Clinical Perspective

Familial dilated cardiomyopathy: from clinical presentation to molecular genetics

Introduction: a patient's story

A 40-year-old male patient is brought to the cardiologist's attention: he complains of asthenia and some dyspnoea, and he’s feeling that something has recently changed in his physical performance. At clinical and echocardiographic evaluation, the heart appears enlarged and hypokinetic. Coronary angiography shows normal coronary arteries and therefore the few elements that are necessary for the diagnosis of dilated cardiomyopathy are all present: left ventricular dilation and dysfunction with angiographically normal coronary arteries. As regards prior or recent inflammatory diseases, the patient does not recall infectious episodes or flu, or gastrointestinal disturbances. Should the cardiologist perform an endomyocardial biopsy? This is routinely conducted in transplant centres so the cardiologist does, with the main aim of excluding disorders contraindicating heart transplantation, and with the hope of obtaining a definite diagnosis, not one saying 'features consistent with cardiomyopathy'. Then the cardiologist starts to explore the patient's family history, and to trace the pedigree. The patient's father died aged 52, with a 'big heart', the patient does not know more. One of four uncles probably had 'some heart disease': he died aged about 55; another one had a son who died young. Both paternal grandparents were healthy and strong; they both died after age 80. Finally, one sister, two brothers, and two children are fine (Fig. 1(a)).

This is enough for the cardiologist to inform the patient that his disease is likely to be familial: informative clinical reports from deceased family members are necessary, and the patient should inform his relatives that they should have a check-up. There is a big research programme going on in the hospital, and the costs will be covered by the research funding. The cardiologist makes the patient understand the importance of his role in being the protagonist of a health care programme involving his family. Family members accept the clinical screening, and the result is that a brother of the patient has an increased end-diastolic volume, and an ejection fraction of 42%; one of the two sons of the patient has an increased end-diastolic diameter with ventricular function within normal ranges. In the meantime, the patient is able to find the record of the 52-year-old father, and the diagnosis of dilated cardiomyopathy is clear; no reports of the patient's uncle are found, and this subject remains uninformative (Fig. 1(b)).

An evidence-based diagnosis of familial dilated cardiomyopathy is obtained (Fig. 2), and constitutes a starting point for further diagnostic progression. Any experienced cardiologist dealing with dilated cardiomyopathy uses such evidence, and he also knows that the diagnosis of familial cardiomyopathy is the start of a troublesome and often frustrating clinical/investigative pathway, that rarely results in a useful contribution to deepen the aetiopathogenetic information.

Familial dilated cardiomyopathy and genetics (Fig. 3)

The first step in characterizing the disease is to trace the familial pedigree and to examine it, to understand the pattern of inheritance. When father and son/daughter are affected, the information is sufficient to suspect an autosomal dominant disorder and to exclude an X-linked or a recessive disease. When mother and son/daughter are affected, the inheritance is likely to be autosomal dominant, if mitochondrial DNA disorders are excluded. If the disease affects mothers and both male and female offspring, but affected males do not transmit the disease, the inheritance is likely matrilinear. If brothers, male cousins, or maternal uncles, all males, are affected, the inheritance is likely to be X-linked recessive. An affected female, especially older than the males (i.e. the
mother of the proband), does not necessarily exclude an X-linked disease: the healthy X chromosome could be inactivated at the myocardial level (lyonism). If a grandmother’s siblings and grandchildren are affected, or if the parents of the proband are consanguineous, the inheritance is probably autosomal recessive; two affected brothers are not enough to assign an inheritance pattern: it is just a sib-pair. This is the first step in a genetic approach to the disease. The mendelian or matrilinear laws are respected. When other information is not available, further steps can be taken to reveal the aetiological background of the disorder.

