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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).

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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

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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