

# Cellular and Biomolecular Mechanisms in Dilated Cardiomyopathy

Beatriz Piva e Mattos

Porto Alegre, RS - Brazil

Dilated cardiomyopathy (DCM) is a primary myocardial disease, characterized by uni- or biventricular dilation associated with a generally progressive contractile dysfunction<sup>1</sup>. It is predominantly diagnosed in the advanced stage, in which it manifests itself by cardiomegaly and heart failure, causing significant morbidity and mortality<sup>2</sup>. Less frequently, it is identified in the initial stage, in which the congestive signals are absent but ventricular dilation and dysfunction in a mild or moderate degree are evident<sup>3,4</sup>.

At first, DCM was considered a disease of obscure etiology. Several decades after being identified as an entity, its complex pathogenesis has gradually become better understood. Systematic studies generated in immunology, genetics, and cellular and molecular biology have contributed to the understanding of the disease mechanism. However, the heterogeneous character of DCM, coupled with the multiplicity of pathologic processes involved, has made this task difficult.

DCM is currently understood as a multifactorial disease, in which viral infections, immunologic mechanisms and genetic factors, acting individually or together, result in a definitive myocardial lesion (fig. 1).

## Role of viral infection

Clinical and experimental evidence suggest the participation of a viral infection in the cellular injury process. Based on this concept, viral myocarditis is considered a precursor of DCM, leading to definitive heart disease in susceptible individuals. The concept of an evolutionary sequence is based on indirect clinical evidence and conclusions from studies developed in experimental models.

Coxsackievirus B, from the *Enteroviridae* family, is considered the main etiological agent of human myocarditis<sup>5</sup> except in the endemic areas of Chagas' disease. These viruses are related to lymphocytic myocarditis, which is a process characterized by diffuse cellular infil-

tration associated with myocytolysis<sup>6</sup>, but in which the microorganism is not usually identified through conventional techniques. If viral participation in myocarditis is difficult to prove, viral action in DCM may still be considered controversial.

Longitudinal studies of a small series of patients demonstrate an evolution to DCM in 40 to 52% of the cases with a histopathologic diagnosis of lymphocytic myocarditis<sup>7,8</sup>. However, the causal relationship between enterovirus infections and further development of cardiomyopathy is difficult to confirm because of the virus' endemic characteristics and the frequent subclinical character of the inflammatory manifestations in the acute phase.

Serology of enterovirus infections is equally complex. Neutralizing antibodies to coxsackievirus B are detected at higher levels in DCM patients than in controls<sup>9</sup>. The fact that most of the population has had contact with the virus, and the possibility of antibody levels remaining high after the acute phase of infection jeopardizes the evaluation of these results. Coxsackie B-specific immunoglobulin M is identified in patients with recent diagnosis and end-stage forms<sup>10,11</sup>. Although this immunoglobulin is found more frequently in the DCM group than in nonselected controls<sup>10</sup>, no difference is seen when serologic data are compared with that obtained from relatives sharing the same domicile<sup>12</sup>. Sequential evaluation of serum specimens revealed antibodies persisting in some cases, as well as late seroconversion after reactions become negative in other cases<sup>11,12</sup>.

Enteroviruses are not commonly isolated from myocardial cells of adult patients with myocarditis and DCM through conventional methods. The advent of new techniques of molecular biology based upon nucleic acid hybridization, made enteroviral RNA detection possible in up to 43% and 47% of the samples containing myocardial tissue of patients with DCM and myocarditis, respectively<sup>13,14</sup>. Later determinations using the polymerase chain reaction (PCR) technique, more sensitive and specific, observed the presence of enteroviral genomes in 0% to 67% of the biopsies evaluated<sup>15-19</sup>. Although significantly high numbers were reported<sup>18</sup>, a similar proportion of enterovirus RNA was also seen in the DCM group in relation to controls<sup>17</sup>. Negative results were also obtained by some researchers<sup>15,16</sup>. However, meta-analysis of data from several studies that used PCR or in situ hybridization suggests a

Hospital de Clínicas de Porto Alegre - UFRGS  
Mailing address: Beatriz Piva e Mattos - Serviço de Cardiologia - Hospital de Clínicas de Porto Alegre - Rua Ramiro Barcelos, 2350 - 90035-003 - Porto Alegre, RS - Brazil