1. If the patient is a male, and his serum creatine phosphokinase (muscular isoform) is increased, then a dystrophin defect has to be excluded. Dystrophin is a large rod-like protein linking intracellular actin with proteins of the extracellular matrix, through a complex of transmembrane proteins (Fig. 4). Dystrophin defects cause Duchenne and Becker muscular dystrophy. Recently, the dominant cardiac involvement with mild/absent skeletal muscle impairment has been defined as X-linked dilated cardiomyopathy (www.dmd.nl). If the patient is a candidate for heart transplantation, and he undergoes endomyocardial biopsy to exclude disorders contraindicating transplantation, then the endomyocardial biopsy samples must be addressed to immunohistochemical study of dystrophin (antibodies to N-terminus, C-terminus, rod domain and mid-rod domain are commercially available and can be usefully applied to frozen sections of endomyocardial biopsy samples). Conventional light microscopy study does not provide diagnostic information. Electron microscopy may support the diagnosis: sarcolemmal damage is a prominent but non-specific finding. Delta lesions, typically seen in the skeletal muscle of Duchenne muscular dystrophy patients, cannot be identified in endomyocardial biopsy samples, which are largely affected by artifactitious contraction bands. In cases of abnormal dystrophin immunostaining, biochemical and molecular diagnosis is necessary. The former is very useful: Western blotting, employing the same antibodies used for immunohistochemistry, shows abnormalities of the banding pattern, and can guide further investigation to identify underlying gene defects. Western blot analysis requires tissue samples large enough to obtain sufficient amount of proteins. Routine endomyocardial biopsy does not provide such a large amount of tissue, but skeletal muscle biopsy can be performed. However, before

Figure 1 (a) Pedigree derived from index patient interview: suspected familial disease (arrow indicated proband/index patient; gray squares represent suspected, not proven affected subjects); (b) the same pedigree obtained after evaluation of clinical reports: the father of the proband was affected and, at clinical and echocardiographic screening of informed and consenting relatives who accepted the clinical evaluation, an older brother is affected, and the end-diastolic volume of one of the two sons of the proband is increased (red square). The paternal uncle as well as other members of the family (II) remains uninformative. DCM=dilated cardiomyopathy.
progressing to further invasive approaches, an extensive molecular investigation can be done on peripheral blood DNA. Given that the so-called X-linked cardiomyopathies are mostly associated with two types of dystrophin gene defects: (1) proximal, involving either muscle promoter and early exons[1-7], or, (2) Becker-type, affecting rod domain exons[8-10], the early phases of molecular diagnostic tests may aim at screening for deletions (the most common defects) in these regions, or for point mutations known to be associated with dilated cardiomyopathy (www.dmd.nl/xldc.html). The screening is done with multiple and single polymerase chain reactions, and with sequencing of given gene regions[11].

When analysing peripheral blood DNA, defects are identified in more than 80% of cases. In patients in whom the defect is not found with the above techniques, the molecular analysis should progress further. If the defect is identified, then the molecular investigation can be easily extended to family members, to diagnose the disease early in younger brothers or maternal uncles and male cousins. Furthermore, female carriers (sisters and maternal aunts and female cousins, while daughters are 'obliged' carriers) can be identified from a microsatellite study. Overall, the molecular and immunohistochemical screening, done in 201 consecutive dilated cardiomyopathy male patients at our centre, identified 13 patients affected by dystrophin defects (6.4%)[11].

2. If the patient is a male with increased creatine phosphokinase and a proven pathological or molecular diagnosis of Becker muscular dystrophy the disease should be considered as a cardiomyopathy in Becker muscular dystrophy[12]. Endomyocardial biopsy is not necessary. Molecular analysis can be performed from peripheral blood DNA.

3. If the patient is male or female, with increased serum creatine phosphokinase, and suspected recessive inheritance, the expression of dystrophin-associated glycoproteins should be immunohistochemically evaluated on endomyocardial biopsy samples with commercially available specific antibodies (alpha, beta, gamma, delta sarcoglycan, beta dystroglycan, merosin, and spectrin) (Fig. 4). If immunostains identify a defect in one of the above glycoproteins, the relative gene can be analysed (Fig. 5). No defect in the dystrophin-associated glycoproteins has been identified to date in our (n=261) and other series of isolated dilated cardiomyopathy patients. However, the recent finding of a deletion affecting the first

Figure 2 Evidenced-based diagnosis of familial dilated cardiomyopathy. Key points for patient evaluation and family history interpretation. DCM=dilated cardiomyopathy. *two major trends in centres dedicated to congestive heart failure: heart transplant tertiary centres; endomyocardial biopsy performed routinely as a part of the pre-transplant protocol; endomyocardial biopsy performed exclusively on clinical hypothesis of myocarditis, or of storage diseases; primary and secondary centres: familial, autosomal dominant inheritance

