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Transgenic mouse model with deficient mitochondrial polymerase exhibits reduced state IV respiration and enhanced cardiac fibrosis

Christopher A Koczor, Rebecca A Torres, Earl Fields, Qianhong Qin, Jade Park, Tomika Ludaway, Rodney Russ and William Lewis

Mitochondria produce the energy required for proper cardiac contractile function, and cardiomyocytes that exhibit reduced mitochondrial electron transport will have reduced energy production and decreased contractility. Mitochondrial DNA (mtDNA) encodes the core subunits for the protein complexes of the electron transport chain (ETC). Reduced mtDNA abundance has been linked to reduced ETC and the development of heart failure in genetically engineered mice and in human diseases. Nucleoside reverse-transcriptase inhibitors for HIV/AIDS are used in antiretroviral regimens, which cause decreased mtDNA abundance by inhibiting the mitochondrial polymerase, pol-γ, as a limiting side effect. We explored consequences of AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) exposure on mtDNA abundance in an established transgenic mouse model (TG) in which a cardiac-targeted mutant form of pol-γ displays a dilated cardiomyopathy (DCM) phenotype with increased left ventricle (LV)-mass and increased LV-end diastolic dimension. TG and wild-type littermate mice received 0.22 mg per day AZT or vehicle for 35 days, and were subsequently analyzed for physiological, histological, and molecular changes. After 35 days, Y955C TGs exhibited cardiac fibrosis independent of AZT. Reduced mtDNA abundance was observed in the Y955C mouse; AZT treatment had no effect on the depletion, suggesting that Y955C was sufficient to reduce mtDNA abundance maximally. Isolated mitochondria from AZT-treated Y955C hearts displayed reduced mitochondrial energetic function by oximetric measurement. AZT treatment of the Y955C mutation further reduced basal mitochondrial respiration and state IV_0 respiration. Together, these results demonstrate that defective pol-γ function promotes cardiomyopathy, cardiac fibrosis, mtDNA depletion, and reduced mitochondrial energy production.

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Mitochondria produce the energy required for proper cardiac function, providing cardiomyocytes the ATP necessary for contractility and cellular maintenance. Energy is produced via oxidative phosphorylation across the mitochondrial membrane. Mitochondrial energy production is diminished in heart failure, suggesting energy starvation as an important mechanism.[1] Mitochondrial DNA (mtDNA) encodes key subunits for the protein complexes of the electron transport chain (ETC), and reduced mtDNA abundance has been linked to the development of heart failure in genetically engineered mice and in human diseases.[2–5]

Current therapeutic protocols for HIV/AIDS include antiretroviral combinations (HAART) with nucleoside reverse-transcriptase inhibitors (NRTIs) that inhibit HIV reverse transcriptase serving as cornerstones. Although NRTIs effectively inhibit HIV replication, limiting side effects relate to mitochondrial polymerase, pol-γ.[6–8] By inhibiting pol-γ, NRTIs decrease mtDNA abundance, reduce mitochondrial energy production, and promote cellular dysfunction and disease.[2] Our findings and those of others strongly implicated dideoxy-NRTIs in the development of mitochondrial and cardiovascular toxicity.[4,9–12] It logically follows that understanding the relationship between NRTIs, mitochondrial dysfunction, and cardiac myocyte dysfunction provides insight into mechanisms of heart failure.

Department of Pathology, Emory University, Atlanta, GA, USA
Correspondence: Dr W Lewis, MD, Department of Pathology, Emory University School of Medicine, 7117 Woodruff Memorial Building, 101 Woodruff Circle, Atlanta, GA 30322, USA.
E-mail: wlewis@emory.edu

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We explored the consequences of AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) exposure on mtDNA abundance in a genetically engineered mouse model that expresses a mutant form of pol-γ targeted to the heart with a commonly employed, robust cardiac promoter.[2,3] In human disease, Y955C pol-γ mutant is associated with the development of progressive external ophthalmoplegia and other defects.[13–15] Biochemically, the Y955C mutation reduces polymerase processivity, creating longer DNA replication times and decreased mtDNA abundance.[13–15] The transgenic mice we generated exhibited DCM with reduced cardiac mtDNA abundance. We congenically backcrossed these mice to C57/Bl6 background. Histological, physiological, and molecular analyses were used to determine the individual and combined effects of this transgene and AZT on cardiac function. Results show decreased cardiac contractile function, increased fibrosis, and reduced mitochondrial function all occur in the Y955C TG. That effect could not be exacerbated by AZT treatment, suggesting that Y955 polymerase function alone was sufficient to cause heart failure.

