respiratory chain abnormalities are also typically found. A number of families have been described.

### 3.4. Mitochondrial inheritance

Finally, mitochondrial inheritance has been identified in FDCM [13,16]. Transmission of the disease is from the mother to her offspring (i.e. maternal inheritance) and all offspring are at risk to develop the disease (Fig. 2). Clinical severity is based on heteroplasmy-that is, the percent of abnormal mitochondria mixed in with normal mitochondria. The higher the percentage of abnormal mitochondria, the more severe the disease. Many of these patients have associated problems including metabolic instability, mental retardation, and other end-organ abnormalities (i.e. skeletal disease, liver disease, etc.).

## 4. Molecular aspects of familial dilated cardiomyopathy

### 4.1. Autosomal dominant dilated cardiomyopathy

#### 4.1.1. Autosomal dominant dilated cardiomyopathy with conduction disturbance

The large six-generation family presenting with conduction defects and subsequent development of DCM reported by Graber et al. [14] in 1986, was recently studied by Kass et al. [19]. In this family, the affected patients presented in the second or third decade with transient arrhythmias which became sustained and commonplace by the third or fourth decade. The abnormal rhythms included second- or third-degree atrioventricular block, atrial fibrillation, or marked bradycardia with heart rates less than 50 beats/min. Pacemaker therapy was required in many of these patients. DCM usually developed in the fourth or fifth decade and was generally out of proportion of the severity of the rhythm disturbance. Sudden death occurred in the late stages of the disease in several family members. On autopsy, marked

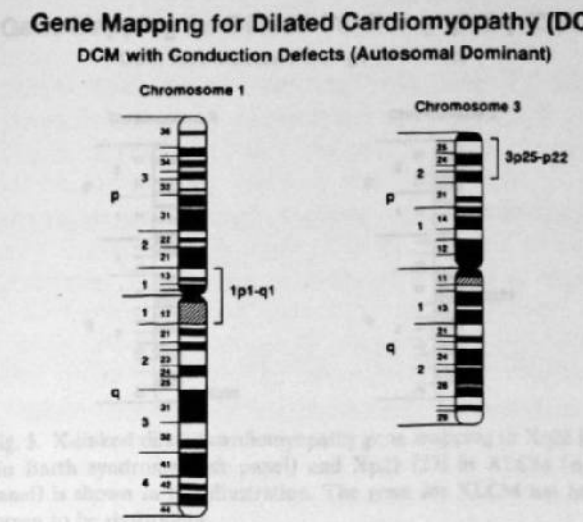


Fig. 3. Gene mapping for autosomal dominant dilated cardiomyopathy with conduction defects. Chromosome 1p1-1q1 linkage was identified by Kass et al. [19] and chromosome 3p25-3p22 linkage was recently defined by Olson and Keating [20] in an unrelated family. The genes have not yet been identified.

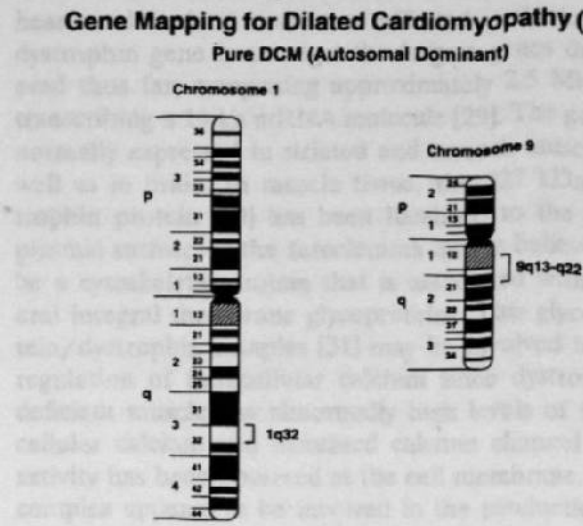


Fig. 4. Gene mapping for autosomal dominantly inherited 'pure' dilated cardiomyopathy. Chromosome 1q32 [22] and chromosome 9q13-9q22 [21] have thus far been identified as disease-causing loci. Neither gene has been discovered at present.

right and left ventricular dilatation, interstitial fibrosis, myocyte degeneration characterized by cytoplasmic vacuolization, and AV nodal cells replaced by fibrous tissue was notable. No coronary artery disease occurred. Kass et al. mapped the gene in this family to 1p1-1q1 (Fig. 3) but the gene and its characteristics have remained elusive [19]. Connexin 40, a gene encoding a protein that forms gap junctions, was mapped to 1cen-1q12 and this was speculated to be a candidate gene. However, this gene has not been found to contain disease-causing mutations. A similar family was also described by Greenlee et al. [15]. Genetic heterogeneity was recently found by Olson and Keating [20] when they mapped the locus for the family described by Greenlee et al. [15] to the 3p25-3p22 region (Fig. 3). The gene has not been identified.

*4.1.2. 'Pure' autosomal dominant dilated cardiomyopathy*

In 1995, the discovery of gene locations for FDCM in families with 'pure' DCM was reported. Krajinovic et al. [21] provided evidence for linkage to 9q13-q22 (Fig. 4) in three families with this disorder (Fig. 4). Durand et al. [22] studied a large family with this disorder and found linkage to 1q32. Although no genes were identified by either research group, they speculated on the possible involvement of structural proteins. Schultz and colleagues [23] reported genetic heterogeneity, demonstrating exclusion of linkage to chromosome 1 and 9. None of the genes have thus far been identified.

*4.1.3. Autosomal dominant arrhythmogenic right ventricular dysplasia*

Familial clusters of ARVD have been reported, demonstrating autosomal dominant inheritance, incomplete penetrance, and a wide spectrum of clinical heterogeneity. Rampazzo et al. [24] mapped the location of the gene responsible for this disease in two families to 14q23-14q24, and to 1q42-q43 in another family [25]. More recently, a third locus was identified by Severini et al. [26] to be on 14q12-q22 in three families. The genes for ARVD have not been identified.

*4.2. X-Linked dilated cardiomyopathy (XLCM)*

Towbin et al. [27] demonstrated linkage of XLCM to the dystrophin locus at Xp21 (Fig. 5) in the family described by Berko and Swift, [17] as well as in a second family. The tightest linkage occurred in the 5′ portion of this gene locus, which is known to cause Duchenne (DMD) and Becker muscular dystrophy (BMD). No deletions were found. Evaluation of the protein defect in XLCM using antiserum against total cardiac protein showed absence (or low abundance) of the N-terminal dystrophin protein (Fig. 6); C-terminal antiserum demonstrated normal staining of cardiac dystrophin, while rod region antisera had abnormally reduced staining. Skeletal muscle total protein was normal using N-terminal, C-terminal, and rod region dystrophin antibody. The 156 kDa dystrophin-associated glycoprotein, a constituent of the dystrophin-associated glycoprotein complex, was decreased in abundance in cardiac tissue from all affected individuals as well [28]. Evaluation of the RNA transcript from heart was also consistent with low abundance message using the most 5′ dystrophin cDNA probe; 3′ cDNA probes produced a band with normal intensity. This has subsequently been con-

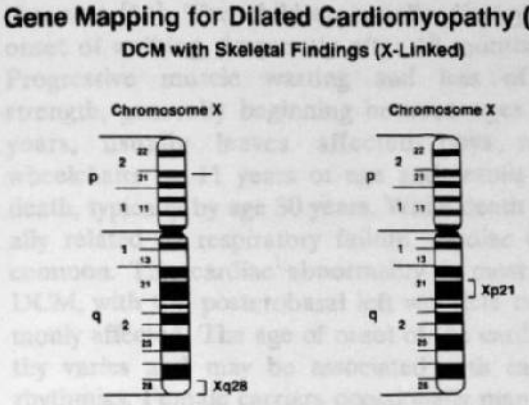


Fig. 5. X-linked dilated cardiomyopathy gene mapping to Xq28 [40] in Barth syndrome (left panel) and Xp21 [27] in XLCM (right panel) is shown in this illustration. The gene for XLCM has been shown to be dystrophin.

firmed. No intragenic deletions were found using multiplex PCR analysis of the 14 common regions of dystrophin in which deletions are seen. This approach eliminated 98% of the usual portions of the dystrophin in which deletions have been reported.

