

The Mitochondrial Organelle and the Heart

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The heart is highly dependent for its function on oxidative energy generated in mitochondria, primarily by fatty acid β -oxidation, respiratory electron chain and oxidative phosphorylation. Defects in mitochondrial structure and function have been found in association with cardiovascular diseases such as dilated and hypertrophy cardiomyopathy, cardiac conduction defects and sudden death, ischemic and alcoholic cardiomyopathy, as well as myocarditis. While a subset of these mitochondrial abnormalities have a defined genetic basis (e.g. mitochondrial DNA changes leading to oxidative phosphorylation dysfunction, fatty acid β -oxidation defects due to specific nuclear DNA mutations), other abnormalities appear to be due to a more sporadic or environmental cardiotoxic insult or have not yet been characterized.

This review focuses on abnormalities in mitochondrial bioenergetic function and mitochondrial DNA defects associated with cardiovascular diseases, their significance in cardiac pathogenesis as well as on the available diagnostic and therapeutic options. A concise background concerning mitochondrial biogenesis and bioenergetic pathways during cardiac growth, development and aging will also be provided.

Key words: *Mitochondria. Dilated cardiomyopathy. Hypertrophic cardiomyopathy. Ischemic cardiomyopathy. Cardiotoxicity. Apoptosis. Stem cells. Gene therapy.*

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INTRODUCTION

Abnormalities in the mitochondrial organelle structure and function have been found with increasing frequency in association with cardiovascular dis-

La mitocondria y el corazón

Para su correcto funcionamiento, el corazón depende estrechamente de la energía oxidativa generada en las mitocondrias, principalmente a partir de la betaoxidación de los ácidos grasos, de la cadena respiratoria de electrones y de la fosforilación oxidativa. Los defectos en la estructura y función mitocondriales se asocian a enfermedades cardiovasculares, como las miocardiopatías hipertrófica y dilatada, defectos en la conducción cardíaca y muerte súbita, miocardiopatías isquémicas y alcohólicas y miocarditis. Aunque una parte de estas anomalías mitocondriales tiene una base genética definida (p. ej., los cambios en el ADN mitocondrial que conducen a una disfunción de la fosforilación oxidativa, o los defectos de la betaoxidación de los ácidos grasos debidos a mutaciones específicas del ADN nuclear), otras anomalías parecen deberse a agresiones cardiotóxicas o ambientales más esporádicas o a causas todavía no identificadas.

Esta revisión se centra en las anomalías de la función bioenergética mitocondrial y en los defectos del ADN mitocondrial asociados a enfermedades cardiovasculares, su significado en la patogenia cardíaca y las opciones diagnósticas y terapéuticas disponibles. También se describen de forma concisa los antecedentes relacionados con la biogénesis mitocondrial y con las vías bioenergéticas durante el crecimiento cardíaco, el desarrollo y el envejecimiento.

Palabras clave: *Mitocondria. Miocardiopatía dilatada. Miocardiopatía hipertrófica. Miocardiopatía isquémica. Cardiotoxicidad. Apoptosis. Células madre. Terapia génica.*

eases such as dilated (DCM) and hypertrophic cardiomyopathy (HCM), cardiac conduction defects and sudden death, ischemic and alcoholic cardiomyopathy, as well as myocarditis. Some of the mitochondrial abnormalities may have a genetic basis (e.g. mitochondrial DNA [mtDNA] changes leading to oxidative phosphorylation [OXPHOS] dysfunction, fatty acid oxidation defects due to specific nuclear DNA mutations) while other abnormalities appear to be due to a more sporadic or environmental cardiotoxic insult or have not yet been characterized.

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In order to fully understand the role that the mitochondrial organelle plays in cardiovascular disease, we provide in this review a brief background describing the biogenesis and function of cardiac mitochondria during normal growth, development and aging. Within this context, we then survey the interaction and characterization of mitochondria/mitochondrial abnormalities in cardiac diseases, their diagnosis, as well as therapeutic options available. While aberrations in the bioenergetic function of the mitochondria are frequently related to the cardiac dysfunction, the specific defect(s) causing the bioenergetic dysfunction often resides in a non-bioenergetic pathway e.g, signaling between the mitochondria and nucleus, or in overall mitochondrial biogenesis and/or degradation pathways. Understanding these pathways and the impact of mitochondrial defects in cardiac pathology is important to improve diagnosis and treatment of mitochondrial-based cardiac diseases.

MITOCHONDRIA AND THE NORMAL HEART

Mitochondrial bioenergetics

Mitochondria are abundant in energy-demanding cardiac tissue constituting 20%-40% of cellular volume. Mitochondrial energy production depends on

both nuclear and mtDNA-encoded genetic factors which modulate normal mitochondrial function including enzyme activity and cofactor availability and on environmental factors including the availability of fuels (e.g. sugars, fats and proteins) and oxygen. Several interacting bioenergetic pathways contribute to mitochondrial energy metabolism (shown in Figure 1) including pyruvate oxidation, the tricarboxylic acid (TCA) cycle, the mitochondrial β -oxidation of fatty acids and the common final pathway OXPHOS which generates 80%-90% of cellular ATP. OXPHOS is performed by complexes of proteins located at the mitochondrial inner membrane including the electron transport chain (ETC) respiratory complexes I-IV, ATP synthase (complex V) and the adenine nucleotide translocator (ANT). Fatty acids are the primary energy substrate for the production of ATP in cardiac muscle by OXPHOS. In order to be utilized for bioenergetic production via mitochondrial fatty acid β -oxidation, fatty acids need to be effectively transported into the cardiomyocyte and subsequently into the mitochondria, a process requiring several transport proteins including the carnitine shuttle (carnitine acyltransferase and two carnitine palmitoyl transferases as well as carnitine). Fatty acid β -oxidation and the oxidation of carbohydrates via the TCA cycle generate the majority of intramito-

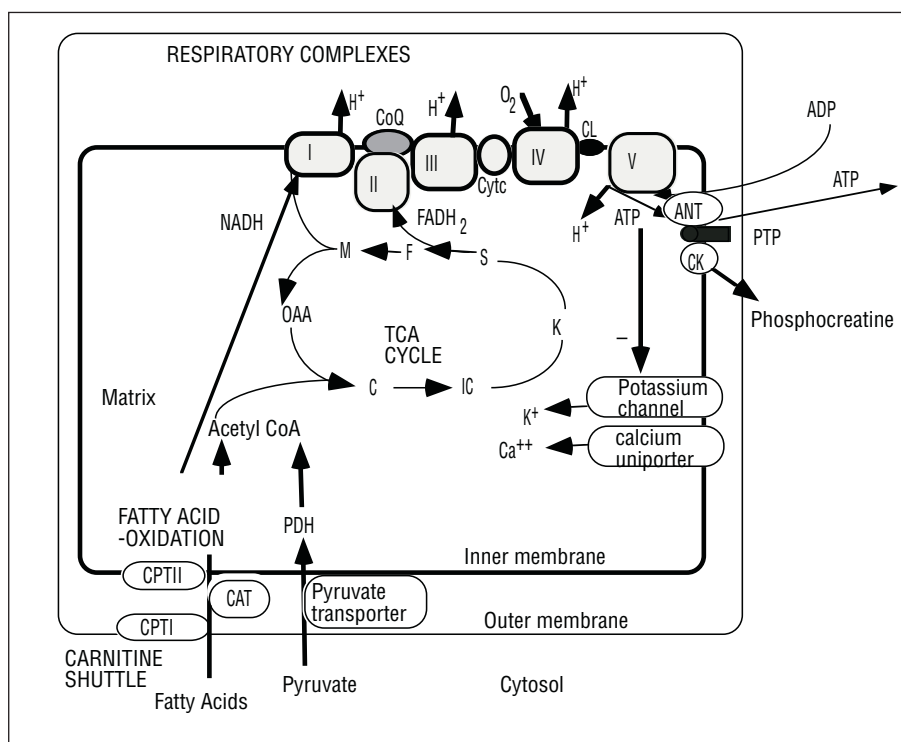
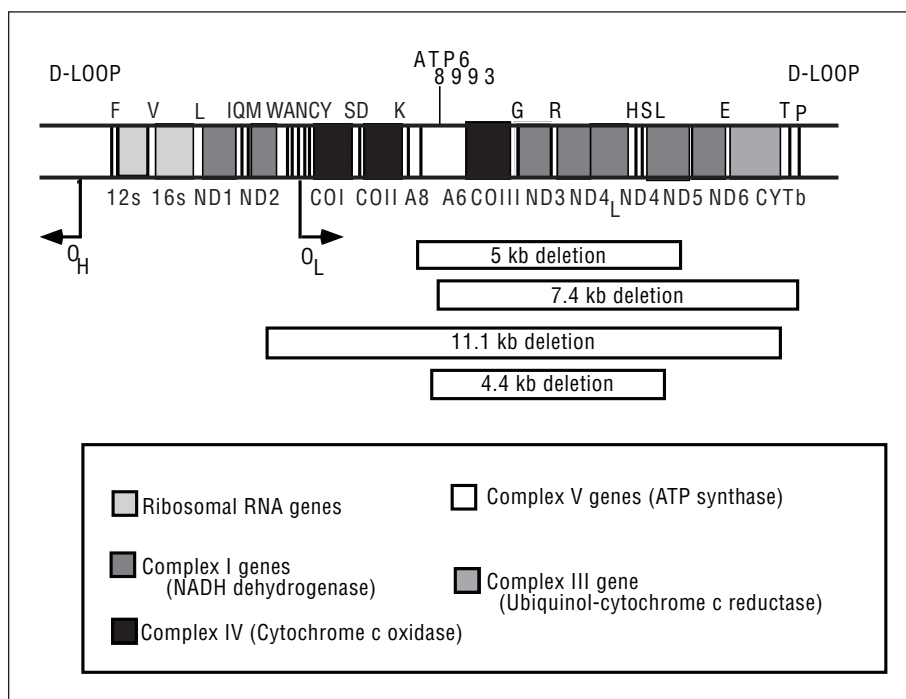


Fig. 1. Mitochondrial bioenergetic pathways. Mitochondrial inner-membrane localized respiratory complexes I-V (hatched circles) with associated electron-transfer components, coenzyme Q (CoQ) and cytochrome c (cyt c). Also shown are the matrix associated pyruvate oxidation, fatty acid β -oxidation and TCA cycle pathways. The apoptosis-associated mitochondrial permeability transition pore (PTP) is shown with two of its many proteins depicted: adenine nucleotide translocator (ANT) and mitochondrial creatine kinase (CK). The carnitine shuttle pathway for the mitochondrial import of fatty acids, the pyruvate transporter, K⁺ channel and Ca⁺⁺ uniporter are located in the mitochondrial membranes.