MATERIALS AND METHODS

Reagents

All reagents were analytical grade and purchased from Sigma Aldrich (St Louis, MO, USA) unless otherwise indicated.

Mice

Transgenic Y955C FVB/N mice were generated as previously described, with cardiac-specific Y955C transgene expression achieved using the cardiac-specific α-myosin heavy-chain promoter.[3] FVB/N Y955C mice were backcrossed with wild-type C57Bl/6 mice (Jackson Labs, Bar Harbor, ME, USA) to produce Y955C C57Bl/6 mice, which were bred to the fifth generation before experimentation. For genotyping, genomic DNA was extracted from mouse tail clippings, and genotype was determined by PCR. PCR primers utilized are as follows: forward, 5′-CGTAGTCGACGATGAGCCGCCTG-3′; reverse, 5′-CTGCGCTGGAAGCTGCTTAGC-3′. All mice were housed at the Emory University in accordance with the IACUC protocols in an AAALAC-certified vivarium according to the NIH guidelines.

Mice were weaned at 3 weeks and genotyped, and experiments on mice were started at 8–12 weeks of age. Mice were administered 0.22 mg per day AZT or 1% CMC vehicle by gavage once daily for 35 days. Mice were analyzed for physiological, histological, and molecular changes after 35 days. All treatments and procedures were conducted in accordance with the approved IACUC protocols.

Echocardiography

Physiological parameters of cardiac function were determined using echocardiography. Mice were anesthetized with avertin (0.25 mg/g of body weight) and weighed to determine body mass. Echocardiography was performed using VisualSonics Vevo 770 (VisualSonics, Toronto, ON, Canada). Results from a two-dimensional M-mode analysis along the short axis (at the level of the largest left ventricle (LV) diameter) were used to determine LV mass, LV-end diastolic dimension (LVEDD), and LV fractional shortening. LV mass values were normalized to mouse body weight.

Histology

Following 35 days of treatment, mice were terminated by cervical dislocation under avertin anesthesia, and hearts were removed, sectioned rapidly with a razor blade (2 mm sections), fixed in 10% neutral buffered formalin and embedded in paraffin. Six micrometer histological sections were processed with Masson's trichome, and images were collected at both $\times 200$ and $\times 400$ magnification using a Nikon Eclipse E800M microscope (Nikon, Melville, NY, USA). Histomorphometric analysis was performed, with the experimenter blinded to treatment or transgenic status. Quantitative microscopic analysis of cardiac fibrosis was determined on the stained tissue sections highlighting the collagen fibers blue. Five to ten randomly selected photomicrographs ($\times 200$) of each LV sample were digitized. The area of fibrosis was measured using color selection with a set tolerance and converted to a binary image using Adobe Photoshop (Adobe, San Jose, CA, USA). Fibrosis was defined as the fraction of blue within the total cellular area using the NIH Image J software (scale = 9.3 pixels/μm). Data are presented as mean ± s.e.m.

Hydroxyproline Analysis

Hydroxyproline quantitation was performed as a biochemical index of collagen abundance as described by others.[16] Approximately 10 mg of frozen LV cardiac tissue from each animal was used. Tissue was acid hydrolyzed overnight at 99 °C in 4 N HCl. Samples were evaporated at 60 °C using a vacufuge (Eppendorf, Hauppauge, NY, USA), resuspended in ddH_2O, vortexed vigorously, centrifuged at 3000 *g* for 10 min, and the supernatant was retained. Each sample was mixed with 1.5 ml ddH_2O, 450 μl 6 mM HCl, and 1 ml 0.05 M chloramine-T, and incubated at room temperature for 20 min. To each sample, 1 ml 3.15 M perchloric acid was added, was allowed to stand for 5 min at room temperature, and 1 ml 20% p-DABA (p-dimethylaminebenzaldehyde) was added to the samples. Samples were incubated for 20 min at 60 °C, cooled to room temperature, and loaded (200 μl) in triplicate into a UV plate to be read in a SpectraMax 190 microplate reader (Molecular Devices, Sunnydale, CA, USA) at the absorbance of 575 nm. Samples were normalized to a standard curve.

