Adenine Nucleotide Translocase 1 Deficiency Results in Dilated Cardiomyopathy With Defects in Myocardial Mechanics, Histopathological Alterations, and Activation of Apoptosis

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OBJECTIVES The aim of this study was to test the hypothesis that chronic mitochondrial energy deficiency causes dilated cardiomyopathy, we characterized the hearts of age-matched young and old adenine nucleotide translocator (ANT)1 mutant and control mice.

BACKGROUND ANT1s export mitochondrial adenosine triphosphate into the cytosol and have a role in the regulation of the intrinsic apoptosis pathway. Mitochondrial energy deficiency has been hypothesized, on the basis of indirect evidence, to be a factor in the pathophysiology of dilated cardiomyopathies. Ant1 inactivation should limit adenosine triphosphate for contraction and calcium transport, thereby resulting in early cardiac dysfunction with later dilatation and heart failure.

METHODS We conducted a multiyear study of 73 mutant (Ant1−/−) and 57 control (Ant1+/+) mice, between the ages of 2 and 21 months. Hearts were characterized by cardiac anatomy, echocardiographic imaging with velocity vector analysis, histopathology, and apoptosis assays.

RESULTS The Ant1−/− mice developed a distinctive concentric dilated cardiomyopathy, characterized by substantial myocardial hypertrophy and ventricular dilation, with cardiac function declining earlier in age as compared to control mice. Left ventricular circumferential, radial, and rotational mechanics were reduced even in the younger mutants with preserved systolic function. Histopathologic analysis demonstrated increased myocyte hypertrophy, fibrosis, and calcification in the mutant mice as compared with control mice. Furthermore, increased cytoplasmic cytochrome c levels and caspase 3 activation were observed in the mutant mice.

CONCLUSIONS Our results demonstrate that mitochondrial energy deficiency is sufficient to cause dilated cardiomyopathy, confirming that energy defects are a factor in this disease. Energy deficiency initially leads to early mechanical dysfunction before a decline in left ventricular systolic function. Chronic energy deficiency with age then leads to heart failure. Our results now allow us to use the Ant1−/− mouse model for testing new therapies for ANT1 mutant patients. (J Am Coll Cardiol Img 2011;4:1–10) © 2011 by the American College of Cardiology Foundation
Mitochondria generate adenosine triphosphate by oxidative phosphorylation. The resulting matrix adenosine triphosphate is exchanged across the mitochondrial membrane for cytosolic deoxyribonucleoside diphosphate by the adenine nucleotide translocators (ANTs) (1). Nuclear-encoded ANTs are 30-kDa transmembrane polypeptides proposed to function as homodimers (2). Certain ANT isoforms also play a role in the regulation of apoptosis through modulating the mitochondrial permeability transition pore (3–5). Animals harbor up to 4 ANT isoforms, which exhibit differential tissue-specific and temporal expression patterns. In both human and mouse, isoform 1 (ANT1, human; Ant1, mouse) is predominantly expressed in high-energy tissues, including heart, muscle, and brain (6–8).

In humans, ANT1 null, autosomal recessive mutations cause mitochondrial myopathy and cardiomyopathy (9). Antibodies against ANT have been commonly observed in patients with dilated cardiomyopathy (10). These observations implicate ANT1 in the etiology of cardiomyopathy associated with dilated, reduced function, and cardiomyocyte loss by apoptosis (11,12). Before the discovery of Ant1-deficient patients, our laboratory genetically inactivated Ant1 in the mouse. The Ant1−/− mice are viable and fertile; however, they develop mitochondrial myopathy with hyperpolarization of abnormal mitochondria, ragged red muscle fibers, and lactic acidosis (13). This mouse Ant1 defect is also associated with increased mitochondrial reactive oxidative species (ROS), antioxidant enzyme induction, and early accumulation of mitochondrial deoxyribonucleic acid (mtDNA) mutations (14).

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**Methods**

**Mice.** We used homozygous Ant1B-geo mutant (Ant1−/−) and control (Ant1+/+) mice on C57BL/6 or 129S4 backgrounds. No clear differences were seen in results for the different backgrounds. The Ant1 mutant mice and polymerase chain reaction genotyping methods were as described (13). All animal procedures were approved by University of California Irvine Institutional Animal Care and Use Committee (#2002-2399).