The dystrophin gene was first localized to the short arm of the X chromosome by inheritance patterns, linkage analysis, and cytogenetically detectable defects in the region of the X chromosome in DMD patients. The Xp21 band in the mid-portion of the short arm was pinpointed as the site of the DMD gene locus, cloned fragments of DNA from this region were used as probes in skeletal muscle to find the mRNA transcribed, and sequence data from the RNA transcript provided the information needed to determine the amino acid sequence of the protein dystrophin. Mutations in the dystrophin gene may cause either low level production of a non-functional protein or complete absence of dystrophin in the heart and skeletal muscle of affected patients. The dystrophin gene is amongst the largest genes discovered thus far, comprising approximately 2.5 Mb and transcribing a 14 kb mRNA molecule [29]. The gene is normally expressed in striated and smooth muscle, as well as in brain. In muscle tissue, the 427 kDa dystrophin protein [30] has been localized to the cytoplasmic surface of the sarcolemma and is believed to be a cytoskeletal protein that is associated with several integral membrane glycoproteins. This glycoprotein/dystrophin complex [31] may be involved in the regulation of intracellular calcium since dystrophin-deficient muscle has abnormally high levels of intracellular calcium and increased calcium channel leak activity has been observed at the cell membrane. This complex appears to be involved in the production of the cardiomyopathic phenotype [32,33].

The dystrophin mRNA transcripts in muscle and brain tissue have sequence differences. The mRNA transcript is alternatively spliced to encode multiple isoforms of the dystrophin protein in a developmental- and tissue-specific manner. Several of these isoforms appear to be unique for the heart; this post-transcriptional modification may result in a functional diversity for the dystrophin protein. Recently, human cardiac purkinje fibers have been shown to express dystrophin isoforms [34] and it is speculated that this may be important in the development of cardiac arrhythmias in this disorder.

DMD, also known as pseudohypertrophic muscular dystrophy, is the most common and most devastating of the human muscular dystrophies. This disease affects approximately 1 in 3500 males born, 1/3 of which results from new mutations. Typically, no obvious clinical manifestations are exhibited until 18 months of age when proximal muscle weakness is first

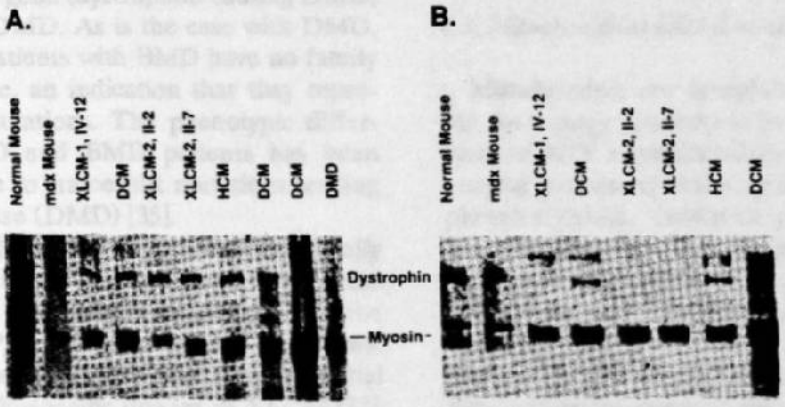


Fig. 6. Western blot analysis of cardiac dystrophin in XLCM. (A) N-terminal dystrophin antibody immunoblotted against cardiac protein from patients with XLCM, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), Duchenne muscular dystrophy (DMD), muscular dystrophy mouse (*mdx* mouse), and normal mouse. The 427 kDa dystrophin protein band is normal in all lanes except the *mdx* mouse and DMD patient, in whom no band is seen. (B) N-terminal dystrophin antibody immunoblotting against cardiac protein obtained from the same patients shown in panel A demonstrates low abundance or non-staining of dystrophin in patients with XLCM, as well as the *mdx* mouse. This is consistent with a cardiac dystrophin mutation in the 5′ portion of the gene which codes for the N-terminal portion of the protein.

observed [13]. The children usually display delayed onset of walking, frequently after 18 months of age. Progressive muscle wasting and loss of muscle strength, generally beginning between ages 3 and 6 years, usually leaves affected boys requiring wheelchairs by 11 years of age and results in early death, typically by age 30 years. While death is generally related to respiratory failure, cardiac failure is common. The cardiac abnormality is most typically DCM, with the posterobasal left ventricle most commonly affected. The age of onset of the cardiomyopathy varies and may be associated with cardiac arrhythmias. Female carriers occasionally manifest clinical disease, but typically this is milder and has later onset than affected males. Histologically, most skeletal muscle groups demonstrate widespread, but focal, degeneration and regeneration of individual muscle fibers. High concentrations of muscle-specific enzymes may be present in the serum of female carriers and affected males before the clinical onset of disease. This has been used clinically as an adjunct to diagnosis; the muscle isoform of creatine kinase (CK-MM) is most commonly measured and is elevated in a substantial percentage of patients. However, false negative results are common.

BMD is a less severe form of X-linked muscle wasting disorder which is similar to DMD. In general, BMD is more benign and less common than DMD, occurring approximately 10% as frequently as DMD. Clinically, BMD has later onset and slower progression of muscle weakness. Cardiac disease, in the form of DCM, may occur, but is also less frequent and typically less severe than that seen in DMD. However, there are occasional patients who demonstrate moderate to severe cardiomyopathy requiring cardiac transplantation. Genetically, the BMD-causing gene is also located at Xp21, and represents a different mutation of the same gene (dystrophin) causing DMD; i.e. it is allelic with DMD. As is the case with DMD, more than 30% of patients with BMD have no family history of the disease, an indication that they represent spontaneous mutations. The phenotypic difference between DMD and BMD patients has been speculated to be due to frameshift mutations leading to more severe disease (DMD) [35].

Muntoni et al. [36] described a mutation in a family with an X-linked dilated cardiomyopathy and mild skeletal disease. In this family, a deletion in the muscle promoter ($P_m$) and exon 1 (E1) was found. These authors subsequently outlined a potential mechanism for cardio-specific disease in XLCM [37]. They suggested that the brain promoter ($P_B$), which usually is not active in skeletal muscle, becomes quite active and hence protects the skeletal muscle from becoming clinically abnormal. Although the authors suggested this mutation to be the common disease-causing mutation in patients with XLCM, it remained unclear why previously reported patients with this same mutation and evidence of mild muscular dystrophy, had no evidence of heart disease. Only mild skeletal muscle symptoms were reported in these patients. Yoshida et al. [38] has reported two patients with exon 1 deletions alone in whom dilated cardiomyopathy was seen, however. Towbin and Ortiz-Lopez [39] showed that the three families evaluated in their laboratory using the same primers as Muntoni et al. had normal muscle promoter and exon 1 sequences. Other mutations in the remaining families are expected to further define the mechanisms of cardio-specific dysfunction.

#### *4.2.1. Barth syndrome*

Molecular mapping of the locus for Barth syndrome has demonstrated linkage to Xq28 [40], the distal portion of the long arm of the X chromosome (Fig. 5). The causative gene has not been found thus far, although two disorders, Emery-Dreifuss muscular dystrophy (EDMD) and X-linked myotubular myopathy, both have clinical overlap and map to this region. It is possible that Barth syndrome and EDMD are related, in a manner similar to that noted for XLCM and DMD/BMD.

EDMD is a rare disease characterized by contractures and severe cardiac manifestations including complete heart block requiring pacemaker implantation early in adolescence, and DCM. EDMD carriers are also at risk to show cardiac abnormalities and sudden death, but typically do not have significant skeletal disease. Since the gene responsible for EDMD is now known to be emerin [41], a serine-rich 254 amino acid protein with uncertain function, analysis of families with Barth syndrome for emerin mutations should soon be reported.

