PPARγ and Aortic Aneurysm

Peroxisome Proliferator–Activated Receptor γ Level Contributes to Structural Integrity and Component Production of Elastic Fibers in the Aorta

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Abstract—Loss of integrity and massive disruption of elastic fibers are key features of abdominal aortic aneurysm (AAA). Peroxisome proliferator–activated receptor γ (PPARγ) has been shown to attenuate AAA through inhibition of inflammation and proteolytic degradation. However, its involvement in elastogenesis during AAA remains unclear. PPARγ was highly expressed in human AAA within all vascular cells, including inflammatory cells and fibroblasts. In the aortas of transgenic mice expressing PPARγ at 25% normal levels (Pparg<sup>−/−</sup> mice), we observed the fragmentation of elastic fibers and reduced expression of vital elastic fiber components of elastin and fibulin-5. These were not observed in mice with 50% normal PPARγ expression (Pparg<sup>−/−</sup> mice). Infusion of a moderate dose of angiotensin II (500 ng/kg per minute) did not induce AAA but Pparg<sup>−/−</sup> aorta developed flattened elastic lamellae, whereas Pparg<sup>−/−</sup> aorta showed severe destruction of elastic fibers. After infusion of angiotensin II at 1000 ng/kg per minute, 73% of Pparg<sup>−/−</sup> mice developed atypical suprarenal aortic aneurysms: superior mesenteric arteries were dilated with extensive collagen deposition in adventitia and infiltrations of inflammatory cells. Although matrix metalloproteinase inhibition by doxycycline somewhat attenuated the dilation of aneurysm, it did not reduce the incidence or elastic lamella deterioration in angiotensin II–infused Pparg<sup>−/−</sup> mice. Furthermore, PPARγ antagonism downregulated elastin and fibulin-5 in fibroblasts, but not in vascular smooth muscle cells. Chromatin immunoprecipitation assay demonstrated PPARγ binding in the genomic sequence of fibulin-5 in fibroblasts. Our results underscore the importance of PPARγ in AAA development through orchestrating proper elastogenesis and preserving elastic fiber integrity.

Key Words: aneurysm ■ angiotensin II ■ collagen ■ doxycycline ■ fibroblasts

Although open surgical or endovascular repair procedures have reduced the mortality of ruptured abdominal aortic aneurysm (AAA) in the past decade,¹ no effective pharmacological treatment has been conducted to inhibit the progression and rupture of human AAAs. Although medical management for hypertension and hyperlipidemia reveals potential benefits for lowering the growth rate of AAA,² directly targeting vessel health is urgently needed. To this end, factors and cell types that are involved in the development of aneurysm should be revisited.

Loss of integrity and massive disruption of elastic architecture are key features of structural changes in AAA.³ Elastic fiber is formed by initial synthesis of soluble precursor tropoelastin and later maturation by cross-linking of fibulins (fibulin-4 and fibulin-5) and fibrillins (fibrillin-1 and fibrillin-2) scaffold with the elastin core.⁴ In contrast, extensive infiltration of macrophages and lymphocytes increases the local expression of proinflammatory cytokines and triggers production of elastolytic proteases. Elastic fibers are degraded by matrix metalloproteinases (MMP-2, MMP-9, MMP-7, and MMP-12) and cysteine proteinases (cathepsin S and cathepsin K).⁵ Aortic fibroblasts in AAA have not been well discussed, but they are documented within the media and adventitia of aneurysm, and express higher levels of several collagens and elastin.⁶ Thus, aortic fibroblasts may actively participate in elastic fiber turnover particularly when vascular smooth muscle cells (VSMCs) and elastic fiber components are compromised in AAA.

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Peroxisome proliferator–activated receptor γ (PPARγ) has been shown to protect from vascular diseases through its anti-inflammatory effect. PPARγ activation in inflammatory cells reduces production of tumor necrosis factor α (TNF-α), interleukin (IL)–1β, and IL-6. PPARγ activation in VSMCs suppresses MMP-9 expression and collagen overproduction. Treatment with PPARγ agonists protects aorta against nicotine-induced calcification and elastic fiber fragmentation and reduces the development and rupture of angiotensin II (Ang II)–induced AAA. However, loss of PPARγ in VSMCs promotes aortic dilatation and elastin degradation in CaCl2-induced AAA. Although these findings indicate a critical role of PPARγ in reduction of inflammation and elastic fiber degradation in AAA, several questions remain unsolved. First, the role of PPARγ in elastic fiber component production remains unclear. Second, the involvement of adventitial fibroblasts in PPARγ protective effect is lacking. Third, although PPARγ gene polymorphism is associated with the incidence and growth of AAA, the threshold levels of PPARγ to maintain integrity and prevent from AAA are not known.