Fig. 2. Human mitochondrial genome. A linear representation of the circular 16 569 base pair human mtDNA molecule showing the location of the 22 tRNAs identified by their cognate amino acid using single letter code (F, V, L, I, Q, M, W, A, N, C, Y, S, D, K, G, R, H, S, L, E, T, P), the two ribosomal RNA genes (12s and 16s) and all 13 protein-encoding genes (ND1-ND6, COI-COIII, cytb, ATPase6 and ATPase8). The key further identifies the functional roles (i.e., the respiratory complexes and associated enzymatic function) played by the proteins encoded by each of the 13 structural genes. The relative locations of the common 5 kb and 7.4 kb deletions, and novel 11.1 kb and 4.4 kb deletions are noted by the boxed regions. The positions of the origin of replication for the heavy strand (OH) contained within the non-coding D-loop region and of the light strand replication origin (OL) are also shown. The numbering is according to Anderson et al.¹



chondrial NADH and FADH which are the direct source of electrons for the ETC. The supply of ATP from other sources (e.g. glycolytic metabolism) is limited in normal cardiac tissue. In addition to these bioenergetic pathways and metabolic intermediates, the heart also maintains stored high-energy phosphates (e.g., phosphocreatine) produced by mitochondrial creatine kinase using ATP from closely-associated ANT and mitochondrial ATP synthase.

Overview of mitochondrial biogenesis

Human mitochondria have their own double-strand DNA circular molecule encompassing 16 569 bp and encoding 13 proteins which constitute a portion of the 5 enzyme complexes involved in electron transport and OXPHOS.¹ The protein-encoding mtDNA genes are transcribed into specific mRNAs which are translated on a mitochondrial specific ribosome/protein synthesis apparatus. The mtDNA also encodes part of the mitochondrial protein synthesis machinery including 2 ribosome RNA (rRNA) and 22 transfer RNA (tRNA) shown in Figure 2. In general, each cardiac cell contains multiple mitochondria (50-100) and each mitochondria contains multiple copies of mtDNA (1-10 molecules/mitochondria). Mitochondrial biogenesis is increased during cardiac hypertrophy, treatment with a variety of agents e.g. thyroxin, xenobiotics, electrical stimulation and exercise.²

Presently, the mechanisms that regulate cardiac-specific mtDNA levels and overall mitochondrial number have not yet been delineated.³

However, it is well established that pathogenic point mutations and large-scale deletions in the mitochondrial genome as well as generalized depletion of mtDNA levels have severe consequences for organs such as heart since ATP derived from OXPHOS is needed to maintain myocardial contractility.

Conversely, the nuclear genome encodes the entire complement of proteins involved in mtDNA replication and transcription, protein components of mitochondrial ribosomes, multiple structural and transport proteins of the mitochondrial membranes and remaining peptide subunits (presently estimated at 71) of the respiratory complexes (other than the 13 mtDNA-encoded peptide subunits). These nuclear-encoded proteins are synthesized on cytosolic ribosomes, targeted to mitochondria and imported by a complex process. Cardiac-specific regulation of a number of the nuclear genes encoding OXPHOS proteins can be mediated by variable gene expression (e.g. ATP synthase β -subunit is expressed at higher levels in heart than other tissues) sensitive to a variety of physiological and developmental stimuli, and by the presence of tissue-specific isoforms for specific peptides (e.g. cardiac specific isoforms exist for genes encoding cytochrome c oxidase subunits VIa,

VIIa and VIII). Therefore, mutations in nuclear genes involved in mitochondrial biogenesis might be expected to contribute in part to the observed mitochondrial cardiac enzyme and mtDNA defects, including increased incidence of large-scale mtDNA deletions and mtDNA depletion associated with cardiac disorders. Thus far, few mutations in nuclear genes affecting mitochondrial biogenesis leading to cardiac disorders have been identified. While the nuclear genome controls mitochondrial biosynthesis, mtDNA genes have a much higher mutation rate, lack histones, have limited DNA repair and are exposed to reactive oxygen species (ROS) generated by the ETC. The search for mutations in both nuclear and mitochondrial genomes as well as mutations that affect cross-talk between the genomes is presently expanding.

Cardiac mitochondrial changes during cardiac growth and development

In the fetal heart which functions in a relatively hypoxic environment, glucose and lactate are the predominant fuel substrates utilized by glycolysis and lactate oxidation respectively. A large endogenous supply of glycogen in the fetal and, to a lesser extent, in the neonatal heart are a significant source of glucose and myocardial ATP. Glycogenolysis is particularly important in conditions of oxygen deprivation, and may allow the fetal heart to be more resistant to the effects of hypoxia and ischemia as compared to the adult heart.⁴ Fetal hearts have lower mitochondrial content and therefore lower levels of both respiratory complex and TCA cycle activities. Fatty acid oxidation provides a small part of the overall ATP production because of low levels of circulating fatty acids and also due to the inhibition of fatty acid oxidation by high lactate levels present in the fetal heart.

Post-natally, a switch occurs so that fatty acids become the primary energy substrate in the heart.⁵ The expression of genes encoding fatty acid enzymes (e.g. mitochondrial carnitine palmitoyltransferase [CPT] enzymes and isoforms) shows a marked change during the early postnatal period which parallels the increased utilization of fatty acids as a energy substrate in the neonatal heart. The specific activity levels of CPT-II increase during the first month of life as does myocardial carnitine levels.⁶ Of the 2 tissue-specific isoforms of CPTI, *CPT-Ia* gene is highly expressed in the fetal heart and declines following birth while *CPT-Ib*, is highly expressed

throughout cardiac development.⁷ Specific mitochondrial membrane-associated and cytoplasmic proteins involved in long-chain fatty acid uptake are co-ordinately regulated during development in cardiac tissue.⁸

The level of cardiac mitochondrial creatine kinase (mtCK) which is undetectable in early fetal heart, is upregulated during neonatal development. The increased expression of mtCK within the first several weeks after birth is coupled to both the availability of ADP to mitochondria as well as to the structural re-organization of cardiac mitochondria from a random arrangement (at 1 day) to an organized network by 3 weeks postnatally.⁹ The maturation of the phosphocreatine shuttle is coordinated with the development of the contractile properties of the myocardium.¹⁰

Post-natal levels of cardiac-mitochondrial OXPHOS enzyme activities and content particularly in humans, need to be clearly established.¹¹ At present, the information available is limited by the small number of subjects studied with normal heart, and by the variability of mitochondrial OXPHOS enzyme activities due to dietary factors, exercise and hormonal stimuli.

A by-product of mitochondrial bioenergetic production is the generation of ROS including superoxide and hydroxyl radicals and hydrogen peroxide (H_2O_2). Side reactions of the mitochondrial ETC with molecular oxygen directly generate the superoxide anion radical. The main sites for mitochondrial ROS generation are complex I and III activities of the respiratory chain; either excessive or diminished electron flux at these sites can stimulate the auto-oxidation of flavins and quinones (including coenzyme Q) producing superoxide radicals. The superoxide radicals can be converted to H_2O_2 (in the presence of the enzyme superoxide dismutase) which can further react to form the hydroxyl radical.

Normally, these toxic by-products which are powerful cell-damaging oxidants are neutralized by antioxidant enzymes some of which are mitochondrially located e.g. Mnsuperoxide dismutase (MnSOD) and glutathione peroxidase (Figure 3) and others are cytosolic e.g. Cu superoxide dismutase and catalase, or scavenged by glutathione. Increased ROS generation resulting from myocardial ischemia/reperfusion, inflammation, impaired antioxidant defenses and aging may cause profound effects on cardiac cells including increased lipid peroxidation effecting primarily membrane phospholipids and proteins. Superoxide is particularly damaging to the Fe-S

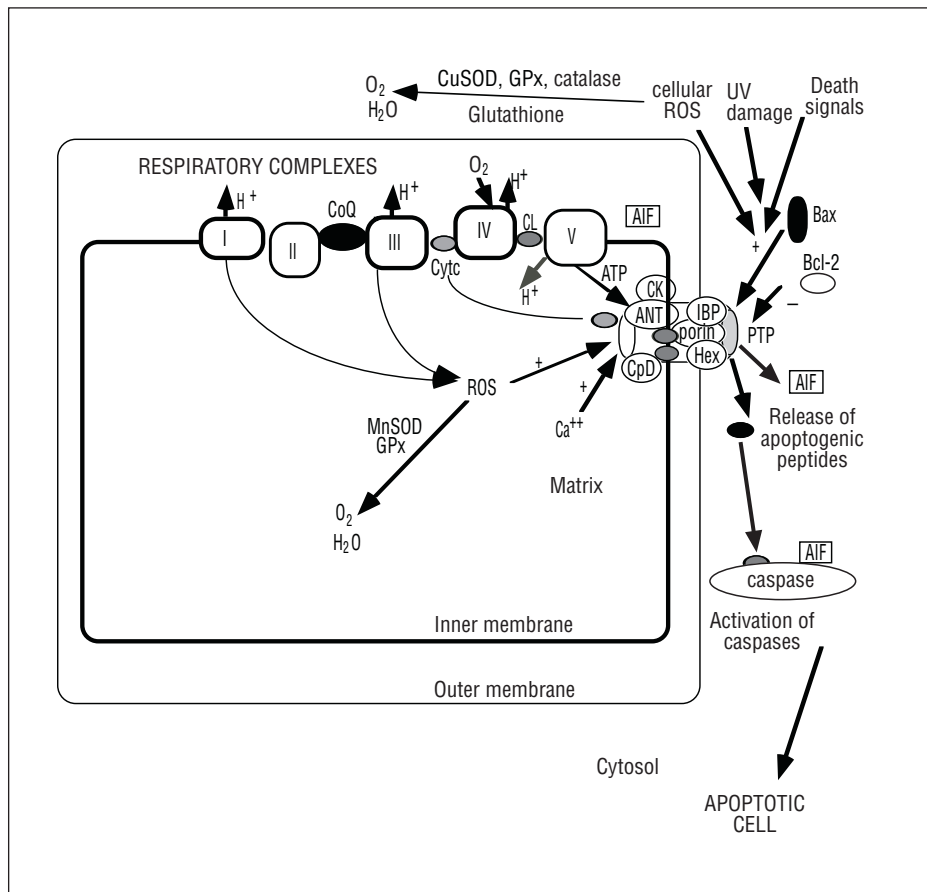


Fig. 3. The mitochondrial pathway of apoptosis. Cellular signals including ROS, and UV-induced DNA damage and a variety of «death signals» trigger the apoptotic pathway by modulation of the binding of proapoptogenic proteins (e.g. BAX) to the PTP causing it to open. Elevated levels of mitochondrial calcium levels as well as excessive ROS (generated at respiratory complex I and III sites) as shown also promote the PTP opening. This is followed by the release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria to the cytosol with subsequent activation of the caspases initiating cell self-digestion and nuclear DNA fragmentation leading to apoptotic cell death. Association of BAX with mitochondria is prevented by anti-apoptogenic Bcl family of proteins. The neutralization of ROS occurs at both intramitochondrial sites by antioxidant enzymes, manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) and within the cytosol by CuSOD, GPx and catalase. Also shown are the major proteins comprising the PTP at contact sites adjoining the inner and outer mitochondrial membranes including hexokinase (HK), adenine nucleotide translocator (ANT), creatine kinase (CK), cyclophosphin D (CpD), porin and the benzodiazepine receptor (IBP) as well as the inner membrane phospholipid cardiolipin (CL).

moieties of enzymes (e.g. complex I, aconitase and succinic dehydrogenase).¹² Oxidative damage also occurs with nucleic acids (particularly targeting mtDNA) including induction of strand breaks, a high incidence of base modification (including the formation of 8-oxoguanosine) and subsequent point mutations and deletions. Mitochondria which are the primary site of ROS generation are therefore the critical target of its damaging effects; the mitochondrial respiratory chain located in the inner membrane is often damaged resulting in a further increase in ROS generation leading to a vicious cycle of diminished mitochondrial function. In addition to the well characterized role of ROS as celldamaging, recent evidence has provided an alternative view concerning increased ROS generation and oxidative stress in its role as an important regulatory phenomena.¹³ Oxidative species (e.g. H_2O_2) can also function as a potent signal sent from mitochondria to other cellular sites rapidly, and reversibly eliciting an array of intracellular cascades leading to different physiological end-points for the cardiomyocyte (e.g.

apoptosis, necrosis, cardio-protection, cell proliferation).

