Cardiac Specific Overexpression of Transglutaminase II (G_h)
Results in a Unique Hypertrophy Phenotype Independent
of Phospholipase C Activation*

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Tissue type transglutaminase (TGII, also known as G_h)
has been considered a multifunctional protein, with
both transglutaminase and GTPase activity. The role of
the latter function, which is proposed as a coupling
mechanism between α₁-adrenergic receptors and phos-
pholipase C (PLC), is not well defined. TGII was overex-
pressed in transgenic mice in a cardiac specific manner
delineated relevant signaling pathways and their con-
sequences in the heart. Cardiac transglutaminase activ-
ity in the highest expressing line was ~ 37-fold greater
than in nontransgenic lines. However, in vivo signaling
to PLC, as assessed by inositol phosphate turnover in
[³H]myoinositol organ bath atrial preparations, was not
increased in the TGII mice at base line or in response to
α₁-adrenergic receptor stimulation; nor was protein ki-
nase Ca (PKCa) or PKCe activity enhanced in the TGII
transgenic mice. This is in contrast to mice moderately
(~5-fold) overexpressing G_q, where inositol phosphate
turnover and PKC activity were found to be clearly en-
hanced. TGII overexpression resulted in a remodeling of
the heart with mild hypertrophy, elevated expression of
β-myosin heavy chain and α-skeletal actin genes, and
diffuse interstitial fibrosis. Resting ventricular function
was depressed, but responsiveness to β-agonist was not
impaired. This set of pathophysiologic findings is dis-
tinct from that evoked by overexpression of G_q. We
conclude that TGII acts in the heart primarily as a trans-
glutaminase, and modulation of this function results in
unique pathologic sequelae. Evidence for TGII acting as
a G-protein-like transducer of receptor signaling to PLC
in the heart is not supported by these studies.

Tissue type transglutaminase (TGII, also known as G_h)
is a multifunctional GTP-binding protein that has been proposed to
mediate both transglutamination and receptor-stimulated PLC
activation (1, 2). As a transglutaminase, TGII catalyzes Ca^{2+} depend-
ent post-translational modification of proteins through
formation of isopeptide bonds between glutamine and lysine
residues (3). However, unlike other transglutaminases, TGII
binds guanine nucleotides in a 1:1 ratio and hydrolyzes GTP (4, 5).
This GTPase activity is independent of transglutaminase
activity (6) and has been shown to mediate α₁-adrenergic re-
ceptor (α₁AR) stimulation of phospholipase C α₁, increasing
inositol phosphate turnover in TGII-transfected cells (7–13).
Numerous in vitro studies implicate TGII in a wide variety of
biological processes, including regulation of cell growth and
derdifferentiation (14–16), apoptosis (17), and tissue repair (18, 19),
as well as signal transduction via α₁AR. The role of TGII,
however, in regulation of these events in relevant target tissues
remains unclear.

Activation of α₁AR expressed on myocytes by catecholamines
stimulates PLC activity, increasing inositol phosphate turn-
over in the heart (20). Traditionally, this signal has been con-
sidered to be transduced by the α subunit of the heterotrimeric
G protein G_q (21). Indeed, a number of other G protein-coupled
receptors that signal via G_q are expressed on myocytes, includ-
ing those for endothelin and angiotensin II (22). Persistent
activation of this pathway, by overexpression of receptor (23) or
G_q (24, 25) or continuous exposure to agonist (26–29), results
in a hypertrophic response in myocytes and intact hearts. This
has prompted the notion that one or more elements of this
pathway are sufficient to trigger the response; thus, this over-
expression strategy in transgenic mice has provided a biochem-
ical mechanism for evoking hypertrophy and ventricular dys-
function in the absence of the systemic effects of continuous
agonist exposure or the hemodynamic loading evoked by vas-
cular banding. Whether TGII also signals via PLC in the heart,
as has been proposed from cell-based studies, is not known.
Interestingly, a potential link between TGII and heart failure
has been suggested by Iwai et al. (30), who showed up-regula-
tion of TGII mRNA in rat models of cardiac hypertrophy and
failure, and Hwang et al. (31), who observed alterations in TGII
protein function and expression in ischemic and dilated car-
diomyopathic human hearts.