In this study, we have tested our hypothesis that PPARγ plays key roles in maintenance of the elastic fiber integrity and prevention of AAA in response to exogenous stimuli, such as increased Ang II. We took advantage of mice genetically altered to have different levels of PPARγ expression from 25% to 100%, and unveiled that fibroblasts express high levels of PPARγ as increased Ang II. We took advantage of mice genetically negative control procedures, which gave consistently negative results. In this study, we have tested our hypothesis that PPARγ plays key roles in maintenance of the elastic fiber integrity and prevention of AAA in response to exogenous stimuli, such as increased Ang II. We took advantage of mice genetically altered to have different levels of PPARγ expression from 25% to 100%, and unveiled that fibroblasts express high levels of PPARγ as increased Ang II. We took advantage of mice genetically negative control procedures, which gave consistently negative results.

**Methods**

**Human Tissue Procurement**

Human AAA samples were collected during open surgical repair of AAA. Samples from 2 patients were used for immunohistochemi- stical staining. The specificity of primary antibody was confirmed by negative control procedures, which gave consistently negative results (Figure S1). One patient had hypertension and the other had diabe- tes mellitus, and the diameters of infrarenal AAs were 7.4 and 8 cm, respectively. Sections of normal human aorta, purchased from Origene, were obtained from a 57-year-old male with heart valve dis- order. Sample diagnosis from pathology verification was not tumor structures and within normal limits. The use of human samples was approved by the Institutional Animal Care and Use Committee of National Cheng Kung University Hospital.

**Mice**

Experimental mice were F1 littermates from the mating of Pparγ−/− mice on a C57BL/6J background with Pparγ+/− mice on a 129S6 background. Two- to 3-month-old male Pparγ−/−, Pparγ+/−, and Pparγ+/+ littermates were used in all experiments. The following groups were studied: (1) no Ang II infusion in Pparγ−/−, Pparγ+/−, and Pparγ+/+ mice; (2) Ang II (500 ng/kg per minute) infusion in Pparγ−/−, Pparγ+/−, and Pparγ+/+ mice; (3) Ang II (1000 ng/kg per minute) infusion in Pparγ−/−, Pparγ+/−, and Pparγ+/+ mice; and (4) Ang II (1000 ng/kg per minute) infusion+doxycycline (30 mg/kg per day) administration in Pparγ−/− mice. All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of National Cheng Kung University.

**Morphological Analysis of Aorta**

The elastic fiber network was evaluated in the paraffin-embedded aortic section (5 μm) with Verhoef stain (Sigma-Aldrich). Thickness of elastic lamella was measured by ImageJ software (National Institutes of Health), and the average was based on ≥24 randomly selected elastic lamellae from 6 different cross-sectional fields per aorta. Waviness of elastic lamellae was graded on a scale from 1 to 5 in 8 to 10 non-overlapping fields of medial section: 1 (<20% of the waviness of a normal lamella), 2 (20%–40% waviness), 3 (40%–60% waviness), 4 (60%–80% waviness), and 5 (>80% waviness); and the average of waviness score was presented.

**Severity Grading of Aneurysm**

The severity grading of mouse AAA was adapted according to the classification by Daugherty et al. We defined our severity grading of AAA by the pronounced form and its covered area of the aorta. Severity of aneurysm was graded on a scale from 0 to 4: 0 (no dilation in suprarenal aorta), 1 (dilation only in suprarenal aorta), 2 (dilation of suprarenal aneurysm that contains the root of CA and SMA), 3 (pronounced dilated aneurysm from suprarenal abdominal aorta [AA] to SMA), and 4 (rupture of aorta or death).

**Data Analysis**

Values are reported as mean±SEM. Statistical analyses were con- ducted by Student t test, 1-way ANOVA followed by Tukey multiple comparison test, or 2-way ANOVA with treatment and genotype as factors. Differences were considered to be statistically significant at P<0.05.