Cardiac mitochondrial changes during aging and senescence

Amongst the multiple metabolic changes that occur in cardiac muscle with advancing age are modifications in membrane fatty acid and lipid composition including increasing levels of saturated fatty acids¹⁴ and reduced levels of polyunsaturated fatty acids¹⁵ and cardiolipin.¹⁶ Cardiolipin, the most unsaturated cellular phospholipid, is a major component of the mitochondrial inner membrane and plays an integral role in cardiac mitochondrial membrane transport function, fluidity and stability, and as a facilitator of key mitochondrial inner membrane bioenergetic enzymes. Marked reduction in carnitine and acetyl carnitine levels has also been reported in older subjects.¹⁷

Increased levels of large-scale deletions and point mutations in cardiac mtDNA, and reduced levels of

mitochondrial enzymatic activities may occur with aging.¹⁸⁻²⁰ These mitochondrial abnormalities have been proposed to be due in large part to the increased mitochondrial production of ROS noted in aging.^{12,21} However, the effect of aging on cardiac OXPHOS enzymatic function has been recently re-evaluated and the extent and role of mitochondrial bioenergetic decline has been put into question.^{11,22} The discrepancies between these findings may be based on the different methodologies employed in measuring mitochondrial activities. If one distinguishes the cardiac mitochondria as distinct populations (requiring the physical separation of the organelle populations *in vitro*) the interfibrillar mitochondria (IFM) located between the myofibers and the subsarcolemmal mitochondria (SSM), located below the plasma membrane, age-dependent reductions are found in IFM and not SSM respiratory complex III and IV specific activities.²³ A host of physiological changes/parameters can also impact on levels of mitochondrial enzymatic activities. Levels of exercise and conditioning as well as increased ischemic stresses can markedly impact on mitochondrial enzyme activities.²⁴

MITOCHONDRIAL DYSFUNCTION IN CARDIOVASCULAR DISEASES

MtDNA and cardiovascular disease

Discrete mitochondrial OXPHOS defects or respiratory chain deficiency have been documented in cardiomyopathies. Both DCM and HCM are frequently accompanied by defective levels of specific OXPHOS/respiratory enzyme activities.²⁵⁻²⁸

Cardiomyopathy (mostly HCM) often occur with specific pathogenic point mutations in mtDNA²⁹⁻³⁵ (an updated list is presented in Table 1). Pathogenic mtDNA mutations are generally located in nucleotides which are highly conserved in evolution, and frequently present in heteroplasmic fashion (a mixed population of both mutant and wildtype mtDNA genomes) albeit recent evidence has indicated that pathogenic mtDNA mutations can also be homoplasmic.³⁶ These mutations are usually accompanied by reduced levels of specific respiratory enzyme activity(s).

Pathogenic mtDNA mutations in a number of mitochondrial tRNA genes have been identified in association with cardiomyopathy. Specific tRNA genes including *LEU*, *ILE* and *LYS* appear to be hot spots for mutation in cardiomyopathic patients. The site of mutations within the generic tRNA cloverleaf structure may impact on the severity of phenotype and possibly

to its tissue-specificity.³⁷ In general, pathogenic mitochondrial tRNA gene mutations negatively affect mitochondrial protein synthesis and multiple specific respiratory enzyme activities. A cardiomyopathy-associated mtDNA mutation has also been found in mitochondrial rRNA.³⁸ Other mtDNA point mutations residing at other mtDNA locations (some occurring in tRNA genes, others in mtDNA protein-encoding genes), which were not present in normal individuals have been reported in patients with DCM. These potentially pathogenic mutations are heteroplasmic, and present in highly conserved sequences.³⁹⁻⁴¹ Moreover, missense mutations in cytochrome *b* have been reported in a wide spectrum of cardiomyopathies including DCM, HCM, histiocytoid and post-partum cardiomyopathy (Table I). It is noteworthy that cytochrome *b*, the sole mitochondrial-encoded subunit of complex III, also represents a hot spot for mutations in patients with cardiomyopathy.

Multi-systemic mitochondrial diseases with cardiac involvement have been reported usually with an expanding spectrum of clinical manifestations. Some are maternally inherited (due to mtDNA mutation) and may present with a variable cardiac phenotype (ventricular hypertrophy, cardiomegaly and dysrhythmias) together with neurological syndromes including Leigh (developmental delay, muscle weakness, ophthalmoplegia and necrosis in the basal ganglia), MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) and MERRF (myoclonic epilepsy and ragged-red fibers).⁴²⁻⁴⁴ Specific mtDNA mutations, found in association with primary cardiomyopathy, can also be present in patients with different combinations of clinical phenotypes. The variability between mitochondrial genotype and phenotype may be explained by the involvement of unidentified genetic or environmental cofactor(s) capable of modulating the effect of mtDNA mutations. Similarly, specific syndrome(s)/phenotype(s) may be caused by entirely different nuclear and/or mtDNA mutations. For example, Leigh syndrome can be caused by mutations in ATPase-6 subunit,⁴³ by point mutation in mitochondrial tRNA *LYS* gene,⁴⁵ by nuclear DNA mutations in pyruvate dehydrogenase,⁴⁶ nuclear-encoded complex II subunits⁴⁷ or by mtDNA depletion.⁴⁸ However, the correlation of cardiac involvement in Leigh disease and the loci affected (whether nuclear or mitochondrial) has not been established.

Sporadic large-scale rearrangements of mtDNA can also be associated with cardiac disorders. In Kearns-Sayre syndrome (KSS), cardiac conduction

TABLE 1. Specific mtDNA point mutations in cardiac disease

Gene	Site	AA change	Cardiac phenotype	Ref.
<i>tRNA mutations</i>				
<i>leu</i>	3243 A->G		HCM	42
<i>leu</i>	3260 A->G		Tachycardia, adult onset HCM	34
<i>leu</i>	3303 C->T		Fatal infantile HCM	30
<i>leu</i>	3254 C->G		HCM	145
<i>leu</i>	12997 T->C		DCM	146
<i>ile</i>	4300 A->G		HCM-adult onset	35
<i>ile</i>	4317 A->G		Fatal infantile HCM/DCM	147
<i>ile</i>	4320 C->T		Fatal infantile HCM	31
<i>ile</i>	4269 A->G		CF at 18 years, adult onset	29
<i>ile</i>	4295 A->G		HCM	148
<i>ile</i>	4284 G->A		CM	149
<i>lys</i>	8363 G->A		HCM	32
<i>lys</i>	8334 A->G		HCM	44
<i>lys</i>	8269 A->G		HCM	150
<i>lys</i>	8348 A->G		HCM	151
<i>gly</i>	9997 T->C		Ventricular arrhythmia HCM	33
<i>cys</i>	5814 A->G		HCM	152
<i>val</i>	1624 C->T		HCM	36
<i>ala</i>	5587 T->C		DCM	40
<i>arg</i>	10415 T->C		DCM	40
<i>arg</i>	10424 T->C		Fatal DCM	41
<i>rRNA mutations</i>				
<i>12s</i>	1555 A->G		CM	38
<i>16s</i>	3093 C->G		CM	153
<i>Structural gene mutations</i>				
<i>Cytb</i>	14927 A->G	Thr->Ala	HCM	154, 56
<i>Cytb</i>	15236 A->G	Ile->Val	DCM	56
<i>Cytb</i>	15508 C->G	Asp->Glu	DCM	41
<i>Cytb</i>	15509 A->C	Asn->His	Fatal post-partum CM	155
<i>Cytb</i>	15243 G->A		HCM	157
<i>Cytb</i>	15498 G->A	Gly->Asp	Histiocytoid CM	156
<i>COI</i>	6860 A->C	Lys->Asn	DCM	40
<i>COII</i>	7923 A->G	Tyr->Cys	DCM	41
<i>COIII</i>	9216 A->G	Gln->Glu	DCM	41
<i>ND5</i>	14069 C->T	Ser->Leu	DCM	41
<i>ATPase6</i>	8993 T->G	Leu->Arg	Leigh syndrome/HCM	43

¹Ref indicates references; CM, Cardiomyopathy; HCM, hypertrophic cardiomyopathy; CF, cardiac insufficiency.

abnormalities typically co-exist with somatic large-scale mtDNA deletions.^{49,50} The majority of mtDNA deletions in KSS are of a single type, not inherited and are detected mostly in skeletal muscle and rarely in blood. One study showed a localized prevalence of mtDNA deletions in the tissues containing the cardiac conduction system i.e. the sinoatrial and atrioventricular nodes as compared to the surrounding myocardium.⁵¹ In contrast, a multiple mtDNA deletion phenotype associated with DCM is due to genetic defects in unidentified autosomal nuclear loci which can be either dominantly or recessively inherited.^{52,53} The mtDNA deletion events detected in KSS

and in autosomal disorders tend to be highly abundant (reaching up to 95% of total mtDNA) either as single-sized discrete deletions or in the aggregate and are detected by Southern blot.^{54,55} Moreover, less abundant, large-scale specific mtDNA deletions are frequently found in cardiac tissues of many primary cardiomyopathies by PCR analysis. These less abundant deletions reflect specific mtDNA damage (presumably a consequence of ROS), occur in an age dependent manner^{54,55} and their role in cardiac disease remains unclear.

Depletion in cardiac mtDNA levels has been reported in patients with isolated cardiomyopathy, ei-

ther DCM and HCM.⁵⁶ In addition, depletion of cardiac mtDNA can be specifically induced by zidovudine (AZT), which inhibits both the DNA polymerase of HIV and mitochondrial DNA polymerase γ interfering with mtDNA replication. In both humans and animals treated with AZT,^{57,58} reduced cardiac mtDNA levels are found associated with a marked reduction in respiratory enzyme activities and a clinical phenotype of cardiac dysfunction. The mtDNA depletion appeared to be reversible since cessation of AZT therapy was associated with improved left ventricular function.

