

Review Article

Guidelines for the study of familial dilated cardiomyopathies

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Project on Familial Dilated Cardiomyopathy**

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Introduction

Dilated cardiomyopathy is a heart muscle disease characterized by 'dilatation and impaired contraction of the left ventricle or both ventricles. It may be idiopathic, familial/genetic, viral and/or immune, alcoholic/toxic, or associated with recognized cardiovascular disease in which the degree of myocardial dysfunction is not explained by the abnormal loading conditions or the extent of ischaemic damage^[1]. Dilated cardiomyopathy represents a significant health problem as it can lead to progressive refractory heart failure, and represents the majority of heart transplantation indications. This condition is also associated with a high rate of sudden death due to ventricular arrhythmias and a high mortality rate of 15% to 50% at 5 years^[2]. The prevalence of dilated cardiomyopathy in the US population, using diagnostic criteria for advanced disease, was estimated to be 36.5 per 100 000 persons^[3]. The aetiology and the pathogenetic mechanisms are unknown in approximately half of the patients^[4]. Moreover, the importance of genetic factors has been underestimated for a long time. In 1981, in a Mayo Clinic retrospective study^[5], the percentage of familial cases was estimated to be 2%. Since then, several prospective studies have clearly demonstrated the exist-

ence of genetic transmission of the disease^[6,7] detectable in at least 25% of dilated cardiomyopathy patients^[8–10]. However, the true frequency is still probably underestimated as, at the moment, except for the familial history, no clinical or histopathological characteristic allows a distinction between familial and non-familial cases. Furthermore, as demonstrated in hypertrophic cardiomyopathy^[11], it is possible that some cases considered to be sporadic are, in fact, due to de novo mutations and potentially transmissible to descendants, to the incomplete and age-related penetrance, or to an insufficient family evaluation^[12].

Familial dilated cardiomyopathy is a heterogeneous disorder^[13,14] as suggested by different patterns of inheritance, with autosomal trait prevailing, and variable clinical features. Accordingly, based on clinical and molecular genetic data, different forms can be distinguished: (1) the autosomal dominant pure familial dilated cardiomyopathy, for which three known chromosomal loci have already been mapped on chromosome 9q13-22^[15], 1q32^[16], and 10q21-23^[17], respectively; (2) the autosomal dominant form in which dilated cardiomyopathy appears after conduction defects, with two disease loci mapping on chromosome 1p1-1q1^[18] and 3p22-p25^[19]; (3) the autosomal dominant form associated with myopathy^[20,21]; (4) the autosomal recessive form; (5) the X-linked dilated cardiomyopathy due to dystrophin gene deletions/mutations both in the critical region of the 5' end^[22–25] and the region of exons 48–49^[26]; (6) mitochondrial dilated cardiomyopathy, characterized by a matrilineal transmission, (7) and,

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See Appendix for the names of participants in the Research Group.

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finally, right ventricular cardiomyopathy which can mimic dilated cardiomyopathy, and other unclassifiable forms of cardiomyopathies.

The detection of a familial trait represents a major advance in the understanding of the aetiology of dilated cardiomyopathy, since this indicates that the cause of the disease is related to a DNA mutation, providing the first clear indication of the pathogenesis of the disease. Molecular genetics offers the tools to detect the DNA defects, such as in the case of long QT syndrome or hypertrophic cardiomyopathy. Pedigree evaluation represents an essential initial step for the performance of molecular genetic studies for the identification of the disease gene.

However, the study of pedigrees of familial dilated cardiomyopathy for genetic analysis remains a difficult task:

1. *The phenotype of affected individuals is not specific for the disease.* At present, the diagnosis of the disease relies mainly on the echocardiographic, radionuclide or angiographic demonstration of a reduced left ventricular ejection fraction and of an increased left ventricular end-diastolic diameter, and on the exclusion of any known cause of myocardial dysfunction. However, the clinical spectrum of the disease is broad and includes supraventricular or ventricular arrhythmias, sudden death, stroke and conduction disease. Given the fact that the chance of inheriting the gene defect is 1:2 in the autosomal dominant trait, the probability of being affected in the presence of mild dilatation and/or hypokinesia, or in the presence of unexplained conduction defects or arrhythmias is high in a given family. However, the clinical identification of subjects with the affected status — a key issue for linkage analysis — may be risky, in particular in elderly generations where the prevalence of other diseases responsible for the same cardiac phenotype, such as hypertension and coronary artery disease, is high.

