Current perspective

New insights into the molecular basis of familial dilated cardiomyopathy

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Key words:
Familial dilated cardiomyopathy; Genetics; Polymorphic variation.

Genetic disease transmission has been identified in a significant proportion of patients with dilated cardiomyopathy (DCM). Variable clinical characteristics and patterns of inheritance, as well as recent molecular genetic data, indicate the existence of several genes causing the disease. Several distinct subtypes of familial DCM have been identified. Autosomal dominant DCM is the most frequent form (56% of our cases), and several candidate disease loci have been identified by linkage analysis. Three disease genes are presently known: the cardiac actin gene, the desmin gene, and the lamin A/C gene. This latter gene has recently been found to be responsible for both the autosomal dominant form of DCM with subclinical skeletal muscle disease (7.7% of cases) and the familial form with conduction defects (2.6% of cases) or the autosomal dominant variant of Emery-Dreifuss muscular dystrophy. The autosomal recessive form of DCM accounts for 16% of cases and is characterized by a worse prognosis. An X-linked form of DCM (10% of cases) manifests in the adult population and is due to mutations in the dystrophin gene. In the rare infantile form of DCM, mutations in the G4.5 gene have been identified. Finally, some of the rare unclassifiable forms (7.7% of cases) may be due to mitochondrial DNA mutations. Clinical and experimental evidence based on animal models suggest that, in a large number of cases, DCMs are diseases of the cytoskeleton. However, other causes, such as alterations in regulatory elements and in signaling molecules, are possible. Moreover, other genes called modifier genes can influence the severity, penetrance, and expression of the disease, and they will be a main objective of future investigations.

Familial DCM is frequent, cannot be predicted on a clinical or morphological basis and requires family screening for identification. The advances in the genetics of familial DCM can allow improved diagnosis, prevention and genetic counseling, and represent the basis for the development of new therapies.

(Ital Heart J 2001; 2 (4): 280-286)

This work was supported by the grant of Telethon, Project no. 1024, 1997.

Received August 29, 2000; revision received November 13, 2000; accepted November 16, 2000.

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Introduction

Dilated cardiomyopathy (DCM) is a heart muscle disease characterized by ventricular dilation and impaired systolic function. DCM is a leading cause of heart failure and arrhythmia and due to its significant prevalence (1 case per 2500 individuals)[1] and high morbidity necessitating frequent hospitalization, it constitutes a relevant medical problem. Moreover, the true incidence of DCM seems to be higher, owing to the fact that subjects may remain asymptomatic and are not identified until marked ventricular dysfunction has developed. Despite improvements in the treatment of heart failure introduced in the last 10 years, including the general availability of cardiac transplantation and better medical treatment, the clinical outcome following the onset of symptoms has not substantially changed. Mortality remains high, the natural history remains progressive, and disability and morbidity are among the highest of any disease or disease syndrome.

In the past DCM was defined by identifying cases of dilated cardiac disease that could not be attributed to known secondary causes (e.g. ischemic or valvular disease). Previously this class of "idiopathic" DCM represented a large fraction of all dilated cardiomyopathy cases. The recent impressive advances in characterizing the molecular genetic contributions to DCM have shrunk the proportion of DCM cases that can still be considered truly "idiopathic"[2]. It is now conceivable that there will soon be a time when

"idiopathic" DCM represents only a small minority of all cases of dilated cardiac disease.

Familial dilated cardiomyopathy

Among the possible etiologic factors, genetic contributions to DCM were considered rare in the past. However, more recent studies have demonstrated that DCM may be inherited in up to 50% of cases when first degree relatives are carefully screened[3-6]. These data show that, in a consistent percentage of DCM patients, the disease might be due to a single or to multiple gene defects (mutations). Analysis of the various phenotypes identifies a wide range of clinical and pathological forms and different patterns of transmission, and indicates that mutations in a number of different genes can cause DCM. Therefore, DCM represents a genetically heterogeneous disease[7,8]. Accordingly, several chromosomal assignments for disease gene locations have been made and recently several genes have been identified. Table I[9-24] summarizes the present knowledge of the genetics of DCM. Several of the identified genes function in the cytoskeleton; the emerging paradigm for familial DCM therefore, is based upon a cytoskeletal model. Unfortunately, there are no reliable clinical or morphologic parameters that clearly distinguish familial DCM from sporadic cases. In our patient population, only more advanced age and worse ventricular function characterized the sporadic versus the familial cases, suggesting different stages of the disease rather than different diseases[8]. Likewise, no differences were found between healthy relatives of sporadic and familial DCM patients. The lack of differential features is probably due to the etiologic heterogeneity of both groups.