Defects in nuclear-DNA encoded mitochondrial proteins and cardiovascular disease

Mutations in a wide array of nuclear genes encoding mitochondrial proteins can cause cardiomyopathy. For example, cardiomyopathy is frequently a consequence of mutations in mitochondrial transport proteins (e.g. carnitine-acylcarnitine translocase) which facilitate the passage of critical metabolites across the inner mitochondrial membrane⁵⁹ and also mutations in a mitochondrial transport protein, frataxin, considered to be involved in mitochondrial iron accumulation cause Friedreich ataxia (primarily HCM).⁶⁰ Moreover, mutations in nuclear genes encoding factors required for the assembly and functioning of the multiple-subunit enzyme respiratory complexes have been implicated in some mitochondrial-based diseases such as Leigh syndrome and mutations in the *SCO2* gene encoding a copper chaperone, involved in the assembly of complex IV (COX), may result in cardiomyopathy.⁶¹ Interestingly, the clinical phenotype in patients with *SCO2* mutations is distinct from that found with mutations in other COX assembly factors (e.g. SURF1) which typically present without cardiac involvement.⁶²

Cardiomyopathy is the primary clinical manifestation in several inherited disorders of mitochondrial fatty acid β -oxidation.⁶³ Deficiencies in very long chain acyl-Co A dehydrogenase (VLCAD),⁶⁴ long chain 3-hydroxylacyl CoA dehydrogenase (LCHAD)⁶⁵ and short-chain acyl CoA dehydrogenase (SCAD)⁶⁶ have been reported to cause cardiomyopathy in young children. Also, defects in carnitine transport into cells as well as in the carnitine-acylcarnitine shuttle mechanism, responsible for fatty acid transport into mitochondria, have been found associated with cardiomyopathy.⁶⁷ Defects in the mitochondrial trifunctional protein (MTP) that affect long chain L-3 hydroxylacyl-CoA protein are often associated with DCM.⁶⁸

The cardiac pathogenesis of these inherited disorders of fatty acid β -oxidation and carnitine metabolism likely includes both a deficient bioenergetic supply to the heart and the accumulation of toxic levels of fatty free acids with subsequent cardiac dysfunction. These disorders primarily occur in early childhood and are usually precipitated by infectious illness or fasting when the heart has an increased dependence on fatty-acid oxidation for energy. Many of the inherited fatty acid disorders described above can result in sudden neonatal death.

Cardiac conduction defects and cardiac arrhythmias are frequently present in patients with specific defects in fatty acid oxidation.⁶⁹ Both ventricular and atrial arrhythmias were associated with deficiencies in CPT-II deficiency, carnitine translocase and trifunctional protein (MTP) deficiency. In contrast, cardiac arrhythmias were absent in patients with deficiencies in CPT-I, the primary carnitine carrier and MCAD suggestive that accumulation of arrhythmogenic metabolites of fatty acids (e.g. long chain acylcarnitines) promote arrhythmias and potentially contribute to CF and sudden death. It has long been known that long chain acylcarnitines possess detergent-like properties and can extensively modify membrane proteins and lipids with toxic effects on the cardiac membrane electrophysiological functions, including ion transport and impair gap-junction channels. Moreover, the accumulation of long chain fatty acid metabolites (e.g. long chain acylcarnitine) plays a pivotal role in the production of ventricular arrhythmias that occur during myocardial ischemia.⁷⁰ The accumulation of potentially toxic long-chain esters during hypoxia/ischemia can be prevented by selective blocking of CPT-I activity with perhexiline or amiodarone reducing both the risk of membrane electrophysiologic disturbances and the incidence of lethal arrhythmias.⁷¹

Abnormal mitochondria and severe DCM has also been reported in Barth syndrome, an X-linked disorder also characterized by cyclic neutropenia with neonatal onset. Arrhythmias and cardiac failure (CF) are frequently present. The protein tafazzin responsible for Barth syndrome⁷² is encoded by the *G4.5* gene and likely belongs to a family of acyltransferases involved in phospholipid synthesis. In patients with the *G4.5* mutation, saturated fatty acid levels increase while unsaturated fatty acid and cardiolipin levels are markedly reduced.^{73,74} Defective acyltransferase function may result in increased fatty acid saturation, decreasing cardiac membrane fluidity and function.

Genes encoding important structural and cytoskeletal proteins have frequently been implicated in the pathogenesis of cardiomyopathy. Specific nuclear DNA mutations in structural and contractile proteins such as actin, desmin, sarcoglycan, and dystrophin have been identified as causal in many cases of DCM and CF. Similarly, cases with familial HCM are due to specific pathogenic mutations in cardiac proteins involved in generating contractile force including β -myosin heavy chain (β -MHC), cardiac troponin T, tropomyosin and myosin-binding protein C. The relationship of mitochondrial defects with mutations in nuclear-encoded structural proteins is worthy of note. Patients with specific β -MHC mutations may develop abnormal mitochondrial number and a marked reduction in mitochondrial respiratory function.^{75,76} A potential interaction of these nuclear pathogenic mutations with specific mtDNA mutations is also suggested by studies showing co-existence in some patients with HCM of mutations in both β -MHC and mtDNA.⁷⁷ In addition, the intracellular distribution of mitochondria can be profoundly altered in patients with defective structural proteins (e.g. desmin)⁷⁸ since both the intracellular position and movement of mitochondria are mediated by cytoskeletal proteins. Defective cellular location of mitochondria with potential downstream effect on cardiac bioenergetic function may play a critical role in cardiac pathophysiology.

Myocardial ischemia

When the supply of oxygen becomes limiting as occurs with myocardial ischemia, OXPHOS and mitochondrial ETC flux decline, creatine phosphate is rapidly depleted, fatty acid and pyruvate oxidation decrease and ATP production is impaired. The hydrolysis of glycolytically derived ATP and accumulation of lactate leads to a decrease in intracellular pH and intracellular acidosis, which has a direct inhibitory effect on contractile function. AMP and other intermediates accumulate with subsequent mitochondrial swelling and progressive degeneration. In addition, myocardial ischemia has been shown to result in reduced levels of respiratory complex IV,⁷⁹ complex V⁸⁰ and increased levels of mtDNA deletions.⁸¹ Sustained ischemia leads to ATP depletion and necrotic cell death.

Paradoxically, functional mitochondria can exacerbate ischemic damage, especially at the onset of reperfusion. A pronounced increase in fatty acid influx and unbalanced fatty acid oxidation predomi-

nates during reperfusion, producing an excess of acetyl CoA saturating the TCA cycle at the expense of glucose and pyruvate oxidation, which is eventually inhibited. Increased OXPHOS causes an increase in the ROS accumulation with increased lipid peroxidation; this results in lower cardiolipin levels in the inner membrane with consequent effect on complex IV activity. Normal respiratory activity can be restored by adding exogenous cardiolipin.⁸² At present, there is evidence that reperfusion injury involves apoptotic cell death in contrast to ischemic injury which primarily involves necrotic cell death.⁸³

Hyperthermic stress can protect against myocardial dysfunction after ischemiareperfusion injury.⁸⁴ Improved levels of complex I-V activities, heat shock protein expression (i.e. Hsp 32, 60, and 72) and ventricular function were attained in heatstressed hearts. In comparison to untreated reperfused myocardium, in which mitochondria were severely disrupted, the reperfused heat-stressed myocardium exhibited higher respiratory complex activities and mitochondria with intact membranes packed with parallel lamellar cristae. This provided evidence of heat-stress-mediated enhancement of mitochondrial energetic capacity associated with increased tolerance to ischemia-reperfusion injury.

A self-defense cardioprotective mechanism in the ischemic heart involves the opening of mitochondrial ATP-sensitive K⁺ (KATP) channels. This cardioprotective effect may be mediated by improved ATP production, lowered Ca⁺⁺ overloading in the mitochondrial matrix and increased ROS generation leading to protein kinase C activation. Several drugs are available that function to specifically activate the mitochondrial K-channel opening, e.g. diazoxide. Evidence for mitochondrial K⁺-ATP channels as effectors of myocardial preconditioning has been demonstrated in human subjects.⁸⁵

Apoptosis and cell death in cardiac disease

Apoptosis (programmed cell death) leading to cardiac cell loss and to extensive left ventricular remodeling has been shown to be a significant feature of CF both in patients with DCM and in animal models of CF. It is increasingly recognized that mitochondria play a pivotal role in the early events of apoptosis.

In the mitochondrial pathway of apoptosis, the release from mitochondria to the cytosol of the apoptogenic proteins, cytochrome *c* and apoptosis inducing factor (AIF) is an integral event in triggering a sub-

sequent cascade of cytoplasmic changes including activation of downstream cysteine-aspartate proteases (caspases) and nuclear endonucleases leading to cell death.^{86,87} The release of mitochondrial peptides is directed by an opening of a megachannel, the mitochondrial permeability transition pore (PTP), a dynamic multiprotein complex located at contact sites between the mitochondrial inner and outer membranes promoted by the binding of proapoptotic proteins such as Bax (Figure 3). The anti-apoptotic protein Bcl-2 (a mitochondrial protein) prevents the association of Bax with the PTP and interferes with the release of the apoptogenic peptides (e.g. cytochrome *c* and AIF) from the mitochondria. Recent evidence has established that PTP opening is a critical early step of apoptosis preceding the caspase cascade. PTP opening is promoted by Ca⁺⁺ influx into mitochondria and excessive mitochondrial ROS production.⁸⁸ Other pro-apoptotic second messengers including pro-oxidants and nitric oxide also induce PTP opening. In addition to its involvement in apoptotic cell death, opening of the PTP has been implicated in the clinical cell death of cardiomyocytes induced by excitotoxins (e.g. glutamate), as well as in necrotic cell death occurring during anoxia and ischemia.⁸⁹ This supports the emerging view that mitochondrial changes are a pivotal step in the commitment to both apoptotic and necrotic types of cell death.

PTP opening is accompanied by dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$)⁸⁸ and mitochondrial membrane depolarization. The changes in membrane potential can be either a cause or the result of PTP opening, since extensive proton influx occurs at this site. Decline in mitochondrial respiratory enzyme activities (particularly complex III) and in OXPHOS can contribute to the onset of apoptosis. The PTP represents a critical site where OXPHOS can integrate with both cell-signalling stimuli and responses. Decreased ATP content promotes the transfer of BAX to the PTP. However, there is a continual dynamic in the balance of the pro-apoptotic BAX and antiapoptotic Bcl factors in modulating the events at the PTP site. Because apoptosis requires energy, the shutdown of OXPHOS either leading or following the PTP opening is not complete. It is important to note that complete mitochondrial de-energization does not favor apoptosis but rather leads to necrosis. It should not be surprising that the early events of apoptosis involves modulation in ATP levels, given the close proximity of the PTP to the respiratory complexes in the mitochondri-

al inner membrane as well as its involvement in the mitochondrial loss of cytochrome *c*, a critical molecule in the ETC. Also present at the PTP site are a number of energy-associated mitochondrial molecules including the adenine-nucleotide exchanger, the glycolytic enzyme, hexokinase, the outer membrane voltage-dependent anion channel protein (VDAC or porin), the mitochondrial creatine kinase and the phospholipid cardiolipin.

Mitochondria and cardiotoxicity

An increasing number of drugs/toxins have been recognized as having deleterious effects on cardiac mitochondrial function. The reader is referred to two recent excellent reviews concerning mitochondria (in general) as a pharmacological target.^{90,91} We limit our discussion to two relatively well-characterized toxins which have cardiac-specific effects associated with mitochondria.

