EDITORIAL VIEWPOINT

Human "Nuclear" Mitochondrial Cardiomyopathy

A Novel Mouse Model Characterizes the Disease*

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Mitochondrial Cardiomyopathies Can Result From Defects of Nuclear and Mitochondrial Genes

Mitochondria generate much of the energy for the cell by oxidative phosphorylation (1); accordingly, mitochondrial diseases preferentially affect tissues with high energy demands, such as the heart, brain, muscle, and endocrine system. The heart is affected either as an isolated organ (cardiomyopathies) or, more frequently, as one of the organs/tissues

involved in more complex systemic disorders/syndromes (including encephalomyopathies and chronic ophthalmoplegia with myopathy). Cardiomyopathy can represent the initial presentation of mitochondrial diseases (2). Because proteins contributing to energy production are encoded by both mitochondrial and nuclear genes, mitochondrial diseases can be inherited both as matrilineal (mitochondrial deoxyribonucleic acid [mtDNA] genes) and Mendelian (nuclear genes) traits (3).

mtDNA-related disorders can result from mutations in protein-encoding genes or in genes encoding transfer ribonucleic acids and ribosomal ribonucleic acids (4). Mendelian mitochondrial diseases can result from mutations in nuclear genes coding subunits of the respiratory chain, proteins involved

in intergenomic nuclear-mitochondrial cross-talk (mtDNA maintenance, replication, or translation), proteins involved in mechanisms of protein assembly and import, synthesis and composition of mitochondrial membrane, and mitochondrial dynamics (5). A complex interplay regulates the functional integration of products coded by nuclear and mitochondrial genes, and several nuclear genes are involved in the control of mtDNA stability. Disorders of mtDNA maintenance frequently show Mendelian inheritance; they are associated with multiple mtDNA deletions (6) and depletion (7). The clinical impact of defects in mtDNA maintenance has recently been shown to go beyond rare Mendelian and matrilineal diseases affecting mitochondrial biogenesis, and extend to multifactorial common conditions such as human heart failure (8). Therefore, models of mitochondrial diseases influencing the maintenance of mtDNA may provide new insights for investigating mechanisms of heart failure.

Adenine nucleotide translocators (ANT). The ANT proteins are adenosine diphosphate (ADP)/adenosine triphosphate (ATP) carriers that belong to the mitochondrial anion carrier protein family (9) and transport solutes across the inner mitochondrial membrane (10). ANT is, in fact, embedded in the inner mitochondrial membrane and constitutes approximately 10% of mitochondrial proteins (11). ANT has a dual function: under physiological conditions, it catalyzes ADP/ATP exchange across the inner mitochondrial membrane, whereas under lethal stimuli, ANT contributes to apoptosis via opening of the mitochondrial permeability pore with translocation of pro-apoptotic proteins such as cytochrome c (9,12).

In its functional homodimeric conformation of 30-kD subunits, ANT forms gated pores through which the ATP synthesized in the mitochondrial matrix by oxidative phosphorylation is exported to

the cytosol, in exchange for ADP (13). Inside of the inner mitochondrial membrane, ATP binds to one of the subunits; outside, ADP binds to the other subunit. The electrochemical gradient of the inner membrane, which is high under physiological conditions, drives the 2 adenine nucleotides to opposite sides of the membrane (13). Recent studies propose a monomeric transport model in which access to a single substrate binding site is controlled by 2 flanking salt bridge networks (14).

The human ANT subfamily is composed of 4 differentially expressed isoforms, namely ANT1 to ANT4, encoded by 4 nuclear genes, with ANT1 to ANT3 sharing approximately 90% homology at the amino acid level (15–18). The ANT1 and ANT3 isoforms induce mitochondrial apoptosis, whereas ANT2 and ANT4 isoforms render cells resistant to death-inducing stimuli (19,20). The human heart-muscle specific isoform ANT1 is coded by a small developmentally regulated nuclear gene (*ANT1; Chr* 4q35-1ter) that has 4 exons and a promoter with the typical *CCAAT* and *TATA* sequences (15). The corresponding mouse *Ant1* gene maps to chromosome 8, syntenic to human chromosome 4q35, and has 4 exons (21,22).