The following discussion will focus on the current knowledge regarding the phenotypes and genotypes of familial DCM.

Genotypes and phenotypes in familial dilated cardiomyopathy. *Autosomal dominant familial dilated cardiomyopathy.* The most common type of familial DCM is an autosomal dominant form with isolated myocardial involvement (56% of our families). Autosomal dominant familial DCM frequently presents as a mild form of DCM (42% of cases). The phenotype is usually characterized by low penetrance (which is the proportion of mutation carriers who manifest the disease phenotype). The onset of clinical manifestations is also age-related and it is estimated that only 20% of gene carriers under the age of 20 present the disease phenotype[8].

Molecular genetic studies based on linkage analysis have led to the identification of seven loci for autosomal dominant familial DCM (Table I). To date, two genes have been associated with this phenotype: the cardiac actin gene[9] and the desmin gene[10]. The exact prevalence of these mutant genes in the affected population is still unknown.

Cardiac alpha-actin gene mutations were found in exons 5 and 6 in two unrelated familial DCM families[9]. This finding was initially surprising since cardiac actin, a sarcomeric gene, was considered a candidate for causing hypertrophic cardiomyopathy. Recently, this has been confirmed by Mogensen et al.[25], who found a cardiac actin gene mutation in a large family with hypertrophic cardiomyopathy. To explain the difference in the

Table I. Phenotypes, known loci and genes for familial dilated cardiomyopathy.

Type	Frequency (%)	Chromosomal location	Known gene
Autosomal dominant	56	1q32[18]	
		2q31[19]	
		2q14-q22[20]	
		2q35[10]	Desmin
		9q13-q22[21]	
		10q21-q23[22]	
		15q14[13]	Cardiac actin
Autosomal recessive	16	Unknown	
X-linked	10	Xq21[11,12]	Dystrophin
Autosomal dominant with subclinical skeletal muscle disease	7.7	1q11-q23[14,15,23]	Lamin A/C
		6q23[24]	
Autosomal dominant with conduction defects	2.6	1q1-q1[14]	Lamin A/C
		3p22-p25[9]	
Unclassifiable	7.7	Unknown	
Autosomal dominant with apical hypertrophy			
Autosomal recessive with retinitis pigmentosa and deafness			
X-linked congenital DCM		Xq28[16]	G4.5 (tafazzin)
Mitochondrial DCM		mtDNA[17]	

DCM = dilated cardiomyopathy; mtDNA = mitochondrial DNA.

phenotypic expression, it has been hypothesized that the mutations involved in familial DCM alter the domains of the protein involved in myocyte force transmission, whereas the mutations occurring in hypertrophic cardiomyopathy alter force generation. In particular, the mutations found in familial DCM are believed to alter the interaction of the sarcomere with the Z-band and the intercalated disks. Further studies based on transgenic animal models carrying different mutations are in progress. The scope is to evaluate the genotype-phenotype correlation and understand the pathogenic mechanisms underlying the two diseases.

Accumulation of the cytoskeletal proteins desmin and alphaB-crystallin in skeletal muscle, and mutations in the corresponding genes are known to cause a myopathy with frequent cardiac involvement. Several familial and sporadic cases have been reported in which desmin gene mutations caused both skeletal muscle dystrophy and variable degrees of cardiomyopathy[26,27]. Recently, however, Li et al.[10] have found a desmin gene mutation in a family with pure familial DCM and no evidence of skeletal muscle involvement. In this case, the mutation involves a domain of the protein (carboxy tail domain) that is critical for filament assembly.

Cardiac actin and desmin are both cytoskeletal proteins. Therefore, these findings strongly support the hypothesis that DCM can be due to mutations in cytoskeletal genes; furthermore, skeletal muscle diseases may represent an important model for the selection of gene candidates for familial DCM.

X-linked dilated cardiomyopathy. The first evidence supporting the hypothesis that DCM is a disease of the cytoskeleton derived from the discovery of dystrophin gene mutations among patients with X-linked DCM and no overt clinical evidence of muscular dystrophy. It was well established that the dystrophin gene was mutated in patients with the severe Duchenne and the milder Becker muscular dystrophies. The dystrophin gene encodes a large cytoskeletal protein that plays a critical role in membrane organization and stability and in force transduction in skeletal and cardiac myocytes. X-linked DCM was previously described in teenage males as a rapidly progressive congestive heart failure without clinical signs of skeletal myopathy but with elevated serum MM-creatine kinase. The disease was X-linked, with typical absence of male-to-male transmission. Females could occasionally be affected, but with later-onset and milder disease[28].