**Cardiac imaging.** Two-dimensional echocardiography with M-mode and velocity vector imaging (VVI) (Amid, Italy, and Siemens, Mountain View, California), were performed on 73 mutant and 57 control mice (ages 2 to 21 months; 63 female and 67 male). The animals were anesthetized and ventilated with 98% oxygen and 2% isoflurane. Electrocardiography leads were applied to monitor heart rate and trigger echo image acquisitions. Ultrasound images were obtained with a 12-MHz frequency linear phased probe (Sequoia, Siemens), in left ventricular (LV) short-axis view at apex level. Zoomed images were digitally captured. M-mode images at mid LV were used to determine left ventricular dimension at end-diastole (LVIDd) and LV dimension at end-systole. The LV ejection fraction (EF) was calculated. Interventricular septal thickness (IVS) and posterior wall thickness were obtained, and IVS/posterior wall ratio was determined. Echocardiograms in DICOM 4.0 format were analyzed with Research Arena (TomTec, Unterschleissheim, Germany) based 2-dimensional VVI package (Version 3.702, Siemens). The endocardial and epicar-
dial borders were manually identified in a single frame of a cine-loop, and the borders in other frames were automatically generated. Segmental peak systolic velocity, diastolic velocity, radial strain, and circumferential strain were obtained by VVI. Accuracy of VVI for testing myocardial deformation in experimental settings has been done previously (17).

**Histopathology.** Of 130 mice imaged, 72 (ages 2 to 19 months; 20 males and 52 females) underwent histopathologic analysis. Cardiac tissue was fixed in either formalin or paraformaldehyde, paraffin-embedded, and sectioned (7 μm). Sections were stained with hematoxylin and eosin. Sections from 69 mice were also stained with Masson’s Trichrome.

**Western blot analysis.** Heart samples (50 mg) from 3 mutants and 3 control mice, ages 14 to 21 months, were diced and solubilized in 210 mmol/l mannitol, 70 mmol/l sucrose, 1 mmol/l ethylene glycol tetraacetic acid, 5 mmol/l HEPES pH 7.4, and 110 μg/μl digitonin for 5 min. The samples were centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant protein concentration was determined by the BioRad Protein Assay reagent (BioRad, Hercules, California). Samples were read spectrophotomically at λ = 595 nm with Spectronic Genesys 2 (Thermo Fisher Scientific, Waltham, Massachusetts). Samples were boiled for 5 min, and proteins were separated on 8-cm length with Tris-glycine polyacrylamide gel (8-cm length) with Tris-glycine buffer. After blotting, membranes were processed with first step antibody. We used mouse antibodies: monoclonal anti–cytochrome-c (PharMingen International, San Diego, California); polyclonal anti-caspase 3 (Biotechnology, Santa Cruz, California); and monoclonal anti–GAPDH (Chemicon International, Temecula, California). Second-step antibody was polyclonal anti-mouse IgG conjugated to horseradish peroxidase (Amersham Biosciences, San Diego, California).

**Statistical analysis.** We used JMP version 7.0 (SAS Institute, Cary, North Carolina) and GRAPHPAD PRISM software (GraphPad Software, La Jolla, California). Student t test was employed for mean values. For 3-group analyses, no corrections were made for multiple comparisons. To compare the histopathologic findings between control mice and mutants, Pearson chi-square tests of 2 × 2 contingency tables were used; a 2 × 3 contingency table was used to compare the 3 categories of hypertrophy (none, mild, and moderate-to-severe). Two-tail p value was used for histopathologic analysis, and 1-tail p value was used for biochemical analysis. We considered values of p < 0.05 as a significant difference. Due to heterogeneity of variance in the biochemical data, both parametric and nonparametric tests and the Welch t test were employed.

Echocardiographic parameters of LV mechanics were correlated with LVEF with Pearson correlation coefficient (R) with p value. The diagnostic accuracy of echocardiographic parameters of LV mechanics for differentiating mutant from control mice was quantified with receiver-operator characteristic analysis. Area under the curve was calculated (18) with binomial confidence interval and compared with the method of DeLong et al. (19) with MedCalc Software (Mariakerke, Gent, Belgium).