Table 1: Diagnosis of familial DCM

<table>
<thead>
<tr>
<th>1st STEP</th>
<th>2nd STEP</th>
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<tbody>
<tr>
<td>Clinical examination</td>
<td>Family pedigree</td>
</tr>
<tr>
<td>Patient history</td>
<td>AFFECTED FIRST AND/OR SECOND-DEGREE RELATIVES</td>
</tr>
<tr>
<td>Electrocardiography</td>
<td>Clinical, non-invasive screening of informed consenting relatives</td>
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<tr>
<td>Coronary angiography</td>
<td>Genetic analysis of affected subjects</td>
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<tr>
<td>Endomyocardial biopsy*</td>
<td>- Linkage analysis (candidate gene screening)</td>
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Figure 3 Critical clinical elements for groups of patients with familial cardiomyopathies. DCM=dilated cardiomyopathy; CPK=creatine phosphokinase; DAG=dystrophin associated glycoproteins.
portion of the gene coding for delta sarcoglycan in the hereditary cardiomyopathic hamster (BIO14.6) is encouraging further studies in this direction\textsuperscript{[13]}.  

4. If the patient is either a male or a female, with signs such as lactacidaemia, hypoacusia, palpebral ptosis, myopathy with ragged red fibres, ophthalmplegia, encephalopathy, retinitis pigmentosa, or other mitochondrial-related phenotype, then a mitochondrial disease can be suspected. Given that mitochondrial disorders can be linked to either mitochondrial or nuclear DNA defects, a mendelian pattern of inheritance does not exclude a mitochondrial disease\textsuperscript{[14]}. Little is known about nuclear DNA defects causing these diseases. In contrast, the information available on mtDNA defects is progressively increasing, as is
the number of mutation/defects associated with mitochondrial diseases\cite{15}. In these latter cases, a matrilineal inheritance may be identified in the familial pedigree. However, it is not unusual for the disease to present clinically as sporadic, especially in small families. Furthermore, the different amount of mutated DNA in maternal relatives, as well as in different tissues of the same subject, complicates the understanding of the clinical phenotype. Moreover, a same defect may show different phenotypes: A1555G mutation, characteristically associated with deafness, has been recently described in a patient with dilated cardiomyopathy\cite{16}. This means that there is a wide phenotypic heterogeneity based on identical gene defects. Ultrastructural study of endomyocardial biopsies from these patients always shows mitochondrial abnormalities: giant mitochondria, concentric cristae, and intramitochondrial inclusions\cite{17}. Light microscopy abnormalities are non-specific; however, histoenzymatic reactions for mitochondrial oxidative enzymes (cytochrome C oxidase and NADH dehydrogenase) may document a reduction of the enzyme activity, if the defect affects mitochondrial genes coding for either enzyme subunits or transfer RNAs\cite{18}. Succinate dehydrogenase activity reduction is extremely rare and may derive from nuclear defects, since all succinate dehydrogenase subunits are coded by nuclear genes.

Analysis of mtDNA defects is made easy by the possibility of screening the entire DNA, which has been entirely coded since 1981\cite{19}. Furthermore, a series of consolidated notions helps in assessing the possible pathological role for any known or novel mutation: heteroplasma, absence of the mutation in healthy control series, defects affecting conserved regions, all contribute to the likelihood that the mutation plays a role in the disease\cite{14,15,17}. Once the defect has been identified, the only clinical implication is for genetic counselling; no specific or effective treatment strategy exists for mitochondrial cardiomyopathies.

5. If the patient is a child presenting with hypoglycaemia without subsequent ketonuria, cardiomegaly, and chronic muscle weakness, carnitine deficiency should be suspected. Carnitine plays a key role in the metabolism of fatty acids, since it is essential for their transfer into the mitochondria. The causes of carnitine deficiency can be grouped as primary defects, caused by mutations of the genes coding carnitine transmembrane transport systems\cite{20,21}, and secondary defects, consequent to errors in mitochondrial fatty acid oxidation, with excess carnitine loss at the kidney level\cite{22}. The disease can also present with arrhythmias and conduction defects\cite{23}, or with endocardial fibroelastosis\cite{24}. Despite the defect heterogeneity in carnitine cardiomyopathy, the disease outcome essentially depends on diagnosis. Increasing serum carnitine concentrations by carnitine supplementation may in fact overcome the carnitine transport defect across the cell membrane\cite{25} and allow adequate intracellular carnitine concentration.

6. If the patient is a male child, with dilated cardiomyopathy, short stature, and granulocytopenia, then a Barth syndrome has to be suspected, and the methylglutaconic acid should be measured in the urine. Barth syndrome is a rare X-linked disease of paediatric age; the gene and its product (recently identified and named ‘tafazzin’) are known, and the molecular diagnosis can be performed\cite{26}.