### *4.3. Mitochondrial dilated cardiomyopathy*

Mitochondria are cytoplasmic organelles responsible for energy production in all cells. Energy in the form of ATP is generated by a series of complicated enzyme processed that together are called oxidative phosphorylation. Oxidative phosphorylation involves five enzyme complexes assembled from polypeptides coded by mitochondrial and nuclear DNA.

The human mitochondrial genome is a small (16 569 bp) circular DNA molecule that is maternally inherited. [42] Mitochondrial DNA (mtDNA) encodes 13 of the 69 proteins required for oxidative metabolism, 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) required for their translation. Since mtDNA has much less redundancy than the nuclear genome (in which essentially identical information is received from both parents), and tRNAs and rRNAs are pre-

sent in multiple copies, the mitochondrial genome is an excellent target for mutations giving rise to human disease. In fact, the rate of mtDNA mutation is estimated to be 10–20 times higher than that of nuclear DNA [43]. Mitochondria enjoy a symbiotic relationship with the cell, but mtDNA replicates independently of the nuclear genome. These subcellular organelles are dependent on nucleocytoplasmic mechanisms for most structural components, but do contribute vital peptides that are central to cellular respiration. Mitochondria contain a permeable outer membrane and a highly restrictive inner membrane that guards the chemical micro-environment of the matrix compartment. Adaptive mechanisms exist for the passage of large and small molecules across the inner membrane. Translocases shuttle monocarboxylic acids, amino acids, acyl-carnitine conjugates, small ions, and other metabolites in and out of the mitochondrial matrix. Energy is required for importation of proteins into the mitochondria since the nuclear gene-synthesized mitochondrial proteins are precursor molecules that require presequence cleavage. The 13 mtDNA genes are located in the respiratory chain and include seven Complex I subunits, (ND1, 2, 3, 4L, 4, 5, and 6); 1 Complex III subunit (cytochrome b); 3 Complex IV subunits (COI, II, III); and 2 Complex V subunits (ATPase 6 and 8). Coordination must exist between nuclear and mitochondrial genomes to permit assembly of the complex holoenzymes. Each cell contains numerous mitochondria and each mitochondria contains multiple copies of mtDNA. This genetic material derives exclusively from the female gamete and any mutation must be passed from female parent to all progeny, male and female. Mitochondria are randomly distributed to daughter cells during cell division so that there is often a heterogeneous population of normal and mutant genomes within a given cell. Replicative segregation occurs when there are mtDNA molecules with different nucleotide sequences within a cell. The normal state for humans is homoplasmy (i.e. all mtDNA molecules have the same nucleotide sequence), but in disease states, at least two different mtDNA sequences are seen within the cells. During cell division, the different types of mtDNA are randomly distributed to daughter cells. This partitioning of mtDNA molecules is called replicative segregation. The replicative segregation of mutant mtDNA copies within the cell determines whether this biological disadvantage is expressed. In most mitochondrial disorders, patients carry a mix of mutant and normal mitochondria-a condition known as heteroplasmy, with the proportions varying from tissue-to-tissue and individual-to-individual within a pedigree, in a manner correlating with severity of phenotype. Clinical manifestations develop when the number of mutant mtDNA molecules causes a decrease in the production of ATP to a level less than the threshold required by the tissue for normal function. Replicative segregation may produce dramatically different quantities of mtDNA mutation within different tissues so a single organ may be more affected than others.

Mitochondrial diseases often produce disturbances of brain and muscle function, presumably because these two organs are so metabolically active, and therefore the metabolic demand is high during growth and development. Cardiac disease is most commonly seen with respiratory chain defects [44]. Ragged red fibers are present in muscle biopsy specimens almost invariably when the molecular defect involves mtDNA. [45] These defects represent the genetics of ATP production. The diverse clinical syndromes associated with various respiratory chain complexes are thought to result from involvement of tissue-specific isoforms in some cases, involvement of tissue-nonspecific (generalized) subunits in other cases, and the residual enzyme activity in affected tissues. The cardiac diseases seen associated with mitochondrial defects include both hypertrophic cardiomyopathy (HCM) and DCM. No theory has thus far been advanced to explain the cause of these phenotypically different cardiac abnormalities. It is possible, however, that the dilated form occurs after an initial hypertrophic response (i.e. is a 'burnt-out' dilated form of HCM).

Mitochondrial DNA mutations fall into three categories: deletions, duplications, and point mutations. All three types of mutations have significant cardiac manifestations [42–46].

## 5. Respiratory chain abnormalities

### 5.1. Complex I deficiency

Approximately 60 cases have been described, approximately 20 of these representing the myopathic syndrome, and 40 cases representing the encephalomyopathic syndrome. The latter includes: (1) a fatal infantile disorder with involvement of brain, muscle, and heart; (2) a milder version of above, with clinical manifestations later in childhood or early adulthood; and (3) MELAS syndrome with Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes thought secondary to NADH CoQ reductase abnormalities. Treatment of these disorders is limited. Riboflavin, succinate supplements (since the metabolite enters the respiratory chain at Complex II), ubiquonone and idebanone have been recommended for therapy in patients with MELAS. A mitochondrial mutation was initially described in a patient with MELAS and fatal infantile cardiomyopathy: this mutation was shown to be due to an A to G transition in mitochondrial DNA of the isoleucine

tRNA gene. Analysis of enzyme activities and subunits in the heart revealed combined defects of Complex I and Complex IV of the respiratory chain. Similar mutations have subsequently been described.

### 5.2. Complex III defects

This results in a myopathic or multi-systemic disorder. Cardiomyopathy has been found both alone or in conjunction with skeletal myopathy. Encephalomyopathy also presents with retinopathy, ataxia, spasticity, dementia, weakness, sensorineural hearing loss, and exercise intolerance.

### 5.3. Complex IV defects

This abnormality is similar clinically to Complex I defects. The mitochondrial genome encodes for three subunits of cytochrome C oxidase, which represents the terminal portion of the respiratory chain and catalyzes conversion of molecular oxygen to water. A benign reversible infantile myopathy which normalizes by early childhood may occur, or a fatal infantile myopathy manifested by profound weakness, hypotonia, respiratory insufficiency, and death. This may occur alone, or in association with severe renal tubular dysfunction or cardiomyopathy with red ragged fibers.

### 5.4. Kearns-Sayre syndrome (KSS)

This mitochondrial myopathy is characterized by ptosis, chronic progressive external ophthalmoplegia, abnormal retinal pigmentation, and cardiac conduction defects, as well as DCM. Approximately 20% of KSS patients have cardiac involvement and, of these, the majority usually have conduction defects causing progressive heart block. These patients generally have large heterogeneous deletions in the mtDNA. Other mutations have also been described.

### 5.5. MERRF syndrome

This syndrome is characterized by Myoclonic Epilepsy with Ragged Red Muscle Fibers and is caused by a single nucleotide substitution in $tRNA^{LYS}$ which apparently interferes with mitochondrial translation. Shoffner et al. [45] showed an A to G transition mutation as the cause of the disease associated with defects in Complexes I and IV. This abnormality causes decline in ATP-generating capacity, with onset of disease that includes cardiomyopathy. Other reports have outlined various disease-causing mutations.

### 5.6. Hypoxemia, mtDNA damage, and cardiac disease

Since cardiac tissue relies on mitochondrial oxidative phosphorylation for energy production, it has been believed that deficiency of portions of this system or its end-product can cause cardiac abnormalities. Hypoxemia has been shown to increase oxygen radical production, which results in elevated mtDNA damage and altered oxidative phosphorylation gene expression. In addition, these enzymes have been shown to decline with age while mtDNA deletions increase with age, especially deletion at nucleotide 4977 bp. Corral-Debrinski et al. [47] hypothesized that ischemic hearts would be likely to have increased chances of mtDNA deletion due to this effect of hypoxemia and, using PCR amplification across the deletion breakpoint of the common $mtDNA^{4977}$ deletion showed mtDNA damage was increased in chronically ischemic hearts, as well as in some hearts with other forms of chronic cardiac disease (i.e. DCM, HCM). It is possible that oxidative phosphorylation gene induction may be part of a general response to chronic cardiac failure. Other reports have supported this view. In addition, there is some support that endomyocardial biopsies should be performed in patients with HCM or DCM to increase the likelihood of detecting mitochondrial abnormalities.