The Ant1 cardiomyopathy hypothesis. In this issue of *iJACC*, Narula et al. (23) have used an experimental *Ant1* beta-geo mutant (Ant1−/−) mouse model (21) to test the hypothesis that chronic mitochondrial energy deficiency causes dilated cardiomyopathy.

Imaging studies characterize the clinical phenotype of ANT1 cardiomyopathy. Narula et al. (23) performed a multi-step in vivo investigation using 2-dimensional echocardiography with M-mode and velocity vector imaging (VVI) in a large series of mutant versus control (*Ant1+/+*) mice 2 to 21 months of age. The imaging studies aimed at evaluating left ventricular (LV) morphology and function in mutant versus normal mice.

The first step of the study was the comparison of all mutant versus control mice. Although the heart weight and heart/body weight ratio, left ventricular dimension at end diastole and end-systole, interventricular septum (IVS), left ventricular posterior wall thickness, and IVS/posterior wall ratio were significantly increased in mutant mice, the fractional shortening and LV ejection fraction (EF) were significantly reduced. The cardiomyopathy developed in the mutant animals was characterized by early LV concentric hypertrophy and late dilation and dysfunction.

The second step of the study addressed the question of variability of contractile parameters in mutant mice and the hypothesis that variable parameters may depend on penetrance or be age-dependant. The results demonstrated that the EF of mutant mice declined with age, being significantly more impaired in older mice (>15 months). Mutant mice were then grouped according to the mean EF and SD on a cutoff value of 56%, which revealed that the EF was below lower normal limits in nearly 60% of mutant animals. By recalculating parameters in the 2 subgroups of mutant mice with normal and abnormal EF, the latter showed larger ventricles. Mutant mice with normal or abnormal EF also showed significant increase in IVS thickness.

Finally, the evaluation of contractile mechanics documented that both normal EF and abnormal EF mutants showed reduction in LV rotational velocities, more pronounced in the latter subgroup. Circumferential strain and radial strain were reduced in mutant as compared to control mice, and the decline was more severe in animals with abnormal EF than those with normal EF. These observations indicate that mutant mice with normal EF show abnormal subclinical contractile indexes. Mutant mice also showed significantly decreased LV apical rotation. The LV apical rotation and twist are significantly influenced by LV configuration (24,25). Interestingly, circumferential strain, radial strain, and rotational velocity in diastole showed better accuracy than EF in differentiating mutants from control mice and mutant mice with normal EF from control mice.

Overall, the extensive echocardiography studies showed that *Ant1−/−* mice develop a "hypertrophic concentric dilated cardiomyopathy." This phenotypical description, which seems to contain contradictory terms, actually reflects the phenotype of human mitochondrial cardiomyopathies, both Mendelian and matrilineal, which are characterized by early concentric hypertrophy and later progression to dilation and dysfunction. In fact, mitochondrial cardiomyopathies in their end-stage often look like dilated cardiomyopathies (26).

Pathology studies confirm the clinical phenotype. Mutant animals showed myocyte hypertrophy and interstitial inflammation, as expected from imaging studies; additional findings were myofibrillar lysis, myocyte calcification, and binucleation. Myocyte hypertrophy was significantly more common in young mutants (<12 months old), and this difference in myocyte hypertrophy was lost in older mice, due to the increased presence of myocyte hypertrophy in older control mice. The increased myocyte

JACC: CARDIOVASCULAR IMAGING, VOL. 4, NO. 1, 2011
JANUARY 2011:11–5

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size in mitochondrial cardiomyopathies is typically due to mitochondrial proliferation (21), and is a hallmark of mitochondrial cardiomyopathies. Fibrosis was also increased in mutant hearts, and was even more evident and significant in older mutants, which also showed replacement fibrosis. Therefore, hypertrophy is an early finding, concordant with functional and morphologic data in vivo, whereas fibrosis appears later and occurs with greater frequency in mutants.