**RESULTS**

**Ant1 cardiomyopathy: concentric hypertrophy with dilation.** M-Mode echocardiographic images of control and mutant hearts are shown in Figure 1A. By averaging the data (Table 1), we observed an increase in heart weight and heart/body-weight ratio and LVIDd, IVS, and LVPW thickness in mutant animals, indicative of LV dilation and concentric myocardial hypertrophy. Fractional shortening and EF were reduced in mutant hearts, indicating presence of LV systolic dysfunction (Table 1).

Mutant mice displayed significant regional defects in myocardial contraction. Examples are shown for control and mutant hearts in Figures 1B and 1C. Circumferential and radial strains, rotation, and rotational velocity for control versus mutant hearts are shown in Table 1. Mutant hearts demonstrated 40% reduction in rotation, 25% reduction in rotational velocity, and more than 50% decrease in circumferential and radial strain development, all highly significant.

**Age-related progression of Ant1 cardiomyopathy.** Our results showed that mutant hearts are in aggregate abnormal in all contractile parameters but with considerable variability. Because we examined animals from ages 2 to 21 months, this variability could be the result of variable penetrance of the mutation or age-related decline in cardiac function in mutant hearts. To investigate this, we analyzed the EF of control and mutant hearts by age (Fig. 2). This suggested that the average EF of control hearts increased throughout life (B coefficient = +0.39, p = 0.056). By contrast, EF of mutant hearts declined with age (B coefficient= −0.79, p = 0.029). There was also significant variability in EF in each age group, greater in mutant than in...
Figure 1. Cardiomyopathy in Ant1−/− Mouse

(A) M-mode echocardiography. Ultrasound images depict left ventricular dimensions in a control (upper panel) and mutant (bottom panel) mouse. Interventricular septum (IVS) thickness is shown as distance between blue and red lines, and left ventricular internal dimension at end-diastole (LVIDd) is shown as distance between red and yellow lines. The LVIDd is increased from 3.3 mm in control to 3.9 mm in mutant. (B) Velocity vector imaging: radial and rotational velocity vectors. Left ventricular short axis in control (a–c) and Ant1 mutant (d–f). Endocardial velocity vectors (a and d) were attenuated at end-ejection in mutant. The 2-dimensional maps of radial velocity and the apical rotational velocity are presented in adjacent panels. In contrast to the uniform pattern of radial velocities seen in control mice (b), marked dyssynchrony is seen in the mutant animal (e). Similarly, the velocity of counterclockwise rotation in systole is biphasic in the control mice (c) but is dysynchronous in the mutant animal (f). (C) Velocity vector imaging: velocity, strain, and strain rates. Images from control (upper panels) and mutant (bottom panels). For each heart, velocity (a and d), strain (b and e), and strain rates (c and f) are depicted by 3-dimensional color mapping. Uniform motion and shortening are observed in control, whereas dysynchronous motion and shortening are delineated in the velocity, strain, and strain rate curves of mutant.
control hearts (Fig. 2). The LVEF in mutants and control mice across all ages correlated with circumferential strain, radial strain, rotation, and rotational velocity in systole and diastole (radial strain, rotational strain, rotation, and rotational velocity of normal EF mutants was decreased only 11%, whereas that of abnormal EF mutants was reduced 60% (Table 2). Our analyses also revealed striking differences in circumferential strain and radial strain for the control and mutant hearts. Compared with control mice, circumferential strain was reduced in normal EF mutants (35%), and this difference was further exaggerated (60%) in abnormal EF mutants (Table 2). Radial strain of normal EF mutants was reduced (43%) and more profound (63%) in abnormal EF mutants, with all differences statistically significant (Table 2).

To further evaluate the decline in cardiac function, we divided the mutant hearts into those that had EF within the range of control hearts (normal EF) and those that fell below the normal range (abnormal EF). We determined the mean and SD of the EF of the control hearts and then selected the EF value that was 2 SDs below the control mean. This lower cutoff value was an EF of 56%. With application of this cutoff, 58% of the mutants demonstrated EF below lower normal limits (Fig. 2, dotted line).

