Vascular Hypertrophy and Hypertension Caused by Transgenic Overexpression of Profilin 1*

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We have overexpressed either the cDNA of human profilin 1 or the mutant (88R/L) in the blood vessels of transgenic FVB/N mice. Reverse transcription-PCR indicated selective overexpression of profilin 1 and 88R/L proteins in transgenic mice compared with control (~30%, p < 0.05). Rhodamine-phalloidin staining revealed increase stress fiber formation in vascular smooth muscle cells of profilin 1 compared with 88R/L and control. Hematoxylin and eosin staining showed clear signs of vascular hypertrophy in the aorta of profilin 1 mice versus 88R/L and control. However, there were no differences between 88R/L and control mice. Western blotting confirmed the activation of the hypertrophic signaling cascades in aortas of profilin 1 mice. Phospho-ERK1/2 was significantly higher in profilin 1 than 88R/L and control (512.3 and 361.7%, respectively, p < 0.05). Profilin 1 mice had significant increases in phospho-JNK as compared with 88R/L and control (371.4 and 346%, respectively, p < 0.05). However, there were no differences between 88R/L and control mice in both kinases. There was a significant increase in ROCK II kinase in the aorta of profilin 1 mice compared with controls (>400%, p < 0.05). Tail cuff and circadian monitoring of blood pressure showed significant increases in systolic and mean arterial blood pressures of profilin 1 mice starting at age 6 months compared with controls (~25 mm Hg, p < 0.05). These results suggest that increased actin polymerization in blood vessels triggers activation of the hypertrophic signaling cascades and results in elevation of blood pressure at advanced age.

Blood vessel walls are subjected to structural alterations through a process known as remodeling. Remodeling is usually an adaptive process in response to long term changes in homeodynamic conditions that occur during development, but it can also contribute to the pathophysiology of vascular and circulatory disease (1, 2). Remodeling is now considered to be a more complex process that includes increased wall thickness, increased cross-sectional area, and decreased lumen diameter (3, 4). Alterations in blood vessel diameter with subsequent changes in blood pressure may lead to heart disease. The cellular events underlying these processes involve changes in vascular smooth muscle cell growth, cell migration, apoptosis, inflammation, and fibrosis (5).

Modulation of smooth muscle phenotype is of major importance for a number of disease states in the cardiovascular, respiratory, and visceral organs, and its molecular mechanisms are rapidly being elucidated (6, 7). Both intrinsic and extrinsic factors contribute to smooth muscle differentiation, marked by the expression of a limited number of proteins, primarily associated with the contractile/cytoskeletal apparatus (8). It is likely that mechanical stress in the walls of hollow organs is one of the key factors that regulate smooth muscle development as well as growth and phenotypic differentiation (9). The effects of wall stress have been extensively studied in blood vessels, which respond to altered transmural pressure with growth and remodeling to normalize the mechanical stress in the tissue (9). Smooth muscle cells have been studied to provide further understanding of biochemical mechanisms involved in hypertension, especially its cytoskeleton proteins such as actin protein (10).

Actin is a highly dynamic network. It is essential for several important activities, such as muscle contraction and transmembrane signaling (11, 12). Actin consists of actin filaments and a variety of associated proteins (13). Many proteins associated with the actin cytoskeleton control actin assembly and disassembly. These proteins regulate actin assembly at multiple levels, including the organization of actin monomers into actin polymers (13). One key actin-regulatory protein is profilin, which associates with polymerization of actin. Profilin is a ubiquitous small (12–15 kDa) actin-binding protein that plays an important role in the regulation of actin polymerization in a number of motility functions (14). The ability of profilin to bind to many ligands suggests that profilin is involved in signal transduction and may link transmembrane signaling to the control of the microfilament system (15, 16). Early biochemical studies...
indicated that profilin interacts with actin in a 1:1 ratio and participates in the addition of monomers at the free barbed end of the filament then disassociates at the barbed end (15). Recent work has suggested several more functions of profilin aside from its monomer-sequestering ability. Profilin promotes the exchange of adenine nucleotide bound to actin monomer and also effectively lowers the critical concentration of monomer actin for polymerization of actin (17, 18). It also promotes nucleotide exchange on an actin monomer by lowering the affinity of the actin monomer for its bound nucleotide by 1000-fold (19). To assess the role of actin dynamics on the vasculature, we have transgenically overexpressed the cDNA of the human profilin 1 gene and the mutant isoform of the gene (88R/L) in the blood vessels of FVB/N mice. This was done using the mouse vascular smooth muscle α-actin promoter.