## 6. Inborn errors of metabolism causing cardiomyopathy

Mitochondrial β-oxidation of fatty acids is the main source of energy for the heart [16]. Inborn errors of myocardial fatty acid oxidation have been increasingly recognized as important causes of inherited cardiomyopathy, skeletal myopathy, metabolic disturbances, and sudden cardiac death in childhood during the past decade [16]. Most cellular fatty acid oxidation occurs via the mitochondrial β-oxidation pathway and inborn defects of the enzymes or transport proteins of this pathway are common causes of inherited metabolic diseases (estimated incidence 1/10000–1/15000 live births). Defects involving oxidation of long chain fatty acids are more likely to cause cardiomyopathy than those involving metabolism of medium-chain or short-chain fatty acids. Cardiomyopathy may result from defects in carnitine transport into cells, carnitine-acylcarnitine shuttling, or fatty acid oxidation enzymes.

All known inborn errors of fatty acid oxidation are autosomal recessive disorders. The molecular basis of several of these are known; these are described below.

### 6.1. Medium chain acyl-CoA dehydrogenase (MCAD) deficiency

This disorder, first described in 1982, appears to be the most common inborn error of fatty acid oxidation, estimated to occur in one per 6000–10000 live Caucasian births. It is characterized by recurrent episodes

of illness, provoked by fasting > 12 h, with the first episode generally occurring between 6–24 months of life. The most common symptoms include vomiting and severe lethargy that can progress to coma, as well as the less striking symptoms of muscle weakness and exercise intolerance. Hypoglycemia is often present between episodes, when patients appear normal. Hepatomegaly and DCM are also seen. Liver biopsy can show marked fatty infiltrate ranging from predominantly microvesicular to a macrovesicular pattern. This autosomal recessive disorder has recently been localized to chromosome 1p31. Human and rat MCAD cDNAs have been cloned and sequenced, as has the entire human MCAD gene. The coding region is 1263 bp and encodes a precursor protein containing 421 amino acids. An A to G nucleotide replacement at position 985 of MCAD cDNA appears to be the most prevalent mutation responsible for MCAD deficiency; a 13 bp tandem repeat insertion at position 999 has also been reported, as have other rare mutations [48]. The common A to G 985 mutation appears to be due to a founder effect.

### 6.2. *Long chain acyl CoA dehydrogenase (LCAD) deficiency / very long chain acyl-CoA dehydrogenase (VLCAD) deficiency*

This disorder was first described in 1985 and clinically manifests with recurrent episodes of coma, vomiting, and hypoglycemia triggered by fasting. Some patients have much more severe illness with notable involvement of cardiac and skeletal muscle. Both DCM and HCM have been seen [16]. Like MCAD, LCAD patients have secondary carnitine/deficiency and their fasting urine organic acid profile is abnormal, with low ketones and increased levels of dicarboxylic acids. The LCAD gene was identified but unlike many of the patients with MCAD deficiency, no mutations or abnormal immunoreactive proteins were identified. In 1992, Izai et al. [49] and Uchida et al. [50] showed that in addition to the well known $\beta$-oxidation enzymes in the mitochondrial matrix, there are two additional membrane-bound enzymes of $\beta$-oxidation. One of these has been called 'very-long-chain acyl-CoA dehydrogenase (VLCAD), while the other is known as a 'trifunctional protein.' This new understanding of the mitochondrial $\beta$-oxidation pathway has led to a new understanding of the disorder thought to be due to LCAD deficiency, but now thought to be VLCAD deficiency.

### 6.3. *Carnitine deficiency*

L-carnitine is a small, water-soluble molecule containing seven carbon atoms, which is excreted in the urine in the free state or esterified to various acyl-conjugates. Carnitine is important in the shuttling of long-chain fatty acids and activated acetate across the inner mitochondrial membrane. A specific translocase facilitates this exchange of long-chain acylcarnitine and acetylcarnitine. Carnitine also serves as the shuttle for the end-products of peroxisomal fatty acid oxidation and for $\alpha$-keto-acids derived from branch chain amino acids. These metabolites are transferred into the mitochondrial matrix for terminal oxidation.

Primary carnitine deficiency syndrome is characterized by a profound decrease in carnitine in affected tissues and plasma. The mechanism underlying the primary disorder is defective transport of carnitine from the plasma into cells and defective transport across kidney tubules resulting in urinary loss of carnitine [51]. Secondary disorders are characterized by less striking decreases in total or free serum carnitine and an increase in esterified: free ratio. The secondary forms are more heterogeneous and include underlying genetically determined metabolic errors, diverse acquired disease, and iatrogenic factors such as drug administration.

The primary form (transport defect) of carnitine deficiency demonstrates multiple tissues affected, including muscle, liver, heart, and plasma. Patients present in infancy or early childhood with episodes of hypoglycemia, hyperammonemia, hepatomegaly, muscle weakness and congestive heart failure. Therapy includes oral carnitine, usually reversing the cardiomyopathy [52]. Once the gene locus and biochemical defect are further elucidated, gene therapy may become a useful endeavor for these patients.

## 7. Conclusion

In summary, the underlying causes of dilated cardiomyopathy are currently an area of investigation using molecular technology. In the years leading up to the 21st century, many of these causes are likely to be identified. This information is expected to improve the ability to diagnose patients pre-symptomatically, as well as enable development of more specific therapies.

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# Evidence for a Dystrophin Missense Mutation as a Cause of X-Linked Dilated Cardiomyopathy

Rocio Ortiz-Lopez, MS; Hua Li, PhD; Jason Su, DO; Veronica Goytia, BS; Jeffrey A. Towbin, MD

***Background*** X-linked dilated cardiomyopathy (XLCM) has previously been shown to be due to mutations in the dystrophin gene, which is located at Xp21. Mutations in the 5′ portion of the gene, including the muscle promoter, exon 1, and the exon 1–intron 1 splice site, have been reported previously. The purpose of this study was to analyze the originally described family with XLCM (and others) for dystrophin mutations.

***Methods and Results*** Polymerase chain reaction (PCR) was used to amplify genomic DNA, and reverse-transcriptase PCR amplified cDNA from RNA obtained from heart and lymphoblastoid cell lines. Primers to the muscle promoter, brain promoter, and Purkinje cell promoter were designed, in addition to the exon 1 to exon 14 regions of dystrophin. Single-strand conformation polymorphism analysis was used for mutation detection, and DNA sequencing defined the mutation. Protein modeling was used for amino acid and secondary structure analysis. A missense mutation in exon 9 at nucleotide 1043 was identified that causes an alanine to be substituted for threonine, a highly conserved amino acid, at position 279 (T279A). This mutation results in a change in polarity in the evolutionarily conserved first hinge region (H1) of the protein and substitution of a β-sheet for α-helix in this portion of the protein, destabilizing the protein.

***Conclusions*** A novel missense mutation in exon 9 of dystrophin causing an abnormality at H1 leads to the cardiospecific phenotype of XLCM. (*Circulation.* 1997;95:2434-2440.)