For total protein, the ninhydrin assay was used as previously described.[17] Hydrolyzed samples above were diluted 1:200 with H_2O. The diluted samples were mixed with 10 × volume of ninhydrin reagent (200 mg ninhydrin, 7.5 ml ethylene glycol, 2.5 ml 4 N sodium acetate buffer, pH 5.5, 250 μl 100 mg/ml stannous chloride) followed by incubation

at 99 °C for 10 min. The samples (200 μl) were loaded in triplicate into a UV plate to be read in a SpectraMax 190 microplate reader at an absorption of 575 nm. Samples absorption was normalized to a standard curve. Hydroxyproline was normalized to total protein and expressed as mg hydroxyproline per mg total protein.

mtDNA and Nuclear DNA Abundance

Methods utilized are similar to those described previously.[3] DNA sequences for primers and probes used for quantitation of mitochondrial and nuclear DNA analyzed the *COX I* gene of the mtDNA and the *POLG2* gene of the nuclear DNA. Amplification was performed using the Lightcycler 480 system (Roche).

Mitochondrial Oximetry

Mitochondria were isolated from mouse hearts, immediately following the killing of the mouse, using differential centrifugation and a commercial mitochondrial isolation kit (Sigma Aldrich). All steps were performed on ice. Heart tissue (~30 mg wet weight) was washed in KHB buffer, minced with a razor blade, trypsinized for 3 min, centrifuged at 16,000 *g* for 1 min, and retrypsinized for 20 min following manufacturer's instructions. Samples were centrifuged at 16 000 *g* for 1 min, resuspended in the proprietary extraction buffer, and homogenized using 15–25 strokes of a Dounce homogenizer. Mitochondria were enriched by differential centrifugation according to the manufacturer's protocol, and resuspended in the proprietary storage buffer. An aliquot was quantitated for protein using the Bradford assay, and 5 μg of protein was diluted into 1 × mitochondrial assay solution (1 × MAS: 70 mM sucrose, 220 mM mannitol, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM HEPES, 1 mM EGTA, 0.2% BSA, pH 7.2 with 1 N KOH) to a final volume of 50 μl, placed into a V7 plate (Seahorse Bioscience, Billerica, MA, USA) and centrifuged at 3400 *g* for 20 min at 4 °C. Following centrifugation, 550 μl of 1 × MAS containing 10 mM pyruvate and 5 mM malate was added to each well, and the mitochondrial respiration was analyzed in an XF24 flux analyzer (Seahorse Bioscience) using the manufacturer's protocol. As defined by others, basal respiration is defined as the oxygen consumption rate of the mitochondria immediately following equilibration. State III respiration was achieved by injecting ADP to a final concentration of 2.5 mM. State IV_0 respiration was achieved by injecting oligomycin to a final concentration of 1.0 μg/ml. Non-mitochondrial respiration (data not shown) was achieved by injecting rotenone to a final concentration of 2 μM at the end and measuring. Oximetric results are provided as pmol O_2/min per μg protein.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 5.0 (Graphpad, La Jolla, CA, USA). Each experiment was analyzed using a one-way or two-way ANOVA where appropriate, with a $P<0.05$ deemed statistically significant. The experiments are displayed as a mean ± s.e.m., with all experiments performed at least three times each. Statistically significant relationships are denoted with an asterisk (*) in each figure.

RESULTS

Transgenic Mice

The Y955C TG congenically expressed in C57Bl/6 had no impact on body weight compared with wild-type littermates over the course of the experiments (Figure 1a). This observation made body weight an acceptable parameter to normalize cardiac echocardiographic measurements. Similarly, AZT exposure had no effect on body weight, either alone or in the Y955C TG (Figure 1a). Y955C TG mice exhibited a significant increase in LV mass compared with wild-type mice (Figure 1b). This result in congenic TGs is in agreement with our previous studies using TGs on the FVB/N background,[2,3] suggesting that strain-specific effects did not exist for this TG. LVEDD measurements show a moderate increase in LVEDD in the Y955C mouse compared with wild-type mice (Figure 1c). AZT itself does not increase LVEDD, but a statistically significant increase in LVEDD was found in the Y955C-AZT-treated mice compared with AZT-treated wild-type mice. This suggests a combined effect on increasing LVEDD and, thus, worsening of the dilation. No change in the fractional shortening was seen (Figure 1d), which is also in agreement with previous studies.[2,3] Together, these results demonstrate the important, but sometimes neglected, point that the inbred background of the mouse can cause specific effects.[18] The inbred strain does not sensitize it to the Y955C transgene and that native pol-γ is crucial in maintaining cardiac performance.