Our hypothesis is that increased actin polymerization in vascular smooth muscle cells will lead to vascular remodeling and subsequent elevation in blood pressure.

**MATERIALS AND METHODS**

**Chemicals**—Unless otherwise stated, all chemicals were purchased from Sigma or Fisher and were of the best grade available. Rhodamine-phalloidin was from Molecular Probes (Eugene, OR).

**Generation of Transgenic Mice**—We have developed transgenic mouse models that overexpress either the cDNA of human profilin 1 gene or express the mutant (88R/L) in FVB/N mice. Vascular smooth muscle α-actin promoter, containing all elements known to be required for optimal transcription of the smooth muscle α-actin gene, was used to induce selective overexpression of the human profilin 1 or the 88R/L profilin mutant in smooth muscle cells. Smp-8 plasmid that contains a 3.6-kb segment of the 5′-region of the mouse smooth muscle α-actin promoter was cut with XhoI and EcoRl, and then the cDNA of human profilin 1 or 88R/L, including its polyadenylation tail (with XhoI and EcoRI ends), was cloned within these sites. The Sphl/EcoRI fragment of the plasmid that has the promoter and the profilin 1 cDNA was isolated and microinjected into the fertilized eggs of mouse FVB/N females. Single cell embryos derived from super-ovulated FVB/N females were used for the microinjection procedure. Surviving microinjected embryos were implanted into pseudo-pregnant FVB/N foster mothers (20). PCR of tail genomic DNA with specific primers for the transgene was used to identify the transgenic founders. Three transgenic founders were selected and confirmed on the basis of positive PCR analysis. Unless otherwise stated, all the experiments were repeated three times and performed with heterozygous transgenic mice (5–6 months old) with age-matched nontransgenic littermates.

**Genotyping**—Profilin 1 and 88R/L transgenic mice were identified using PCR. Genomic DNA was isolated from tail clips and incubated in 500 μl of lysis buffer overnight at 60 °C. The lysis buffer contained 50 mM Tris, pH 8, 50 mM NaCl, 25 mM EDTA, and 37.5 μl of proteinase K. The lysate was cleaned in triplicate with phenol/chloroform (1:1) and once with chloroform. The supernatant was recovered, and DNA was precipitated with 2 volumes of ethanol and spooled out in a clean tube. PCR was run using specific primers for the human profilin 1 gene. The forward primer (TCT CTG CAG AAC CCT GAG AC) was derived from the smooth muscle α-actin promoter located at the 5′ end of the human profilin 1 cDNA. The reverse primer sequence (GAG GTC GAC GGT ATC GAT AGG CTT G) was derived from the 3′ end of the human profilin 1 cDNA.

**Reverse Transcription-PCR**—Tissues isolated from profilin 1, 88R/L, and nontransgenic control mice were frozen in liquid nitrogen. The frozen tissues were pulverized in liquid nitrogen by a mortar and pestle. Total cellular RNA was extracted from various frozen mouse tissues using TRIzol reagent (Invitrogen), and the integrity of total RNA was checked on an agarose gel. Total RNA samples from different tissues were treated for 15 min with DNase I (1 unit/1 μg of total RNA) to eliminate any possible genomic DNA contamination. The reaction was inactivated by the addition of 1 μl of 25 mM EDTA to the reaction mixture, and the mixture was then heated for 10 min at 65 °C. The first strand cDNA was synthesized utilizing Superscript II (Invitrogen). It was then amplified using Taq DNA polymerase with 35 cycles of denaturation (94 °C, 45 s), primer annealing (55 °C, 30 s), and extension (72 °C, 2 min). The profilin-specific primers (as shown above) were used for PCR. On each sample, a negative control (DNase I, no primers or irrelevant primers) and positive control (no DNase I) were performed. The absence of any signal with the negative control showed that the DNase I digestion eliminated all DNA-based synthesis pathways/contamination, and thus the signal was RNA-based.