The anticancer anthraquinone glycoside drugs (e.g. adriamycin/doxorubicin) have long been known to affect myocardial function. Molecular and biochemical myocardial changes occur with doxorubicin-induced cardiomyopathy including striking changes in mitochondrial structure and function. Mitochondrial changes include markedly increased free radical production through abnormal NADH dehydrogenase activity; increased mtDNA damage, including accumulation of 8 OHdG adducts and large-scale mtDNA deletions; alterations in mitochondrial Ca⁺⁺ homeostasis; changes in enzyme activities involved in fatty acid oxidation (e.g. CPTI) and decreased ANT activity.⁹²⁻⁹⁶ The majority of deleterious biochemical effects of doxorubicin can be counteracted by supplementation with any of the following: MnSOD, carnitine, adenosine, metallothionein, which provide varying levels of cardioprotection.⁹⁷⁻⁹⁹ There is also evidence that doxorubicin impacts events at the mitochondrial PTP modulating Ca⁺⁺ flux, free O₂-radical generation, ANT activity, carnitine transport and metabolism and mtDNA integrity. The involvement of doxorubicin with cardiac apoptotic pathway induction is also consistent with the doxorubicin-stimulated release of cytochrome *c* in cardiomyocytes.¹⁰⁰ In this regard, it is noteworthy that patients with viral myocarditis also tends to have decreased ANT activity and content¹⁰¹ suggesting that the mitochondrial PTP may represent a common target site for cardiac toxins/ infectious agents. Moreover, there is evidence that the PTP site of the apoptotic pathway may be a primary target for toxic

agents (i.e. salicylate and valproate) involved in the pathophysiology of Reyes disease.⁸⁸

Intake of moderate amount of alcohol may exert beneficial effects on the cardiovascular system;¹⁰² this may be manifested by moderate increases in myocardial mitochondrial bioenergetics (i.e. increased levels of respiratory enzyme activities and mtDNA) suggesting increased cardiac-specific mitochondrial biogenesis.¹⁰³ On the contrary, intake of high dosage of alcohol has been found to have detrimental effects on myocardial tissues characterized at the subcellular level by mitochondrial structural and functional abnormalities and mtDNA depletion.¹⁰⁴⁻¹⁰⁶

Animal models of mitochondrial-associated cardiovascular disease

Gene ablation in mice (i.e. generation of null mutations or gene «knock-outs») targeting a relatively wide spectrum of genes encoding mitochondrial proteins results in severe cardiac dysfunction. Targeted genes include ANT,¹⁰⁷ MnSOD,^{108,109} factors involved in fatty acid metabolism, e.g. PPAR, MTP subunits^{110,111}, the mitochondrial transcription factor mt-TFA (also termed TFAM)¹¹² and frataxin, e.g. the protein responsible for Friedreich ataxia.¹¹³ In addition to examining the specific effects on cardiac function by eliminating specific genes of mitochondrial function, tissue-specific knockout mice with mitochondrial cardiomyopathy have been used to identify modifying genes of potential therapeutic value.¹¹⁴ At present, there is limited information about the impact on myocardium of knocking-out nuclear genes involved directly in mitochondrial OXPHOS. A recent report described cardiac dysfunction in mice lacking cytochrome c oxidase subunit VIa-H, the heart isoform.¹¹⁵ Little information is currently available concerning mtDNA gene ablation since generation of mtDNA gene knock-outs represents a difficult technical challenge requiring a novel approach for the creation of a mitochondrial transgenic animal; several approaches are discussed below.

A recent breakthrough in the creation of a mitochondrial transgenic animal was achieved with the construction of a mouse strain containing high levels of a single largescale mtDNA deletion (4696 bp).¹¹⁶ Myocardial mitochondria from mice with very high levels (>85%) of the mtDNA4696 deletion tended to have abnormal cristae and marked COX deficiency;¹¹⁷ however, unlike human patients with KSS and its analogous mtDNA deletion, mice with high proportions of the mtDNA deletion died with kidney

disease. Further studies with mice harboring the mtDNA4696 deletion also revealed that single cells (in most tissues) either harbored mutant or wild-type mitochondria; both types of mitochondria did not co-exist in the same cell.¹¹⁸ It was proposed that the existence of inter-mitochondrial complementation makes the expression of a deletion phenotype unlikely in the presence of full-length wildtype mtDNA. If the mouse model is relevant to human disorders with mtDNA deletions, this finding could have important ramifications for the potential treatment of deletion-based KSS using full-length mtDNA.

The use of cardiac-specific overexpression of specific genes has also been highly informative in our understanding of the role of mitochondria in cardiac dysfunction. This technique involves fusing a regulatory region from a cardiac-specific gene with a candidate gene of interest and introduction into the transgenic mice which will express the candidate gene specifically in cardiac muscle cells. Overexpression of a number of genes which mediate expression and control of cardiac energy metabolism (e.g. *PGC1*, *PPAR*, *TNF- α*) have been shown to lead to cardiomyopathy with severe cardiac dysfunction and marked changes in mitochondrial structure and function.^{119,120}

The development of animal models of mitochondrial-based cardiac dysfunction offers the possibility of direct testing for potential treatments. For example, the demonstration that MnSOD deficient animals developed ROS toxicity and DCM prompted speculation that effective treatment with anti-oxidants could ameliorate the cardiac phenotype; indeed, peritoneal injection of MnSOD-deficient mice with the anti-oxidant MnTBAP eliminated the cardiac dysfunction and reverses the ROS accumulation.¹²¹

Diagnosis and treatment of mitochondrial-based cardiac diseases

The diagnosis of mitochondrial dysfunction in cardiac disease has largely come from studies of endomyocardial biopsies using histochemical, ultrastructure and OXPHOS and respiratory enzyme analysis. Recently, the diagnostic utility of skeletal muscle enzyme analysis in patients with cardiomyopathy was demonstrated¹²² and muscle biopsy may eventually replace the need for endomyocardial biopsy in evaluation and follow-up of mitochondrial-based cardiomyopathy. DNA analysis used to identify specific pathogenic mtDNA mutations, large-scale mtDNA deletions and evaluation of mtDNA levels

can be informative; however, in our experience, the overall incidence of pathogenic mtDNA mutations is low in patients with cardiac disease including those patients with definitive OXPHOS enzymatic defects. After their initial identification, the functional significance of specific mtDNA mutations can be confirmed by the use of cybrid technology. In this methodology, patient's cytoplasts (enucleated cells containing the patient's mitochondria and mtDNA) are fused with mtDNA-deficient recipient rho⁰ cells (nucleated cultured cells whose mtDNA has been eliminated through prolonged growth in ethidium bromide). The resulting cytoplasmic hybrid («cybrid») cell lines which can be grown under appropriate selective conditions, possess a wild-type nuclear background derived from the rho⁰ recipient cells and mtDNA derived entirely from the patient and can be studied for growth, oxygen consumption, ATP production, mitochondrial protein synthesis and evaluation of specific enzymatic activities.¹²³ For patient's with suspected nuclear gene defects, cybrids can also be constructed with a wild-type mitochondrial genome and the patient's nuclear genome.

While evaluation of fatty acids using a profile of blood-acylcarnitine levels by mass spectroscopy analysis appears to be the most reliable method of assessing a mitochondrial-based fatty acid metabolism disorder,¹²⁴ further biochemical analysis can be used for the determination of levels of other key mitochondrial intermediates including coenzyme Q10, cardiolipin and carnitine.

A precise knowledge of the specific molecular and biochemical defect may allow treatment with metabolic intermediates (e.g. succinate), coenzymes and vitamins serving as electron donors, transporters and co-factors for electron transport (e.g. vitamin K, thiamine, ascorbate and riboflavin) by bypassing specific defects in OXPHOS and increasing ATP production.¹²⁵ For instance, coenzyme Q10 has been reported to have beneficial effects in the treatment of stroke-like episodes, lactic acidosis and fatigability in MELAS, in the cardiac conduction disturbances associated with KSS¹²⁵ and in the management of other cardiomyopathies¹²⁶. Recently, idebenone, a synthetic analogue of coenzyme Q has been reported to be effective in the treatment of the cardiomyopathy associated with Friedreich ataxia.^{127,128} Coenzyme Q10 also is potentially useful in its role as an anti-oxidant.¹²⁵

Mitochondrial long-chain fatty acid oxidation disorders (including CPT II deficiency) are effectively treated with long-term dietary therapy, with the re-

placement of normal dietary fat by medium-chain triglycerides and increased carbohydrates particularly, in the acute cardiomyopathy associated with VLCAD and LCHAD deficiencies.^{129,130} Prevention of these disorders also involves the strict avoidance of fasting, particularly in infancy. Patients with cardiomyopathy secondary to primary carnitine deficiency can be treated with oral L-carnitine. In a number of carnitine-treated cases, cardiomyopathy was resolved with recurrence prevented for over 5 years.¹³¹ There is also increasing evidence that free polyunsaturated fatty acids in the diet can provide a significant cardioprotective effect against both ischemia-related ventricular fibrillation and arrhythmias, increase mitochondrial cardiolipin content and can facilitate cardiac tolerance of ischemia and reperfusion stresses.¹³²

Deficiencies in CPT-II, carnitine acylcarnitine translocase or MTP that can result in increased incidence of cardiac conduction defects/arrhythmias can be treated with targeted drugs which would enhance glucose use and pyruvate oxidation energy, at the expense of fatty acid oxidation, in order to prevent the accumulation of long-chain acylcarnitines.¹³³ Other examples of metabolic intervention include the reversal of the rise in fatty acid oxidation that occurs during reperfusion using trimetazidine and ranolazine. Dichloroacetate has shown promise in stemming the rise in lactic acidosis and reversing the decrease in pyruvate dehydrogenase activity noted in myocardial ischemia and reperfusion injury.

Carnitine therapy also can be used to lower acyl-CoA accumulation. Moreover, treatment of patients in CF with carvedilol, a β -adrenoreceptor blocker, can lead to marked improvement in myocardial energy efficiency by employing a shift of myocardial oxidative substrates from fatty acid to glucose.¹³⁴

The road ahead: embryonic stem cells and gene-therapy

The formation of functioning myocardial cells in mouse and human cells has been achieved with the use of embryonic stem (ES) derived from blastocyst-stage preembryos.¹³⁵⁻¹³⁷ This exciting technology has numerous potential applications in the treatment of cardiac diseases such as cardiomyopathy by augmentation, regeneration or replacement of defective cardiomyocytes. It may also be employed in pharmacological testing for the evaluation of cardiotoxic compounds.¹³⁸ Presently, limited information con-

cerning mitochondrial structure and function in ES cells is available, nor has its use been applied to cardiac diseases with extensive mitochondrial abnormalities or for testing of mitochondrial cardiotoxicity.

Recently, mtDNAs from a respiratory-deficient chloramphenicol-resistant (CAPR) cell line from mice were introduced as enucleated cytoplasts into ES cells (treated with rhodamine 6G to eliminate ES cell mitochondria) using cell fusion; the resultant cybrids were injected into blastocysts and chimeric mice were obtained.¹³⁹ The CAPR phenotype which is due to a mtDNA point mutation at nucleotide near the 3' end of the 16S rRNA gene was shown to be transmitted maternally to the progeny. CAPR chimeric mice developed an array of ocular abnormalities, including congenital cataracts, and decreased retinal function as well as severe growth retardation, myopathy and dilated cardiomyopathy. Mitochondria were abnormally enlarged in skeletal and cardiac muscle of the CAPR mice and contained numerous electron-dense granules. While the cardiopathogenic mechanism of CAPR has not yet been fully elucidated, it is noteworthy that the CAPR mutation results in marked deficiency of mitochondrial protein synthesis and shares the severity and cardiac pathogenicity manifested by other mtDNA mutations affecting mitochondrial protein synthesis (e.g. the forementioned pathogenic tRNA and rRNA mutations).