2. *The families tend to be small.* In most families reported in the literature^[6,7,10] and observed in clinical practice, the size of the single families is too small to allow a classical linkage analysis for the identification of the disease locus and, then, of the disease gene. Typically, these families include only two to four affected individuals and are insufficient to provide adequate statistical power for the identification of the disease locus. This raises the need for collecting a large number of families, and of accurate diagnostic criteria, with the possibility of undergoing other genetic strategies, such as non-parametric approaches.

3. *The penetrance of the disease is variable.* Another characteristic of the disease is its variable penetrance, which was assumed to be 80% in families with an autosomal dominant pattern of transmission^[12]. However, the clinical manifestation of the disease appears to be typically age-dependent and, in a series of Italian families, it was estimated to be 10% for young, i.e. below 20 years, 34% in those aged 20–30 years, 60% in those aged 30–40 years and 90% for subjects older than 40 years^[12]. This finding points out the difficulties of estab-

lishing the phenotype, in particular for young individuals, who can be carriers of the disease without any clinical manifestation.

4. *There is no consensus on diagnostic criteria.* Most scientific groups working in the field of familial dilated cardiomyopathy have adapted criteria from the World Health Organization. If there is general agreement for obviously affected individuals with marked cardiac dilatation and profoundly reduced ejection fraction, differences exist for less typical clinical situations^[27]. In that context, a European working group was established in order to reach consensus on major and minor criteria.

Based on the idea that the presence of mild cardiac abnormalities would have a high probability of being the expression of a gene disease in the context of a family, the European Collaborators propose that the diagnosis of familial dilated cardiomyopathy would be fulfilled in a first-degree relative in the presence of:

- one major criterion
- or left ventricular dilatation + one minor criterion
- or three minor criteria

The status 'unknown' would be fulfilled by the presence of one or two minor criteria.

Identification of patients at high risk and the assessment of the clinical status of each family member is crucial for family counselling as well as for genetic analysis. With this in mind, the aim of the present work is to define the diagnostic criteria of familial dilated cardiomyopathy.

Criteria of diagnosis of idiopathic dilated cardiomyopathy (Table 1)

The diagnosis of dilated cardiomyopathy is based on the presence of the criteria given below (Manolio *et al.*, modified^[28]).

1. Ejection fraction of the left ventricle <0.45 (>2 SD) and/or fractional shortening $<25\%$ (>2 SD), as ascertained by echocardiography radionuclide scanning or angiography.

2. Left ventricular end-diastolic diameter $>117\%$ of the predicted value corrected for age and body surface area^[29], which corresponds to 2 SD of the predicted normal limit + 5%.

Comment: A diagnostic assessment for clinical and molecular genetic studies requires simple and, whenever possible, quantitative criteria. For these reasons, the criteria provided by Manolio *et al.*^[28] were chosen. However, the method for evaluating left ventricular dilatation was changed from the indicized left ventricular end-diastolic diameter to the equation of Henry^[29]. In fact, the limit of $2.7 \text{ cm} \cdot \text{m}^{-1}$ ^[30] appeared to lack sufficient specificity, whereas the Henry equation $(45.3(\text{BSA})^{1/3} - 0.03(\text{age}) - 7.2)$ allows a joint estimation of the adult and paediatric population, a major problem in family studies. As a more conservative approach, a limit of 117% of the value predicted for age

Table 1 Check list of the laboratory and histological examinations suggested as baseline evaluation and in special conditions