**In Situ Reverse Transcription-PCR**—The aortas from transgenic or control mice were fixed in 10% buffered formalin and then embedded in paraffin. Three 4-μm sections were placed on individual silane-coated glass slides to facilitate the performance of the in situ PCR. In situ RT-PCR3 was performed on aortic sections using primers specific for profilin 1 transgene (as mentioned above). The protocol used for these experiments has been described previously (21, 22). Briefly, optimal protease digestion time was first determined using as the guide nonspecific incorporation of the reporter nucleotide (10 μM digoxigenin dUTP). Optimal protease digestion was followed by overnight incubation in RNase-free DNase (10 units per sample) (Roche Applied Science) and one step RT-PCR using digoxigenin dUTP. After 20 cycles, the slides were washed at high stringency (60 °C for 10 min in 15 mM salt with 2% bovine serum albumin). The digoxigenin-labeled target-specific cDNA was detected using the anti-digoxigenin-alkaline phosphatase conjugate (Roche Applied Science, 1:200 in PBS for 30 min at 37 °C) followed by exposure to the chromogens nitro blue tetrazolium and bromochloroindolyl phosphate (EnzoBiochemicals, Farmingdale, NY). Nuclear fast red, which stains negative cells pink, served as the counter-stain. As negative controls, in situ RT-PCR was performed similarly either with irrelevant human papillomavirus-specific primers or with RNase pretreatment of the slides prior to the in situ RT-PCR with specific profilin primers.

3 The abbreviations used are: RT, reverse transcription; c-Jun, c-Jun N-terminal kinase; VSMC, vascular smooth muscle cells; IHC, immunohistochemistry; SBP, systolic blood pressure; ERK, extracellular signal-regulated protein kinase; MAP, mean arterial pressure; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; WASP, Wiskott-Aldrich syndrome protein.
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For the positive control, the same human papillomavirus primers were used without prior DNase treatment on the slides. Specimens from transgenic mice were run concurrently with nontransgenic mice.

Preparation of Aortic Vascular Smooth Muscle Cells (VSMCs)—We developed primary aortic VSMCs from thoracic aortas isolated from profilin 1, 88R/L transgenic mice, and nontransgenic control mice. These VSMCs were explanted from the medial layer of the thoracic aorta and expanded for experiments up to passage 6. The aortas were treated with collagenase type II (2 mg/ml) for 10 min to free the adventitia, and then both endothelial and adventitial layer were carefully removed by gentle peeling with two pairs of fine forceps under a dissecting microscope. The muscle layers were transferred to fresh collagenase, cut into small pieces in a gently stirred orbital shaker for 1 h at 37 °C, and dispersed into single cells. The cells were incubated in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and were passage twice a week by harvesting with trypsin/EDTA and seeding into 75-cm² flasks. Smooth muscle cell analysis by flow cytometry after vascular smooth muscle α-actin staining confirmed the purity of the isolated aortic VSM cells.

Rhodamine-Phalloidin Staining—Aortic smooth muscle cells from profilin 1, 88R/L, and nontransgenic controls were grown on coverslips placed in 35-mm plates. The cells were fixed in 3.7% formaldehyde in PBS for 10 min at room temperature after reaching 60–70% confluency. Triton X-100 in PBS was then added to the coverslips for 10 min at room temperature. Rhodamine-phalloidin (100 μl in 1 ml of PBS) was added to each sample and incubated for a half-hour in the dark. The coverslips were washed with PBS for 30 min. A Nikon Eclipse 800 fluorescence microscope was used to visualize and assess the actin filaments stained with rhodamine-phalloidin. The 800 fluorescence microscope was used to visualize the intensity of stress fiber formation was assessed by the intensity of rhodamine-phalloidin staining/cell in more than 300 different cell types using the MetaMorph (version 4.6) software.