Next, we divided the mutant hearts into those with “normal EF” (n = 31) and those with “abnormal EF” (n = 42). We then recalculated the major parameters (Table 2). The mean EF for control mice, normal EF mutants, and abnormal EF mutants was 71%, 65%, and 41%, respectively. The LVIDd of normal EF mutants and control mice were similar, whereas LVIDd of abnormal EF mutants was greater than control mice by 12% (Table 2). Normal EF mutants showed 40% increase in IVS thickness, whereas abnormal EF mutants showed 44% increase in IVS thickness (Table 2).

We further characterized contraction mechanics of LVs. Compared with control mice, both normal EF mutants and abnormal EF mutants showed reduction in LV rotational velocities, more marked for abnormal EF mutants (Table 2). Rotational velocity of normal EF mutants was decreased only 11%, whereas that of abnormal EF mutants was reduced 60% (Table 2). Our analyses also revealed striking differences in circumferential strain and radial strain for the control and mutant hearts. Compared with control mice, circumferential strain was reduced in normal EF mutants (35%), and this difference was further exaggerated (60%) in abnormal EF mutants (Table 2). Radial strain of normal EF mutants was reduced (43%) and more profound (63%) in abnormal EF mutants, with all differences statistically significant (Table 2).
Table 2. Echocardiographic Parameters of LV Mechanics in Control and Ant1 Deficient Mice With and Without Obvious Systolic Dysfunction

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 57)</th>
<th>Normal EF (n = 31)</th>
<th>Abnormal EF (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF %</td>
<td>70.90 ± 7.44</td>
<td>64.79 ± 5.76*</td>
<td>40.90 ± 11.09†</td>
</tr>
<tr>
<td>Age</td>
<td>10.03 ± 4.82</td>
<td>10.18 ± 3.79</td>
<td>12.95 ± 5.26§</td>
</tr>
<tr>
<td>LVIDd (0.1 mm)</td>
<td>37.53 ± 3.87</td>
<td>37.52 ± 3.98</td>
<td>42.10 ± 6.21¶</td>
</tr>
<tr>
<td>IVS (0.1 mm)</td>
<td>7.69 ± 0.92</td>
<td>10.77 ± 1.50*</td>
<td>11.00 ± 1.61*</td>
</tr>
<tr>
<td>Rotation (degree)</td>
<td>1.68 ± 0.36</td>
<td>1.50 ± 0.26†</td>
<td>0.67 ± 0.44‡</td>
</tr>
<tr>
<td>Rotation velocity (degree/s)</td>
<td>45.68 ± 9.35</td>
<td>44.39 ± 4.70</td>
<td>26.36 ± 6.90†</td>
</tr>
<tr>
<td>CS %</td>
<td>−12.01 ± 2.12</td>
<td>−7.81 ± 1.99*</td>
<td>−4.75 ± 1.66‡</td>
</tr>
<tr>
<td>RS %</td>
<td>11.96 ± 2.60</td>
<td>6.80 ± 2.09*</td>
<td>4.38 ± 1.72‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *Compared with normal group, p < 0.0001; †compared with normal EF group, p < 0.0001; ‡compared with normal group, p < 0.01; §compared with mutant normal EF group, p < 0.05; ¶compared with mutant normal EF group, p < 0.001.

Abbreviations as in Table 1.

In summary, mutant mice with normal EF, despite having normal LVIDd, show subclinical perturbations in contractile indexes. Although LV rotation has been previously shown to remain unaltered in hypertrophied hearts (20), LV hypertrophy in Ant1−/− mutant hearts with preserved LVEF was associated with significantly reduced LV apical rotation. This might suggest transmural disease (Table 2). Furthermore, all cardiac dysfunction parameters declined with age in the mutant animals.

Table 3 shows the diagnostic accuracy of various parameters of LV function for differentiating mutant from control mice. Circumferential strain, radial strain, and rotational velocity in diastole showed greater area under the curve than EF for differentiating mutants from control mice (p < 0.02) (Table 3). For hearts with normal EF (>56%), all 3 parameters showed good diagnostic accuracy for differentiating mutants from control mice.