Basic examination	Comments
Complete blood count	
Glycaemia	
Serum creatinine	
Serum electrolytes	
Hepatic enzymes (ALT, AST)	
Bilirubin	
Cholesterol (total HDL)	
Triglycerides	
Serum creatine phosphokinase (CK)	Expressed in time/normal
Lactate and pyruvate	If matrilinear pattern of inheritance
Sedimentation rate	In children with suspect inflammation
C-reactive protein	In children with suspect inflammation
Thiamine	In children
Urinary and serum carnitine and acyl carnitine	In children
Urinary organic acids and amino acids	In patients under 16 years of age or recessive inheritance
Special investigations	
Ophthalmological examination	If mitochondrial disease suspected and multiorgan disease
Endomyocardial biopsy and skeletal muscle biopsy	
Haematoxylin and eosin	EMB, SMB
Verhoff–Van Gieson	EMB, SMB
Tricromica di Gomori	EMB, SMB: rods structure and abnormal mitochondria
Cytochrome C oxidase	SMB: distinction between fibres type I–II
PAS	EMB, SMB: glycogen
Oil red O or Sudan Black	EMB, SMB: lipids
Special histological investigations	
Mitochondrial DNA	EMB, SMB: if abnormal cytochrome C oxidase
Nicotinamide dehydrogenase	EMB, SMB: if abnormal cytochrome C oxidase
Succinyl dehydrogenase	EMB, SMB: if abnormal cytochrome C oxidase
Acyl CoA dehydrogenase	SMB: if abnormal cytochrome C oxidase
ATPase	SMB: if abnormal cytochrome C oxidase
Special immunocytochemical investigations	
Dystrophin (C-ter, N-ter, rod domain)	EMB, SMB
HLA I and II	EMB, SMB: inflammation
Fetal myosin	SMB: to identify regenerative fibres
δ -sarcoglycan	SMB: in recessive cases or skeletal muscle involvement

EMB=endomyocardial biopsy; SMB=skeletal muscle biopsy. C ter=terminal; N ter=N terminal.

and body surface area was selected, corresponding to $2\text{ SD} \pm 5\%$.

Exclusion criteria

Exclusion criteria were considered:

- systemic arterial hypertension ($>160/100$ mmHg documented and confirmed at repeated measurements and/or evidence of target-organ disease^[31]);
- coronary heart disease (obstruction $>50\%$ of the luminal diameter in a major branch);
- history of chronic excess of alcohol consumption (>40 g \cdot day⁻¹ for female and >80 g \cdot day⁻¹ for males for more than 5 years, according to the WHO criteria^[32]), with remission of dilated cardiomyopathy after 6 months of abstinence;
- clinical, sustained and rapid supraventricular arrhythmias;
- systemic diseases;
- pericardial diseases;
- congenital heart disease;
- cor pulmonale.

Comment: All the above can lead to phenocopies and must be taken into account in a genetic study. Hypertension is a particularly important problem: mild or borderline systemic hypertension, which is very frequent in the overall population, cannot explain significant left ventricular dysfunction. However, moderate to severe and sustained hypertension can lead to phenocopy.

Likewise, a high alcohol intake is considered a potential independent cause of myocardial damage: therefore, it is suggested that the clinical status be assessed after 6 months of abstinence.

Criteria for the diagnosis of familial dilated cardiomyopathy

The diagnosis of familial dilated cardiomyopathy is made:

1. in the presence of two or more affected individuals in a single family

2. or in the presence of a first-degree relative of a dilated cardiomyopathy patient, with well documented unexplained sudden death^[1] at <35 years of age.

Definition of the clinical status of members of families with familial dilated cardiomyopathy

Major criteria

1. Defined criteria for dilated cardiomyopathy (as stated above).

Minor criteria

1. Unexplained supraventricular (atrial fibrillation or sustained arrhythmias) or ventricular arrhythmias, frequent ($>1000 \cdot 24 \text{ h}^{-1}$) or repetitive (three or more beats with $>120 \text{ beats} \cdot \text{min}^{-1}$) before the age of 50;
2. Left ventricular dilatation $>112\%$ of the predicted value^[29];
3. Left ventricular dysfunction: ejection fraction $<50\%$ or fractional shortening $<28\%$;
4. Unexplained conduction disease: II or III atrioventricular conduction defects, complete left-ventricular bundle branch block, sinus nodal dysfunction;
5. Unexplained sudden death or stroke before 50 years of age;
6. Segmental wall motion abnormalities (<1 segment, or 1 if not previously present) in the absence of intra-ventricular conduction defect or ischaemic heart disease.