While the mouse ES cell-cybrid approach offers a potential means to generate and examine animal models of mitochondrial disease, the use of ES cells technology to study human pathogenic mtDNA mutations involved in cardiac disease has not yet been attempted. A recent report proposed the possibility of introducing «mtDNA repaired» ES cells into a patient harboring a mtDNA defect, potentially transforming a diseased myocardium into a healthy one.¹⁴⁰ The «mtDNA-repaired» cell could likely be derived from the patient's own cells (bypassing immune rejection), which have undergone treatment with ethidium-bromide to remove the endogenous defective mtDNA genomes, and which have been newly retro-fitted to contain entirely wild-type mtDNA genes. In a similar vein, treatment of a patient's nuclear DNA defects might be envisioned as involving either a specific genetic replacement of the nuclear genetic defect (site-specific homologous recombination can be readily undertaken in ES cells) or if the precise site of the nuclear defect is not known, by replacement of the patient's entire nucle-

us with a wild-type nucleus and subsequent generation of an ES cells construct. The successful development of cell engineering using ES cells therapy may have a dramatic impact on the treatment of mitochondrial-based cardiac diseases.

The effective use of gene therapy for mtDNA-encoded OXPHOS defects awaited the development of a methodology for mitochondrial gene replacement in human cells. Towards this end, several laboratories have reported progress in the creation of cationic vectors (e.g. liposomes), to carry signal peptide-targeting sequences covalently attached to mitochondrial oligonucleotides into the mitochondria of living cells.¹⁴¹⁻¹⁴³ This type of mitochondrial transfection approach can be combined with an antisense strategy aimed at reducing the expression of the defective mitochondrial allele in the patient's tissue. In addition, genetic correction of a specific mitochondrial DNA deficiency in ATP synthase has been recently demonstrated.¹⁴⁴ Using directed expression of an engineered nuclear-localized version of a mtDNA-encoded polypeptide (ATPase δ) in human cybrids, both the biochemical and growth phenotype resulting from a mutant 8993 allele were corrected. A similar genetic approach may prove successful in the treatment of cardiac disorders due to defined mtDNA point mutations.

REFERENCES

1. Anderson S, Bankier AT, Barrell BG, De Bruijn MHL, Coulson AR, Drouin J, et al. Sequence and organization of human mitochondrial genome. *Nature* 1981;290:457-65.
2. Attardi G, Schatz G. Biogenesis of mitochondria. *Annu Rev Cell Biol* 1988;4:289-333.
3. Shadel GS, Clayton DA. Mitochondrial DNA maintenance in vertebrates. *Annu Rev Biochem* 1997;66:409-35.
4. Lopaschuk GD, Collins-Nakai RL, Itoi T. Developmental changes in energy substrate use by the heart. *Cardiovasc Res* 1992; 26:1172-80.
5. Sack MN, Harrington LS, Jonassen AK, Mjos OD, Yellon DM. Coordinate regulation of metabolic enzyme encoding genes during cardiac development and following carvedilol therapy in spontaneously hypertensive rats. *Cardiovasc Drugs Ther* 2000; 14:31-9.
6. Brown NF, Weis BC, Husti JE, Foster DW, McGarry JD. Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart. *J Biol Chem* 1995;270: 8952-7.
7. Cook GA, Edwards TL, Jansen MS, Bahouth SW, Wilcox HG, Park EA. Differential regulation of carnitine palmitoyltransferase-I gene isoforms (CPT-I alpha and CPT-I beta) in the rat heart. *J Mol Cell Cardiol* 2001;33:317-29.

8. Van Nieuwenhoven FA, Willemsen PH, Van der Vusse GJ, Glatz JF. Co-expression in rat heart and skeletal muscle of four genes coding for proteins implicated in long-chain fatty acid uptake. *Int J Biochem Cell Biol* 1999;31:489-98.
9. Tiivel T, Kadaya L, Kuznetsov A, Kaambre T, Peet N, Sikk P, et al. Developmental changes in regulation of mitochondrial respiration by ADP and creatine in rat heart *in vivo*. *Mol Cell Biochem* 2000;208:119-28.
10. Hoerter JA, Kuznetsov A, Ventura-Clapier R. Functional development of the creatine kinase system in perinatal rabbit heart. *Circ Res* 1991;69:665-76.
11. Marín-García J, Ananthakrishnan R, Goldenthal MJ. Human mitochondrial function during cardiac growth and development. *Mol Cell Biochem* 1998;179:21-6.
12. Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci* 2000;25:502-8.
13. Nulton-Persson AC, Szweda LI. Modulation of mitochondrial function by hydrogen peroxide. *J Biol Chem* 2001;276:23357-61.
14. Paradies G, Ruggiero FM. Age-related changes in the activity of the pyruvate carrier and in the lipid composition in rat-heart mitochondria. *Biochim Biophys Acta* 1990;1016:207-21.
15. McMillin JB, Taffet GE, Taegtmeier H, Hudson EK, Tate CA. Mitochondrial metabolism and substrate competition in the aging Fischer rat heart. *Cardiovasc Res* 1993;27:2222-8.
16. López Jiménez JA, Bordoni A, Lorenzini A, Rossi CA, Biagi PL, Hrelia S. Linoleic acid metabolism in primary cultures of adult rat cardiomyocytes is impaired by aging. *Biochem Biophys Res Commun* 1997;237:142-5.
17. Costell M, O'Connor JE, Grisolia S. Age-dependent decrease of carnitine content in muscle of mice and humans. *Biochem Biophys Res Commun* 1989 161:1135-43.
18. Linnane AW, Marzuki S, Ozawa T, Tanaka M. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* 1989;1:642-5.
19. Hattori K, Tanaka M, Sugiyama S, Obayashi T, Ito T, Satake T, et al. Age-dependent increase in deleted mitochondrial DNA in the human heart: possible contributory factor in presbycardia. *Am Heart J* 1991;121:1735-42.
20. Muller-Hocker J. Cytochrome-c-oxidase deficient cardiomyocytes in the human heart – an age-related phenomenon. A histochemical ultracytochemical study. *Am J Pathol* 1989;134:1167-73.
21. Ames BN, Shigenaga MK, Hagen TM. Mitochondrial decay in aging. *Biochim Biophys Acta* 1995;1272:165-70.
22. Miro O, Casademont J, Casals E, Perea M, Urbano-Márquez A, Rustin P, et al. Aging is associated with increased lipid peroxidation in human hearts, but not with mitochondrial respiratory chain enzyme defects. *Cardiovasc Res* 2000;47:624-31.
23. Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia – reperfusion, aging, and heart failure. *J Mol Cell Cardiol* 2001;33: 1065-89.
24. Lucas DT, Szweda LI. Declines in mitochondrial respiration during cardiac reperfusion: age-dependent inactivation of alpha-ketoglutarate dehydrogenase. *Proc Natl Acad Sci USA* 1999; 96:6689-9.
25. Rustin P, Lebidois J, Chretien D, Bourgeron T, Piechaud JF, Rotig A, et al. Endomyocardial biopsies for early detection of mitochondrial disorders in hypertrophic cardiomyopathies. *J Pediatr* 1994;124:224-8.
26. Buchwald A, Till H, Unterberg C, Oberschmidt R, Figulla HR, Wiegand V. Alterations of the mitochondrial respiratory chain in human dilated cardiomyopathy. *Eur Heart J* 1990;11:509-16.
27. Marín-García J, Goldenthal MJ, Pierpont ME, Ananthakrishnan R. Impaired mitochondrial function in idiopathic dilated cardiomyopathy: biochemical and molecular analysis. *J Card Fail* 1995;1:285-91.
28. Marín-García J, Ananthakrishnan R, Goldenthal MJ, Filiano JJ, Pérez-Atayde A. Cardiac mitochondrial dysfunction and DNA depletion in children with hypertrophic cardiomyopathy. *J Inherit Metab Dis* 1997;20:674-80.
29. Taniike M, Fukushima H, Yanagihara I, Tsukamoto H, Tanaka J, Fujimura H, et al. Mitochondrial tRNA Ile mutation in fatal cardiomyopathy. *Biochem Biophys Res Commun* 1992;186:47-53.
30. Silvestri G, Santorelli FM, Shanske S, Whitley CB, Schimmenti LA, Smith SA, et al. A new mtDNA mutation in the tRNA LEU(UUR) gene associated with maternally inherited cardiomyopathy. *Hum Mutat* 1994;3:37-43.
31. Santorelli FM, Mak SC, Vázquez-Acevedo M, González-Astiazaran A, Ridaura-Sanz C, González-Halphen D, et al. A novel mtDNA point mutation associated with mitochondrial encephalocardiomyopathy. *Biochem Biophys Res Commun* 1995;216:835-40.
32. Santorelli FM, Mak SC, El-Schahawi M, Casali C, Shanske S, Baram TZ, et al. Maternally inherited cardiomyopathy and hearing loss associated with a novel mutation in the mitochondrial tRNA Lys gene (G8363). *Am J Hum Genet* 1996;58:933-9.
33. Merante F, Tein I, Benson L, Robinson BH. Maternally inherited cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA Gly gene. *Am J Hum Genet* 1994;55:437-46.
34. Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, et al. Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNA Leu. *Lancet* 1991;338:143-7.
35. Casali C, Santorelli FM, D'Amati G, Bernucci P, DeBiase L, DiMauro S. A novel mtDNA point mutation in maternally inherited cardiomyopathy. *Biochem Biophys Res Commun* 1995;213:588-93.
36. McFarland R, Clark KM, Morris AA, Taylor RW, Macphail S, Lightowers RN, et al. Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. *Nat Genet* 2002;30: 145-6.
37. Schon EA, Bonilla E, DiMauro S. Mitochondrial DNA mutations and pathogenesis. *J Bioenerg Biomembr* 1997;29:131-49.
38. Santorelli FM, Tanji K, Manta P, Casali C, Krishna S, Hays AP, et al. Maternally inherited cardiomyopathy: an atypical presentation of the mtDNA 12S rRNA gene A1555G mutation. *Am J Hum Genet* 1999;64:295-300.
39. Arbustini E, Diegoli M, Fasani R, Grasso M, Morbini P, Banchieri N, et al. Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. *Am J Pathol* 1998;153:1501-10.
40. Li YY, Maisch B, Rose ML, Hengstenberg C. Point mutations in mitochondrial DNA of patients with dilated cardiomyopathy. *J Mol Cell Cardiol* 1997;29:2699-709.
41. Marín-García J, Goldenthal MJ, Ananthakrishnan R, Pierpont ME. The complete sequence of mtDNA novel in idiopathic dilated cardiomyopathy shows novel missense and tRNA mutations. *J Card Fail* 2000;6:321-9.
42. Shoffner JM, Wallace DC. Heart disease and mitochondrial DNA mutations. *Heart Dis Stroke* 1992;1:235-41.