Cardiac morphology and histopathology. Increased heart/body-weight ratios, IVS, and LVPW of mutants all point to myocardial hypertrophy. Our detailed hematoxylin and eosin analysis on 25 control mice (ages 2 to 19 months) and 47 mutant mice (ages 3 to 18.5 months) (Fig. 3) confirmed the presence of myocyte hypertrophy. In addition, we found evidence of interstitial edema (Figs. 3A to 3D), interstitial inflammation, myocyte calcification, binucleation, and myofibrillar lysis.

Myocyte hypertrophy was significantly more common in mutants than in control mice [chi-square(2) = 25.6, p < 0.0001] (Fig. 4A). This was also true of mutant mice <12 months old [chi-square(2) = 19.5, p < 0.0001] (Fig. 4B). The difference in myocyte hypertrophy was lost in older mice due to the increased presence of myocyte hypertrophy in older (>12 months) control mice. Hypertrophy seems to be an early finding of the Ant1−/− heart.

### Table 3. Diagnostic Accuracy of Echocardiographic Parameters of LV Mechanics

<table>
<thead>
<tr>
<th>Overall Group</th>
<th>AUC</th>
<th>SE</th>
<th>95% CI</th>
<th>p Value</th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF &lt;56%</td>
<td>0.89</td>
<td>0.03</td>
<td>0.62 to 0.94</td>
<td>&lt;0.001</td>
<td>64%</td>
<td>79.5</td>
<td>89.5</td>
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<tr>
<td>C5*</td>
<td>0.96*</td>
<td>0.01</td>
<td>0.91 to 0.98</td>
<td>&lt;0.001</td>
<td>−8.7%</td>
<td>87.7</td>
<td>96.5</td>
</tr>
<tr>
<td>RS</td>
<td>0.96*</td>
<td>0.01</td>
<td>0.92 to 0.99</td>
<td>&lt;0.001</td>
<td>7.7%</td>
<td>87.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Rotation</td>
<td>0.81*</td>
<td>0.03</td>
<td>0.73 to 0.87</td>
<td>&lt;0.001</td>
<td>1°</td>
<td>49.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Rotation velocity</td>
<td>0.77*</td>
<td>0.04</td>
<td>0.69 to 0.84</td>
<td>&lt;0.001</td>
<td>31½/3</td>
<td>46.6</td>
<td>96.5</td>
</tr>
<tr>
<td>Reverse rotation velocity</td>
<td>0.94*</td>
<td>0.02</td>
<td>0.89 to 0.97</td>
<td>&lt;0.001</td>
<td>39½/3</td>
<td>94.5</td>
<td>78.5</td>
</tr>
<tr>
<td>EF &gt;56%</td>
<td>0.91</td>
<td>0.03</td>
<td>0.84 to 0.96</td>
<td>&lt;0.001</td>
<td>−9%</td>
<td>77.4</td>
<td>94.7</td>
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<tr>
<td>C5</td>
<td>0.93</td>
<td>0.02</td>
<td>0.86 to 0.97</td>
<td>&lt;0.001</td>
<td>7.7%</td>
<td>77.4</td>
<td>100.0</td>
</tr>
<tr>
<td>RS</td>
<td>0.63</td>
<td>0.06</td>
<td>0.52 to 0.73</td>
<td>&lt;0.001</td>
<td>1.6%</td>
<td>77.4</td>
<td>49.1</td>
</tr>
<tr>
<td>Rotation</td>
<td>0.52</td>
<td>0.06</td>
<td>0.41 to 0.62</td>
<td>0.02</td>
<td>0.74</td>
<td>52½/3</td>
<td>100.0</td>
</tr>
<tr>
<td>Rotation velocity</td>
<td>0.88</td>
<td>0.03</td>
<td>0.79 to 0.94</td>
<td>&lt;0.001</td>
<td>39½/3</td>
<td>87.1</td>
<td>78.9</td>
</tr>
</tbody>
</table>

*Versus EF, p < 0.02.