Quantitative Analysis of Profilin with Polyproline-Sepharose Beads—Equal amounts of proteins isolated from primary culture of aortic VSM cells of profilin 1, 88R/L, or nontransgenic control mice were mixed with 50 μl of poly-L-proline-Sepharose beads and kept on a shaker for 1 h at 4 °C. The tubes were centrifuged at 1000 rpm at 4 °C, and the supernatant was discarded. The beads were washed twice with PBS buffer. Thirty microliters of sample buffer was added to each sample tube and then boiled for 30 s in water. The samples were centrifuged, and the supernatant was collected, and profilin 1 was separated on SDS-PAGE. Lysates isolated from platelets were used as a positive control. Protein bands were visualized on the SDS-polyacrylamide gel using Coomassie Blue staining as described previously (23).

Analysis of F-actin/G-actin Ratio—Cells were lysed with 200 μl of F-actin stabilization buffer (50 mM PIPES, pH 6.9, 50 mM NaCl, 5 mM MgCl₂, 5 mM EGTA, 5% glycerol, 0.1% Triton X-100, 0.1% Nonidet P-40, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.0011% antifoam, 1 mM ATP, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 10 μg/ml benzamidine, 500 μg/ml tosyl arginine methyl ester). The cell lysates were homogenized using 26.5-gauge syringes and then centrifuged at 100,000 × g for 60 min at 37 °C. The supernatants (G-actin) were separated from the pellets (F-actin) and were immediately placed on ice. The pellets were resuspended using 26.5-gauge syringes to the same volume as the supernatants by using ice-cold distilled H₂O plus 20 μM cytochalasin B and were incubated on ice for 60 min. The resuspended pellets were gently mixed every 15 min. Equal amounts of the samples (20 μg of the G-actin fractions and the corresponding amounts of the F-actin fractions) were loaded to each lane and analyzed by Western blotting with α-actin antibody. The amount of F-actin and G-actin was determined by scanning densitometry.

Western Blot Analysis—Aortic extracts or VSM cells isolated from aortas were separated on SDS-polyacrylamide (4–20%) gels. Western blot was performed using specific antibodies. The bands were visualized with ECL system (Amersham Biosciences). The following antibodies were used: rabbit polyclonal anti-Rho/Rock, extracellular signal-regulated protein kinase (Erk1/2), and c-Jun N-terminal kinase (JNK) mouse monoclonal antibodies (Santa Cruz Biotechnologies).

Immunohistochemistry (IHC)—IHC for aortas of profilin 1 transgenic mice was performed on 4-μm sections from formalin-fixed and paraffin-embedded tissue using ABC kit according to the manufacturer’s instructions (Vector Laboratories). Hematoxylin and eosin staining procedures were performed according to standard protocols. Hypertrophy was quantified by measuring the medial thickness in cross-sections of aortas after hematoxylin and eosin staining. We have measured 30 aortic cross-sections from profilin 1, 88R/L, or control mice using the MetaMorph (version 4.6) software.

Blood Pressure Measurements—Systolic blood pressure (SBP) was monitored by tail cuff measurement in profilin 1, 88R/L, transgenic mice as compared with control littermates (n = 8 for each group). The experiment started when the mice were 3 months old and continued until they were 9 months old. The blood pressure was checked once a month. We also assessed the circadian blood pressure in transgenic and matching control littermates at age 13–14 months (n = 8 for each group). The mice were anesthetized using a 5:2 ketamine/xylazine mixture. Carotid arterial catheters (Micro-Renathane®, Braintree Scientific, MA) were inserted as described previously (24). The catheter was protected by a stainless steel spring and connected to a swivel located on top of the cage, which allowed the animal to move freely. A continuous infusion of 60 units/ml of heparinized saline (25 μl/h) was used to maintain catheter patency. At 3–4 days post-surgery, the mice displayed a stabilized pattern of behavior, food consumption, and water intake.