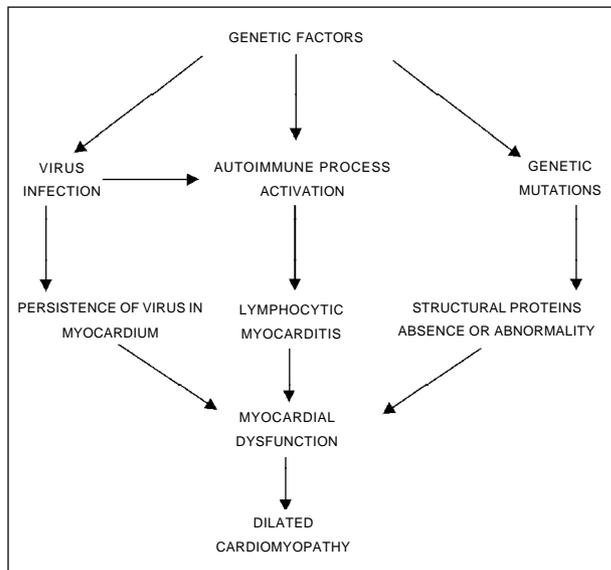


Fig. 1- Hypotheses on dilated cardiomyopathy pathogenesis.

possible association between enterovirus infections and DCM<sup>20</sup>. Interpretation of these data should take into account methodological differences related to the technique applied and selection of patients and control groups. Small outbreaks of epidemics caused by enteroviruses may cause results to become positive in normal controls exposed to the same environmental factors, considering the cardiotropic characteristics of these agents.

The presence of enteroviral genomes combined with the persistence of serologic reactions suggests the possibility of the virus remaining in the myocardium after the acute phase, with no evidence of protein synthesis but nonetheless interference with cellular functions<sup>12</sup>. This hypothesis becomes questionable because the enteroviral RNA proportion usually detected through PCR in these hearts is small and localized<sup>19</sup>. On the other hand, experimental myocarditis models show that some coxsackievirus B-3 strains have greater cardiovirulence<sup>20</sup>. It is predicted that methods will be developed in the future that will be able to distinguish whether or not the identified genomes are related to strains with greater pathogenic potential.

### Activation of immunologic mechanisms

It is estimated that, in one third of the cases, DCM is an autoimmune disease<sup>21</sup>. Immunologic organ-specific reactions depend on genetic predisposition and are modulated by environmental factors. Enterovirus infections are thought to play a fundamental role in triggering autoimmunity in this disease. In an initial step, the virus may cause myocytolytic necrosis by direct action, depending on its capacity for intracellular replication. As this process evolves, activation of cytotoxic T lymphocytes occurs, which intensifies the injury of infected and intact cells during lysis<sup>22</sup>. Persistence of infection could result in exacerbation of the cellular and humoral immunologic

response, which would perpetuate aggression against myocytes<sup>12</sup>. Autoimmune reaction would result from molecular interactions between viral and host proteins or from neoantigen expression<sup>23</sup>. Other factors may be responsible for triggering an autoimmune process, such as gestation and alcoholism.

Immunoregulatory disorders are seen in DCM. A decrease in the activity of natural killer cells<sup>24</sup> and reduction of the suppressor function of T lymphocytes<sup>25</sup> may indicate greater susceptibility to viral infections. Experimental models of viral and autoimmune myocarditis show the decisive participation of cellular immunity in the myocardial injury process through T lymphocyte activation and cytokine release<sup>22</sup>. In DCM, a correlation of the expression of perforin and T cell intracellular antigen-1 with the degree of interstitial fibrosis revealed at endomyocardial biopsy<sup>26</sup> was observed.

Autoantibodies to cardiac proteins are identified in 30 to 40% of DCM cases<sup>21</sup>. Organ-specific autoantibodies, which interact with beta-adrenergic receptors<sup>27</sup>, cholinergic muscarinic receptors<sup>28</sup>, mitochondrial antigens<sup>29</sup> and alpha and beta cardiac myosin heavy chains<sup>30</sup>, have already been described elsewhere. The clinical importance of autoantibodies and their pathogenic meaning are not fully understood, because they are usually identified in an evolutionary phase, in which the disease is established. It is possible that not all of them have the same clinical meaning. Negative results obtained in most of the patients could be justified by the fact that, similarly to other autoimmune processes, the autoantibodies become undetectable as the disease progresses<sup>21</sup>.

