Efficiency of metabolic screening in childhood cardiomyopathies

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Aim To estimate the efficiency of metabolic screening in children’s cardiomyopathy.

Methods and Results Blood glucose, lactate, pyruvate and ketone body, and carnitine levels were measured in 58 children referred with a cardiomyopathy of unknown origin. Organic acids, amino acids, oxidation of [1-14C] fatty acids to CO₂ and dehydrogenation of [9,10-3H] fatty acids by lymphocytes were measured. Mitochondrial respiratory chain complex activity was measured in skeletal muscle and in endomyocardial biopsies. Acid α-glucosidase activity was measured in infants with hypertrophic cardiomyopathy. The prevalence of metabolic disorders was 22.4% (13/58–CL95%: 11.4–33.3%): four infants had a storage disease (Pompe’s disease (3), Hurler’s disease (1); two patients had a fatty acid β-oxidation defect (systemic carnitine deficiency (1) and very-long chain acyl-CoA dehydrogenase deficiency (1)); respiratory enzyme deficiency was diagnosed in seven patients. This defect was confined to the myocardium in six. In the remaining 45 patients, metabolic screening was unrevealing.

Conclusion Metabolic screening should be performed in all children with cardiomyopathy as the prevalence of metabolic disorders is high in this population. This may help to define therapeutic strategy and to improve genetic counselling.

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Key Words: Cardiomyopathy, metabolism, children, mitochondria.

See page 682 for the Editorial comment on this article

Introduction

Cardiomyopathy has been recognized as the chief clinical manifestation of a variety of inherited disorders of cardiac energy metabolism[1–5]. During the past decade, new insights into the clinical, biochemical and molecular delineation of these disorders have emerged[6,7]. While myocardial diseases often develop in the course of such metabolic disorders, cardiomyopathy can be the presenting symptom[8,9]. Indeed, the clinical presentation in either fatty acid oxidation disorders or respiratory enzyme deficiencies may be puzzling, masquerading as acute myocarditis or apparently isolated cardiomyopathy. It was necessary to assess the prevalence of metabolic diseases in childhood cardiomyopathies, to define therapeutic strategies and improve genetic counselling. We thus undertook a metabolic screening programme in children referred to our Institution with cardiomyopathy of unknown origin to detect storage diseases, fatty acid β-oxidation disorders and mitochondrial respiratory chain deficiencies.

Methods

Population

Over a period of 3 years, 58 consecutive children aged 1.4 ± 2.3 years (range 2 weeks–16 years) referred for an apparently idiopathic cardiomyopathy were prospectively included in the study. Children exhibiting cardiomyopathy of known cause were excluded: namely previously diagnosed metabolic disorders, neuromuscular diseases (Friedreich’s ataxia, dystrophinopathies, Emery–Dreifus disease), and syndromic cardiomyopathies (Noonan syndrome, Sotos syndrome). Subjects with hypertensive, ischaemic, arrhythmic cardiomyopathies and proven myocarditis were also excluded. In addition, familial hypertrophic cardiomyopathies were excluded when the family history was suggestive of an autosomal dominant inheritance.
Screening for extra-cardiac involvement included studying renal and liver function as well as ophthalmological and neurological examinations.

**Metabolic, molecular and histopathologic investigations**

Blood glucose, lactate, pyruvate and ketone body levels and their molar ratio were determined after deproteinization by perchloric acid in both fasted and fed individuals. The length of the fast was variable according to age and haemodynamic condition ranging from 6 h in infants to 12 h in children. Total and free carnitine levels were measured in serum and in urine. These procedures enable the detection of post-absorptive hyperlactataemia, elevated lactate/pyruvate and ketone body molar ratios, in relation to impaired oxidative phosphorylation, and fasting hypoketotic hypoglycaemia in relation to fatty-acid β-oxidation defects. Organic acid (urine) and amino-acid (plasma) determination were performed by chromatography/mass spectrophotometry. Oxidation of [1-14C] butyric, palmitic and octanoic acids to CO₂ and dehydrogenation of [9,10-3H] myristic and palmitic acids to H₂O by lymphocytes were measured. Acid α-glucosidase activity was measured in infants with hypertrophic cardiomyopathy and other leukocyte enzymes activities were measured in the last 12 cases.