Ant1−/− hearts and activation of apoptosis pathway. The Western blot analysis showed 6-fold higher Cytochrome c levels, 4-fold increase of full-length caspase-3, and a significant proportion of cleaved caspase-3 in the older mutants as compared with older control mice; further, negligible levels of activated caspase 3 were observed in control mice. ANT1 plays a major role in promoting apoptosis. In fact, it is a component of the mitochondrial permeability transition pore (mtPTP) (27) that mediates the mitochondrial membrane permeabilization, a rate-limiting step of apoptosis. Opening of mtPTP dissipates the electrochemical gradient and inhibits ATP synthesis; the mitochondrial matrix swells, and the outer membrane ruptures, with release of cytochrome c and other proapoptotic proteins into the cytosol. Mitochondria from livers of *Ant1−/−* mice still possess mtPTP activity, as

ANT is a structural component of mtPTP and substantially contributes to its regulation (28). This property makes the *Ant1−/−* model especially interesting to test the effects of molecules modulating the apoptotic cascade (29).

Human diseases associated with mutations of the ANT1 gene. The number of mutations of *ANT1* reported to date in humans is limited (Fig. 1): most *ANT1* defects are associated with autosomal dominant Chronic Progressive External Ophthalmoplegia without cardiomyopathy (30–32); the sole cardiomyopathy reported in humans to date is autosomal recessive and is characterized by concentric left ventricular hypertrophy, dilation, and dysfunction (33), a phenotype that is reproduced by the *Ant1−/−* mouse model in the present study (23). The low number of reported cases can be due to the still-limited screening studies or to the true rarity of *ANT1* mutations as cause of cardiomyopathy. The "hypertrophic dilated phenotype" might be clinically confounding, and the recessive inheritance does not help, especially in small families in which consanguinity might not be apparent or there are no markers to guide genetic testing to *ANT1*. The *Ant1−/−* mouse model highlights the relevance of knowing the entire natural history of cardiomyopathies or investigating large series to establish the natural course of the disease. A patient seen in the early phase of the disease may show concentric LV hypertrophy without dilation, whereas a patient seen in
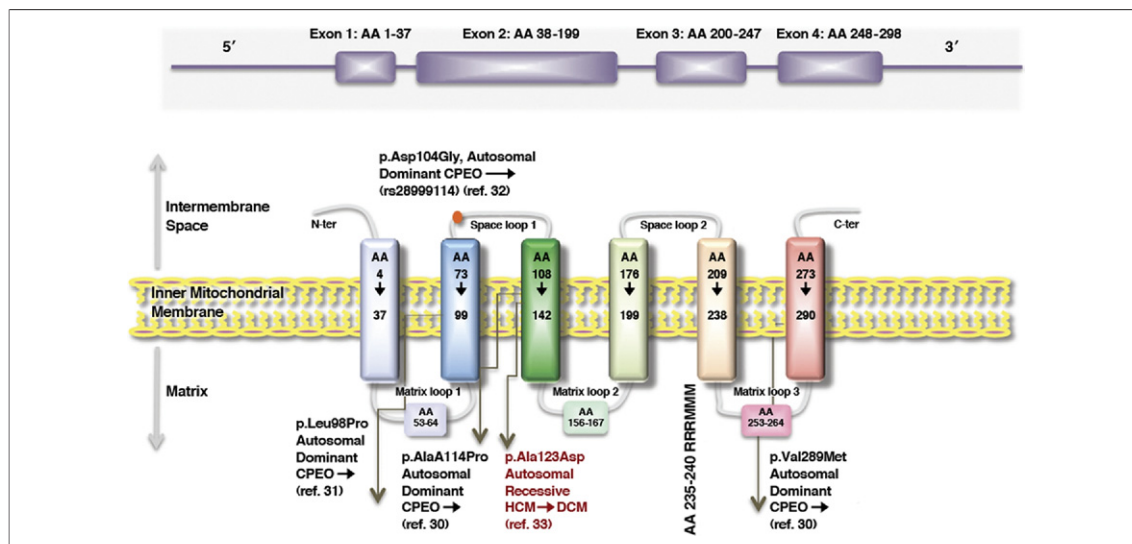