Assessment of the clinical status (Table 1)

1. Affected

- presence of the major criteria (left ventricular dilatation and systolic dysfunction)
- or left ventricular dilatation ($>117\%$)+one minor criterion
- or three minor criteria

2. Unknown

- presence of one or two minor criteria

3. Unaffected

- individuals with normal hearts
- the presence of other causes of myocardial disease

Comment: A major problem in family studies is the assessment of minor cardiological abnormalities and the definition of the limits between normal and affected. Moreover, very little is known about early signs of disease. Finally, the full criteria required for the diagnosis of dilated cardiomyopathy in a proband appear too restrictive in the context of a familial dilated cardiomyopathy, where the potential risk of disease among relatives is higher than in the normal population.

The proposed minor criteria, based on the experience from family studies may be stringent, but less conservative criteria could seriously affect further genetic studies, such as linkage analysis.

Methods of investigation

Protocol for the probands evaluation (Fig. 1)

1. Basic evaluation

- Accurate (three or more) family history with pedigree construction;
- Physical examination, with particular attention to neuromuscular characteristics;
- chest X-ray (cardiothoracic ratio);
- standard and ambulatory electrocardiography;
- echocardiography: M-mode, 2D and Doppler and colour flow;
- effort test: treadmill or bicycle maximal, symptom limited, with increments of $10 \text{ Watt} \cdot \text{min}^{-1}$ (modified Bruce protocol) is suggested;
- Standard laboratory examination, including serum creatine kinase (Table 1).

2. Suggested studies

- Haemodynamic, ventriculographic and coronary angiographic study, in particular in the subject with suspected ischaemic heart disease^[33], modified); male >40 years, risk factors for ischaemic heart disease, chest pain on exertion, positive effort test for ischaemia, suspected ischaemic left ventricular dysfunction;
- Non-invasive evaluation of myocardial ischaemia, such as dobutamine echocardiography or thallium scintigraphy;
- Right and/or left endomyocardial biopsy: morphology, immunohistochemistry, tissue bank (\geq two samples, stored at -80°C) for molecular virology and molecular biology studies;
- radionuclide ventriculography;
- Signal averaged electrocardiography;
- Serum bank to analyse cardiac organ-specific auto-antibodies^[34];
- Enzyme dosage (troponin T);
- Clinical evaluation by a neuromuscular specialist;
- CT scan for muscular involvement;
- $\text{VO}_2 \text{ max.}$;
- In the case of abnormalities of the neuromuscular apparatus: (a) needle skeletal muscle biopsy (usually quadriceps), samples for histology and immunohistochemistry; (b) when metabolic disorder suspected: electron microscopy, carnitine, carnitin-palmitil-transferase; (c) suspected dystrophinopathy, based on high MM-creatine kinase or pathological immunocytochemistry: multiple PCR amplification of the dystrophin gene, Western blot, mutational analysis;
- Study of viral persistence.

