

Dystrophin analysis in idiopathic dilated cardiomyopathy

Virginia V Michels, Gregory M Pastores, Patricia P Moll, David J Driscoll, Fletcher A Miller, John C Burnett, Richard J Rodeheffer, Jamil A Tajik, Alan H Beggs, Louis M Kunkel, Stephen N Thibodeau

Abstract
Idiopathic dilated cardiomyopathy (DCM) is characterised by ventricular dilatation and impaired systolic function resulting in congestive heart failure and frequently death. A dilated cardiomyopathy is common in patients with symptomatic Duchenne/Becker muscular dystrophy, a disease caused by dystrophin gene defects. However, cardiomyopathy is rarely the predominant clinical feature of this form of muscular dystrophy. To determine whether dystrophin gene defects might account for a significant number of patients with apparently isolated idiopathic DCM, we performed dystrophin gene analysis in 27 DCM patients, who were ascertained as part of a prospective study on idiopathic DCM. No dystrophin gene defects were found in our patients, whose average age was 50 years. These data suggest that dystrophin defects are not a common cause of idiopathic DCM in this age group in the absence of skeletal muscle cramps or weakness.

(*J Med Genet* 1993;30:955-7)

Idiopathic dilated cardiomyopathy (DCM) is a serious heart disorder characterised by ventricular dilatation and impaired systolic function. The diagnosis may be made when other causes of heart disease, such as coronary atherosclerosis and haemochromatosis, have been excluded.¹ Clinical manifestations of DCM may include congestive heart failure, cardiac arrhythmia, and sudden unexpected death.² Our recent studies indicated that 20% of DCM cases are familial when first degree relatives are investigated using echocardiography.³ Other than family history, no differences in the clinical, electrocardiographic, or echocardiographic findings have been detected that distinguish patients with familial DCM from those with sporadic DCM.³ Specifically excluded from this group of patients with idiopathic DCM were those with known mitochondrial or other systemic diseases, or with skeletal myopathies such as Duchenne/Becker muscular dystrophy.

Dystrophin is expressed at similar levels in both cardiac and skeletal muscle. Patients with Duchenne/Becker muscular dystrophy may have cardiac dilatation similar to patients with idiopathic DCM.^{4,5} Recently, a female heterozygote for Duchenne muscular dystrophy was reported who first presented with a severe dilated cardiomyopathy.⁶ Furthermore, dilated cardiomyopathy is occasionally a major com-

plication in patients with Becker muscular dystrophy, even when symptomatic skeletal myopathy is not present.⁷⁻⁹ In addition, two families with X linked DCM have been described who had tight linkage to dystrophin and low abundance of the dystrophin RNA transcript in cardiac tissue, although no gene defect was identified.¹⁰ Beggs *et al*,¹¹ in a study exploring the molecular basis of variability among patients with Becker muscular dystrophy, described one patient with a typical dystrophin deletion who died of cardiomyopathy at the age of 15 years and who had no neuromuscular symptoms except for large calves. Deletion analysis in patients with Becker muscular dystrophy has shown a paucity of in frame deletions in the region of exons 14-44,¹² and the few deletions that have been characterised are in patients with mild or no skeletal muscular weakness.¹¹ It is possible that mutations in this region could result in a different phenotype, with predominately dilated cardiomyopathy rather than skeletal muscle disease.

Therefore, we used several PCR based assays and Southern blot analysis to investigate the possibility that intragenic mutations resulting in altered cardiac dystrophin might account for DCM in some patients. Analysis for dystrophin gene defects was performed in 27 patients with idiopathic DCM.

Materials and methods

PATIENT SELECTION

The patients were identified through a prospective Mayo Clinic study of a sequential series of DCM patients.³ After exclusion of other causes of heart disease and known systemic disease,³ the diagnosis of DCM was based on the demonstration by echocardiography or heart catheterisation or both of left ventricular dimension above the normal limits for the patient's age and body surface area, and a left ventricular ejection fraction of <50%. Cardiac catheterisations had been performed on all patients >40 years of age and on selected younger patients to exclude coronary atherosclerosis and other causes of heart disease. Informed consent was obtained from each participant, and the study was approved by the Mayo Clinic Institutional Review Board.