Cardiac Fibrosis

Cardiac fibrosis is an important histological marker of the DCM phenotype.[19,20] Cardiac fibrosis was determined quantitatively and parametrically in mice using two independent methods. Methods were chosen because they offered robust results morphologically and biochemically. Results obtained from each corroborated the other. For morphological studies, paraffin-embedded sections of cardiac tissue stained with Masson's trichrome highlighted fibrosis microscopically in hearts. Utilizing image analysis, the area of fibrosis from LV tissue sections was defined. The fibrosis pattern appears interstitial and perimyseal (Figure 2). Histological analysis revealed a three-fold increase in the area of fibrosis in the hearts of Y955C transgenic mice, independent of AZT treatment (Figure 2).

We measured the abundance of hydroxyproline in cardiac tissue samples as a parallel biochemical marker of fibrosis.[16] Those results were normalized to total protein using ninhydrin quantitation of the same hydrolyzed samples. A 60% increase in hydroxyproline was found in the Y955C LVs compared with wild-type mouse LVs (Figure 3); however,

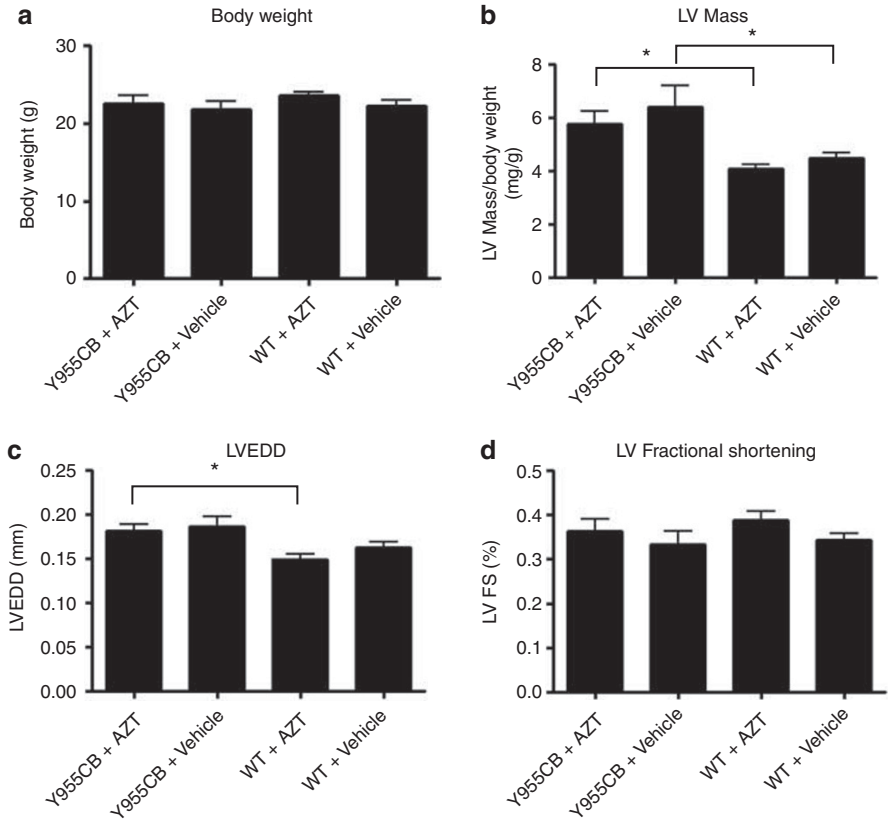

Figure 1 Physiological measurement of cardiac function. Wild-type and Y955C pol-γ transgenic mice were exposed to AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) or vehicle for 35 days. Following treatment, the mice were analyzed for echocardiographic changes. (**a**) No change in body weight was observed. (**b**) Y955C transgenic mice displayed an increase in left ventricle (LV) mass, with AZT having no appreciable effect by itself or in combination with the Y955C transgene. (**c**) LV-end diastolic dimension (LVEDD) was increased in Y955C AZT-treated mice compared with AZT-treated wild-type mice. (**d**) There was no significant change in fractional shortening observed in any treatment group compared with controls.

AZT reduced the fibrosis in the Y955C mouse by 50% compared with Y955C with vehicle. These results support the hypothesis of a mitochondrially driven increase in fibrosis and suggest that AZT treatment may promote a cellular response that reduces fibrosis formation.[21–23]

mtDNA Abundance

Y955C mice displayed a 50% decrease in mtDNA abundance compared with wild-type controls (Figure 4). In contrast, AZT exposure caused a statistically significant 20% increase in mtDNA abundance, suggesting that the doses utilized in this experiment may provoke a compensatory mechanism. However, the observed increase in mtDNA abundance provided by AZT was blunted by the Y955C transgene. Results indicate the Y955C transgene reduces mtDNA abundance in a robust manner that negates any compensatory mechanism AZT may elicit.