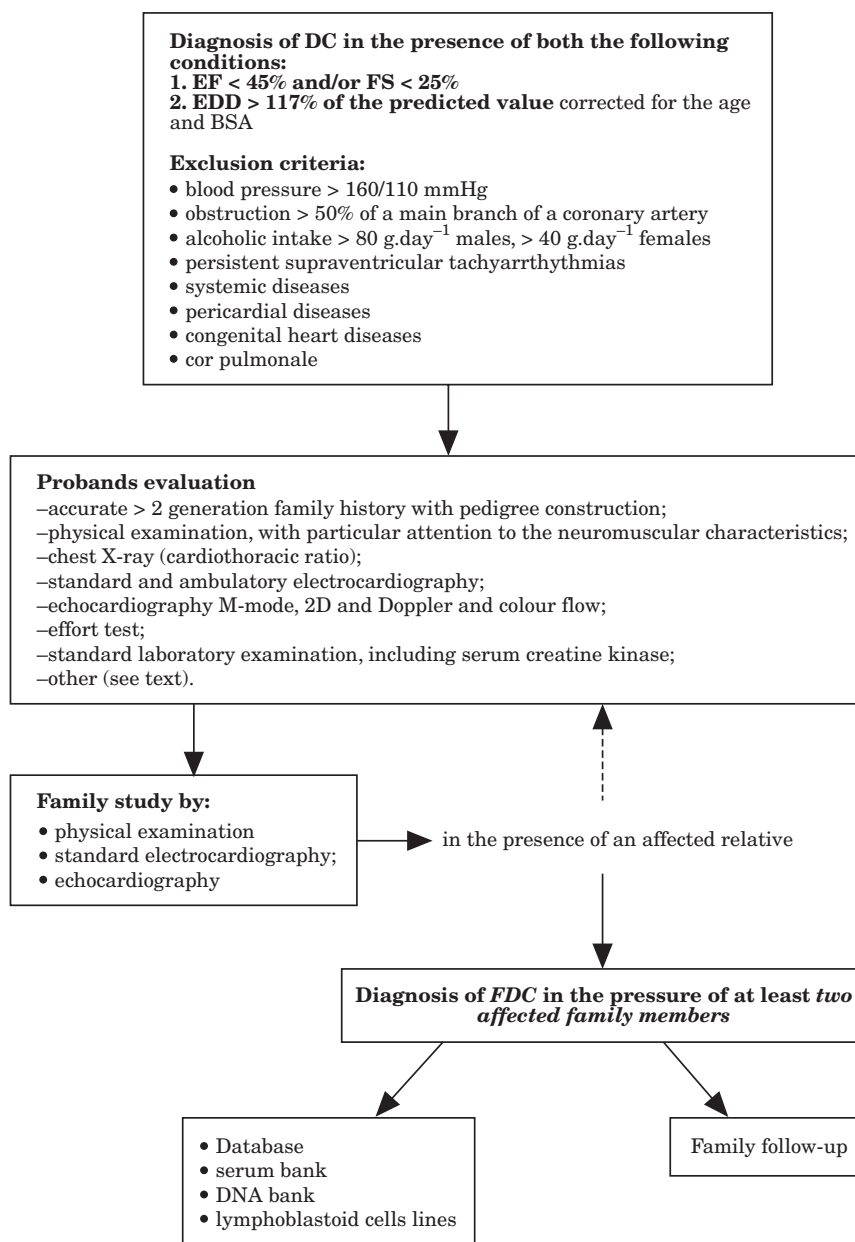


Figure 1 Protocol for the clinical and molecular genetic studies of familial dilated cardiomyopathy (DC). EF=ejection fraction; FS=fractional shortening; EDD=end diastolic diameter; BSA=body surface area.

Comment: The study of the proband reproduces the consensus criteria for the diagnosis of dilated cardiomyopathy.

An invasive evaluation with coronary angiography and endomyocardial biopsy should be performed in probands over 35 years of age, particularly in the presence of risk factors. Therefore, in affected relatives, it is suggested that an invasive study be performed only when strictly needed, taking into account the growing difficulties in performing catheterizations and the risk/benefit ratio in terms of accuracy of diagnosis^[33]. On the other hand, when a coronary angiogram cannot be performed, a non-invasive test for induction of ischaemia should be conducted.

Chronic inflammation of the myocardium due to viral infection and/or autoimmune reactions has long been suspected of having pathogenetic significance, leading to ventricular dilatation and dysfunction. Interstitial lymphocytic infiltrates are often found in endomyocardial biopsies, supporting the view that dilated cardiomyopathy is a residue to active myocarditis^[35,36]. Viral genome has been reported to be present in endomyocardial biopsies of patients with dilated cardiomyopathy. However, its relevance is still controversial: in particular, it is not clear whether viral persistence causes myocardial damage directly or by means of an autoimmune reaction^[37-41].