AUC = area under the curve; CI = confidence interval; other abbreviations as in Table 1.
To examine fibrosis, we stained myocardial specimens from 27 control mice (ages 2 to 19 months) and 42 mutants (ages 3 to 18.5 months) with Masson’s Trichrome (Figs. 5A to 5F). Fibrosis was classified as focal or multifocal and interstitial, perivascular, or replacement (Figs. 4C and 4D). Overall, fibrosis was increased in mutant hearts (Fig. 4C), which became more evident and significant in older hearts (≥12 months) [χ²(1) = 4.5, p < 0.05] (Fig. 4D). Moreover, multifocal interstitial fibrosis was significantly more frequent in older mutants, and multifocal replacement fibrosis was exclusively observed in mutants (Figs. 4C and 4D). Fibrosis seems to be a later-stage phenomenon and occurs with greater frequency in mutants.

Calcification was also strikingly increased in mutants [χ²(1) = 20.8, p < 0.0001] (Figs. 4A and 4B); occurrence of calcification was independent of age.

Activation of apoptotic pathway in Ant1 mutant mice. Cytochrome c levels were found to be 6-fold higher in the older mutants as compared with older control mice (Fig. 6A). This could be due to the upregulation of oxidative phosphorylation to compensate for the chronic energy deficiency (16) or the synthesis and release of mitochondrial cytochrome c or both. Caspase activation was evaluated on the basis of the relative levels of the full length inactive caspase 3 and the shorter cleaved and activated caspase 3. Mutant hearts exhibited a 4-fold increase in amount of full length caspase 3. In contrast to the negligible levels in control myocardium, a large proportion of cleaved and thus activated caspase 3 was observed in the mutant mice. The difference was statistically significant for cytochrome c release as well as active caspase 3 by application of parametric tests; however, due to small sample size and heterogeneity of variance, the results were not statistically significant when nonparametric or Welch t tests were applied (Fig. 6B).

**DISCUSSION**

Genetic inactivation of the Ant1 gene in the mouse has been found to cause a unique cardiomyopathy characterized by substantial myocardial hypertrophy and ventricular dilation. Echocardiographic analyses of circumferential, radial, and rotational mechanics permitted detection of cardiac dysfunction in Ant1-deficient mice when global determinants of contractility were still preserved, even as early as the age of 3 months. Although LV rotation is usually increased or remains unaltered in hypertrophied hearts, LV hypertrophy in Ant1−/− mutants with preserved LVEF was associated with reduced LV apical rotation, suggesting presence of transmural disease. Early evidence of cardiac defects suggests that cardiac dysfunction might already be present in the neonatal or prenatal period. This “latent” cardiac dysfunction was confirmed by demonstration of histological changes in all mutant hearts, regardless of age. Mutant hearts had myocyte hypertrophy, myofibrillar lysis, edema, inflammation, and calcification at all ages. Although none of these features are individually pathognomonic, collectively they have been reported in idiopathic dilated cardiomyopathy. This pathology is associated with increased activated-caspase 3, indicating that Ant1-deficiency is accompanied by activation of apoptotic pathways; apoptosis has been reported in cardiomyopathic hearts. This Ant1 deficiency cardiomyopathy seems unique, because it produces dilated cardiomyopathy, which is associated with a substantial degree of concentric myocardial hypertrophy. Furthermore, this cardiomyopathy shows onset of mechanical dysfunction early on even when hypertrophy is the predominant feature and global systolic function is normal.

The clinical relevance of our Ant1−/− mutant mouse model for human disease has been shown by the recent report of a patient with a homozygous null missense (A123D) mutation in human ANT1 gene. This individual experienced mitochondrial myopathy with ragged red fibers and exercise intolerance together with a hypertrophic cardiomyopa-
thy. On echocardiography the patient was seen to have a concentric LV hypertrophy with comparably thickened IVS and LVPW. The LV was not dilated, and EF was still normal (9). In contrast to this human case resulting from a homozygous null mutation (9), other cases of human disease resulting from \textit{ANT1} gene missense mutations are inherited with autosomal dominance; these mutations present with mitochondrial myopathy with progressive external ophthalmoplegia, in absence of cardiomyopathy (21,22). Neither the \textit{ANT1} (A123D) patient (9) nor our mouse (23) exhibited evidence of ophthalmoplegia and ptosis. Hence, the dominant \textit{ANT1} missense mutations in autosomal dominant progressive external ophthalmoplegia seem to be acting as dominant negative mutations and are genetically and phenotypically different from the homozygous null mutations for cardiomyopathy. Perhaps the biochemical influence of the dominant \textit{ANT1} missense mutations is amplified through