There is no confirmation in regard to the ability of autoantibodies producing myocytolysis. They may interfere with cellular functions, as previously shown for beta-adrenergic receptors<sup>27</sup> and calcium channels, by cross-reaction with autoantibodies to ADP/ATP carriers<sup>29</sup>. Although there may be a correlation between disease severity and autoantibody presence, the autoantibodies may constitute mere cellular lesion markers. Their detection in 20% of asymptomatic individuals, 1<sup>st</sup>-degree relatives of DCM patients, indicates greater propensity to the disease in this subgroup<sup>31</sup>.

Other evidence suggests the participation of two immunologic mechanisms in DCM: 1) presence of abnormal serologic concentrations of interleukin-2 and interleukin-10<sup>32</sup>, and 2) abnormal expression of MHC class II antigens in endothelial cells<sup>33</sup>.

### Genetic factors and familiar forms

Recent studies introduced the concept that the development of DCM depends on genetic factors, which modulate sensitivity to viral infections and subsequent activation of immunologic reactions. Multiple genes may be involved in this process. Myocardial lesion depends on the interaction between genetic and environmental factors. Clinical and experimental studies suggest the association

with HLA genes, which modulate the immunologic response, and the genes of T cell receptors<sup>34</sup>. HLA genes regulate the immunologic system and the predisposition to autoimmune disease, through the modulation of T cell receptors, and the selection and presentation of antigenic peptides. A higher frequency of class II HLA antigens, DR4 and, possibly, DQw-4 loci, is identified in DCM patients when compared with controls<sup>35</sup>, even though a further analysis restricted to patients in a heart transplant program did not confirm these results<sup>36</sup>. Elevation of HLA-specific haplotypes is seen in the subgroup with deficiency in natural-killer cells<sup>37</sup>. Association of HLA-D alleles with auto-antibodies to beta-adrenergic receptors is also referred to<sup>38</sup>. These findings indicate that the disease development may be related to immunologic response and genes controlling this process.

Studies performed during the past decade to determine the frequency of familial forms of DCM reveal that this entity, initially interpreted as an acquired process, has a genetic basis in a significant number of patients. In preliminary investigations, familial forms were sporadically identified. Further, prospective analyses reveal an increasing prevalence of these forms, corresponding to 20% to 35% of the cases<sup>39-42</sup>. These figures may be underestimated, considering the difficulty in evaluating all members of an affected family and the reduced gene penetrance, which makes the carriers of the disease look apparently healthy.

In a recent prospective evaluation of 200 1<sup>st</sup>-degree relatives of 100 patients, McKenna et al<sup>41</sup> identified familial nature and tendency in 25% and 45% of the cases, respectively. HLA antigen was detected in 2/3 of the affected families and a greater prevalence of this antigen was noted in familial forms when compared to nonfamilial ones.

DCM has several forms of genetic transmission. Keeling et al<sup>40</sup>, in a prospective study of 236 individuals from 40 affected families, identified the predominance of autosomal dominant inheritance with incomplete penetrance. Early forms of the disease in asymptomatic members of the families were observed: 18% exhibited a slight increase of the left ventricle and 4% exhibited reduction in the fractional shortening when compared with controls. Other inherited forms are recognized: autosomal recessive, X chromosome-linked and mitochondrial<sup>43</sup>.

From the genetic viewpoint, DCM also has heterogeneous characteristics. Distinct genetic mutations produce multiple phenotypes, which determine similar clinical, histopathologic and hemodynamic manifestations. Familial forms are, however, diagnosed in earlier ages than sporadic ones<sup>42</sup>. It is estimated that 20% of the 1<sup>st</sup>-degree relatives have a risk of developing the disease at more advanced ages, as gene penetrance is related to age range<sup>40</sup>.

Lately, the progress made in genetics and molecular biology has allowed great advances in the study of the familial forms of the disease. The autosomal dominant form manifests itself in the 2<sup>nd</sup> or 3<sup>rd</sup> decade of life, with ventricular dilation and dysfunction, expressed by heart failure or arrhythmias<sup>43</sup>. The first genetic locus was identified in

chromosome 9q13-q22<sup>44</sup>. Later, a second locus was described in chromosome 1q32<sup>45</sup>. Forms associated with mitral valve prolapse were mapped to the short arm of chromosome 10<sup>46</sup>. These chromosomal regions are related to genes codifying regulatory and cytoskeletal proteins.