Recent evidence indicates that autoimmunity may play a particular role in familial dilated cardiomyopathy, since sera of affected and unaffected family members contain organ-specific antibodies^[42,43]. This finding may reflect a genetically determined susceptibility for cardiac inflammation due to microbial agents, leading to a specific autoimmune reaction. Corresponding antigens so far identified include mitochondrial, sarcolemmal or cytosolic proteins^[34,43–47].

Abnormalities of the neuromuscular apparatus should be suspected in the presence of: elevated creatine kinase serum levels, a familial history of muscle disease, X-linked or maternal transmission, pathological electromyography, calf hypertrophy, muscular hypotrophy, cramps, rigidity or muscle weakness. Many neuromyopathic diseases are known to show cardiac involvement, such as the Duchenne or Becker muscular dystrophy, which are the result of mutations of the dystrophin gene, the limb-girdle dystrophies, due to mutations of dystrophin associated glycoproteins, mitochondrial myopathies, Friedreich's ataxia or myasthenia gravis. As previously suggested^[21,48], preliminary data indicate the presence of discrete clinical symptoms of myopathy in a significant number of dilated cardiomyopathy patients^[20,21], and Wilke A., Richter A., Braune H. J., Kuchelmeister D., Portig I., Maisch B. unpublished data, 1998. Taking into account that cardiac symptoms can be the first clinical presentation of the neuromuscular disease^[49], these data stress the importance of an extensive neurophysiological examination, of the electromyogram and of the skeletal muscle biopsy in patients with familial dilated cardiomyopathy.

Family study

1. Evaluation of the proband's relatives

- (a) physical examination
- (b) standard electrocardiography
- (c) echocardiography (M-mode and 2D; colour flow optional)
- (d) signal averaged electrocardiography (optional)
- (e) blood sampling

2. Evaluation of the affected relatives

- Pedigree extension (study of first-degree relatives of new affected family members);
- Non-invasive examinations and, when indicated, invasive examinations (see protocol for the probands evaluation);
- Review of previous hospital records;
- genetic counselling.

Comment: In evaluating a genetic disorder, a simple, reproducible and inexpensive test is required. The physical examination (including an accurate family history) electrocardiography and echocardiography provide a sensitive and specific tool for screening for dilated cardiomyopathy. As in any other inherited disorder, all first-degree relatives at least should be screened.

For affected individuals identified during the family screening, invasive investigations (haemodynamic

studies, ventriculography, coronary angiography, and endomyocardial biopsy) should be performed when strictly indicated by the risk of phenocopies, in particular ischaemic heart disease. Cardiac catheterization can be replaced in these cases by non-invasive tests for the induction of ischaemia.

3. Analysis of retrospective cases

- Review of hospital records;
- Interview of family physicians;
- Interview of multiple informants among close relatives.

Definition of the familial dilated cardiomyopathy subgroups

1. Analysis of the pattern of transmission

- Autosomal dominant
- Autosomal recessive
- X-linked
- Matrilineal

2. Analysis of the special clinical features

- Isolated myocardial forms
- Dominant conduction defects
- Neuromuscular involvement, high MM-creatine kinase
- Multi-organ involvement.

Material sampling

- Clinical database;
- DNA: extracted from 10–40 ml of peripheral blood in EDTA, according to standard procedures;
- Serum bank, according to standard procedures, stored at -20°C ;
- Lymphoblastoid cell line (or isolated lymphocytes): extracted from 10–20 ml of peripheral blood in acetate-citrate-dextrose or heparin^[50];
- if available, tissue bank (endomyocardial biopsy, skeletal muscle biopsies).

Comment: The material sampling should be performed in any examined family member. The collection of the data in a database represents the basis for any further multicentric study on the epidemiology, natural history and treatment of familial dilated cardiomyopathy. The development of a database for dilated cardiomyopathies, particularly addressed to the study of the familial forms is currently in progress and will be available soon (A. Di Lenarda: dilenar@uts.univ.trieste.it). Programs for the construction of pedigrees are available both commercially or by internet.