Blood pressure and heart rate were recorded continuously using a data acquisition system (Biopac, Santa Barbara, CA) with a sampling rate of 100 Hz. Circadian rhythm analysis was obtained by converting data to text files for plotting and analysis using the Circadia program, specific for biological rhythms analysis (Behavioral Cybernetics, Boston). Mean arterial pressure (MAP) was calculated from systolic and diastolic blood pressure and reported in mm Hg.

Echocardiography—Profilin 1 (n = 8) and 88R/L (n = 8) transgenic mice were compared with nontransgenic control (n = 8) matched for age and gender. Mice were anesthetized
with isoflurane by inhalation. A warming pad was used to maintain normothermia. Once the chest of the mouse was shaved and a layer of acoustic gel was applied to the thorax, two-dimensional and M-mode echocardiographic studies were performed with a commercially available 15-MHz linear array transducer system (Hewlett Packard, Sonos 5500).

**Statistical Analysis**—Data are presented as means ± S.E. Statistical significance was analyzed with a Student’s t test or 1-way analysis of variance, followed by Bonferroni correction for post hoc pairwise comparisons. A value of p < 0.05 was considered significant.

**RESULTS**

**Generation of Transgenic Mice**—We engineered transgenic mouse models overexpressing either the cDNA of profilin 1 gene or expressing the mutant (88R/L) in FVB/N mice under the control of the mouse smooth muscle α-actin promoter. Transgenic mice were identified using PCR analysis of tail genomic DNA and were used to establish three stable transgenic lines by breeding them with nontransgenic FVB/N mates (Fig. 1A). Three transgenic founders were identified from each model based on the levels of profilin 1 expression in the aortas of F1 generations as shown below. These transgenic founders were used to establish stable transgenic lines by breeding them with nontransgenic FVB/N littermates. All transgenic lines essentially displayed a similar phenotype.

**Expression of Profilin 1 in Transgenic Mice**—To detect the selective expression of profilin 1 in transgenic mice, various tissues were analyzed by RT-PCR using primers that selectively recognize the transgene. RT-PCR analysis revealed selective transcription of profilin 1 in smooth muscle such as in aorta, gut, and spleen. However, profilin 1 was not detected in organs of transgenic mice that express little of the smooth muscle cells such as the liver and brain (Fig. 1B). Furthermore, we were able to confirm the selective overexpression and the localization of profilin 1 transcript in the aorta of profilin 1 transgenic mice using in situ RT-PCR (Fig. 1C). Similar results were obtained using aortic sections from 88R/L transgenic mice (data not shown).

**Quantitative Analysis of Profilin 1 and 88R/L Proteins with Polyproline-Sepharose Beads**—Because of the lack of specific profilin 1 antibody at the time of the experiment, we took advantage of the high binding affinity between profilin 1 protein and polyproline to assess the expression of profilin 1 or 88R/L proteins in the aortas of transgenic mice as described under “Materials and Methods.” Our results showed that smooth muscle cells of profilin 1 or 88R/L transgenic mice have significantly (p < 0.05) higher amounts (~30% or more) of human profilin 1 and 88R/L proteins in their aortic VSMC compared with the nontransgenic control mice (Fig. 2).

**Overexpression of Profilin 1-induced Actin Polymerization**—We stained confluent monolayers of smooth muscle cells from transgenic mice or nontransgenic control with rhodamine-phalloidin to determine stress fibers formation. There was more than a 2-fold increase in the intensity of rhodamine-phalloidin staining/cell in profilin 1 compared with nontransgenic control and 88R/L. Our results showed higher expression of stress fibers and membrane ruffling in vascular smooth muscle cells from profilin 1 transgenic mice compared with nontransgenic control and 88R/L. The 88R/L cells, however, showed lower expression of stress fiber formation and ruffling than the nontransgenic controls (Fig. 3A).