Rare forms, characterized by conduction system of the disease of the heart beginning in the 2<sup>nd</sup> or 3<sup>rd</sup> decade of life, followed by ventricular dilation and dysfunction some years after, are also transmitted by autosomal dominant inheritance through genes mapped to the centromere of chromosome 1<sup>47</sup> and to the short arm of chromosome 3<sup>48</sup>. Association of DCM with complex diseases that affect the mitochondrial DNA and are transmitted by maternal inheritance has also been described<sup>43</sup>.

So far, only two types of genes related to DCM have been identified<sup>49</sup>: the 1<sup>st</sup> encodes dystrophin and associated muscular proteins, among them LIM, and the 2<sup>nd</sup> encodes transcription factors regulating gene expression of myocardial cell genes.

Dystrophin is a protein present in the sarcolemma; it establishes the linkage of the cytoskeleton to the extracellular matrix. Anomalies affecting the gene codifying this substance cause Duchenne's dystrophy and Becker type muscular dystrophy. In rare cases, mutations involving this gene produce myocytolysis and cardiomyopathy development<sup>50</sup>. In males, the disease develops during adolescence and evolves quickly, but in females it develops later and with a slow progression. These patients do not usually have neuromuscular compromise but lack dystrophin in the myocardium and have generally high serum levels of creatine kinase<sup>43</sup>. Inheritance is linked to the X chromosome and can be dominant or recessive<sup>51</sup>. Sporadic cases are reported<sup>50</sup>. Prevalence of disorders involving dystrophin in DCM patients is still unknown.

Mutations involving the gene encoding the muscle LIM protein produce severe cardiomyopathy in animal models. This protein is related to cellular maintenance and viability<sup>49</sup>.

Identification of genetically transmitted DCM forms is of fundamental clinical importance. Systematic evaluation of affected families may establish the actual prevalence of familial forms and allow early diagnosis of the disease. The clinical meaning and evolutionary potential of early manifestations, such as conduction disturbances, arrhythmias, incipient ventricular dilation and dysfunction, can be thus cleared up, as well as the effect of early pharmacological intervention.

Individualization of affected genes and their respective mutations represents the next step to the elucidation of genetic and molecular bases of familial forms of DCM. Applying techniques developed under the orientation of these concepts may make possible the pre-clinical diagnosis in patients who still do not exhibit the disease phenotype. Genetic therapy may allow, in the future, the correction of molecular disorders through the substitution of anomalous structural proteins.

In conclusion, several processes participate in DCM

pathogenesis, justifying its multifactorial character. Elucidation of cellular and biomolecular mechanisms responsible for myocardial dysfunction will help with the intro-

duction of diagnostic methods and therapeutic modalities that will disrupt the development and evolution of this disease.