\begin{figure}
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\includegraphics[width=\textwidth]{Figure4}
\caption{Quantification of Histopathologic Features}
\begin{description}
\item[(A and B)] Hematoxylin and eosin stained; (C and D) Masson’s trichrome stained; (A and C) aggregate of data from all ages; (B) aggregate of data from mice \textless 12 months old; (D) aggregate of data from mice \textgreater=12 months old. Binuc1 = binucleation; Calc = calcification; F = focal; Hyp = hypertrophy; Infl = inflammation; Int = interstitial; M = multifocal; MFL = myofibrillar lysis; Per = perivascular; Rep = replacement.
\end{description}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure5}
\caption{Cardiac Fibrosis by Masson’s Trichrome Staining}
\begin{description}
\item[(A)] Control, 8.5-month-old, shows multifocal interstitial fibrosis; (B) mutant, 5.5-month-old, shows multifocal replacement fibrosis; (C) control, 14.5-month-old, shows no fibrosis; (D) mutant, 13.5-month-old, shows multifocal interstitial fibrosis; (E) control, 19-month-old, shows focal perivascular fibrosis and focal interstitial fibrosis (not seen here); (F) mutant, 18.5-month-old, shows focal replacement fibrosis and multiple foci of interstitial fibrosis.
\end{description}
\end{figure}
their inactivation in dimers with mutant subunits incorporated. Alternatively, dominant missense mutations might change ANT1 function, perhaps making it leakier for protons and thus reducing energy metabolism (24).

One of the most striking features of both the autosomal recessive and autosomal dominant ANT1 diseases, in man and mouse, is the age-related accumulation of multiple mtDNA deletions. The accumulation of multiple mtDNA deletions has been documented in muscle for both the human dominant and recessive cases (9,21) and in heart in the Ant1/H11002/H11002 mouse (14). These mtDNA rearrangements have been proposed to result from nucleotide imbalance (21) or excessive mtDNA ROS damage (13). Whatever the mechanism, the age-related accumulation of mtDNA damage might progressively erode mitochondrial energy production, exacerbating the inherited ANT1 defect and leading to the age-related progression of the cardiomyopathy. If so, one therapeutic approach might be administration of mitochondrially targeted antioxidants to inhibit mtDNA damage, thus slowing disease progression.

We demonstrated initiation of intrinsic apoptosis pathway leading to the activation of caspase 3 in mutant hearts. This might indicate that apoptotic processes contribute to the progression of the AN1-deficient cardiomyopathy (25). Expression of a caspase-8 fusion protein in the hearts of transgenic mice resulted in activation of apoptosis and cardiomyopathy (26), and cardiomyocyte apoptosis has been associated with dilated cardiomyopathy (27). Therefore, these observations suggest that therapies that stabilize the mitochondrial permeability transition pore, such as cyclosporin A-derivatives, might play a role in reducing progression of Ant1−/− cardiomyopathy (28,29).

The most direct approach to treating the Ant1−/− cardiomyopathy would be to replace the missing Ant1 gene. We have been successful in partially restoring ANT1 function in Ant1−/− mouse muscle through transduction of the mouse Ant1 complementary deoxyribonucleic acid with adeno-associated virus. After transduction of the muscle with the Ant1−adeno-associated virus, there was a striking amelioration of the severe aspects of skeletal muscle pathology (30). By developing the mouse Ant1−/− model and performing detailed characterization of the cardiomyopathy, we are now in position to evaluate relative benefits of various therapeutic modalities.

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REFERENCES


Key Words: adenine nucleotide translocator • aging • apoptosis • cardiomyopathy • mitochondria.