In addition, we confirmed these findings by assessing the ratio of F-actin/G-actin in the aortic smooth muscle cells from profilin 1. Our results showed a significant increase in F/G-actin ratio in the aortic smooth muscle cells from profilin 1 mice (301 ± 4%) compared with the nontransgenic controls (153 ± 5%; p < 0.0001) (Fig. 3B).
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FIGURE 3. Overexpression of profilin 1 induced actin polymerization and led to vascular hypertrophy. Rhodamine-phalloidin staining of smooth muscle cell confluent monolayers shows increased stress fibers in vascular smooth muscle cells from profilin 1 transgenic mouse as compared with nontransgenic control (A). The 88R/L cells, however, show lower expression of stress fiber formation than the control (A). Analysis of the F-actin/G-actin ratio shows significant increase in F-actin/G-actin (F/G) ratio in the aortic smooth muscle cells from profilin 1 mice (301 ± 4%) compared with the nontransgenic controls (153 ± 5%; p < 0.0001) (B). Hematoxylin and eosin staining shows clear signs of remodeling and vascular hypertrophy in the aorta of profilin 1 transgenic mice as compared with nontransgenic control (yellow arrows). There are no differences, however, between 88R/L and nontransgenic control aortic sections (C).

Overexpression of Profilin 1 Resulted in Vascular Hypertrophy—We used IHC to examine the integrity of the blood vessels in profilin 1 and 88R/L transgenic mice compared with matched nontransgenic control littermates. The hematoxylin and eosin staining showed clear signs of remodeling and vascular hypertrophy in the aorta of profilin 1 transgenic mice compared with nontransgenic controls. Vascular remodeling was evident by enlargement of vascular smooth muscle cells and increases in nuclei size in these cells. Our results showed significant increase in the medial thickness of profilin 1 aortas (138 ± 4 μm) as compared with control 90 ± 3 μm; p < 0.05). There were no differences, however, between 88R/L and nontransgenic control aortic sections (Fig. 3C).

Activation of the Hypertrophic Signaling in Smooth Muscle Cells of Profilin 1 Mice—We assessed activation of the hypertrophic signaling cascade in transgenic mice as compared with nontransgenic controls using Western blot analysis. We assayed two important kinases, ERK1/2 and JNK, in the hypertrophy pathway. Western blot analysis was performed on protein extracts isolated from vascular smooth muscle cells isolated from the aortas of profilin 1, 88R/L, and nontransgenic controls using antiphosphotyrosine-ERK1/2 and antiphospho-JNK antibodies. Our results showed that profilin 1 mice have significantly (p < 0.05) higher levels of activated ERK1/2 than nontransgenic controls and 88R/L (361.7 and 512.3%, respectively). However, no significant difference was observed between 88R/L transgenic mice and nontransgenic controls. In addition, there was no significant difference among groups in total ERK1/2 when we used anti-ERK1/2 antibody (Fig. 4A). Western blotting analysis of phospho-JNK indicated that profilin 1 mice have significantly (p < 0.05) higher levels of phosphorylated JNK than nontransgenic controls and 88R/L (346.7 and 371.4%, respectively). There was no significant difference between nontransgenic controls and 88R/L mice. In addition, Western blots performed using anti-JNK antibody showed no changes in total JNK protein levels among groups (Fig. 4B). In addition, we have confirmed the expression of ERK and JNK in the aortas of transgenic and control mice (age, 5–6 months and 12–15 months) and found no significant differences in the expression among different ages and the expression in VSMC cells (data not shown).

Blood Pressure Is Elevated in Older Profilin 1 Transgenic Mice—Systolic blood pressure (SBP) was monitored by tail cuff measurements in profilin 1 and 88R/L transgenic mice compared with control littermates (n = 8 for each group). The experiment started when the mice were 3 months old and continued until they were 9 months old. Blood pressure was checked once a month. Our results showed significant elevations in SBP (p < 0.05) in profilin 1 transgenic mice when the mice became 6 months old and the SBP continued to increase until the end of the experiment when the mice became 9 months old. However, there was no significant difference in SBP between nontransgenic controls and 88R/L mice (Fig. 5A). In addition, we monitored the circadian pattern of blood pressure in profilin 1 mice compared with nontransgenic controls. Continuous monitoring of blood pressure in conscious, nonstressed mice was performed by cannulation of the external carotid artery in eight profilin 1 male transgenic mice (13–14 months old) and eight nontransgenic control littermates matched for age and gender. MAP of profilin 1 mice was significantly (p < 0.05) and consistently higher (~25 mm Hg increase) compared with MAP of the nontransgenic control mice (Fig. 5B). MAP in profilin 1 mice fluctuated within 130–134 mm Hg, whereas MAP in the nontransgenic control mice fluctuated within 104–112 mm Hg (Fig. 5B). These results clearly indicate that vascular hypertrophy in profilin 1 transgenic mice led to the development of hypertension at an advanced age.