The current study was undertaken to investigate the signal-
ing of TGII to its proposed intracellular pathways of transglu-
tamination and activation of the PLC-inositol phosphate-PKC
cascade, in a relevant target tissue, the heart. Our recent
creation of transgenic mice overexpressing G_q in the heart has
provided an animal with well documented activation of PLC-
inositol phosphate-PKC for anatomic, biochemical, and physi-
ologic comparison (24). To this end, the myosin heavy chain
promoter was used to drive cardiac specific expression of the
wild-type TGII gene in transgenic mice (32). Our results show
that overexpression of TGII in mouse heart results in unique
pathologic consequences and depressed systolic function without enhanced inositol phosphate turnover, α1AR function, or PKC activation. Thus, TGI in the heart appears to function primarily as a transglutaminase.

**EXPERIMENTAL PROCEDURES**

**Transgenic Mice**—Cardiac specific expression of TGI in transgenic mice was achieved using the murine α-myosin heavy chain (MHC) promoter that directs expression to atria and ventricles. Briefly, the 2.1-kb coding region of the wild-type rat TGI gene (11) was blunt end-ligated into the NotI site of a plasmid containing the full-length (4.5-kb) murine α-MHC promoter (38). The resulting recombinant α-MHC-TGI, was confirmed by restriction mapping and sequencing. The transgene construct was digested with NotI to release a 8.3-kb fragment that was isolated, purified, and used for microinjection into the male pronuclei of FVB/N embryos. Injected embryos were implanted into oviducts of pseudopregnant females. Pups were screened for the presence of the transgene by Southern blot analysis performed on genomic DNA digested with 

**Transgene Expression**—Mouse hearts were homogenized in 1 ml of 20 mM HEPES buffer (pH 7.4) containing 1 mM EGTA, 1 mM dithiothreitol, 10% glycerol, and protease inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml bacitracin, 100 μg/ml benzamidine, 2 μg/ml soybean trypsin inhibitor, and 20 μg/ml antipain) and stored at –80 °C until use. Western blots were performed as described previously (31) using a TGI-specific antibody, G44T9. Transglutaminase activity was determined by evaluating the incorporation of [3H]putrescine into N,N′-dimethylene casein in HSD buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol) containing 0.05% sucrose monolaurate (33, 34). Briefly, 5 μg of heart lysate was preincubated with 2 mM MgCl₂ in a 100-μl total volume at room temperature for 30 min. Samples were then transferred to an ice-water bath where 1% casein, 1 μCi of [3H]putrescine, and 0.5 mM CaCl₂ (final concentrations) were added. Following a 30-min incubation at 30 °C, reactions were iced and stopped by the addition of 100 μl of cold 50% trichloroacetic acid containing 0.1% putrescine. The precipitates were trapped on GF/F glass fiber filters (Whatman), washed six times with ice-cold 5% trichloroacetic acid, and counted. GTP-mediated inhibition of transglutaminase activity was determined under the same conditions after preincubation of the samples in the presence of 0.5 mM GTP (final) at room temperature for 30 min. The GTP binding activity of TGI was determined by direct photoaffinity labeling of [3H]GTP as described previously (7–11). Heart lysate preparations (10 μg/assay) were incubated in HSD buffer containing 0.05% sucrose monolaurate in the presence of 10 μCi of [α-32P]GTP, 100 μM MgCl₂, and 0.5 mM CaCl₂ (final concentrations) at room temperature for 20 min, the samples were subjected to UV irradiation in an ice-water bath for 10 min. Samples were then fractionated by SDS-polyacrylamide gel electrophoresis and subjected to autoradiography.

**Cardiac TGII Signaling**—Western blot analysis of cardiac hypertrophy gene expression was performed by RNA dot blotting as described previously (40). Filters were scanned using a PhosphorImager (Molecular Dynamics, Inc.), and the radioactivity of each hybridizing signal was quantitated using ImageQuant software. Results were normalized to glyceraldehyde-3-phosphate dehydrogenase expression.