***Key Words*** • cardiomyopathy • genetics • dystrophin • mutation

Dilated cardiomyopathy, the most common form of primary myocardial disease, is a major cause of morbidity and mortality and a leading indication for cardiac transplantation.[1-3] Approximately 20% to 30% of all cases of DCM are inherited,[4,5] with autosomal dominant transmission being most common; autosomal recessive, X-linked, and mitochondrial inheritance patterns have also been described.[6,7]

XLCM is a severe and rapidly progressive myocardial disease that affects young men in their teens or early twenties.[8] Typically, affected males present with severe congestive heart failure that results in death or cardiac transplantation within 1 to 2 years of diagnosis. Clinically, XLCM appears to be identical to other causes of DCM except for its X-linked transmission[6,8,9] and elevated serum CK-MM without evidence of clinical skeletal myopathy. Female carriers with XLCM may manifest symptoms later in life, usually in the fifth decade, but the disease is mild and progresses slowly.[8]

Molecular genetic linkage to the 5′ end of the dystrophin gene at Xp21 was previously reported by Towbin et al.[9] In addition, N-terminal dystrophin antibody against cardiac tissue protein extracts showed decreased abundance (or absence) of dystrophin, whereas C-terminal antibody immunoblots were normal.[9] Dystrophin antibody against the rod domain also demonstrated low abundance of protein.[10] Immunoblots using N-terminal, C-terminal, and rod domain dystrophin antibodies against skeletal muscle protein demonstrated normal dystrophin, however.[9,10]

See p 2344

The dystrophin gene, which causes DMD and the milder allelic form, BMD, is the largest gene identified in humans thus far, covering >2.5 Mb and having 79 exons.[11,12] The corresponding 14-kb dystrophin mRNA is expressed predominantly in skeletal, cardiac, and smooth muscle, although lower levels also appear in brain. Transcription of dystrophin in different tissues is regulated from either the $P_B$,[13-15] which is active predominantly in neuronal cells; the $P_M$,[16] which is active in differentiated myogenic cells and glial cells; and the $P_P$,[17,18] which is active in cerebellum. The resultant protein, dystrophin,[12,19] is a 427-kD protein localized to the cytoplasmic face of the sarcolemma, colocalizing with β-spectrin and vinculin at the sarcolemma.[20] The C-terminal region of dystrophin is bound to the protoplasmic half of the plasmalemma. Thus, dystrophin forms an intricate part of the muscle cytoskeleton and may function to link the normal contractile apparatus to the sarcolemma. In skeletal and cardiac muscle, the C-terminal domain is bound to a large oligomeric glycoprotein complex of six novel proteins localized to the sarcolemma[21-24]; the N-terminus attaches to actin.

In DMD, the levels of all members of the oligomeric glycoprotein complex are reduced.[25] In BMD, however, there is only a general mild reduction in these proteins.[26] It is believed that dystrophin localizes or stabilizes this oligomeric protein complex.[27] Without dystrophin, the complex is unable to organize properly, and consequently the linkage between the extracellular matrix and sarcolemma is disrupted.[28]

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From the Departments of Pediatrics (R.O.-L., H.L., J.S., V.G., J.A.T.) and Molecular and Human Genetics (J.A.T.), Baylor College of Medicine, Houston, Tex.

Guest editor for this article was Christine Seidman, *MD, Harvard* Medical School, Boston, Mass.

Reprint requests to Jeffrey A. Towbin, MD, Pediatric Cardiology, Baylor College of Medicine, One Baylor Plaza, Room 333E, Houston, TX 77030 E-mail jtowbin@bcm.tmc.edu

**Selected Abbreviations and Acronyms**

BMD = Becker muscular dystrophy
C-terminal = carboxy-terminal
CK-MM = creatine kinase muscle isoforms
DCM = dilated cardiomyopathy
DMD = Duchenne muscular dystrophy
E1 = exon 1
H1 = first hinge region
I1 = intron 1
N-terminal = amino-terminal
$P_B$ = *brain promoter*
PCR = polymerase chain reaction
$P_M$ = muscle promoter
$P_P$ = Purkinje-cell promoter
RT = reverse-transcriptase
SSCP = single-strand conformation polymorphism
XLCM = X-linked dilated cardiomyopathy

*Muntoni et al*[29] *reported a deletion within the* $P_M$ *and* E1 of dystrophin in a family with DCM, X-linked inheritance, and mild abnormalities of skeletal muscle histology and dystrophin immunohistochemical staining. They showed this mutation to be the cause of disease in this family and speculated that this deletion was the responsible mutation in all patients with XLCM. More recently, these authors[30,31] also confirmed the dystrophin protein findings of Towbin et al.[9,10] Holder et al[32] described a mutation in the muscle promoter *that potentially affects* cardiac-specific regulatory sequences and therefore could selectively cause DCM without significant skeletal myopathy. This mutation is quite uncommon in BMD and DMD patients screened for dystrophin deletions,[33] however. Since previous reports of $P_M$-E1 mutations in other unrelated patients were usually associated only with mild skeletal muscle disease without cardiac disease,[13,34] doubt arose that this mutation was the only mutation leading to XLCM. *In fact, only the patient reported by* Yoshida et al[35] with an E1 deletion and normal $P_M$ had DCM. In addition, Towbin and Ortiz-Lopez[36] reported on three families with documented XLCM in whom $P_M$-E1 was normal. More recently, Milasin et al[37] reported a point mutation in the 5′ splice site of dystrophin E1-I1 boundary causing XLCM clinically and abolishing expression of dystrophin in cardiac tissue. Therefore, XLCM appears to be due to various different mutations in the dystrophin *gene. In this report, we describe* a novel missense mutation in the 5′ end of dystrophin in the originally described family with XLCM,[8] and we also provide further data that suggest that there is allelic heterogeneity for XLCM, because two other families with XLCM studied here have none of the reported dystrophin mutations.

## Methods

### Families and Linkage Analysis

Clinical evaluation and linkage analysis of the three families (XLCM-1, XLCM-2, and XLCM-3) were previously described.[8,9]

### Control Individuals

One hundred unrelated and unaffected individuals (50 male, 50 female; 50 black and 50 white), as determined by history, physical examination, and echocardiography, were analyzed. After informed consent was obtained, blood for lymphoblastoid cell line immortalization[38] was obtained.

### Procedures

***RT-PCR.*** RNA was prepared from lymphoblastoid cell lines[38] or cardiac tissue as previously described.[39] Briefly, 2 μg of total RNA was reverse transcribed in a 20-μL reaction with 5 μg of random hexamer primers (Gibco-BRL) and 100 U murine Moloney leukemia virus RT at 37°C in the buffer provided by the supplier (Gibco-BRL). Of the RT reaction, 2 μL was used for each PCR reaction in 25 μL of 1.5 mmol/L $MgCl_2$, 67 mmol/L Tris-HCl (pH 8.8), 16.6 mmol/L ammonium sulfate, 0.01% Tween-20, 200 μmol/L of each dNTP, 25 pmol of each primer, and 1 U *Taq* DNA polymerase (Promega). PCR conditions included 94°C for 3 minutes, 30 cycles of 92°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute, and a final extension of 72°C for 5 minutes. The PCR products were visualized on 2% agarose gel. The primers used for the amplification of the first 14 exons of dystrophin cDNA were derived from the reported dystrophin sequence[1]; primers used for RT-PCR were exon 1F, 5′-TGGGAAGAAGTAGAGGACTGTTATG-3′; exon 5R, 5′-TTGACCTGCCAGTGGAGGAT-3′; exon 4F, 5′-GCA CTGCGGGTTTTGCAGAA-3′; exon 7R, 5′-GAATGC ATTCCAGTCGTTGTGT-3′; exon 6F, 5′-TGAATGCTCTC ATCCAGTCATAG-3′; exon 10R, 5′-CTCTCCATCA ATGAACTGCC-3′; exon 10F, 5′-CATTGCAAGCACAAG GAGAG-3′; and exon 13R, 5′-CAGTTGCGTGATCTCCACTA GATTC-3′.