Expression of Rho/Rock Pathway in the Aorta of Profilin 1 Transgenic Mice—The Rho protein, through its downstream effector ROCK, mediates cytoskeletal reorganization as well as smooth muscle cell contraction and is implicated in the pathophysiology of hypertension. Spontaneously hypertensive rats demonstrated a significant increase in Rho expression and activity (25). In addition, specific inhibition of ROCK prevents agonist-induced smooth muscle cell contraction and decreases blood pressure in spontaneously hypertensive rats (40). Therefore, we assayed the expression of ROCKII kinase in aortas of
profilin 1 or 88R/L transgenic mice as compared with nontransgenic controls. Our results showed that overexpression of profilin 1 in vascular smooth muscle cells led to significant increases (\( p < 0.05 \)) in the protein expression of ROCK II kinase in profilin 1 transgenic mice as compared with nontransgenic controls and 88R/L mice (400% increase) (Fig. 6). These results indicate that overexpression of profilin 1 in smooth muscle cells leads to increased actin polymerization, which may induce mechanical stress and a subsequent activation of the Rho/Rock pathway resulting in elevated blood pressure at old age in the profilin 1 transgenic mice.

**Cardiovascular Parameters in Profilin 1 Transgenic Mice**—Two-dimensional echocardiographic analysis using left ventricular guided M-mode and Doppler aortic flow velocities was performed on profilin 1, 88R/L, and the control littermates. The experiment started when the mice were 3 months old and continued until they were 9 months old. As seen in Fig. 7, profilin 1 mice had a small but significant (\( p < 0.05 \)) increase in cardiac output and stroke volume (determined by aortic flow velocities) compared with nontransgenic controls. This may be related to preload recruitment at an early age prior to arterial blood pressure increases at advanced age. No significant changes were evident in heart rate or left ventricular fractional shortening (determined by M-mode) between the ages of 3 and 9 months.

**DISCUSSION**

The goal of our study was to assess the effects of altering smooth muscle contractility because of changes in actin polymerization.
Enhanced actin polymerization by overexpression of profilin 1 was evident by increased the F-actin/G-actin ratio in aortic smooth muscle cells from profilin 1 mice. IHC analyses of aortic sections from profilin 1 transgenic mice showed structural changes with clear signs of medial hypertrophy.

It is well established that hypertension leads to remodeling of large and small arteries (31, 32). Vascular remodeling is believed to be an adaptive process in response to chronic changes in hemodynamic conditions during development and vascular pathologies (1, 2, 33, 34). The primary aim of anti-hypertensive drugs, particularly angiotensin-converting enzyme inhibitors and angiotensin receptor subtype 1 antagonists, is to lower the blood pressure (mechanical stress) with the hope of reversing this remodeling (35). In this profilin 1 model we demonstrate that the reverse can be true as well, i.e. alteration in cytoskeleton dynamics favoring increased actin polymerization can contribute to vascular adaptations (hypertrophy) with aging resulting in increased systolic blood pressure. Our preliminary results in microvessels (tail arteries) of profilin 1 mice demonstrated increased medial cross-section area and increased medial/lumen ratio in profilin 1 transgenic mice compared with matching littermate controls.4