**Physiologic Measurements**—Echocardiography was performed in anesthetized mice as described (24, 41). Calculations for left ventricle (LV) mass and wall thickness/radius ratio (H/r) were as follows: LV mass = (EDD + (SWT + PWT)² – (EDD)²) × 1.06 and H/r = PWT/EDD. Closed chest invasive hemodynamic studies were performed on 12–16-week-old nontransgenic and TGI-8.3 littermates as described previously (42). Briefly, animals were anesthetized with ketamine (50 mg/kg, body weight) and thiobutabarbitol (100 μg/kg, body weight). A tracheotomy was performed to maintain airway patency, and mice were maintained on a thermally controlled surgical table. Catheters were placed in the right femoral artery and vein for measurement of systemic arterial pressure and infusion of drugs. A Millar Mikrotip transducer was advanced to the LV via the right carotid artery for measurement of LV pressure and dP/dt. Incremental doses of dobutamine were infused for 3 min each, and steady-state measurements were made during the

![Fig. 1. TGI transgene expression](image-url)

Mouse heart lysates were prepared and analyzed as described under "Experimental Procedures." A, the relative level of transglutaminase activity reported as pmol/min/mg of heart lysate was determined at basal levels, in the presence of 0.5 mM Ca²⁺, and in the presence of 0.5 mM Ca²⁺ plus 0.5 mM GTP for NTG, TGI-1.1, and TGI-8.3 mice. Each bar represents the average ± S.E. from three mice. *p < 0.05 difference from basal levels. B, Western analysis of cardiac homogenates from transgenic and nontransgenic mice shows increased expression of the 74-kDa TGI protein in TGI-1.1 and TGI-8.3 transgenic mice. Purified guinea pig liver TGI (0.5 μg) was used as a control (lane 1) along with samples from three nontransgenic mice (100 μg, lanes 2–4), three TGI-1.1 mice (10 μg, lanes 5–7), and three TGI-8.3 mice (5 μg, lanes 7–10). C, [α-32P]GTP photoaffinity labeling shows increased GTP binding by TGI in TGI-1.1 and TGI-8.3 transgenic mice. Purified guinea pig liver TGI (0.5 μg) serves as a positive control (lane 1).
Statistical Analysis—Data are reported as means ± S.E. Statistical comparisons were performed with two-tailed Student’s t tests comparing transgenic values to nontransgenic littermates. For in vivo hemodynamic measurements, data were analyzed by one factor (within) or by mixed, two-factor analysis of variance using SUPERANOVA software by Abacus. Differences between individual means were further analyzed using single degree-of-freedom contrasts. Differences were regarded as significant at the p < 0.05 probability level.

RESULTS AND DISCUSSION

Two transgenic mouse lines (TGII-1.1 and TGII-8.3) that stably expressed the cMHC-TGII transgene were established. Mice from both lines were viable and reproduced normally. To quantitate functional TGII expression, transglutaminase activity of TGII was measured in transgenic and nontransgenic heart lysates (Fig. 1A). In the absence of Ca\(^{2+}\), basal myocardial transglutaminase activity was similar between nontransgenic \((0.43 ± 0.04 \text{ pmol/min/mg})\), TGII-1.1 transgenic mice \((0.38 ± 0.08 \text{ pmol/min/mg})\), and TGII-8.3 transgenic mice \((0.51 ± 0.06 \text{ pmol/min/mg})\). Upon Ca\(^{2+}\)-stimulation, nontransgenic animals exhibited a modest 1.5-fold increase in transglutaminase activity over basal levels. Ca\(^{2+}\)-stimulated activities of TGII-1.1 and TGII-8.3 lysates showed increases to 2.5 ± 0.50 pmol/min/mg \((−6.6\text{-fold over basal})\) and to 19 ± 1.7 pmol/min/mg \((−37\text{-fold over basal})\), respectively (Fig. 1A), indicating functional overexpression of the transgene. As would be predicted by the proposed model (1, 4), this Ca\(^{2+}\)-stimulated transglutaminase activity was inhibited by the addition of 0.5 mM GTP in the assays from both transgenic mouse lines and nontransgenic littermates. Western blots showed migration of the overexpressed TGII at the expected molecular mass \((−74 \text{ kDa})\) of the mature protein (Fig. 1B). A previously described assay that was optimized to show TGII GTP-binding activity by photoaffinity labeling in vitro (31) was also utilized to confirm the integrity of the transgenic TGII protein. Increased radiolabeling of an 
\(~74\text{-kDa}\) protein corresponding to TGII was found in heart lysates from both TGII-1.1 and TGII-8.3 transgenic lines showing 5.2- and 12-fold increases in GTP binding, respectively, compared with nontransgenic littermates (Fig. 1C).