7. If the patient is diagnosed with cardiomyopathy and associated atrioventricular block, a series of known disorders has to be ruled out:

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### Table 1 Results of gene defect screening in the overall series of 1277 dilated cardiomyopathy patients at the Cardiology and Pathology Departments of the IRCCS Policlinico S. Matteo, Pavia from 1985 to 1999

<table>
<thead>
<tr>
<th>Defect/disease</th>
<th>Number of screened cases</th>
<th>Number of defects identified</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystrophin</td>
<td>228†</td>
<td>16</td>
<td>Screening done in consecutive series</td>
</tr>
<tr>
<td>Dystrophin-associated glycoproteins</td>
<td>261</td>
<td>0*</td>
<td>Screening done in consecutive series</td>
</tr>
<tr>
<td>MtDNA</td>
<td>150</td>
<td>24</td>
<td>Patients selected the overall endomyocardial biopsy investigated with electron microscopy</td>
</tr>
<tr>
<td>Emerin</td>
<td>210</td>
<td>0†</td>
<td>Immunohistochemical screening</td>
</tr>
<tr>
<td>Desmin gene defect in dilated cardiomyopathy</td>
<td>52</td>
<td>0</td>
<td>Screening done in consecutive series</td>
</tr>
<tr>
<td>Actin</td>
<td>52</td>
<td>0</td>
<td>Screening done in consecutive series</td>
</tr>
</tbody>
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*Data upgraded at January 2000.
†Present series is mostly constituted of isolated dilated cardiomyopathy.
(a) haemochromatosis: the A–V block may be the first clinical marker of this disease; left ventricular dysfunction comes later, often in the end-stage disease [27]. Serum ferritin levels and serum transferrin saturation play a diagnostic role. The study may extend to the identification of the known molecular defects of hereditary haemochromatosis (HFE/HLA-H) gene (Cys282Tyr, His63Asp and Ser65Cys mutations). In young patients, the disease is unlikely to be related to the HFE gene [28], for instance, only the Cys282Tyr seems to carry a causative link with the disease. On the other hand, older patients are more likely to carry HFE gene defects. The paramount role played by correct and early diagnosis (early treatment with venesection) makes it mandatory to exclude this disease.

(b) X-linked emerin defects [29]: patients characteristically present with Emery–Dreifuss disease, and cardiologists are ‘secondarily’ involved in the management of the cardiac dysfunction, once the diagnosis has been done in the neuromyology clinical setting. The pathological approach to the diagnosis is easy: emerin is a nuclear membrane protein [30], which can be identified by immunostaining endomyocardial biopsy sections with specific antibodies [31]. The gene is known, and molecular diagnosis can be obtained and usefully employed for family studies [29].

(c) idiopathic cardiomyopathy with A–V block: this is the case of familial dilated cardiomyopathy with a slowly progressing ‘benign’ phenotype. From the onset of A–V block to the development of dilated cardiomyopathy there is an interval of decades. In a consecutive series of 104 patients, only one (a sib-pair) of 26 familial dilated cardiomyopathy cases consecutively evaluated in 4 years presented first with A–V block which later evolved to dilated cardiomyopathy. Two loci associated with this subset of dilated cardiomyopathies have been identified [32,33].

One of them (1p1–1q1) contains the gene for the nuclear envelope proteins lamin A and C (LMNA gene) [34]. Mutations in the head and tail domains of this gene are known to cause the autosomal form of Emery–Dreifuss muscular dystrophy [35]. A recent study identified five novel mutations in the rodomain of the lamin A/C gene, associated with progressive conduction system disease and familial dilated cardiomyopathy [34]. Screening index patients with early A–V block and late dilated cardiomyopathy from our historical database, we identified, in three unrelated patients, three novel mutations in the lamin A/C gene causally linked with the disease.

(d) A–V block is the rule in desmin storage disease with or without restrictive cardiomyopathy. The disorder is autosomal dominant, often associated with skeletal muscle involvement of variable extent/severity. Desmin accumulations in cardiac and skeletal muscle myocytes are easily diagnosed and characterized at electron and immunoelectron microscopy: the typical granulofilamentous material immunoreacting with antisemin antibodies represents the diagnostic marker of the disease [36]. A diagnostic role is played by light microscopy immunohistochemistry only for skeletal muscle biopsy, where desmin accumulations typically localize in the subsarcolemmal areas; for endomyocardial biopsy samples, light microscopy immunohistochemical study is not informative and may be similar to that of other cardiomyopathies [36].