Increased actin polymerization and stress fiber formation generate mechanical force that may play a role in the modulation of cellular morphology and function. Mechanical force (such as increased blood pressure) is an important modulator of cellular morphology and function in a variety of tissues and is an important contributor to hypertrophy in the cardiovascular system (36). It has been demonstrated that mechanical stretch such as in hypertension can rapidly induce activation of JNKs and ERKs in vascular walls after balloon injury or angioplasty in animal models (37, 38), suggesting that mechanical stretch is closely related to JNK and ERK1/2 activation. Activation of JNKs and ERKs signaling pathways leads to increased c-fos and c-jun gene expression and enhanced transcription factor AP-1 DNA binding activity contributing to increased gene expression and protein synthesis (37). Overexpression of a dominant negative form of the small G protein Rac could block ERK activation in response to stretch in smooth muscle cells. In addition, both suramin and PD98059 could not only inhibit ERK1/2 activation but also block AP1 DNA binding activities, suggesting that ERK1/2 signaling mediates stretch-induced AP1 activation and smooth muscle cell protein synthesis (37). Therefore, mechanical stress resulting from increased stress fiber formation is associated with activation of the signaling cascades of JNK and ERK1/2. This pathway is also activated by angiotensin II and has been implicated in the pathogenesis of cardiovascular diseases (39). These cascades play an important role in remodeling of blood vessels. We examined activation of the hypertrophy pathway in aorta of profilin 1 hypertensive mice as compared with 88R/L hypotensive mice and nontransgenic controls. Up-regulation of the hypertrophy pathway was evident by the activation of ERK1/2 and JNK kinases. Furthermore, vascular hypertrophy in profilin 1 transgenic mice led to elevated blood pressure in these mice at the age of 6 months and

4 H. Hassanain, unpublished observations.
older as compared with control mice. However, there was no change in blood pressure in 88R/L transgenic mice.

The small GTP-binding protein Rho, a member of the Ras superfamily, and Rho kinase ROCK play an important role in blood pressure regulation, particularly hypertension (40). Activated Rho elevates myosin light chain phosphorylation by (i) directly phosphorylating myosin light chain and (ii) phosphorylation and inhibition of the myosin-binding subunit of myosin light chain phosphatase (41, 42). This increases myosin contractility and tension contributing to stress fibers. Effectors of Rho also include LIM kinase, which phosphorylate and inhibit the actin-depolymerizing protein cofilin, leading to actin filament stability in the form of stress fibers (41). Our results showed a significant increase in the expression of the Rho kinase ROCK in aortas of profilin 1 mice compared with controls, which is expected to increase Rho expression. This increase was absent in the aortas of 88R/L transgenic mice, which also lack any increase in stress fiber formation and hypertrophy. These results suggest that elevated Rho-ROCK activity contributes to increased smooth muscle cell contractility. Indeed, profilin 1 transgenic mice showed increased mean arterial pressure. The blood pressure in the profilin 1 mice was elevated 25–30 mm Hg higher than nontransgenic controls. In contrast, the blood pressure in 88R/L mice was below the control littermates; however, it did not reach statistical significance. In other words, the transgenic profilin 1 mouse is a model of vascular hypertrophy that leads to hypertension. The absence of a hypertensive phenotype in the 88R/L mice could be due to the lack of significant vascular remodeling as a result of decreased actin polymerization. Our results showed a decrease in stress fibers formation in 88R/L mice; however, these changes did not translate into significant alterations in the vasculature. This might be due to an activation of a compensatory mechanism to maintain the integrity of vessel structure and thus keep the blood pressure at a survival level.

It is not entirely clear how profilin 1 may affect cardiac output and stroke volume, as well as preload recruitment. The best data on the physiologic cellular effects of profilin 1 showed that profilin increases adhesion of cells (43), most likely through integrins. Hence, the integrin-linked kinase could be activated leading to known effects such as increased contractility and stiffness (44). Such effects are under investigation and will be part of future reports.

Taken together, increased actin dynamics in VSMC favoring F-actin induces stress fiber formation and plays an important role in vascular hypertrophy by inducing internal mechanical stress and triggering the hypertrophic signaling pathway resulting in vascular remodeling. In addition, stress fiber formation could affect the relaxation/contraction process of the smooth muscles, making it more constrictive and/or less responsive to vasodilators such nitric oxide. That could be an important factor contributing to hypertension besides the vascular hypertrophy in the profilin 1 transgenic mice.

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