In vitro reconstitution and coimmunoprecipitation studies have defined PLC\(\alpha\)1 as the effector of TGII G-protein signaling (10, 12). Western blot data confirm that, in mice, TGII and PLC\(\alpha\)1 are indeed coexpressed in both the atria and ventricles of the heart and that overexpression of TGII does not appear to alter PLC\(\alpha\)1 expression in either tissue (Fig. 2). Therefore, to investigate whether overexpression of TGII enhanced PLC activation in live tissue, total inositol phosphate levels were examined in organ cultured atria from TGII-8.3- and G\(_{\alpha}\)q-overexpressing mice and their nontransgenic littermates (Fig. 3A). Despite abundant expression of PLC\(\alpha\)1 in the mouse heart, basal inositol phosphate levels from TGII-8.3 transgenic mice were similar to those of nontransgenic littermates \((71.1 ± 8.45 \text{ cpm} \text{versus} 92.1 ± 19.6 \text{ cpm/mg}, \text{respectively})\). These results contrast with parallel studies using G\(_{\alpha}\)q transgenic mice atria in which basal inositol phosphate levels were increased 4.4-fold \((p < 0.01)\) over those of nontransgenic littermates (Fig. 3A). Furthermore, with the addition of the full \(\alpha\)\(_{1}\)AR agonist norepinephrine for 10 min (in the presence of the \(\beta\)AR antagonist propanolol), the increase in inositol phosphate levels with atria from TGII-8.3 mice to 191 ± 6.00 cpm/mg \((2.7\text{-fold over basal})\) was the same as that observed with nontransgenic littermates, which increased to 236 ± 23.6 cpm/mg \((2.6\text{-fold over basal})\).

Taken together, these results do not support coupling of TGII to inositol phosphate hydrolysis in mouse heart.

We have recently shown that transgenic overexpression of G\(_{\alpha}\)q in the heart results in translocation of PKC\(\varepsilon\) from cytosolic to particulate fractions (24) and that total PKC activity is increased 2.6-fold in G\(_{\alpha}\)q hearts as well.\(^2\) Thus, it appears that chronic cardiac PKC activation is an expected outcome of enhanced signaling via PLC in cardiomyocytes. Activation of PKC in TGII-8.3 and nontransgenic littermates was assessed by quantitating the expression of PKC\(\alpha\)s and PKC\(\varepsilon\)s (the two dominant diacylglycerol-regulated isoforms in mouse heart) in cardiac particulate versus cytosolic fractions. As shown in Fig. 3B, neither PKC isoform expression nor distribution was altered by TGII overexpression. Thus, two measurements of PLC coupling (inositol phosphate hydrolysis and PKC activation) failed to show increases in TGII transgenic mice despite −37-fold overexpression of transglutaminase activity. From these studies, then, it appears that TGII does not couple (or couples very weakly) to PLC, PLC\(\alpha\)1, or PKC\(\varepsilon\) in atrial and ventricular lysates isolated from TGII-8.3 mice and nontransgenic littermates. Therefore, the 236 ± 23.6 cpm/mg (2.6-fold over basal) increase in inositol phosphate levels with atria from TGII-8.3 mice to 191 ± 6.00 cpm/mg (2.7-fold over basal) was the same as that observed with nontransgenic littermates, which increased to 236 ± 23.6 cpm/mg (2.6-fold over basal).

\(^2\) G. W. Dorn, unpublished data.

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Fig. 2. Western blot analysis confirms coexpression of TGII and PLC\(\alpha\)1 in atria and ventricle. Western analysis of TGII (top) and and PLC\(\alpha\)1 (bottom) in atrial and ventricular lysates isolated from nontransgenic (N) and TGII-8.3 (+) mice shows coexpression of TGII (74 kDa) and PLC\(\alpha\)1 (revealed as a 90- and 100-kDa doublet) in both chambers of the heart. In the TGII Western blot, 100 μg of protein from each nontransgenic lysate as well as 50 and 10 μg each from atrial and ventricular lysate from TGII-8.3 mice was analyzed, respectively, and for PLC\(\alpha\)1, 50 μg of protein from each lysate was analyzed.