Mitochondrial Function

Reduced mtDNA abundance will lead to reduced ETC activity by limiting the abundance of ETC polypeptides. In experiments here, preparations of isolated mitochondria from the murine LVs displayed high coupling and good response to ETC-specific compounds. Basal respiration (oxygen consumption of the isolated mitochondria after pyruvate/malate addition, but before ADP addition) served as an indicator of mitochondrial quality. Operationally, basal measurements greater than 600 pmol/min per 5 μg protein were associated with proton leakage and low-quality mitochondria. The Y955C transgene and AZT each reduced basal respiration (Figure 5a). Interestingly, both parameters acted to reduce basal respiration further than individual effects. AZT reduced basal respiration by 20%, whereas Y955C reduced it by 50%. Together, Y955C and AZT reduced basal respiration by over 60% compared with wild-type controls. These results suggest that Y955C and AZT may not be working via an identical mechanism (ie, mtDNA depletion by reduced pol-γ function) to yield the observed reduction in mitochondrial function. Measurements of state III respiration showed no change among the experimental sets (Figure 5b), indicating that mitochondria from all treatment groups demonstrate the same capacity to produce ATP when

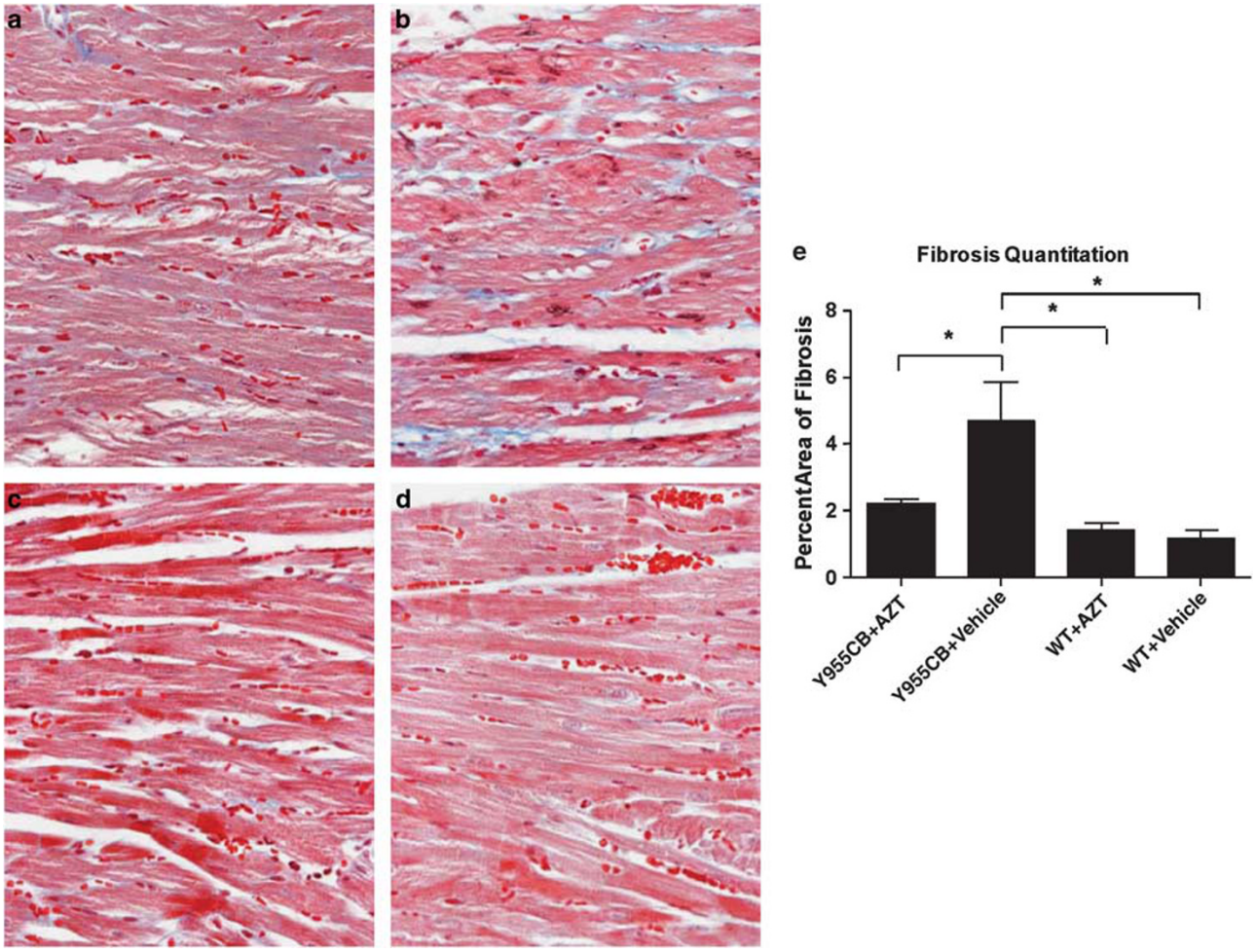

Figure 2 Cardiac fibrosis. Cardiac fibrosis was evaluated using histological sections stained by Masson's trichrome. Presented are representative images (× 400) depicting cardiac fibrosis from Y955CB transgenic mice treated with AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) (**a**), vehicle (**b**), or wild-type mice treated with AZT (**c**) or vehicle (**d**). The level of cardiac fibrosis was determined by calculating the area of blue as a percentage of the total cellular area (**e**).