A total of 27 males with idiopathic DCM participated in the study. Of these, five had familial disease, without male to male transmission in four families. In the fifth family, there was no evidence of male to male transmission when the family was entered into the

Department of
 Medical Genetics,
 Mayo
 Clinic/Foundation,
 Rochester, Minnesota,
 55905, USA.
 V V Michels
 G M Pastores*

Department of
 Epidemiology, Ann
 Arbor, MI 48109, USA.
 P P Moll

Department of
 Pediatrics, Mayo
 Clinic, Rochester,
 Minnesota, USA.
 D J Driscoll

Department of
 Internal Medicine,
 Mayo Clinic,
 Rochester, Minnesota,
 USA.
 F A Miller
 J C Burnett
 R J Rodeheffer
 J A Tajik

Division of Genetics,
 Children's Hospital,
 Boston, MA 02115,
 USA.
 A H Beggs
 L M Kunkel

Department of
 Laboratory Medicine
 and Pathology, Mayo
 Clinic, Rochester,
 Minnesota, USA.
 S N Thibodeau

Correspondence to
 Dr Thibodeau, Molecular
 Genetics Laboratory, 970
 Hilton Building, Mayo
 Clinic, 200 First Street, SW,
 Rochester, MN 55905,
 USA.

* Present address: The
 Mount Sinai School of
 Medicine, New York, NY
 10029, USA.

Received 21 May 1993.
 Revised version accepted
 22 June 1993.

study, but on follow up the index patient's son was found to have developed the disease. None of the patients had clinical evidence of skeletal muscle disease or any systemic illness that could cause heart disease. A detailed three to four generation pedigree was constructed for each family studied. All patients had a physical examination, electrocardiogram, and 2D and M mode echocardiogram. The mean age of the patients was 50.2 years (range 5 to 72 years, median 54 years). All had left ventricular dilatation with a mean ejection fraction of 27% (range 13% to 47%) by echocardiogram.

SPECIMEN COLLECTION AND PROCESSING

High molecular weight DNA was isolated from peripheral blood leucocytes using an Applied Biosystems 340A Nucleic Acid Extractor (ABI, Inc) according to the manufacturer's instructions.

DYSTROPHIN GENE ANALYSIS

Analysis of the dystrophin gene was performed using several different multiplex PCR reactions and Southern blot analysis. For the PCR reaction, 250 ng genomic DNA was amplified in 50 µl reactions as previously described.¹³ The assays performed involved examination of exons 16, 32, 34, 41, and 42 in one reaction,¹⁴ and 4, 8, 12, 17, 19, 44, 45, 48, and 51 in another.¹⁵

For the Southern blot analysis, DNA was digested with the restriction endonuclease *HindIII*, separated by agarose gel electrophoresis (0.8%), and transferred to nylon membrane (magnagraph, MSI, Inc) according to the method of Southern.¹⁶ Hybridisation was performed with the cDNA probes 1-2a, 2b-3, 4-5a, 5b-7, 8, 9-10, and 11-14 which recognises the entire coding region plus some of the 3' untranslated region of the dystrophin gene.

Results

None of the 27 male patients was found to have a deletion or evidence to suggest regions of duplication within the coding region or 3' untranslated region of the dystrophin gene either by PCR or by Southern blot analyses.

Discussion

Idiopathic dilated cardiomyopathy has a prevalence of 36.5/100 000. It is the most common reason for heart transplantation, resulting in an economic burden of \$177 million/year.¹ The biochemical and molecular basis of idiopathic DCM remains unknown. Based upon our recent investigations, 20% of patients with DCM have familial disease.³ Even among familial cases, the disease is genetically heterogeneous, as shown by different patterns of inheritance in unrelated families.¹⁷ Most pedigrees described show segregation patterns compatible with autosomal dominant inheritance, but X linked recessive and autosomal recessive inheritance have also been reported.^{3 18-20} However, most patients

reported to have autosomal recessive disease have not had systematic investigation of asymptomatic parents to exclude the possibility of autosomal dominant disease with variable expression. In four of the five patients with familial DCM in the present study, male to male transmission had not occurred through at least two affected generations compatible with autosomal dominant, X linked, or multifactorial inheritance.

Several lines of evidence suggest that idiopathic DCM could be the result of X chromosome dystrophin gene defects in some cases. A dilated cardiomyopathy is a component of Duchenne/Becker muscular dystrophy, and occasional patients have this as the predominant symptom.^{6 11} One of these patients was a 15 year old male with fatal dilated cardiomyopathy whose only evidence of skeletal muscle involvement was calf hypertrophy. This patient had a deletion of exons 45-53, similar to other patients who presented with skeletal muscle involvement.¹¹ In addition, two families with X linked DCM show linkage to the dystrophin gene and have low abundance cardiac dystrophin by antidystrophin antibody, while skeletal muscle dystrophin appeared normal. In these families, cardiac tissue showed low abundance of dystrophin RNA transcript but no gene deletions were detected. In this study, analysis of the dystrophin gene did not show any detectable intragenic duplications or deletions for our 27 DCM patients or for 24 DCM patients analysed in a similar study.¹⁴ However, these results do not exclude the possibility that some patients with idiopathic DCM may have disease as a result of dystrophin gene mutations. Other types of mutations (point mutations, etc) separate from deletion/duplication events may be important in the disorder, or mutations may exist in other critical regions of the gene such as the promoter.