***PCR amplification of genomic DNA.*** The primers used to amplify sequences of the $P_B$, $P_M$, $P_P$, and exon-intron junctions were designed with the previously reported sequences[13-17] and conditions. The primers used for promoter amplification included the following: $P_M$, 5′-GAAGATCTAGACAGTGGAT ACATAACAAATGCATG-3′; $E1_R$, 5′-TTCTCCGAAG GTAATTGCCTCCCAGATCTGAGTCC-3′; $P_B$F, 5′-GAA GATCTATATTTTACAACGCAGAAATGTGG-3′; $P_B$R, 5′-CTTCCATGCCAGCTGTTTTTCCTGTCACTC-3′; $P_P$F, 5′-CAGCCTCCGCAGAATTTGAAATG-3′; and exon 2R, 5′-CTTAGAAAATTGTGCATTTACCCA-3′.

***Multiplex PCR.*** Multiplex PCR was performed with the five primer pairs described by Beggs et al[40] and nine primer pairs described by Chamberlain et al[41]; an additional set of primers designed to amplify exon 9 (F, 5′-GAATTCCTCTCGCAGAT CACG-3′; R, 5′-GTAAATGTTGACAGACCTGTG-3′) was also added to each. This multiplex PCR was performed under the following conditions: 94°C *for 6 minutes*, 94°C *for 30 seconds*, 54°C for 30 seconds, 65°C for 4 minutes (25 cycles), and 65°C for 7 minutes. Amplification products were visualized on a 3% agarose gel with ethidium bromide under ultraviolet light.

***SSCP.*** Mutation analysis was performed by the method of Orita et al.[42] *PCR primers were designed to amplify a region* including the 3′ portion of intron 8 and the 3′ end of exon 9. This region flanked the normal, polymorphic, or mutated sequence of exon 9 (Fig 1A, primers a through d). Radioactive PCR was performed with 100 ng genomic DNA in a 10-μL reaction containing 2.5 *mmol/L* $MgCl_2$, 10 *pmol of each primer*, 0.05 μCi [γ-$^{32}$P]dCTP, 0.5 U *Taq* polymerase, and 30 rounds of amplification (92°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1 minute). After PCR amplification, the samples were denatured by addition of 5 μL formamide dye (95% formamide, 20 *mmol/L EDTA*, 0.05% bromophenol blue, and 0.05% xylene cyanol) at 85°C for 5 minutes. After 10 minutes of cooling on ice, 3 μL of sample was electrophoresed on a nondenaturing 10% polyacrylamide:bisacrylamide (50:1) gel at 8 W over 24 hours in a 4°C cold room. Bands were visualized by exposure of the *dried gels to* Kodak X-AR film.

***Sequencing.*** Normal and aberrant SSCP conformers were cut directly from dried gels and eluted in 100 μL distilled water

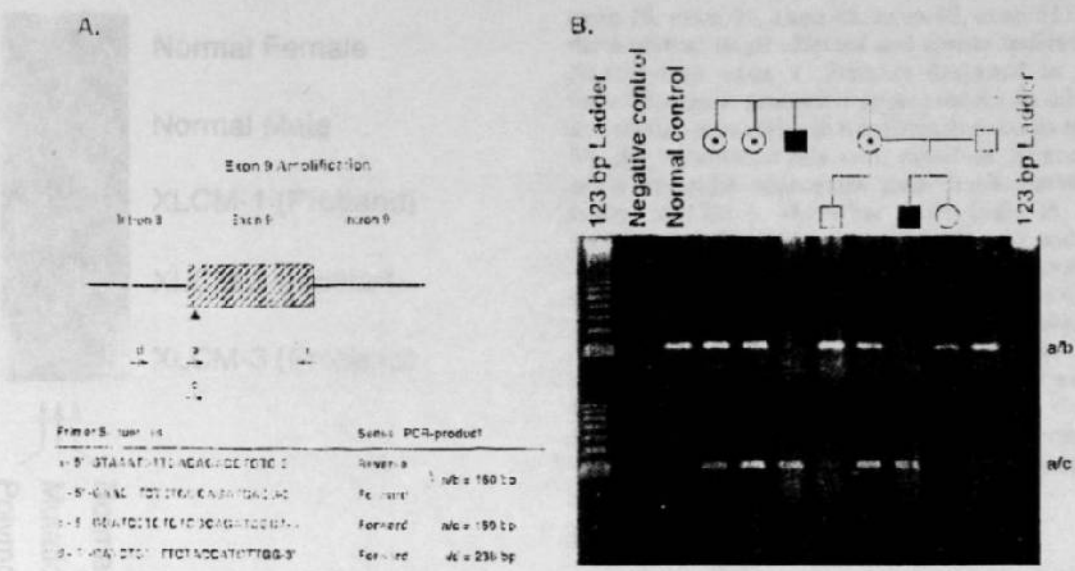


FIG 1. A, PCR analysis of dystrophin exon 9. Top, Position of primers (a through d) relative to intron 8 and exon 9 and position of mutation (denoted by ▲) found in affected and carrier members of XLCM-1. Bottom, Sequences of primers a through d and expected PCR products. Primers a/d were used to PCR-amplify normal exon 9 sequences; primers a/c were used to amplify mutated exon 9 sequences. Primers a/d were used for SSCP analysis. B, PCR analysis of eight members of XLCM-1 family. XLCM-1 family members analyzed (top) and resultant PCR products (bottom) are shown. With primers (a/b) designed to amplify normal dystrophin exon 9 sequence (top band) and primers (a/c) designed to amplify mutated sequence (lower band), PCR products were electrophoresed on a 2% agarose gel. Note that affected males amplify only mutated sequences; carrier females are heterozygous. All members of this family were evaluated with both primer pairs (data not shown), with expected results (based on clinical status) occurring in all cases. Genomic DNA was used as template for this experiment.

(65°C for 30 minutes), and the eluted DNA (10 μL) was used as the template in a second PCR using the original primer pair. The PCR products were sequenced directly after fractionation in 2% low-melting agarose gel (FMC Corp) and purified by Qiaquick columns (Qiagen). Purified PCR products (200 ng) were used for each sequencing reaction with cycle sequencing.[43] Alternatively, purified PCR products were cloned into pBluescript II SK(+) (Stratagene) by the T-vector method as described previously.[44] Manual sequencing was performed according to conditions suggested by the supplier (United States Biochemical), and automated sequences were performed on an ABI Automated Sequencer (ABI model 373).

***Protein modeling.*** Dystrophin sequences were analyzed for species conservation by use of GenBank. Analysis of amino acid sequences was performed by the MOSAIC computer program.[45,46] Hydrophobicity and plot structure predictions were performed according to the Garnier-Osguthorpe-Robson prediction method.[47]

## Results

### Phenotypic Analysis of XLCM Families

To identify the disease-causing mutations in XLCM, three previously described multigenerational families with familial DCM, X-linked inheritance, and no clinical evidence of skeletal disease were studied.[8-10] These families were not related, and all were of North American descent. Two of the families were black (XLCM-1, XLCM-3) and one family was white (XLCM-2). All three families were previously found to have linkage to the 5′ portion of the dystrophin gene at Xp21, with maximal logarithm-of-the-odds scores at intron 7 within DXS206.[9,10]

### PCR Amplification of Promoter-Specific Transcripts

With oligonucleotide primers designed to amplify sequences of the $P_B$,[13] $P_M$,[16,29] $P_P$,[17] and E1-I1[11,29,37] from DNA and RNA isolated from lymphoblastoid cell lines and cardiac tissue, normal amplification of all promoters and E1-I1 was seen in all individuals. DNA sequencing revealed normal sequences of all amplified products.