In our X-linked DCM patients[8], we observed an older age of onset, a less severe prognosis and, in one case, normal serum creatine kinase, suggesting variable expression. Several studies have recently shown that mutations in the 5' end of the dystrophin gene (deletions, point mutations, a duplication mutation, and rearrangements of Alu-like sequences or mutations in the region of the exon 48-49) can selectively cause severe DCM[11,12,29-32]. Interestingly, in our families, expression studies showed that dystrophin was absent in the heart and partially replaced by the compensatory production of other isoforms (brain- and Purkinje-) in skeletal muscle[30,33].

These findings were clinically relevant: they indicated the need for systematic and accurate skeletal muscle examination in familial DCM, even in the absence of overt signs of myopathy. Furthermore, in X-linked DCM, skeletal muscle biopsy is diagnostic and identification of the molecular defect is possible.

Autosomal dominant dilated cardiomyopathy with conduction disease and familial dilated cardiomyopathy with variable skeletal muscle involvement. Familial DCM with conduction defects was rare in our series (2.6%). A peculiarity of this form is the onset with severe conduction defects, arrhythmia, and only minor ventricular dysfunction, subsequently followed by progressive heart failure. In this form, two disease loci have been reported, one on chromosome 1p1-q1[34] and the other on chromosome 3p22-p25[13].

Linkage to the chromosome one locus is accounted for by the lamin A/C (LMNA) gene. Missense mutations in LMNA have recently been shown to be responsible for inherited DCM with conduction system disease[14], while a single nucleotide deletion in LMNA has been shown to cause DCM with variable skeletal muscle involvement[15]. In DCM with variable skeletal muscle involvement, the phenotype of the affected relatives can be very variable, ranging from pure DCM to mild Emery-Dreifuss-like or limb-girdle-like muscle dystrophy[15]. Mild abnormalities in creatine kinase levels are found in both phenotypic variants and suggest skeletal muscle involvement, even in the absence of overt clinical signs.

The lamin A/C gene encodes two proteins: lamin A and lamin C. These are members of the intermediate filament class of cytoskeletal proteins. Lamins A and C are components of the nuclear lamina, which is a meshwork of proteins lining the inner surface of the nuclear membrane.

Missense mutations in the LMNA gene have also been shown to cause autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD). Patients with autosomal dominant EDMD frequently display cardiac conduction defects. It is of interest to note that lamin A/C gene mutations can also result in disease phenotypes that do not include muscle abnormalities. Mutations in exon 8 have been shown to cause partial lipodystrophy, a disease characterized by regional fat loss and variable insulin resistance[35]. Patients with partial lipodystrophy do not present with signs of cardiac involvement.

The observation that LMNA mutations can result in DCM with conduction defects, associated with limb-girdle- or Emery-Dreifuss-like skeletal muscle dystrophy or otherwise, as well as with partial lipodystrophy points

to the fact that the lamin A and C proteins contain a number of distinct functional domains. Mutations in these different domains can lead to tissue-specific disease phenotypes. Mutations in the LMNA gene may result in mechanical weakening of the nuclear envelope, in altered gene expression resulting from aberrant interactions between the lamins and chromatin and/or in altered transport of messenger molecules through the nuclear envelope. The identification of the mechanism by which a subset of LMNA mutations result in DCM will require elucidation of the effects of these mutations on lamin expression and function. Furthermore, very recent studies[36] have identified the occurrence of *de novo* dominant mutations in a very high number of cases with EDMD2 (> 70%). These data indirectly suggest that *de novo* mutations in this gene could also be responsible for sporadic cases of DCM.

Other forms of familial dilated cardiomyopathy. The autosomal recessive form of the disease is less frequent (16% of cases in our population). In these families, both parents of the index patient are found to be unaffected. In our survey[8], the recessive form was characterized by a significantly younger age of onset and a worse prognosis compared to the dominant form.

Also rare in our study population were families with unclassifiable familial DCM (7.7%). Two of these families showed a peculiar phenotype characterized by apical hypertrophy and autosomal dominant transmission. A third family was characterized by a complex phenotype including hearing loss, retinitis pigmentosa and apparently autosomal recessive transmission. This phenotype, also observed in other familial DCM patient populations[5], has been reported in rare mitochondrial disorders and in autosomal recessive syndromes such as the Alstrom syndrome and the Usher syndrome, suggesting mutations in genes (autosomal or mitochondrial) with critical functions in the heart, eye and ear.