The disease gene is intriguing: since original linkage analysis had excluded desmin as the disease gene [37], the defect was thought to derive from post-transcriptional events, and other genes have been proposed as potential candidates. In particular, a missense mutation in the alpha-B-crystallin gene has been identified in the affected members of one family with demin storage disease [38]. The role of alpha-B-crystallin in desmin accumulation has been ascribed to the interaction between the two proteins in intermediate filament network assembly, which is negatively affected by the mutation [38]. However, a heterozygous missense mutation in the desmin gene in one affected family, and compound heterozygosity for two other mutations in another family, have been reported [39]. Very interestingly, a recent study has shown a further desmin gene defect in familial dilated cardiomyopathy without desmin accumulation [40]. Since desmin is an essential intermediate filament linking Z bands, its gene represents an optimal candidate for dilated cardiomyopathy as well as for other types of cardiomyopathy.

8. The majority of dilated cardiomyopathy patients with exclusive cardiac disease, familial or not, does not present increased creatine phosphokinase, A–V block, or other non-cardiac signs useful in addressing molecular investigation. No peculiar marker is associated with their cardiac dysfunction, and no clinical sign offers a guide for molecular genetic studies: all patients look alike.

For familial diseases that do not fit the above seven phenotypes and relative genetics, several disease loci, and three disease genes have been identified in the last 5 years by linkage analysis [41–46]. Actin [47] and desmin gene [40] defects have been recently described in a few unrelated families. In dilated cardiomyopathy disease loci, genes, mutations are emerging as private loci, genes, mutations, as in hypertrophic cardiomyopathy. Without linkage analysis, the identified defects are likely to play a role, but their causative link
is not fully proven. The intriguing involvement of a same gene (e.g. actin) in different phenotypes (dilated cardiomyopathy[47] and hypertrophic cardiomyopathy[48]) is providing the bases for screening in familial cardiomyopathies. Two recent studies have been dedicated to actin gene screening: one was done in 136 Japanese patients and one in 57 patients, most of these latter of black African origin[49,50]. Both were negative. Therefore, actin gene mutations must be very rare and some useful marker (morphological and immunohistochemical) should be identified to guide the screening itself before progressing with further studies. A further Caucasian series of dilated cardiomyopathy patients, collected in Paris and Pavia, screened for desmin and actin gene defects have given negative results (Eur Heart J, in press).

What strategy of investigation should be followed: molecular screening of candidate genes or linkage analysis in large families to add further chromosome loci to the existing list? Or, given that neither linkage nor candidate gene analysis are providing consistent data, none of them? Or, in order to progress with knowledge, both of them? This latter working hypothesis is likely the most useful, with the major aim of identifying the genetic bases of the disease to provide molecular diagnosis. At present, however, information for patients should not rely on molecular genetics, but rather on clinical examination, assessment of the familial condition of the disease, identification of pre-clinical signs and of asymptomatic patients, prevention of life-threatening ventricular arrhythmias, and counselling.

Conclusions

Although the knowledge on molecular genetics in dilated cardiomyopathy is progressively increasing, the number of molecular diagnoses that can be provided to patients is limited to a few X-linked, autosomal dominant, and matrilinear dilated cardiomyopathies, as well as to extremely rare myopathies with heart involvement. Large molecular screening studies for candidate genes are partially justified by preliminary reports of candidate gene defects cosegregating with the disease in a few dilated cardiomyopathy families. In case of some positive results from a wide-screening approach, ‘mutated healthy subjects’ will soon ask clinicians for some ‘protection’ from the disease. When ‘potential patients’ bearing a molecular diagnosis of a gene defect know that nothing can be done, they could regret being informed, or they will ask to be strictly followed-up and/or treated to prevent cardiac events and disease evolution. Decades of follow-up will be necessary to learn more: either other disease genes, or other mechanisms of disease will come out.

Cardiologists will have to play a difficult role in the management of their patients and relatives: how to handle the problem, who will pay for screenings, who will take the responsibility of assigning a predictive diagnostic value to pathological mutations? What type of projects will be planned by the public health institutions, and who will provide numbers to calculate the need, when literature is publishing few ‘scientifically fascinating cases’? Major multicentric research projects are necessary to plan useful molecular genetic analysis for the prevention and care of dilated cardiomyopathy. International networks of reference centres should be created, converging in large databases, which may be thought of as collective memory tools of evidence-based genetic-cardiology. The major Scientific Societies should promote such networking and, as Europeans, we would see the European Society of Cardiology as the natural promoter of aggregated research in this field. Only large multinational research projects are likely to provide the information needed to plan useful prevention and care strategy for dilated cardiomyopathy.

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References