### 5′ Dystrophin Mutation Analysis

Primers designed to individually amplify exons 2 through 14 of dystrophin[11] were used to analyze the region with tightest linkage in XLCM-1, XLCM-2, and XLCM-3. In XLCM-2 and XLCM-3, all exons were able to be amplified, and the resultant amplimers were the predicted sizes. SSCP analysis was also normal (ie, no abnormal conformers) in XLCM-2 and XLCM-3, as were the sequences of all amplimers. In XLCM-1, however, exon 9 could not be amplified with normal

**Exon 9 Sequence Conservation**

| Species | Sequence | | |
|---|---|---|---|
| Human | ATC (Ile) | ACG (Thr) | GTC (Val) |
| Chicken | ATC (Ile) | ACG (Thr) | GTC (Val) |
| Dog | ATC (Ile) | ACG (Thr) | GTC (Val) |
| Mouse | ATC (Ile) | ACG (Thr) | GTC (Val) |
| XLCM-1 | ATC (Ile) | GCA (Ala) | GTC (Val) |

Dystrophin exon 9 sequence conservation between human and other species is shown. The sequence change in exon 9 of patients in XLCM-1 results in an amino acid substitution of alanine (Ala) for threonine (Thr). This substitution changes a polar, hydrophilic amino acid (threonine) to a nonpolar, hydrophobic amino acid (alanine). The flanking sequences are conserved for isoleucine (Ile) and valine (Val).

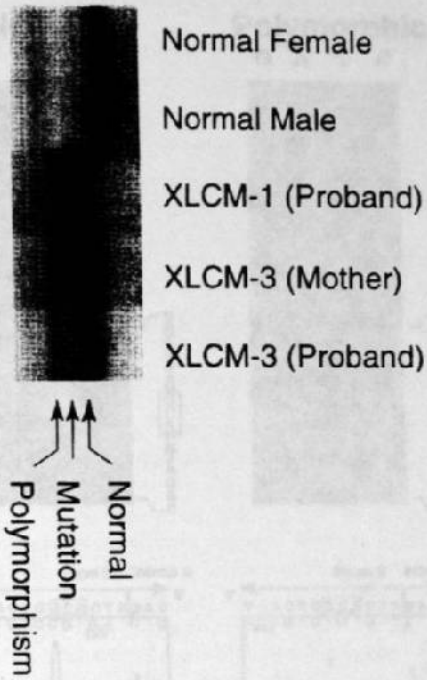


FIG 2. SSCP analysis of dystrophin exon 9 in genomic DNA from normal male and female individuals and individuals from XLCM-1 (proband) and XLCM-3 (mother and proband) with primers a through d. In XLCM-1 (lane 3), note aberrant conformer (ie, "mutation") that differs from normal and polymorphic bands seen in normal individuals (lanes 1 and 2) and XLCM-3 patients (lanes 4 and 5). All XLCM-1 affected and carrier individuals were found to have "mutation"; none of 100 normal control subjects or XLCM-2 or XLCM-3 patients had this abnormal conformer.

sequence primers (ie, primers a/b). SSCP analysis of affected and carrier individuals from XLCM-1 demonstrated an abnormal conformer at exon 9 in all affected individuals (hemizygous); a heterozygous result (an abnormal conformer and a normal conformer) was found in all carrier females (Fig 2). Evaluation of exon 9 sequence (GenBank reference No. X14298) identified a mutation at nucleotide 1043, with substitution of A→G only in affected and carrier individuals (Fig 3). The A→G mutation changes the conserved amino acid threonine at amino acid position 279 to alanine (T279A). This amino acid, which is within the H1 of dystrophin,[48,49] is conserved across species[50-54] (Table). Oligonucleotide primers containing the mutated sequence within the primers (primers a/c, Fig 1A) were able to amplify a PCR product in affected and carrier XLCM-1 patients but not in unaffected individuals (Fig 1B), whereas PCR primers designed to the normal sequence (primers a/b, Fig 1A) were able to amplify the normal exon 9 sequence in unaffected individuals but were unable to amplify this portion of exon 9 in affected patients in XLCM-1 (Fig 1B). This selective amplification of the mutated sequence in affected individuals by use of mutation-specific primers occurred because of the use of stringent PCR conditions and sequence-specific oligonucleotides. Multiplex PCR[40,41] using 6 primer pairs ($P_M$, exon 9, exon 13, exon 43, exon 50, exon 52) and 10 primer pairs (exon 4, exon 8, exon 9, exon 12, exon 17, exon 19, exon 44, exon 45, exon 48, exon 51) identified the mutation in all affected and carrier individuals from XLCM-1 at exon 9. Primers designed to flank the mutated region amplified appropriately in all individuals; sequence analysis in the affected patients in pedigree XLCM-1 identified this same mutation. Hence, the missense mutation segregates with disease status within family XLCM-1. No other individuals in XLCM-1, XLCM-2, or XLCM-3 had the A→G mutation at nucleotide 1043; none of 100 unrelated control individuals analyzed had the mutation as seen by PCR, SSCP, and mutation analysis. However, a G→A polymorphism in the third nucleotide of this codon was commonly detected at nucleotide 1045, consistent with codon redundancy (degenerate code). In fact, the G→A polymorphism at 1045 was seen in 8% of individuals in the three families as well as in 8% of the 100 unrelated normal control patients analyzed.

### Dystrophin Protein Analysis

Analysis of amino acid sequences was performed by the MOSAIC computer program[45,46]; prediction of α-helix and β-sheet was performed by a segment-oriented method designed to locate secondary structure elements. In addition, hydrophobicity and plot structure predictions were performed by use of the Garnier-Osguthorpe-Robson prediction.[47] The T279A amino acid substitution induces a change in the polarity in this critical region of the dystrophin protein because threonine is a neutral-polar amino acid and alanine is neutral-nonpolar. This, in turn, changes the secondary and tertiary structure of dystrophin by substituting a β-sheet for α-helix at the H1 between the N-terminal domain and rod domain of dystrophin[49,55] (Fig 4).

## Discussion

XLCM is the result of mutations within the dystrophin gene at the Xp21 locus. The missense mutation in dystrophin reported here in the originally described family with XLCM initially described by Berko and Swift[8] is found within exon 9 (A→G at position 1043) and is within a critical structural region (H1) of this cytoskeletal protein.[19,47-49,55,56] The resultant amino acid substitution (threonine→alanine) at amino acid 279 is within a highly conserved portion of the protein,[50-54] causing a change in polarity in this part of the protein and leading to a change in the dystrophin secondary and tertiary structure. The end result is conversion of a segment of α-helix to a β-sheet in the H1 between the N-terminal domain and the rod domain of dystrophin[49]; this abnormality could potentially destabilize the protein. This mutation was not found in the other two XLCM families (XLCM-2, XLCM-3) studied.

Dystrophin is predicted to fold into four domains,[19,49,56] including the N-terminal domain, a long repeat domain (rod domain), a cysteine-rich domain, and a C-terminal domain. The N-terminal domain has significant similarity to the N-terminal domain of α-actinin and β-spectrin,[57,58] possibly reflecting a common actin-binding function. The large midportion of the protein is formed by repeat elements predicted to adopt a triple-helical conformation,[59] and this region is quite similar to that seen in α-actinin and spectrin as well. In dystrophin, this repeat domain is composed of 24 repeat units (≈109