43. Pastores GM, Santorelli FM, Shanske S, Gelb BD, Fyfe B, Wolfe D, et al. Leigh syndrome and hypertrophic cardiomyopathy in an infant with a mitochondrial point mutation (T8993G). *Am J Med Genet* 1994;50:265-71.
44. Anan R, Nakagawa M, Miyata M, Higuchi I, Nakao S, Suehara M, et al. Cardiac involvement in mitochondrial diseases: a study of 17 patients with mitochondrial DNA defects. *Circulation* 1995;91:955-61.
45. Graf WD, Marín-García J, Gao HG, Pizzo S, Naviaux RK, Markusic D, et al. Autism associated with the mitochondrial DNA G8363A transfer RNA(Lys)mutation. *J Child Neurol* 2000;15:357-61.
46. Matthews PM, Marchington DR, Squier M, Land J, Brown R, Brown GK. Molecular genetic characterization of an X-linked form of Leigh's syndrome. *Ann Neurol* 1993;33:652-5.
47. Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Pequignot E, et al. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* 1995;11:144-9.
48. Filiano JJ, Goldenthal MJ, Mamourian AC, Hall CC, Marín-García J. Mitochondrial DNA depletion in Leigh syndrome. *Pediatr Neurol* 2002;26:239-42.
49. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of mtDNA in patients with mitochondrial myopathies. *Nature* 1988;331: 717-9.
50. Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, et al. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988;38:1339-46.
51. Muller-Hocker J, Jacob U, Seibel P. The common 4977 base pair deletion of mitochondrial DNA preferentially accumulates in the cardiac conduction system of patients with Kearns-Sayre syndrome. *Mod Pathol* 1998;11:295-301.
52. Bohlega S, Tanji K, Santorelli FM, Hirano M, al-Jishi A, DiMauro S. Multiple mitochondrial DNA deletions associated with autosomal recessive ophthalmoplegia and severe cardiomyopathy. *Neurology* 1996;46:1329-34.
53. Suomalainen A, Paetau A, Leinonen H, Majander A, Peltonen L, Somer H. Inherited idiopathic dilated cardiomyopathy with multiple deletions of mitochondrial DNA. *Lancet* 1992;340:1319-20.
54. Marín-García J, Goldenthal MJ, Ananthkrishnan R, Pierpont ME, Fricker FJ, Lipshultz S, et al. Specific mitochondrial DNA deletions in idiopathic dilated cardiomyopathy. *Cardiovasc Res* 1996;31:306-14.
55. Simonetti S, Chen X, DiMauro S, Schon E. Accumulation of deletions in human mitochondrial DNA during normal aging: analysis by quantitative PCR. *Biochim Biophys Acta* 1992; 1180:113-22.
56. Marín-García J, Ananthkrishnan R, Goldenthal MJ, Pierpont ME. Biochemical and molecular basis for mitochondrial cardiomyopathy in neonates and children. *J Inherit Metab Dis* 2000;23:625-33.
57. Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. *Nature Med* 1995;1:417-22.
58. Herskowitz A, Willoughby SB, Baughman KL, Schulman SP, Bartlett JD. Cardiomyopathy associated with antiretroviral therapy in patients with HIV infection: a report of 6 cases. *Ann Intern Med* 1992;116:311-13.
59. Huizing M, Iacobazzi V, Ijlst L, Savelkoul P, Ruitenbeek W, van den Heuvel L, et al. Cloning of the human carnitine-acylcarnitine carrier cDNA and identification of the molecular defect in a patient. *Am J Hum Genet* 1997;61:1239-45.
60. Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 1996;271:1423-7.
61. Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, et al. Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. *Nat Genet* 1999;23:333-7.
62. Sue CM, Karadimas C, Checcarelli N, Tanji K, Papadopoulou LC, Pallotti F, et al. Differential features of patients with mutations in two COX assembly genes, SURF1 and SCO2. *Ann Neurol* 2000;47:589-95.
63. Kelly DP, Strauss AW. Inherited Cardiomyopathies. *N Eng J Med* 1994;330:913-9.
64. Strauss AW, Powell CK, Hale DE, Anderson MM, Ahuja A, Brackett JC, et al. Molecular basis of human mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency: causing cardiomyopathy and sudden death in childhood. *Proc Natl Acad Sci USA* 1995 92:10496-500.
65. Rocchiccioli F, Wanders RJ, Aubourg P, Vianey-Liaud C, Ijlst L, Fabre M, et al. Deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase: a cause of lethal myopathy and cardiomyopathy in early childhood. *Pediatr Res* 1990;28:657-62.
66. Tein I, Haslam RH, Rhead WJ, Bennett MJ, Becker LE, Vockley J. Short-chain acyl-CoA dehydrogenase deficiency: a cause of ophthalmoplegia and multicore myopathy. *Neurology* 1999;52:366-72.
67. Stanley CA, Treem WR, Hale DE, Coates PM. A genetic defect in carnitine transport causing primary carnitine deficiency. *Prog Clin Biol Res* 1990;321:457-64.
68. Brackett JC, Sims HF, Rinaldo P, Shapiro S, Powell CK, Bennett MJ, et al. Two subunit donor splice site mutations cause human trifunctional protein deficiency. *J Clin Invest* 1995;95: 2076-82.
69. Bonnet D, Martin D, de Lonlay P, Villain E, Jouviet P, Rabier D, et al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation* 1999;100:2248-53.
70. Corr PB, Creer MH, Yamada KA, Saffitz JE, Sobel BE. Prophylaxis of early ventricular fibrillation by inhibition of acylcarnitine accumulation. *J Clin Invest* 1989;83:927-36.
71. Tripp ME. Developmental cardiac metabolism in health and disease. *Pediatr Cardiol* 1989;10:150-8.
72. D'Adamo P, Fassone L, Gedeon A, Janssen EA, Bione S, Bolhuis PA, et al. The X-linked gene G4.5 is responsible for different infantile dilated cardiomyopathies. *Am J Hum Genet* 1997;61:862-7.
73. Bissler JJ, Tsoras M, Goring HH, Hug P, Chuck G, Tombragel E, et al. Infantile dilated x-linked cardiomyopathy, g4.5 mutations, altered lipids, and ultrastructural malformations of mitochondria in heart, liver, and skeletal muscle. *Lab Invest* 2002;82:335-44.
74. Vreken P, Valianpour F, Nijtmans LG, Grivell LA, Plecko B, Wanders RJ, et al. Defective remodeling of cardiolipin and phosphatidylglycerol in Barth syndrome. *Biochem Biophys Res Commun* 2000;279:378-82.
75. Fananapazir L, Dalakas MC, Cyran F, Cohn G, Epstein ND. Missense mutations in the beta-myosin heavy-chain gene cause central core disease in hypertrophic cardiomyopathy. *Proc Natl Acad Sci USA* 1993;90:3993-7.
76. Thompson CH, Kemp GJ, Taylor DJ, Conway M, Rajagopalan B, O'Donoghue A, et al. Abnormal skeletal muscle bioenergetics in familial hypertrophic cardiomyopathy. *Heart* 1997;78:177-81.

77. Arbustini E, Fasani R, Morbini P, Diegoli M, Grasso M, Dal Bello B, et al. Coexistence of mitochondrial DNA and beta myosin heavy chain mutations in hypertrophic cardiomyopathy with late congestive heart failure. *Heart* 1998;80:548-58.
78. Milner DJ, Mavroidis M, Weisleder N, Capetanaki Y. Desmin cytoskeleton linked to muscle mitochondrial distribution and respiratory function. *J Cell Biol* 2000;150:1283-98.
79. Corbucci GG. Adaptive changes in response to acute hypoxia, ischemia and reperfusion in human cardiac cell. *Minerva Anestesiol* 2000;66:523-30.
80. Ylitalo K, Ala-Rami A, Vuorinen K, Peuhkurinen K, Lepojarvi M, Kaukoranta P, et al. Reversible ischemic inhibition of F(1)F(0)-ATPase in rat and human myocardium. *Biochim Biophys Acta* 2001 1504:329-39.
81. Corral-Debrinski M, Stepien G, Shoffner JM, Lott MT, Kanter K, Wallace DC. Hypoxemia is associated with mitochondrial DNA damage and gene induction. Implications for cardiac disease. *JAMA* 1991;266:1812-6.
82. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. *Free Radic Biol Med* 1999;27:42-50.
83. Gottlieb RA, Burlison KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 1994;94:1621-28.
84. Sammut IA, Jayakumar J, Latif N, Rothery S, Severs NJ, Smolenski RT, et al. Heat stress contributes to the enhancement of cardiac mitochondrial complex activity. *Am J Pathol* 2001;158:1821-31.
85. Ghosh S, Standen NB, Galinanes M. Evidence for mitochondrial K ATP channels as effectors of human myocardial preconditioning. *Cardiovasc Res* 2000;45:934-40.
86. Marzo I, Brenner C, Zamzami N, Susin SA, Beutner G, Brdiczka D, et al. The permeability transition pore complex: a target for apoptosis regulation by caspases and Bcl-2 related proteins. *J Exp Med* 1998;187:1261-71.
87. Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* 1998;281:2027-31.
88. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta* 1998;1366:177-96.
89. Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. *J Neurosci* 1996;16:6125-33.
90. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. *Pharmacol Rev* 2002;54:101-27.
91. Wallace KB, Starkov AA. Mitochondrial targets of drug toxicity. *Annu Rev Pharmacol Toxicol* 2000;40:353-88.
92. Abdel-Aleem S, el-Merzabani MM, Sayed-Ahmed M, Taylor DA, Lowe JE. Acute and chronic effects of adriamycin on fatty acid oxidation in isolated cardiac myocytes. *J Mol Cell Cardiol* 1997;29:789-97.
93. Serrano J, Palmeira CM, Kuehl DW, Wallace KB. Cardioselective and cumulative oxidation of mitochondrial DNA following subchronic doxorubicin administration. *Biochim Biophys Acta* 1999;1411:201-5.
94. Zhou S, Heller LJ, Wallace KB. Interference with calcium-dependent mitochondrial bioenergetics in cardiac myocytes isolated from doxorubicin-treated rats. *Toxicol Appl Pharmacol* 2001;175:60-7.
95. Solem LE, Henry TR, Wallace KB. Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration. *Toxicol Appl Pharmacol* 1994;129:214-22.
96. Adachi K, Fujiura Y, Mayumi F, Nozuhara A, Sugiy Y, Sakanashi T, et al. A deletion of mitochondrial DNA in murine doxorubicin-induced cardiotoxicity. *Biochem Biophys Res Commun* 1993;195:945-51.
97. Yen HC, Oberley TD, Vichitbandha S, Ho YS, St Clair DK. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. *J Clin Invest* 1996;98:1253-60.
98. Shneyvays V, Mamedova L, Zinman T, Jacobson K, Shainberg A. Activation of A (3) adenosine receptor protects against doxorubicin-induced cardiotoxicity. *J Mol Cell Cardiol* 2001;33:1249-61.
99. Sayed-Ahmed MM, Salman TM, Gaballah HE, Abou El-Naga SA, Nicolai R, Calvani M. Propionyl-L-carnitine as protector against adriamycin-induced cardiomyopathy. *Pharmacol Res* 2001;43:513-20.
100. Wang GW, Klein JB, Kang YJ. Metallothionein inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3 activation in cardiomyocytes. *J Pharmacol Exp Ther* 2001;298: 461-8.
101. Schulze K, Schultheiss HP. The role of the ADP/ATP carrier in the pathogenesis of viral heart disease. *Eur Heart J* 1995;16 (Suppl O):64-7.
102. Fuchs CS, Stampfer MJ, Colditz GA, Giovannucci EL, Manson JE, Kawachi I, et al. Alcohol consumption and mortality among women. *N Engl J Med* 1995;332:1245-50.
103. Marín-García J, Ananthakrishnan R, Goldenthal MJ. Heart mitochondria response to alcohol is different than brain and liver. *Alcohol Clin Exp Res* 1995;19:1463-6.
104. Siddiq T, Salisbury JR, Richardson PJ, Preedy VR. Synthesis of ventricular mitochondrial proteins *in vivo*: effect of acute ethanol toxicity. *Alcohol Clin Exp Res* 1993;17:894-9.
105. Beckemeier ME, Bora PS. Fatty acid ethyl esters: potentially toxic products of myocardial ethanol metabolism. *J Mol Cell Cardiol* 1998;30:2487-94.
106. Schoppet M, Maisch B. Alcohol and the heart. *Herz* 2001;26:345-52.
107. Graham BH, Waymire KG, Cottrell B, Trounce IA, MacGregor GR, Wallace DC. A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat Genet* 1997;16:226-34.
108. Lebovitz RM, Zhang H, Vogel H, Cartwright Jr, Dionne L, Lu N, et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci USA* 1996;93:9782-7.
109. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995;11:376-81.
110. Djouadi F, Brandt JM, Weinheimer CJ, Leone TC, González FJ, Kelly DP. The role of the peroxisome proliferator-activated receptor alpha (PPAR α) in control of cardiac lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids* 1999;60:339-43.
111. Ibdah JA, Paul H, Zhao Y, Binford S, Salleng K, Cline M, et al. Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and sudden death. *J Clin Invest* 2001;107:1403-9.
112. Wang J, Wilhelmsson H, Graff C, Li H, Oldfors A, Rustin P, et al. Dilated cardiomyopathy and atrioventricular con-