## References

1. Richardson P, McKenna W, Bristow M, et al – Report of the 1995 World Health Organization/International Society and Federation of Cardiology task force on the definition and classification of cardiomyopathies. *Circulation* 1996; 93: 841-2.
2. Goodwin JF – The frontiers of cardiomyopathy. *Br Heart J* 1982; 48:1-18.
3. Kuhn H, Knierem HJ, Breithardt G, et al – Prognosis and possible pre-symptomatic manifestations of congestive cardiomyopathy. *Post Grad Med J* 1978; 54: 451-9.
4. Mattos BP, Zettler CG, Pinotti AFF, Raudales JC, Zago AJ – Left ventricular function and endomyocardial biopsy in early and advanced dilated cardiomyopathy. *Int J Cardiol* 1998; 63: 141-9.
5. Woodruff JF – Viral myocarditis: a review. *Am J Pathol* 1980; 101: 425-84.
6. Aretz HT, Billingham ME, Edwards WD, et al – Myocarditis. A histopathological definition and classification. *Am J Cardiovasc Pathol* 1986; 1: 3-14.
7. Billingham ME, Tazelaar HD – The morphological progression of viral myocarditis. *Post Grad Med J* 1986; 62: 581-4.
8. Quigley RJ, Richardson PJ, Many BT, et al – Long-term follow-up of acute myocarditis. *Eur Heart J* 1987; 8(suppl J): 39-42.
9. Cambridge G, MacArthur CGC, Waterson AP, Goodwin JF, Oakley CM – Antibody to coxsackie B viruses in congestive cardiomyopathy. *Br Heart J* 1979; 41: 692-6.
10. Keeling PJ, Lucaszuk A, Poloniecki J, et al – A prospective case-control study of antibodies to coxsackie B virus in idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 1994; 23: 593-8.
11. Muir P, Nicholson F, Tilzey AJ, Signy M, English TA, Banatvala JE – Chronic relapsing pericarditis and dilated cardiomyopathy: serological evidence of persistent enterovirus infection. *Lancet* 1989; i: 804-7.
12. Keeling PJ, Tracy S - Link between enteroviruses and dilated cardiomyopathy: serological and molecular data. *Br Heart J* 1994; 72(suppl): S25-S9.
13. Bowles NE, Rose MI, Taylor P, et al – End-stage dilated cardiomyopathy. Persistence of enterovirus RNA in myocardium at cardiac transplantation and lack of immune response. *Circulation* 1989; 80: 1128-36.
14. Archard LC, Bowles NE, Cunningham L, et al – Molecular probes for detection of persisting enterovirus infection of human heart and their prognostic value. *Eur Heart J* 1991; 12: 56-9.
15. Weiss LM, Movahh LA, Billingham ME, Claery ML – Detection of coxsackie virus B<sub>3</sub> RNA in myocardial tissues by the polymerase chain reaction. *Am J Pathol* 1991; 138: 497-503.
16. Grasso M, Arbustini E, Silini E, et al – Search for coxsackie B<sub>3</sub> RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction. *Am J Cardiol* 1992; 69: 658-64.
17. Keeling PJ, Jeffery S, Caforio ALP, et al – Similar prevalence of enteroviral genome within the myocardium from patients with idiopathic dilated cardiomyopathy and controls by the polymerase chain reaction. *Br Heart J* 1992; 68: 554-9.
18. Petitjean JJ, Kopecka H, Freymuth F, et al – Detection of enterovirus in endomyocardial biopsy by molecular approach. *J Med Virol* 1992; 37: 76-82.
19. Giacca M, Severini GM, Mestroni L, et al – Low frequency of detection by nested polymerase chain reaction of enterovirus ribonucleic acid in endomyocardial tissue in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 1994; 24: 1033-40.
20. Baboonian C, Treasure T – Meta-analysis of the association of enteroviruses with human heart disease. *Heart* 1997; 78: 539-43.
21. Caforio ALP – Role of autoimmunity in dilated cardiomyopathy. *Br Heart J* 1994; 72(suppl): S30-S4.
22. Huber AS, Lodge PA – Coxsackie B-3 myocarditis in Balb/c mice. Evidence for autoimmunity to myocyte antigens. *Am J Pathol* 1984; 116: 21-30.
23. Herskowitz A, Neumann DA, Ansari AA – Concepts of autoimmunity applied to dilated cardiomyopathy. *J Am Coll Cardiol* 1993; 22: 1385-8.
24. Anderson JL, Carlquist JF, Higashikubo R – Quantitation of lymphocyte subset by immunofluorescence flow cytometry in idiopathic dilated cardiomyopathy. *Am J Cardiol* 1985; 55: 1550-4.
25. Eckstein R, Mempel W, Bolte HD – Reduced suppressor cell activity in congestive cardiomyopathy. *Circulation* 1982; 65: 1224-9.