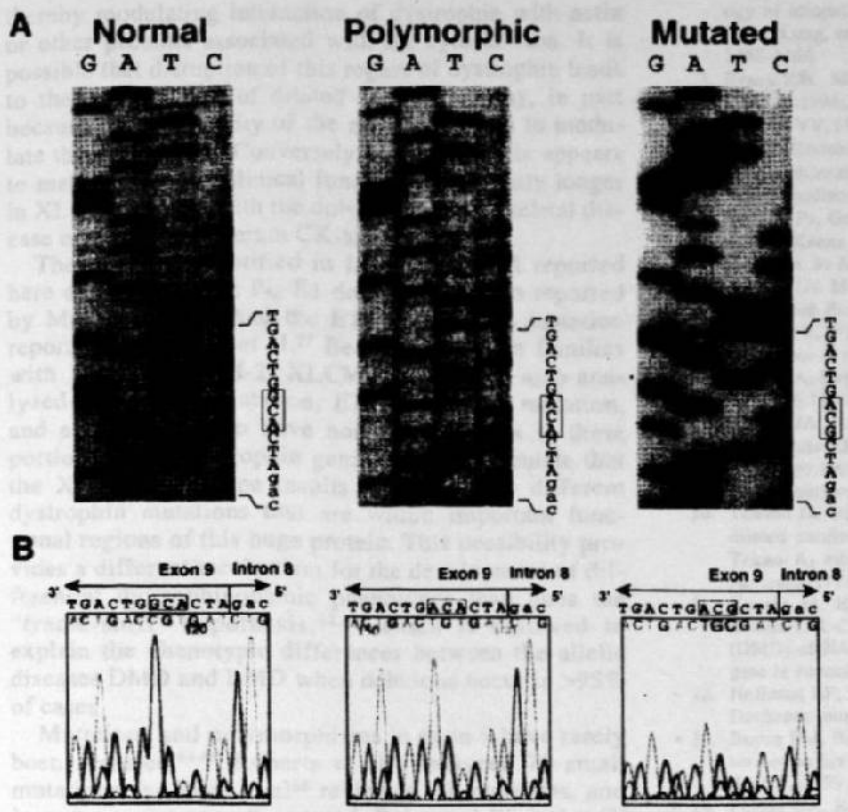


FIG 3. Manual (A) and automated (B) sequencing of normal, polymorphic, and mutated conformers obtained by SSCP. Mutated sequence A→G at nucleotide 1043, which occurred in proband and all affected and carrier individuals from XLCM-1, changes conserved amino acid threonine (at position 279) to alanine. Polymorphism seen in normal population and XLCM-2 and XLCM-3 patients is also shown.

amino acid repeats) and shows evolutionary conservation.[60] In addition, Koenig and Kunkel[49] showed that four hinge segments are interspersed along the dystrophin molecule, and these hinges appear to confer flexibility to the membrane-associated network of dystrophin, resulting in membrane resilience. The existence of these flexible hinges at precise positions within the elongated dystrophin molecule is thought to be important for the mechanical properties of the membrane cytoskeleton. It is possible that flexibility is needed during contraction-relaxation of the muscle fibers. These hinge regions are found at positions within the protein encoded by exon 9 and part of exon 8 (hinge 1, amino acid 253 to 327), exon 17 (hinge 2, amino acid 667 to 717), and exon 50 to 51 (hinge 3, amino acid 2424 to 2470); the hinge 4 sequence (amino acid 3041 to 3112) has not been definitively identified.[49] The mutation identified in family XLCM-1 is in exon 9, within the H1.

It appears that dystrophin performs a number of distinct cytoskeletal functions. Three related but conceptually distinct roles of dystrophin function have been suggested: (1) membrane stability (dystrophin may stabilize the membrane during repeated cycles of muscle contraction), (2) force transduction (dystrophin may link the contractile force produced in the intracellular domain to the extracellular environment), and (3) organization of membrane specializations. The biochemistry and function of the cardiac membrane and T tubules appear to be different from those of skeletal muscle. In the heart, this exon 9 mutation affecting the H1 portion of the protein is likely to result in loss of membrane integrity and eventual loss of function, probably due to the continual stress placed on the beating pump. In addition, this portion of the protein has been shown to have significant sequence similarity to troponin I[61] in the region that functionally enables binding with calcium binding proteins. It has also been speculated that dystrophin binds to calmodulin in this region,

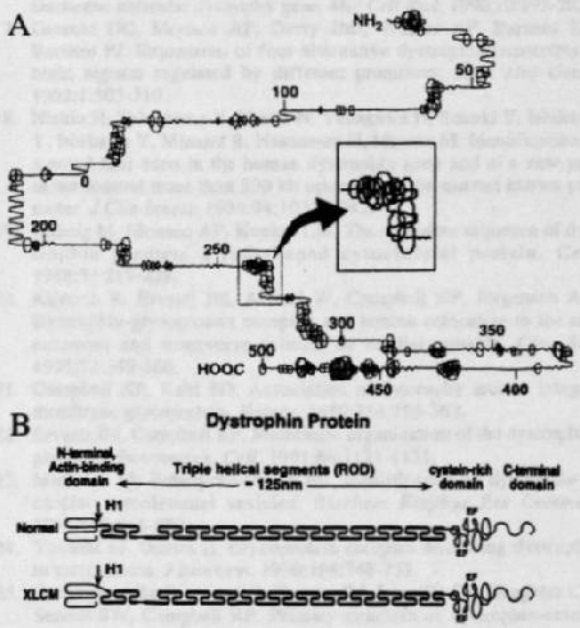


FIG 4. Plot structure prediction of mutant dystrophin protein compared with normal dystrophin. A, Normal dystrophin protein and mutant dystrophin (inset). Note structural change between amino acids 250 and 300 (arrow), thus causing a change in secondary structure. B, Idealized dystrophin protein structure. Change in structure due to T279A mutation at H1 is shown. This change is predicted to functionally destabilize the protein.

thereby modulating interaction of dystrophin with actin or other proteins associated with the cytoskeleton. It is possible that disruption of this region of dystrophin leads to the development of dilated cardiomyopathy, in part because of the inability of the mutated protein to modulate this interaction. Conversely, skeletal muscle appears to maintain normal clinical function significantly longer in XLCM patients, with the only evidence of skeletal disease being elevated serum CK-MM.

The mutation identified in family XLCM-1 reported here differs from the $P_M$-E1 deletion mutation reported by Muntoni et al[29] and the E1-I1 splice site mutation reported by Milasin et al.[37] Because two other families with XLCM (XLCM-2, XLCM-3) that were also analyzed for the $P_M$ mutation, E1-I1 boundary mutation, and exon 9 mutation have normal sequences in these portions of the dystrophin gene, one can speculate that the XLCM phenotype results from multiple different dystrophin mutations that are within important functional regions of this huge protein. This possibility provides a different mechanism for the development of differential dystrophinopathic phenotypes than does the "frame-shift" hypothesis,[62,63] which is believed to explain the phenotypic differences between the allelic diseases DMD and BMD when deletions occur in >95% of cases.

Mutations and polymorphisms in exon 9 have rarely been reported.[64-66] Roberts et al[65] analyzed 70 small mutations and Prior et al[66] reviewed 29 mutations, and in no case was exon 9 mutated. Reiss and Rinnosland[64] described a constitutive exon 9 cassette-splicing phenomenon found in peripheral blood lymphocyte studies. These authors found that in approximately half of the transcripts of the dystrophin gene isolated from lymphocytes, exon 9 was omitted. This exon skipping was found to occur to a variable extent in all tissues not specifically expressing dystrophin but was rarely seen in muscle, heart, or brain. When seen, omission of exon 9 did not disrupt the reading frame of the mRNA. They also suggested that when exon 9 mutations occur, BMD results (as long as the correct reading frame is maintained), consistent with the observations of Koenig et al.[63]

Once the mutations are identified in other families with XLCM (ie, XLCM-2 and XLCM-3), more insight will be gained as to the important cardiac functional regions of dystrophin. Animal models (such as transgenic mice) with these "cardiospecific mutations" could open doors to our knowledge of the function of the dystrophin protein as well as its relationship to other interrelated proteins.

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# RESUMEN AUTOBIOGRÁFICO


## Rocío Ortíz López

**Candidato para el grado de DOCTOR en Ciencias con especialidad en Biología Molecular e Ingeniería Genética**


**Tesis:** **ANÁLISIS MOLECULAR Y CARACTERIZACIÓN DE LA CARDIOMIOPATÍA DILATADA LIGADA AL CROMOSOMA X.**

**Campo de Estudio: Ciencias Básicas**

**BIOGRAFÍA:**

**Datos Personales**: Nacida en Jalapa, Veracrúz el 21 de Marzo de 1963, hija de Andrés Ortíz (†) y Dora López de Ortíz.

**Educación**:

Egresada de la Facultad de Ciencias Químicas, de la Universidad Veracruzana, en Jalapa, Veracrúz.
Grado obtenido: *Licenciatura en* Químico Farmacéutico Biólogo en 1988.

Facultad de Medicina de la UANL. Monterrey, N.L
Grado obtenido: Maestro en Ciencias con especialidad en Biología Molecular en ingeniería genética en 1992.