Finally, in the pediatric population, X-linked familial DCM is caused by mutations in the G4.5 gene (tafazzin), particularly if associated with other signs (such as endocardial fibroelastosis, neutropenia, short stature or skeletal muscle abnormalities)[16]. The function of the G4.5 gene is still unknown.

Dilated cardiomyopathy: a disease of the cytoskeleton

Cardiac actin, dystrophin, desmin and lamin A/C are all cytoskeletal proteins. A number of additional observations also support the hypothesis that, in a significant percentage of cases, DCM is a disease of the cytoskeleton. First, the absence of the cytoskeletal protein metavinculin in the myocardium was reported in one familial DCM patient[37]. Second, mild myocardial dysfunction is frequently found in limb-girdle muscular dystrophies caused by alpha-, beta-, and gamma-sarcoglycan mutations (components of the sarcolemma-dystrophin complex)[38]. Finally, the DCM phenotype can be associated with emerin gene mutations in X-linked Emery-Dreifuss muscular dystrophy.

However, other molecular mechanisms cannot be ruled out. Mitochondrial DNA (mtDNA) mutations can lead to DCM. In these cases, myocardial dysfunction is usually associated with multiorgan involvement (encephalopathy, lactic acidosis, skeletal muscle abnormalities, retinitis pigmentosa, etc.). Arbustini et al.[17] have shown that in DCM patients with histologic signs of mitochondrial abnormalities, more than 20% of cases have evidence of pathological mtDNA mutations, suggesting an important role of mtDNA in the pathogenesis of idiopathic DCM and heart failure.

Lessons from animal models of dilated cardiomyopathy

There are several naturally occurring animal models of DCM (Table II). However, the only one for which the responsible gene is known occurs in the Syrian hamster (BIO14.6). In this model, gene mapping by linkage analysis led to the identification of mutations of the delta-sarcoglycan gene, which encodes a cytoskeletal protein of the dystrophin complex[39].

The ability to genetically manipulate the cardiovascular system has made it possible to investigate the role of a number of genes in the murine heart. Myosin heavy

Table II. Animal models of dilated cardiomyopathy.

Naturally occurring mutants	Gene/function
Syrian hamster	Gamma-sarcoglycan
Canine	Unknown
Bovine	Unknown
Turkey	Unknown
Feline	Unknown
Transgenic mice	
Overexpression	
Tropomodulin	Cytoskeletal
RhoA	Signaling molecule
Retinoids	Signaling molecule
FKBP12	Signaling molecule
Galphaq	Signaling molecule
Human β_1 adrenoreceptor	Signaling molecule
Knockout	
Muscle LIM protein	Cytoskeletal
Gamma-sarcoglycan	Cytoskeletal
Desmin	Cytoskeletal
Lamin	Cytoskeletal
gP130	Signaling molecule (cytokine receptor)
MnSOD	Mitochondrial enzyme
Ant1	Mitochondrial carrier
Dominant negative	
CREB	Mitochondrial carrier

chain gene mutations, causing hypertrophic cardiomyopathy in humans, lead to hypertrophy in mice, but in adult males result in late decompensation and ventricular dilation[40]. These findings suggest the existence of important epigenetic factors (modifiers of gene expression) in the development of DCM. The *myf* 5 mice have been generated by activation of a skeletal muscle genetic program in the heart[41]. These mice have a DCM phenotype characterized by progressive myocardial dysfunction and dilation, heart failure and high mortality. Overexpression of components of the β_1-adrenergic receptor pathway causes both systolic and diastolic dysfunction as well as increased mortality secondary to heart failure[42]. The MLP deficient mouse lacks MLP (muscle LIM protein), a regulator of myogenic differentiation involved in maintaining the integrity of the actin cytoskeleton[43]. Targeted ablation of the MLP gene results in hypertrophy and in a cardiomyopathy.

These data and a number of other animal models show that apparently diverse signals can culminate in the same phenotype, presumably by converging on final common pathways. They confirm the importance of the cytoskeleton in maintaining both cardiac structure and function and also suggest that other mechanisms, such as altered metabolic or regulatory pathways or other structural defects possibly under the effect of epigenetic factors, can be involved in the pathogenesis of DCM. Whatever the molecular basis, mouse models represent an invaluable tool for the study of the mechanisms that lead to the development and progression of myocardial failure.