Molecular genetic studies require the collection of DNA from total blood. The advantage of developing continuous cell lines resides in the availability of a virtually endless source of both DNA and RNA. A tissue bank can be of value for further diagnostic purposes (for instance immunohistochemistry) or the study of gene expression. The serum bank can provide

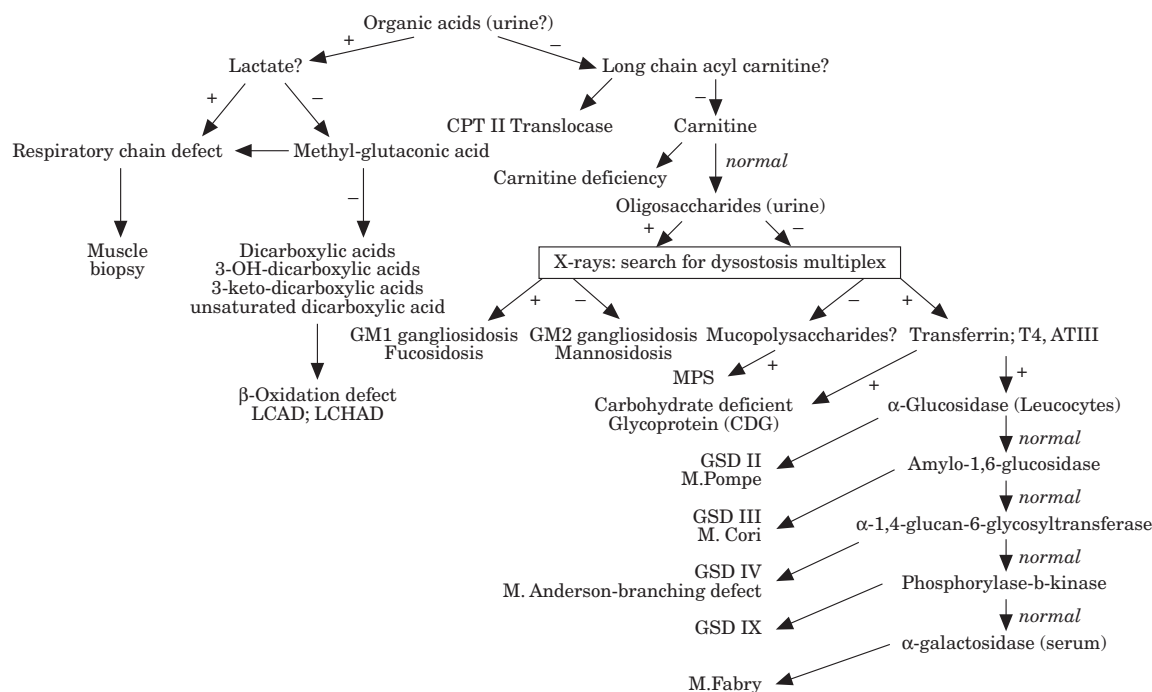


Figure 2 Diagnostic diagram of metabolic cardiomyopathies, to be specially considered in a paediatric population (from Böhles *et al.*, modified^[71]).

important information about immunological aspects of familial dilated cardiomyopathy.

Family follow-up studies

The clinical follow-up of the family should be performed every 2–3 years, by means of:

- Physical examination
- Electrocardiography.
- Echocardiography.

Comment: The follow-up is recommended in both affected and unaffected family members. Very little is known about the evolution of the disease in familial forms: in particular, it is not clear if familial forms have a worse prognosis^[51] and if there is any difference in prognosis or progression among the various forms of familial dilated cardiomyopathies. Another important aspect, as already mentioned, is the possibility of studying the early phases of the disease. For these reasons, a long-term follow-up of both affected and unaffected family members is critical.

Finally, once the molecular defect has been identified, a study of the relationship between the gene defect and the clinical features as well as the prognosis, as observed in hypertrophic cardiomyopathy, could lead to important information which may form the platform for genetic counselling.