Procedures for endomyocardial biopsies have been previously reported and are widely used for the diagnosis of rejection after cardiac transplantation in our group[10]. Five endomyocardial biopsy samples (1 mg) were obtained from the right ventricle septum or apex in 38/58 patients, of which two were used to analyse tissue morphology, and three were freeze-dried for enzyme activities (cytochrome-c oxidase, succinate cytochrome-c reductase, and α-ketoglutarate dehydrogenase) were performed by chromatography/mass spectrometry. Oxidation of [1-14C] butyric, palmitic and octanoic acids to CO₂ and dehydrogenation of [9,10-3H] myristic and palmitic acids to H₂O by lymphocytes were measured. Acid α-glucosidase activity was measured in infants with hypertrophic cardiomyopathy and other leukocyte enzymes activities were measured in the last 12 cases.

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**Results**

The prevalence of identified metabolic disorders was 22.4% (13/58–confidence limits 95%: 11.4–33.3%) in our series. In addition, four children in whom all the biochemical investigations were negative, had histopathological findings on endomyocardial biopsy suggestive of a metabolic disorder with mild lipidosis.

Four patients had a storage disease: three glycosogenase type II (Pompe's disease, α-glucosidase activity=0) and one patient had type I mucopolysaccharidosis presenting as an isolated cardiomyopathy at 2 months of age (Hurler's disease, α-L-Iduronidase=0.3 nmol . h⁻¹ . mg⁻¹ proteins (n=1–5 nmol . h⁻¹ . mg⁻¹ proteins)). Two patients had a fatty acid β-oxidation disorder: systemic carnitine deficiency in a 12-year-old boy, and very-long chain acyl-CoA dehydrogenase deficiency in a 9-month-old infant. Seven patients exhibited respiratory enzyme deficiency in myocardium: two complex I deficiencies, two complex III deficiencies, one complex IV deficiency, and two complex I+IV deficiencies. This defect was confined to the myocardium in 6/7 patients. One patient expressed cytochrome-c oxidase deficiency in skeletal muscle while her muscular testing was normal at time of diagnosis. No mtDNA rearrangement was found in 38/38 endomyocardial biopsies and none of the point mutations reported in cardiomyopathy were observed in our series. Finally, metabolic screening was unremarkable in the remaining 45 patients.

**Discussion**

We report on a metabolic screening programme in children presenting with apparent idiopathic cardiomyopathy. Our study was designed to diagnose metabolic disorders most frequently responsible for
cardiomyopathy in childhood: namely storage diseases, fatty acid β-oxidation disorders, amino acid metabolism deficiencies and mitochondrial respiratory enzyme deficiencies. The prevalence of these disorders was high in our series since 1/5 patients exhibited such a deficiency.

**Therapy**

Assessing the diagnosis of such deficiencies in cardiac energy metabolism has therapeutic implications. In systemic carnitine deficiency, carnitine therapy is dramatically successful and an almost complete and stable clinical remission can be achieved in these patients. In our patient, oral supplementation with L-carnitine enables complete recovery of the myocardial function within 6 months. Along the same lines, once a diagnosis of fatty acid oxidation is confirmed, the mainstay of treatment is the avoidance of prolonged fasting and administration of intravenous glucose to prevent fatty acid mobilization until the child is able to take oral feeds. Our Hurler patient is on the waiting list for bone marrow transplantation as improvement of cardiomyopathy has been demonstrated in such cases. Finally mitochondrial diseases have been proven to have a poor prognosis. Currently, there is no effective treatment. Further, cardiac involvement worsens the outcome and multi-systemic metabolic disorders are usually a contra-indication for heart transplantation as the enzymological defect is ubiquitous and the expression of the disease multi-systemic. Consequently, one may argue, transplanting the hearts of patients with mitochondrial respiratory chain defects does not prevent extra-cardiac complications related to this defect. However, the peculiar genetic origin of respiratory chain enzymes means that mitochondrial respiratory chain defects may be specifically expressed into myocardium and might remain restricted to this tissue all life long. However, mitochondrial respiratory chain defects might potentially affect any organ or tissue. Specific involvement of one given tissue or organ might result from the occurrence of tissue-specific isoforms of several nuclear-encoded subunits of respiratory chain complexes. On the other hand, it might also result from a potential mitochondrial DNA heteroplasmy (i.e., coexistence of both mutated and normal mitochondrial genomes in cells). Indeed, variable proportions of both mutated and normal mitochondrial genomes, with tissue to tissue and cell to cell differences, are often observed in affected patients. Finally, heart-specific involvement might originate from organ-specific regulation of electron fluxes in the respiratory chain, or from the myocardial-specific requirements of energy supply. In our series, the mitochondrial respiratory chain disorder was apparently heart-specific in some patients and it would have remained undetected if endomyocardial biopsy was not routinely performed in the metabolic screening of severe cardiomyopathies in our Institution. Without data on biochemical expression in tissues other than skeletal muscle, lymphocytes or skin fibroblasts, we cannot rule out that the defect is latent in these tissues. Nevertheless, we did not observe clinically patent extra-cardiac expression of the mitochondrial defect during follow-up in our series. Since mitochondrial respiratory chain defects may be confined to the myocardium, as in 6/7 patients in our series, orthotopic heart transplantation can be proposed in children with proven myocardial oxidative phosphorylation defects and severe isolated cardiomyopathy.