provided adequate substrates. Y955C mice treated with AZT exhibited a 55% reduction in state IV_o respiration compared with untreated controls (Figure 5c). Although both AZT and Y955C reduced state IV_o, AZT alone did not diminish the Y955C-induced state IV_o respiratory reduction. These latter results suggest AZT and Y955C both promote mitochondrial coupling, with Y955C achieving near maximal coupling with little additional coupling achieved when combined with AZT treatment. Finally, analysis of the respiratory control ratio (RCR) revealed that both Y955C and AZT promote tighter coupling of the mitochondrial membrane (Figure 5d). A higher RCR is associated with increased capability of producing ATP and decreased proton leakage across the mitochondrial membrane. Data from these experiments suggest that the Y955C transgene and AZT may promote more efficient ATP production in the face of reduced ETC capacity, and that some subcellular compensatory mechanism to energetic dysfunction may be operating.

DISCUSSION

The Y955C mutation reduces the processivity of pol-γ, resulting in increased replication time and decreased mtDNA abundance in human patients.[13,24,25] Similarly, AZT has been demonstrated to reduce pol-γ function in humans and mice, providing both a genetic and chemical method of measuring reduced pol-γ function.[6,7] Previous experiments with cardiac-specific Y955C transgene expression on the FVB/N background demonstrated decreased mtDNA abundance and reduced cardiac contractility.[2,3] Here we congenically backcrossed these mice to the ubiquitously used C57Bl/6 mouse background, and physiological changes were determined by echocardiography.

Findings here characterize the contribution of mitochondrial dysfunction to defective cardiac performance and the resultant development of cardiomyopathy from mtDNA depletion and ETC changes. Generation of a stable Y955C transgene on the C57Bl/6 background demonstrated no strain-specific changes, a point that bears emphasis.

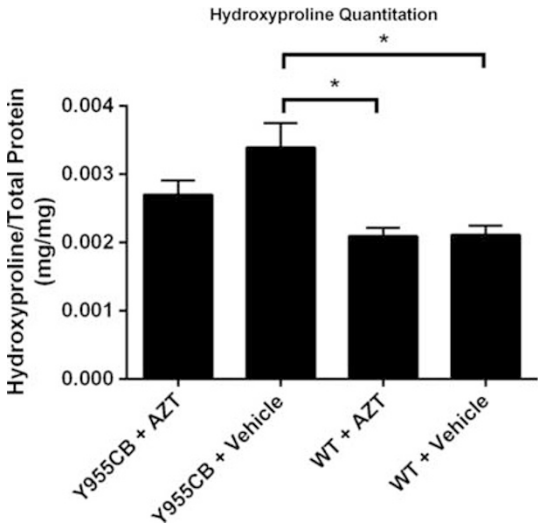

Figure 3 Hydroxyproline quantitation. Hydroxyproline was detected using a colorimetric assay on hydrolyzed tissue samples, with results normalized to total protein via ninhydrin assay. Y955C mice displayed a significant increase in hydroxyproline. Interestingly, AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) treatment reduced the amount of hydroxyproline, and therefore fibrosis, in the Y955C mice.