Particular forms of familial dilated cardiomyopathy

The following chapters concern forms of left ventricular dilatation and dysfunction which need special considera-

tion and approaches, and can only partially be evaluated by the aforementioned protocol.

Paediatric familial dilated cardiomyopathies

The diagnostic evaluation of children with dilated cardiomyopathy of genetic origin is complicated by the large number of rare genetic causes, the broad range of clinical presentations, and the array of specialized diagnostic tests and biochemical assays (reviewed in detail in Schwartz *et al.*^[52]).

Therefore, in the case of young probands (under the age of 20 years) of families with dilated cardiomyopathy, it is important to take into account some particular features. The diagnosis of dilated cardiomyopathy in childhood is one of exclusion, as for the adult form. In the paediatric population in particular, differential diagnosis includes inborn errors of metabolism, such as amino acidopathies, defects in fatty acid metabolism^[53], glycogen storage diseases^[54], glycoproteinoses, mucopolysaccharidoses and disorders of the extracellular matrix, such as Marfan's and Ehlers-Danlos syndromes, and the Pseudoxanthoma elasticum^[55], as well as inborn heart defects. The diagnostic procedures include electrocardiography, echocardiography and laboratory methods (Table 1, Fig. 2).

Moreover, in children presenting with dilated cardiomyopathy with no identifiable cause^[56–59] an occult viral infection must be considered. The utilization of molecular biology, including polymerase chain reaction, in addition to the clinical symptoms has strengthened this presumption^[59]. Viral cytotoxicity, autoimmune response and virus persistence could be

involved in the disease process. Even if never described before, a familial predisposition to viral infection should be considered as another possible cause of genetic heart muscle disease.

Cardiac involvement in mitochondrial diseases

Mitochondrial disorders may be due to primary defects either in nuclear or in mitochondrial DNA^[60]. The rate of mutation in the mitochondrial DNA is expected to be much higher than that of nuclear DNA^[61]. Mitochondrial diseases usually involve many but not all organ systems of the body: this is mainly due to the different energy requirements and heteroplasmy. Cardiac involvement in mitochondrial diseases has been described in several such diseases^[62–65], Kearns Sayre Syndrome, MERRF, and MELAS. Moreover, mutations/deletions of mtDNA may also be an important contributor to familial dilated cardiomyopathy^[63,64].

In surveys of patients with dilated cardiomyopathy, deletions and point mutations were significantly more frequent compared with the normal controls^[66–68]. These mutations could be secondary to ageing, or a co-factor in the development of the disease, or finally, could primarily cause the disease in a subset of patients (the so-called mitochondrial cardiomyopathy)^[69].

As mitochondrial DNA is inherited through maternal lineage, its mutations should always be considered in case of maternal inheritance and high lactate and mitochondrial respiratory chain abnormalities.

Conclusions

The project of developing a common protocol for the study of familial dilated cardiomyopathy, with defined diagnostic criteria and simple but sufficiently sensitive and specific methods, originated from the need to define a common base to undergo clinical and molecular genetic studies in familial dilated cardiomyopathy.

Except for rare cases, most of the families with this disease are small, or anyway not sufficiently informative to undergo a positional cloning project. As already mentioned, in the case of molecular genetic analysis for mapping the disease gene, a large number of small and well characterized pedigrees is required^[70]. Moreover, some characteristics of familial dilated cardiomyopathy that are found in complex trait, such as reduced penetrance, environmental influences, and genetic heterogeneity, require larger family samples and strict diagnostic criteria.

These guidelines are limited by some arbitrary values used to discriminate between the presence and absence of the disease. They are related, on the one hand, to the need for stringent and specific diagnostic criteria to undergo molecular genetic studies, and, on the other, to the absence of knowledge about the initial manifestations of the disease.

Finally, specific forms of familial dilated cardiomyopathy have been mentioned, to underline the need

for special attention in neuromuscular disorders, mitochondrial defects and in the particular problems of the paediatric population. In our view, this protocol could represent the first step towards a multicentre study on the difficult task of elucidating the molecular basis of dilated cardiomyopathy.

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Appendix

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