**Prognosis**

The outcome of apparent idiopathic cardiomyopathy in children is uncertain. Therefore, the precise identification of the cause of the disease may guide therapeutic strategies and even lead to abstinence where no treatment is available. Indeed, life expectancy is less than 1 year in glycogenosis type II with infantile onset. However, the cardiomyopathy is just the tip of the iceberg as there is a massive accumulation of glycogen in brain, skeletal muscle and liver. Similarly, the short-term prognosis of fatty acid oxidation disorders with cardiomyopathy is poor.

**Genetic counselling**

Genetic counselling of affected families with an inherited metabolic disease has major consequences. The molecular basis of the various metabolic deficiencies has been characterized and most of them are transmitted as a mendelian trait. All known inborn errors of fatty acid oxidation are autosomal recessive disorders. The molecular basis of the vast majority of these defects is now known and if the mutation in the gene encoding the defective enzyme can be identified in the proband, pre-natal diagnosis can be proposed for a subsequent pregnancy. Equally, the gene encoding the acid α-gluco-cosidase (Pompe’s disease) has been mapped to chromosome 17q23 and a variety of mutations have been described. Pre-natal diagnosis is also available for this autosomal recessive condition.

Genetic counselling is more complex in respiratory enzymes deficiencies. Familial history of cardiomyophathy or sudden death were found in three pedigrees in our series. The structure of these pedigrees did not allow us to ascribe a specific and unambiguous pattern of inheritance for any of them. Indeed, the unique transmission and genetics of mitochondrial defects may account for various patterns of transmission, namely maternal inheritance, X-linked or autosomal inheritance for nuclear encoded respiratory chain peptides. In our series, the absence of mtDNA rearrangements or known point mutation does not preclude occurrence of other mtDNA mutations. Moreover, since the mitochondrial genome encodes only a small proportion of respiratory chain proteins compared to the nuclear genome, it seems...
likely that a significant proportion of mitochondrial oxidative phosphorylation defects is due to mutations in nuclear DNA. According to this and to the confined expression of the defect in the myocardium in most of our patients, pre-natal diagnosis appears speculative in these families.

Conclusion

The metabolic evaluation of paediatric cardiomyopathy is aided by keeping in mind that the heart is often only one of many organs affected in what should be seen as a systemic disease. When an extensive biochemical study is planned in an isolated cardiomyopathy, the first step should be to investigate the possibility of abnormal oxido-reduction status, related to impaired oxidative phosphorylation, and fasting hypokinetic hypoglycaemia in connection with fatty-acid β-oxidation defects. Thereafter, carnitine levels, chromatographies of organic acids and amino acids, and lysosomal enzymes have to be determined. Finally, if these investigations remain unremarkable, endomyocardial biopsy is required. However, careful planning is needed to allocate samples for a wide variety of histochemical, enzymological, and ultrastructural studies. Indeed, the incidence of metabolic cardiomyopathy remains a function of the effort with which it is sought.

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References