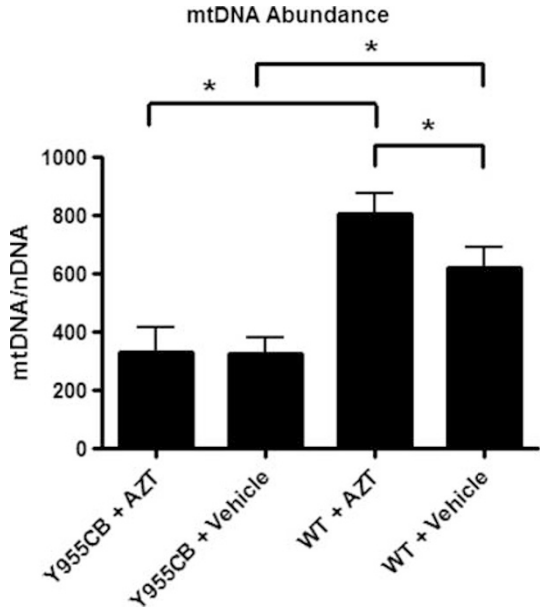

Figure 4 Mitochondrial DNA (mtDNA) abundance. Abundance of mtDNA was quantitated in heart samples from each experimental group. Y955C transgenic mice displayed a 50% decrease in mtDNA abundance compared with wild-type untreated groups. AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) alone elicited a 30% increase in mtDNA abundance compared with vehicle-treated wild-type mice, though Y955C–AZT mice displayed a 50% reduction in mtDNA abundance compared with vehicle-treated wild-type mice.

The Y955C transgene diminished LV function and caused the development of cardiomyopathy in C57Bl/6 mice as shown in FVB/N mice previously.[2,3] In addition, Y955C transgenic mice display a 50% decrease in mtDNA abundance in both backgrounds. These results implicate pol-γ as crucial in maintenance of cardiac function. It may be possible in future studies to approach congenic expression of the Y955C mutant in other inbred strains, or in genetic recombinant strains, but such studies are beyond the nature of the present one.

Our study utilized two independent approaches to determine cardiac fibrosis. The first morphometrically quantitates fibrosis using histological stains and computerized examination of slides. The second method uses quantitation of hydroxyproline in extracted cardiac samples.[16] Importantly, both robust methods yielded similar results that corroborated one another. The Y955C transgenic mice display a significant increase in cardiac fibrosis without AZT treatment (Figures 2 and 3), which is consistent with other models of heart failure. Interestingly, AZT treatment was found to reduce cardiac fibrosis in the Y955C transgenic mice, though levels of fibrosis remained elevated compared with controls. These results may suggest that AZT can attenuate the generation of fibrosis, but the subcellular mechanism requires further study. It may be possible to consider that nucleotide pool levels and TK1/TK2 competition and/or inhibition have been demonstrated as important additional considerations in AZT metabolism and utilization. Similarly, further studies may be useful in determining these effects with the Y955C transgenic mouse to enable clarification of these mechanisms of fibrosis.[9,26,27]

The analysis of mitochondrial function in Y955C transgenic and AZT-treated mice provides novel insight into the effects of mtDNA changes on mitochondrial function. Utilizing oximetric analysis on isolated mitochondria following a 35-week-AZT or vehicle treatment, we noted a decrease in the basal respiration of the mitochondria from Y955C and AZT treatments. This decrease in basal respiration implicated abnormal mitochondrial function in both Y955C transgenic mitochondria and AZT exposure. However, both the Y955C transgene and AZT decreased the basal respiration, again suggesting a secondary effect of AZT that promotes mitochondrial dysfunction. We found no change in state III respiration, demonstrating that the mitochondria from both Y955C transgenic and AZT-treated mice have a similar capacity to generate ATP under ADP-stimulated conditions (Figure 5b). The change in mitochondrial function was determined to be a decrease in state IV_o respiration (Figure 5c). These data suggest that the isolated mitochondria from the Y955C mice and AZT-treated mice may be more tightly coupled than the wild-type untreated mitochondria, and exhibit a lower proton leak. This novel finding may be explained by the following hypotheses. First, the Y955C transgene or AZT-treated cardiomyocytes are only retaining mitochondria that have the highest energy production and eliminating damaged mitochondria. Such an effect would lead to a population that is more tightly coupled to maintain ATP production to meet cardiomyocyte energetic demands and reduce proton leakage across the mitochondria membrane. The second hypothesis suggests that enhanced