Fig. 3. Cell signal activation in TGII-8.3 mice. A. isolated atria were incubated in a physiologic organ bath in the presence of \([\text{H}]\)myoinositol. Total \([\text{H}]\)inositol phosphate production was subsequently measured by HPLC anion exchange chromatography as described under “Experimental Procedures.” Inositol phosphate production per mg of atria weight is shown for NTG and transgenic littermates from TGII-8.3 and G\(_{\alpha}\)q transgenic mouse lines. Each bar represents the average ± S.E. for three mice. *, p < 0.05 difference from NTG (control) mice. B. Western analysis of PKC\(\alpha\)s and PKC\(\varepsilon\)s in membrane and cytosolic fractions (50 μg each) was performed using ventricular extracts from nontransgenic (lanes 1–3) and TGII-8.3 mice (lanes 4–6) (see “Experimental Procedures”). Bands were quantitated using ScanAnalysis software. For cytosolic PKC\(\varepsilon\), the top band corresponding to this isoform was quantitated. No significant differences in PKC expression or the ratios of PKC in cardiac membranes/cytosol were found for either isoforms between TGII-8.3 mice and nontransgenic littermates.
in mice in each group. are typical of sections obtained from four trate or myocyte disorganization. Results shown. The latter shows diffuse interstitial fibrosis without inflammatory infiltrate or myocyte disorganization. Results are typical of sections obtained from four mice in each group.

inefficiently) to PLC in the heart. This conclusion is particularly compelling given that only ~5-fold overexpression of Gαq, a known activator of PLC, clearly enhances inositol phosphate hydrolysis and PKC activation in the heart.

Thus, the anatomic, biochemical, and functional consequences, if any, of overexpressing TGII in the heart would be expected to have little similarity to those of transgenic mice, such as those overexpressing Gαq, where PLC activation is indeed enhanced. TGII-8.3 transgenic mice showed a mild cardiomegaly with an ~10% increase in whole heart weight indexed to body weight compared with nontransgenic littermates (6.79 ± 0.30, n = 14 versus 6.06 ± 0.15, n = 16, p < 0.03). Lung/body weight ratios were not different. Histologic examination of 12–16-week-old mice revealed normal cardiomyocyte morphology, with a moderate degree of diffuse interstitial fibrosis (Fig. 4). Further examination of a limited number of 7-month-old mice suggested that neither the cardiomegaly nor the interstitial fibrosis appears to worsen with age (data not shown). Since animal models of hypertrophy and the human disease are associated with increased expression of fetal cardiac genes, such as atrial natriuretic factor, α-skeletal actin, and the β isoform of the myosin heavy chain (β-MHC) (43–48), we considered that the mice with increased transglutaminase activity might be associated with increased levels of these genes, with potentially a different pattern than observed with models where PLC signaling is enhanced. TGII-8.3 mice indeed showed some modulation in fetal gene expression, with a 4.7- and a 22-fold increase in β-MHC and α-skeletal actin expression, respectively (Fig. 5). Significantly, atrial natriuretic factor gene expression, which is elevated in most models of hypertrophy, was not increased in TGII-8.3 mice. This pattern of fetal gene expression contrasts with that of Gαq transgenic mice in which increased β-MHC and α-skeletal actin expression is accompanied by a dramatic (55-fold) increase in atrial natriuretic factor expression (24).

The findings of mild cardiomegaly, increased molecular markers of pathologic hypertrophy, and interstitial fibrosis in TGII-8.3 transgenics suggested the potential for physiological consequences of TGII overexpression. This was assessed in vivo using echocardiography and catheterization-derived hemodynamic measurements. Ventricular systolic function, as determined by echocardiographical fractional shortening, was significantly decreased in TGII-8.3 transgenic mice compared with nontransgenic littermates (32 ± 1%, n = 5, versus 51 ± 3%, n = 13, p < 0.0001, Fig. 6A). Calculated left ventricular mass was also significantly increased in TGII-8.3 mice (Table I), in agreement with the increased heart/body weight ratios found in these mice. Furthermore, Hr/r, the ratio of wall thickness to heart radius at diastole was not different from that observed in nontransgenic (NTG) mice, suggesting an eccentric pattern of enlargement (Table I). Consistent with the echocardiographic data, invasive hemodynamic studies also showed depressed ventricular function. Base-line (resting) left ventricular contractility (dP/dt max, Fig. 6B) was depressed ~30% in TGII-8.3 mice as compared with nontransgenic littermates (6400 ± 870 versus 9400 ± 390 mm Hg/s, n = 5 each, p < 0.04);
however, the magnitude of the dP/dt_max responses to dobutamine was not different between TGII transgenic and nontransgenic littermates. Similarly, base-line left ventricular relaxation (dP/dt_min) was less in the TGII mice, while the dP/dt_min responses were not significantly different between the two lines (Fig. 6C). Heart rates and left ventricular systolic and diastolic pressures were the same at base line and in response to dobutamine (data not shown).