26. Badorf C, Noutsias M, Kühl U, Schultheiss HP - Cell-mediated cytotoxicity in hearts with dilated cardiomyopathy: correlation with interstitial fibrosis and foci of activated T lymphocytes. *J Am Coll Cardiol* 1997; 29: 429-34.
27. Limas JL, Goldenberg IF, Limas C – Effects of antireceptor antibodies in dilated cardiomyopathy in cycling of cardiac beta-receptor. *Am Heart J* 1991; 122: 108-14.
28. Fu LX, Magnusson Y, Bergh CH, et al – Localisation of a functional autoimmune epitope on the muscarinic acetylcholine receptor-2 in patients with idiopathic dilated cardiomyopathy. *J Clin Invest* 1993; 91: 1964-8.
29. Schultheiss HP – Disturbance of the myocardial energy metabolism in dilated cardiomyopathy due to auto-immunological mechanism. *Circulation* 1993; 87(suppl IV): IV-43-IV-8.
30. Caforio ALP, Grazzini M, Mann JM, et al – Identification of  $\alpha$  and  $\beta$  - cardiac myosin heavy chain isoforms as major auto-antigens in dilated cardiomyopathy. *Circulation* 1992; 85: 1734-42.
31. Caforio ALP, Keeling PJ, Zaccara E, et al – Evidence from family studies for autoimmunity in dilated cardiomyopathy. *Lancet* 1994; 344: 773-7.
32. Marriott JB, Goldman JH, Keeling PJ, Baig MK, Dalgleish AG, McKenna W – Abnormal cytokine profiles in patients with idiopathic dilated cardiomyopathy and their asymptomatic relatives. *Heart* 1996; 75: 287-90.
33. Hufnagel G, Maisch B – Expression of MHC class I and II antigens and the IL-2 receptor in rejection, myocarditis and dilated cardiomyopathy. *Eur Heart J* 1991; 12(suppl D): 137-40.
34. Limas CJ – Autoimmunity in dilated cardiomyopathy and the major histocompatibility complex. *Int J Cardiol* 1996; 54: 113-6.
35. Carlquist JF, Menlove RL, Murray MB, O'Connell JB, Anderson IL – HLA class II (DR and DQ) antigens associations in idiopathic dilated cardiomyopathy: validation study and meta-analysis of published HLA – associations in idiopathic dilated cardiomyopathy. *Circulation* 1992; 83: 515-22.
36. Grant SCD, Sheldon S, Levy RD, Brooks NH – Do specific HLA antigens predispose to ischaemic disease or idiopathic dilated cardiomyopathy? *Br Heart J* 1994; 71: 76-8.
37. Anderson JL, Carlquist JF, Hammond EH – Deficient natural killer cell activity in patients with idiopathic dilated cardiomyopathy. *Lancet* 1982; ii: 1124-7.
38. Limas CJ, Limas C – HLA-DR antigen linkage of anti-  $\beta$  receptors antibodies in idiopathic dilated cardiomyopathy. *Br Heart J* 1992; 67: 402-5.
39. Michels VV, Moll PP, Miller FA, et al – The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. *N Engl J Med* 1992; 326: 77-82.
40. Keeling PJ, Gang Y, Smith G, et al – Familial dilated cardiomyopathy in the United Kingdom. *Br Heart J* 1995; 73: 417-21.
41. McKenna CJ, Codd MB, McCann HA, Sugrue DD – Idiopathic dilated cardiomyopathy: familial prevalence and HLA distribution. *Heart* 1997; 77: 185-8.
42. Grünig G, Tasman JA, Kücherer H, Franz W, Kübler W, Katus HA – Frequency and phenotypes of familial dilated cardiomyopathy. *J Am Coll Cardiol* 1998; 31: 186-94.
43. Mestroni L, Giacca M – Molecular genetics of dilated cardiomyopathy. *Curr Op Cardiol* 1997; 12: 303-9.
44. Krajcinovic M, Pinamonti B, Sinagra G, et al – Linkage of familial dilated cardiomyopathy to chromosome 9. *Am J Hum Genet* 1995; 57: 846-52.
45. Durand JB, Bachinski LL, Bieling LC, Czernuszewicz GZ, Abchee AB, Yu QT – Localization of a gene responsible for familial dilated cardiomyopathy to chromosome 1q32. *Circulation* 1995; 92: 3387-9.
46. Bowles KL, Gajarski R, Porter P, et al – Gene mapping of familial autosomal dominant dilated cardiomyopathy to chromosome 10q21-23. *J Clin Invest* 1996; 98: 1355-60.
47. Kass S, MacRae C, Graber HL, et al – A gene defect that causes conduction system disease and dilated cardiomyopathy. *Nature Genet* 1994; 7: 546-51.
48. Olson TM, Keating MT – Mapping a cardiomyopathy locus to chromosome 3p22-p25. *J Clin Invest* 1996; 97: 528-32.
49. Leinden JM – The genetics of dilated cardiomyopathy: emerging clues to the puzzle. *N Engl J Med* 1997; 337: 1080-1.
50. Muntoni F, Di Lenarda A, Porcu M, et al – Dystrophin gene abnormalities in two patients with idiopathic dilated cardiomyopathy. *Heart* 1997; 78: 608-12.
51. Berko BA, Swift M.X-linked dilated cardiomyopathy. *N Engl J Med* 1987; 316: 1186-91.