The patients selected for this study had a mean age of 52 years, similar to the mean age of 49 years of patients with idiopathic DCM in the general population.³ Idiopathic DCM is more common in males (M:F ratio 2.4:1). Among the patients included in the present study, 21% had familial disease, similar to that in the general population of idiopathic DCM patients of 20%. Additional investigations of a large number of patients with sporadic, idiopathic DCM and those with a family history suggestive of X linked inheritance need to be performed. However, our data suggest that deletion/duplication type mutations within the coding region of the dystrophin gene that cause most cases of Duchenne/Becker muscular dystrophy are not a common cause of idiopathic DCM in adult males, in whom idiopathic DCM is most prevalent.

1 Manolio TA, Baughman KL, Rodeheffer R, *et al.* The prevalence and etiology of idiopathic dilated cardiomyopathy: summary of an NHLBI workshop. *Am J Cardiol* 1992;69:1458-66.

2 Rodeheffer RJ, Gersh BJ, Kennel AJ. Myocarditis, dilated cardiomyopathy and specific myocardial disease. In: Guilianeri ER, Fuster V, Gersh BJ, McGoon MD, McGoon DC, eds. *Cardiology*. 2nd ed. St Louis: Mosby, 1991:1792-801.

3 Michels VV, Moll PP, Miller FA, *et al.* The frequency of familial dilated cardiomyopathy in a series of patients

- with idiopathic cardiomyopathy. *N Engl J Med* 1992;326:77-82.
- 4 Perloff JK, De Leon AC, O'Doherty D. The cardiomyopathy of progressive muscular dystrophy. *Circulation* 1966;33:625-48.
 - 5 Emery AEH. *Duchenne muscular dystrophy*. 2nd ed. New York: Oxford University Press, 1988.
 - 6 Kamakura K, Kawai M, Arahata K, Koizumi H, Watanabe K, Sugita H. A manifesting carrier of Duchenne muscular dystrophy with severe myocardial symptoms. *J Neurol* 1990;237:483-5.
 - 7 Ueda K, Okada R, Harumi K, Tsuyuki H, Ueda H. Myocardial involvement in benign Duchenne type of muscular dystrophy. *Jpn Heart J* 1970;11:26-35.
 - 8 Katiyar BC, Misra S, Somani PN, Chaterji AM. Congestive cardiomyopathy in a family of Becker's X linked muscular dystrophy. *Postgrad Med J* 1977;53:12-15.
 - 9 Kuhn E, Fiehn W, Schroder M, Assmus H, Wagner A. Early myocardial disease and cramping myalgia in Becker-type muscular dystrophy: a kindred. *Neurology* 1979;29:1144-9.
 - 10 Towbin JA, Zhu XM, Gelb B, et al. X-linked dilated cardiomyopathy (XLCM): molecular characterization. *Am J Hum Genet* 1991;49:421(suppl A2385).
 - 11 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.
 - 12 Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 1989;45:498-506.
 - 13 Beggs AH, Koenig M, Boyce FM, Kunkel LM. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. *Hum Genet* 1990;86:45-8.
 - 14 Kunkel LM, Snyder JR, Beggs AH, Boyce FM, Feener CA. Searching for dystrophin gene deletions in patients with atypical presentations. In: Lindsten J, Pettersson U, eds. *Etiology of human disease at the DNA level*. New York: Raven Press, 1991:51-60.
 - 15 Chamberlain JS, Gibbs RA, Ranier JE, Caskey CT. Multiplex PCR for the diagnosis of Duchenne muscular dystrophy. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press, 1990:272-81.
 - 16 Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;98:503-17.
 - 17 Emanuel R, Withers R, O'Brien K. Dominant and recessive modes of inheritance of idiopathic dilated cardiomyopathy. *Lancet* 1971;ii:1065-7.
 - 18 Berko BA, Swift EP. X-linked dilated cardiomyopathy. *N Engl J Med* 1987;316:1186-91.
 - 19 Goldblatt J, Melmed J, Rose AG. Autosomal recessive inheritance of idiopathic dilated cardiomyopathy in a Madeira Portuguese kindred. *Clin Genet* 1987;31:249-54.
 - 20 Michels VV, Driscoll DJ, Miller FA. Familial aggregation of idiopathic dilated cardiomyopathy. *Am J Cardiol* 1985;55:1232-3.