In many forms of heart failure or in hypertrophy with ventricular dysfunction, cardiac βAR function is impaired (35). Given that a subtle alteration might not be evident in the in vivo agonist infusion studies, expression and coupling of βAR was examined in the TGII-8.3 mice. In membranes from TGII-8.3 and nontransgenic mice (n = 4 each), βAR density was not different (29 ± 4 versus 32 ± 8 fmol/mg), nor were basal (36 ± 5 versus 30 ± 2 pmol/min/mg) or maximal isoproterenol-stimulated adenyl cyclase activities (71 ± 5 versus 68 ± 7 pmol/min/mg). Again, these data are in marked contrast with the impaired cardiac function in G88-overexpressing mice, which exhibit an absence of in vivo βAR stimulation of cardiac contractility and depressed receptor coupling to adenyl cyclase, probably due to, among other mechanisms, βAR phosphorylation by PKC.2

In summary, we have overexpressed TGII in cardiomyocytes of transgenic mice to delineate both biochemical and physiologically applicable signaling pathways in the heart. With such overexpression, we were unable to detect enhanced basal or agonist-stimulated coupling of α1-AR to inositol phosphate hydrolysis; nor was there evidence of cardiac PKC activation. This finding strongly suggests that TGII signaling, if present at all, represents a minor regulator of PLC activity in the heart. The TGII overexpressing mice do have, however, cardiac hypertrophy, diffuse interstitial fibrosis, and depressed ventricular function at rest with normal responsiveness to βAR stimulation. The remodeling observed is consistent with the transglutaminase activity of TGII, which in other systems has been implicated in cell growth (15), matrix formation (36), and tissue repair processes (18, 19). As has been suggested by studies in heart failure (30, 31), alterations in expression or function of TGII may play a role in pathogenesis of the syndrome; based on the current body of work, we conclude that this is probably due to the transglutaminase activity of TGII rather than signaling through PLC.

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TABLE I

|               | NTG     | TGII-8.3 | p     |
|---------------|---------|----------|-------|
| n             | 5       | 13       |       |
| FS (%)        | 50 ± 3  | 32 ± 1   | <0.0001 |
| PWT (mm)      | 0.62 ± 0.03 | 0.58 ± 0.02 | NS#   |
| ESD (mm)      | 1.8 ± 0.1 | 2.8 ± 1  | <0.0001 |
| EDD (mm)      | 3.7 ± 0.1 | 4.1 ± 1  | <0.0008 |
| LV mass       | 68 ± 5  | 88 ± 4   | <0.015 |
| HR (bpm)      | 0.032 ± 0.001 | 0.031 ± 0.02 | NS   |
|               | 372 ± 15 | 383 ± 11 | NS    |

# Fractional shortening.
# NS, not significant.
# End systolic dimension.
# Heart rate (beats/min).

FIG. 6. In vivo cardiac function in TGII transgenic mice. A, echocardiography shows depressed fractional shortening in TGII-8.3 mice. Bars represent mean values ± S.E. for nontransgenic (n = 5) and TGII-8.3 (n = 13) mice. B and C, catheterization-derived hemodynamic measurements show cardiac contractility (dP/dt_max) and relaxation (dP/dt_min) of the TGII-8.3 mice are depressed at base line. The responses to dobutamine were not impaired. Shown are results from experiments conducted with five mice in each group, *p < 0.05 difference from NTG (control) mice.

Echocardiography results of mice overexpressing TGII versus values obtained in nontransgenic littermates
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