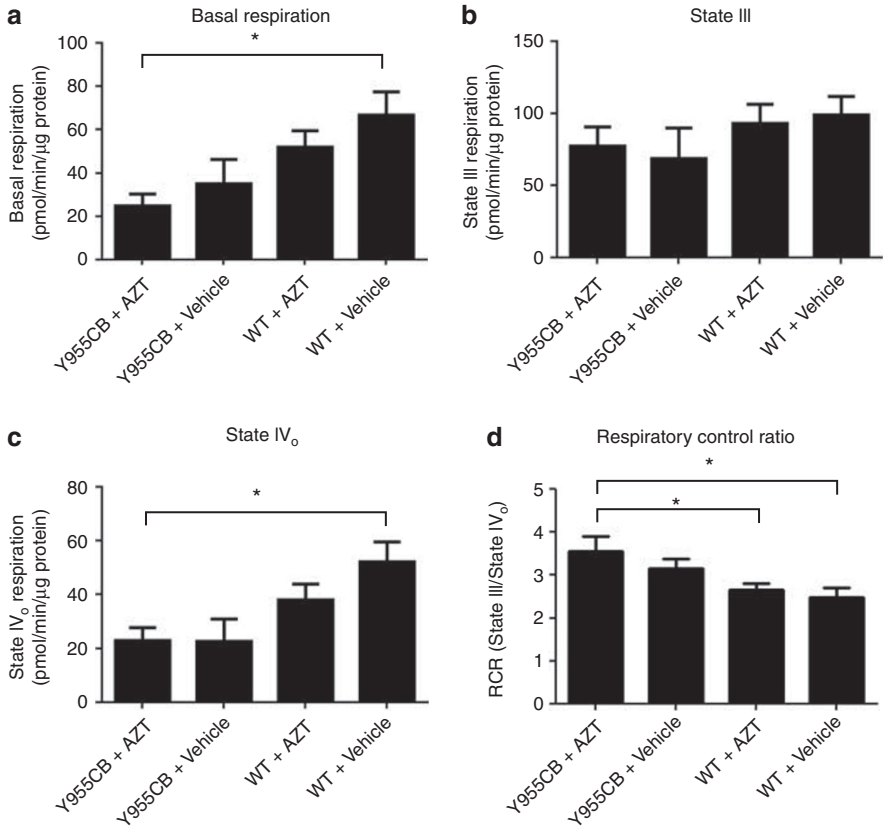

Figure 5 Mitochondrial function. (**a**) Basal respiration of the mitochondria (in the presence of substrates, but absence of excess ADP) was suppressed in all treatment groups, with the Y955C transgene and AZT (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) working cumulatively. (**b**) State III respiration was unchanged in any experimental group, suggesting similar capabilities of ATP production in each group. (**c**) State IV_o respiration was reduced in each treatment group, with Y955C–AZT mice displaying a 55% reduction in proton leakage compared with vehicle-treated wild-type mice. (**d**) Respiratory control ratios (RCRs) demonstrate enhanced coupling in the Y955C and AZT treatment groups, with Y955C and AZT cumulatively promoting increased RCR.

coupling of the mitochondria by an adaptive process in the cardiomyocytes increases energy-production efficiency in light of reduced mtDNA abundance. The mechanism of such a process is unknown, though oxidative stress from the Y955C transgene or AZT treatment may be involved.[3,28] Finally, the increase in coupled mitochondria may be the result of the mitochondrial isolation process *per se*. Our methods for producing an enriched mitochondrial preparation select the best mitochondria from the Y955C or AZT-treated mice. Although it cannot be ruled out, enhanced state III respiration would also be expected to be observed in better prepared mitochondria. The RCR can be used to normalize modulation of state III and state IV_o and substantiate observed changes observed in state IV_o respiration.

These studies provide insights into the effects of reduced mtDNA abundance by pol-γ inhibition. Results suggest the Y955C mutation promotes mtDNA depletion and increases mitochondrial coupling, whereas AZT elicits an increase in mitochondrial coupling and increases mtDNA abundance possibly in a compensatory way. This mitochondrial dysfunction, via increased oxidative stress or altered mitochondrial functionality, promotes the development of cardiac fibrosis, with the observed fibrosis intimately linked to the mitochondria, because of the models utilized. These studies support a theory of 'mitocentric' heart failure with energy starvation at its core.[1] They implicate mitochondrial dysfunction in the progression of cardiomyopathy and other cardiovascular diseases.

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DISCLOSURE/CONFLICT OF INTEREST
The authors declare no conflict of interest.

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