Figure 1. Human *ANT1* Gene and Mutations Reported to Date

The schematic shows the structure of the human *ANT1* gene and the mutations reported to date in humans that cause autosomal dominant Chronic Progressive External Ophthalmoplegia (CPEO) (31–33) and autosomal recessive cardiomyopathy and myopathy. The human *ANT1* gene maps at chromosome 4q35; it has 4 exons, as does the mouse *Ant1* gene. Each *ANT1* monomer has 6 transmembrane helixes and contains the highly conserved consensus sequence RRRMMM, with the 3 arginine residues lying at the base of a conical well in which the adenine nucleotides bind. The promoter beta-geo cassette of the *Ant1−/−* mice is inserted into the exon 2; the only *ANT1* mutation causing cardiomyopathy predicts a p.Ala123Asp substitution in exon 2. AA = amino-acid residues; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy.

the late phases of the diseases may show dilation and dysfunction, and the hypertrophy markers—both electrocardiographic and echocardiographic—may be lost. Unless other family members are diagnosed in their early phases, the disease can be labeled as dilated cardiomyopathy. This late phenotype might limit the investigation of nonsarcomeric genes that cause hypertrophic cardiomyopathy, especially when parental status is unknown.

Translational impact of the animal model: when should cardiologists suspect "nuclear" mitochondrial cardiomyopathies? Human mitochondrial cardiomyopathies, both matrilineal and Mendelian, typically show progressive evolution though LV dilation and end-stage heart failure (26), independent of the possible early hypertrophic concentric phenotype. The *Ant1−/−* model reflects the phenotype and evolution of mitochondrial cardiomyopathies (23). Rarely, the heart is the only affected organ/tissue, especially at onset (2): the "hypertrophic dilated cardiomyopathy" phenotype recurs in several mitochondrial diseases caused by defects of nuclear genes. Most of them share autosomal recessive inheritance (Leigh Syndrome [34], Sengers Syndrome [35], dilated cardiomyopathy and ataxia [36]) or are X-linked recessive, such as Barth Syndrome (37). The family data and clinical markers that may guide diagnostic investigation include the nonmatrilineal inheritance; the involvement of skeletal muscle, brain, eyes, and liver; and the presence of lactic acidosis either after effort or at rest. Common clinical markers will be highlighted as far as the number of reported cases increase, thus providing a clinical guide for suspecting nuclear mitochondrial cardiomyopathies.

Future considerations. Mitochondrial dysfunction accounts for impaired myocardial energetics and increased cell death during myocardial injury and the development of heart failure. Animal models reproducing human mitochondrial dysfunction, both monogenic cardiomyopathy phenotypes observed in rare diseases and multifactorial disorders associated with loss of mtDNA, are of extraordinary importance to investigate pathogenetic mechanisms and explore the effects of existing or novel drugs/molecules for possible disease-specific treatments, potentially recovering energy production and interfering with the loss of energy. The *Ant-/-* mouse model, reported by Narula et al. (23), very elegantly reproduces the clinical phenotype of cardiomyopathies caused by homozygous *ANT1* gene defects in humans and should be potentially useful for developing novel pharmacologic interventions. Immediate advantages are related to the application of the best imaging approaches and novel imaging tools, particularly in the early, subclinical phases of the disease.

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