- duction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. *Nat Genet* 1999;21:133-7.
113. Puccio H, Simon D, Cossee M, Criqui-Filipe P, Tiziano F, Melki J, et al. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat Genet* 2001;27:181-6.
 114. Li H, Wilhelmsson H, Hanson A, Thoren P, Duffy J, Rustin P, et al. Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. *Proc Natl Acad Sci USA* 2000;97:3467-72.
 115. Radford NB, Wan B, Richman A, Szczepaniak LS, Li JL, Li K, et al. Cardiac dysfunction in mice lacking cytochrome-c oxidase subunit VIaH. *Am J Physiol Heart Circ Physiol* 2002;282:H726-33.
 116. Inoue K, Nakada K, Ogura A, Isobe K, Goto Y, Nonaka I, et al. Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. *Nat Genet* 2000;26:176-81.
 117. Nakada K, Inoue K, Chen CS, Nonaka I, Goto Y, Ogura A, et al. Correlation of functional and ultrastructural abnormalities of mitochondria in mouse heart carrying a pathogenic mutant mtDNA with a 4696-bp deletion. *Biochem Biophys Res Commun* 2001;288:901-7.
 118. Nakada K, Inoue K, Ono T, Isobe K, Ogura A, Goto YI, et al. Inter-mitochondrial complementation: Mitochondria-specific system preventing mice from expression of disease phenotypes by mutant mtDNA. *Nat Med* 2001;7:934-40.
 119. Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, et al. The cardiac phenotype induced by PPAR- α overexpression mimics that caused by diabetes mellitus. *J Clin Invest* 2002;109:121-30.
 120. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 2000;106:847-56.
 121. Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS, et al. A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat Genet* 1998;18:159-63.
 122. Marín-García J, Ananthakrishnan R, Goldenthal MJ, Filiano JJ, Pérez-Atayde A. Mitochondrial dysfunction in skeletal muscle of children with cardiomyopathy. *Pediatrics* 1999;103:456-9.
 123. Chomyn A, Meola G, Bresolin N, Lai ST, Scarlato G, Attardi G. *In vitro* genetic transfer of protein synthesis and respiration defects to mitochondrial DNA-less cells with myopathy patient mitochondria. *Mol Cell Biol* 1991;11:2236-44.
 124. Saudubray JM, Martin D, de Lonlay P, Touati G, Poggi-Travert F, Bonnet D, et al. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999;22:488-502.
 125. Shoffner JM, Wallace DC. Oxidative phosphorylation diseases and mitochondrial DNA mutations: diagnosis and treatment. *Annu Rev Nutr* 1994;14:535-68.
 126. Langsjuen P, Vadhanavikit S, Folkers K. Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and cross-over trial with coenzyme Q10. *Proc Natl Acad Sci USA* 1985;82:4240-4.
 127. Rustin P, von Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A, Rotig A. Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study. *Lancet* 1999;354:477-9.
 128. Lerman-Sagie T, Rustin P, Lev D, Yanoov M, Leshinsky-Silver E, Sagie A, et al. Dramatic improvement in mitochondrial cardiomyopathy following treatment with idebenone. *J Inherit Metab Dis* 2001;24:28-34.
 129. Bonnefont JP, Demaugre F, Prip-Buus C, Saudubray JM, Brivet M, Abadi N, et al. Carnitine palmitoyltransferase deficiencies. *Mol Genet Metab* 1999;68:424-40.
 130. Brown-Harrison MC, Nada MA, Sprecher H, Vianey-Saban C, Farquhar J Jr, Gilladoga AC, et al. Very long chain acyl-CoA dehydrogenase deficiency: successful treatment of acute cardiomyopathy. *Biochem Mol Med* 1996;58:59-65.
 131. Pierpont ME, Breningstall GN, Stanley CA, Singh A. Familial carnitine transporter defect: a treatable cause of cardiomyopathy in children. *Am Heart J* 2000;139:S96-S106.
 132. Pepe S, Tsuchiya N, Lakatta EG, Hansford RG. PUFA and aging modulate cardiac mitochondrial membrane lipid composition and Ca²⁺ activation of PDH. *Am J Physiol* 1999; 276:H149-58.
 133. Pollitt RJ. Disorders of mitochondrial long-chain fatty acid oxidation. *J Inherit Metab Dis* 1995;18:473-90.
 134. Wallhaus TR, Taylor M, DeGrado TR, Russell DC, Stancko P, Nickles RJ, et al. Myocardial free fatty acid and glucose use after carvedilol treatment in patients with congestive heart failure. *Circulation* 2001;103:2441-6.
 135. Doetschman T, Shull M, Kier A, Coffin JD. Embryonic stem cell model systems for vascular morphogenesis and cardiac disorders. *Hypertension* 1993;22:618-29.
 136. Muller M, Fleischmann BK, Selbert S, Ji GJ, Endl E, Middel G, et al. Selection of ventricular-like cardiomyocytes from ES cells *in vitro*. *FASEB J* 2000;14:2540-8.
 137. Wobus AM, Kaomei G, Shan J, Wellner MC, Rohwedel J, Ji G, et al. Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes. *J Mol Cell Cardiol* 1997;29:1525-39.
 138. Bremer S, Worth AP, Paparella M, Bigot K, Kolosov E, Fleischmann BK, et al. Establishment of an *in vitro* reporter gene assay for developmental cardiac toxicity. *Toxicol In Vitro* 2001;15:215-23.
 139. Sligh JE, Levy SE, Waymire KG, Allard P, Dillehay DL, Nusinowitz S, et al. Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice. *Proc Natl Acad Sci USA* 2000;97:14461-6.
 140. Zullo SJ. Gene therapy of mitochondrial DNA mutations: a brief, biased history of allotopic expression in mammalian cells. *Semin Neurol* 2001;21:327-35.
 141. Geromel V, Cao A, Briane D, Vassy J, Rotig A, Rustin P, et al. Mitochondria transfection by oligonucleotides containing a signal peptide and vectorized by cationic liposomes. *Antisense Nucleic Acid Drug Dev* 2001;11:175-80.
 142. Taylor RW, Wardell TM, Lightowlers RN, Turnbull DM. Molecular basis for treatment of mitochondrial myopathies. *Neurol Sci* 2000;21:S909-12.
 143. Weissig V, Torchilin VP. Towards mitochondrial gene therapy: DQAsomes as a strategy. *J Drug Target* 2001;9:1-13.
 144. Manfredi G, Fu J, Ojaimi J, Sadlock JE, Kwong JQ, Guy J, et al. Rescue of a deficiency in ATP synthesis by transfer of MTATP6, a mitochondrial DNA-encoded gene, to the nucleus. *Nat Genet* 2002;30:394-9.
 145. Kawarai T, Kawakami H, Kozuka K, Izumi Y, Matsuyama Z, Watanabe C, et al. A new mitochondrial DNA mutation associated with mitochondrial myopathy: tRNA (Leu) (UUR) 3254C-to-G. *Neurology* 1997;49:598-600.
 146. Grasso M, Diegoli M, Brega A, Campana C, Tavazzi L, Arbustini E. The mitochondrial DNA mutation T12297C

- affects a highly conserved nucleotide of tRNA (Leu (CUN)) and is associated with dilated cardiomyopathy. *Eur J Hum Genet* 2001;9:311-5.
147. Tanaka M, Ino H, Ohno K, Hattori K, Sato W, Ozawa T, et al. Mitochondrial mutation in fatal infantile cardiomyopathy. *Lancet* 1990;336:1452.
 148. Merante F, Myint T, Tein I, Benson L, Robinson BH. An additional mitochondrial tRNA (Ile)point mutation (A-to-G at nucleotide 4295) causing hypertrophic cardiomyopathy. *Hum Mutat* 1996;8:216-22.
 149. Corona P, Lamantea E, Greco M, Carrara F, Agostino A, Guidetti D, et al. Novel heteroplasmic mtDNA mutation in a family with heterogeneous clinical presentations. *Ann Neurol* 2002;51:118-22.
 150. Akita Y, Koga Y, Iwanaga R, Wada N, Tsubone J, Fukuda S, et al. Fatal hypertrophic cardiomyopathy associated with an A8296G mutation in the mitochondrial tRNA (Lys)gene. *Hum Mutat* 2000;15:382.
 151. Terasaki F, Tanaka M, Kawamura K, Kanzaki Y, Okabe M, Hayashi T, et al. A case of cardiomyopathy showing progression from the hypertrophic to the dilated form: association of Mt 8348A- >G mutation in the mitochondrial tRNA (Lys) gene with severe ultrastructural alterations of mitochondria in cardiomyocytes. *Jpn Circ J* 2001;65:691-4.
 152. Karadimas C, Tanji K, Geremek M, Chronopoulou P, Vu T, Krishna S, et al. A5814G mutation in mitochondrial DNA can cause mitochondrial myopathy and cardiomyopathy. *J Child Neurol* 2001;16:531-3.
 153. Hsieh RH, Li JY, Pang CY, Wei YH. A novel mutation in the mitochondrial 16S rRNA gene in a patient with MELAS syndrome, diabetes mellitus, hyperthyroidism and cardiomyopathy. *J Biomed Sci* 2001;8:328-35.
 154. Obayashi T, Hattori K, Sugiyama S, Tanaka M, Tanaka T, Itoyama S, et al. Point mutations in mitochondrial DNA in patients with hypertrophic cardiomyopathy. *Am Heart J* 1992;124:1263-9.
 155. Marin-García J, Ananthkrishnan R, Gonzalvo A, Goldenthal MJ. Novel mutations in mitochondrial cytochrome b in a case of fatal post-partum cardiomyopathy. *J Inher Metab Dis* 1995;18:77-8.
 156. Andreu AL, Checcarelli N, Iwata S, Shanske S, DiMauro S. A missense mutation in the mitochondrial cytochrome b gene in a revisited case with histiocytoid cardiomyopathy. *Pediatr Res* 2000;48:311-4.
 157. Valnot I, Kassis J, Chretien D, de Lonlay P, Parfait B, Munnich A, et al. A mitochondrial cytochrome b mutation but no mutations of nuclearly encoded subunits in ubiquinol cytochrome c reductase deficiency. *Hum Genet* 1999; 104:460-6.