Links with the viral and the autoimmune hypotheses of dilated cardiomyopathy

A clinical and pathologic syndrome similar to DCM may develop after resolution of viral myocarditis in animal models and biopsy-proven myocarditis in human subjects. This has led to speculation that DCM may develop as a result of viral myocarditis. This hypothesis is still controversial since it has never been possible to isolate an infectious virus and since studies on the presence of viral antigens in the myocardium of patients with DCM led to very discrepant results. However, recent experimental data have shown *in vitro* and *in vivo* that the enteroviral protease 2A is able to cleave dystrophin and disrupt the cytoskeleton in cardiac myocytes, providing a link between viral infection and a genetic model of the disease[44].

A number of immune regulatory abnormalities have been identified in DCM. These include humoral and cellular autoimmune reactivity. Such anomalies have been considered as potentially important etiologic factors in the development of the disease. In our survey, organ-specific cardiac autoantibodies were detected in 72% of affected individuals with the autosomal dominant form[8] compared to 15% of sporadic cases and 3.5% of controls. The identification of a high frequency of cardiac autoantibodies suggested activation of an immune response, which was independent of the histocompatibility gene (HLA) DR4. There is no conclusive evidence that these antibodies are directly pathogenic, but they may have a functional role or predict early disease among relatives at risk of developing DCM.

There has been great interest in HLAs in DCM since HLA antigens are known to be associated with immune regulatory functions, many autoimmune diseases are found to have positive HLA antigenic associations and several HLA associations (in particular HLA-DR4) have also been identified in DCM. Genetic abnormalities in the HLA region could alter the immune response and thereby increase disease susceptibility to infectious agents such as enteroviruses. Despite the fact that in our studies[8,45] the HLA locus (chromosome 6) was excluded as a disease locus, and that the HLA-DR4 antigen did not correlate with the presence of autoantibodies and with disease status in the familial DCM population, a contribution of HLA genes to disease susceptibility in DCM cannot be ruled out.

Other genetic mechanisms: polymorphic variations in modifier genes

Genes exhibit polymorphic variations, which result in the expression of proteins that differ only slightly in their amino acid sequences and molecular weights. Polymorphisms can be pathological, normal variants of genes or can be associated with differences in the function of the expressed protein product. These polymorphisms account for some of the "biological variations" routinely encountered in population studies of disease susceptibility or of clinical response to treatment. Thus, besides the major disease genes, even the variable penetrance and expression of familial DCM and the differences in gender susceptibility suggest the existence of modifier genes that could influence disease manifestation and severity.

Examples of "modifier" genes which may modify the natural history of a cardiomyopathy include genes of the renin-angiotensin system that seem to influence the severity of heart failure in ischemic and idiopathic DCM[46] and are also believed to influence the development of hypertrophy in hypertrophic cardiomyopathy. Moreover, a complex genetic basis could account for the autoimmune subset of familial DCM. Finally, the increased male/female ratio observed in forms with evidence of autosomal transmission suggests the existence of susceptibility factors associated with gender. The elucidation of the mechanisms of these susceptibility or modifier genes could provide excellent tools for the management of DCM.

Clinical implications

Even if still incomplete, the new knowledge on the genetics of DCM has relevant clinical implications. The frequency of familial forms indicates the need for family screening, which can allow for genetic counseling, early detection of the disease and early therapeutic interventions in affected relatives[47]. The complexity of the phenotype requires accurate skeletal muscle investigation that directs the diagnosis towards a specific type of familial DCM. Finally, family investigations require the development of more sensitive diagnostic criteria[48] that can characterize minor cardiac abnormalities such as initial dilation without marked systolic dysfunction, arrhythmia and isolated muscular abnormalities[6,15,49] as early signs of the disease. In the absence of clinical evaluation, the potential risk of misclassification error (12.5% in our population) stresses the need of screening at least for first-degree relatives of DCM patients regardless of family history. The disease can be asymptomatic or clinically not evident due to the reduced and age-related penetrance. In individuals of unknown status, family screening can detect initial cardiac abnormalities, which may represent early manifestations of the disease. These abnormalities, particularly frequent in the autosomal dominant form (20% of the patients' relatives), have been shown to progress to overt DCM in 27% of subjects[6].

Identification of the contribution of genetic factors to cardiac illness represents a new frontier in the study of cardiomyopathies as well as in all fields of medicine. The elucidation of these factors will almost certainly lead to new therapeutic and diagnostic approaches in the treatment of cardiomyopathies. In the near future, molecular genetic testing will be routinely performed for many cardiomyopathies, both in order to identify single gene defects and to test the influence of polymorphic genetic variation on the natural history of the disease and the selection of specific medical therapy.

Appendix

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