**Results**

**Upregulation of PPARγ in Both Human and Mouse AAA**

To evaluate the involvement of PPARγ in AAA, we first deter- mined the expression of PPARγ in human AAA by immu- nohistochemistry. Normal human aortic samples scarcely expressed PPARγ (Figure 1A and 1B). Some PPARγ-positive cells with a high nuclear–cytoplasmic ratio were present in the adventitia (Figure 1C). In human AAA sections, the intima was thickened with typical atherosclerotic changes, whereas media was significantly thinned. PPARγ expression was marked- edly increased within the atheroma under the fibrous caps as well as deeper near the media (Figure 1D). Within the media of aneurysm, PPARγ was increased in elongated cells with tapering ends, the characteristics of VSMCs (Figure 1E). Within the adventitia of aneurysm, PPARγ was expressed markedly in cells with a high nuclear–cytoplasmic ratio with the charac- teristics of inflammatory cells (black arrows, Figure 1F). The bulk of inflammatory cells with high nuclear–cytoplasmic ratio in AAA have been reported as CD3+ T lymphocytes and CD19+ B lymphocytes. Consistently, only a small portion of inflammatory cells was positive for CD68 staining (Figure S2). PPARγ was also expressed in endothelial cells associated with vasa vasorum, and occasionally in spindle-shaped cells indicative of fibroblasts (white arrowheads, Figure 1F).

PPARγ expression in the normal AA of wild-type (WT) mice was sparsely detected in VSMCs (Figure 1G through 1I). Although AAA occurs rarely in WT mice even after infusion of Ang II at 1000 ng/kg per minute for 4 weeks, we observed in one of the treated mice a large bulge of upper suprarenal aorta with a dissecting AAA histology (Figure S3) similar to
those documented previously. PPARγ expression was markedly elevated compared with the intact vessels particularly in which the tunica media is torn and bordered by the organizing intramural hematoma (Figure 1J and 1K), as well as in the adventitia (Figure 1L). Consistent with that in human AAA, PPARγ was mainly expressed in inflammatory cells with a high nuclear–cytoplasmic ratio in the luminal surface of vessels and in adventitia. PPARγ was also expressed in cells with a spindle shape present in relatively intact medial layers (Figure 1K) and in adventitia (Figure 1L), respectively, representing VSMCs and fibroblasts. These results indicate PPARγ is upregulated in all vascular cell types in atherosclerosis-associated AAA in humans as well as in Ang II–induced AAA in mice.

Increased Elastic Fiber Fragmentation in PpargC−/− Aorta

To examine the functional significance of PPARγ upregulation in AAA development, we took advantage of mice with genetic reduction in the Pparg gene expression down to 25% normal (PpargC−/−) generated by crossing mice having a PPARγ deletion allele (Pparg−/−) with mice bearing an allele of c-fos AU-rich element sequence inserted into the PPARγ 3′-untranslated region (PpargC−/−).14 mRNA and protein levels of PPARγ in the descending aorta of PpargC−/− mice were decreased to 40% and 29% of the WT levels, respectively (Figure S4A; Figure 2A); and the reduction was confirmed by the immunohistochemical staining (Figure S4B). In addition, the ratio to PPARγ level of Ser82 phosphorylation, which is known to inhibit its transactivation, was higher in PpargC−/− aorta (Figure S5), suggesting that PPARγ activity may be even repressed in PpargC−/− aorta. PpargC−/− mice have normal plasma cholesterol levels, but, consistent with our previous findings, they are hypertensive showing higher systolic blood pressure (BP) in both light and dark cycles during the telemetric monitoring and by a tail-cuff method (Figure 2B and 2C). No gross abnormality in the vascular dimensions was present in PpargC−/− mice (Table S1; Figure S4C). However, under light microscopic examination, occasional breaks of medial elastic fibers were noted in the closer look of the aortas of PpargC−/− mice (Figure 2D). Expression of genes coding for elastin-degradation enzymes MMP-9 and cathepsin S (Ctss) was increased in PpargC−/− aorta (Figure 2E). MMP-9, but not cathepsin S, protein level was elevated in PpargC−/− aorta, particularly in the medial layer near luminal side (Figure 2A and 2F). We also found a significantly increased expression of TNF-α and F4/80 (Adgre1) and a trend toward increased expression of IL-1β in PpargC−/− aorta (Figure 2G). These results suggest that the elastic fiber fragmentation in PpargC−/− aorta is associated with increased elastolytic enzymes and inflammation in the aorta.

Reduced Production of Elastic Fiber Components in PpargC−/− Aorta

Defective elastic fiber formation can also lead to its fragmentation. We therefore examined elastic fiber components by
immunoblotting in the soluble fraction of aortic lysate, which reveals free monomers before assembly into mature fibers. In PpargC/− aorta, tropoelastin level was tended to be less and fibulin-5 level was significantly reduced (Figure 2H). The levels of collagen type I and fibulin-4 were not different between genotypes. To determine the insoluble fraction of aortic lysate, which directly reflects the functional scaffold of mature fibers, we applied liquid chromatography/mass spectrometer–based label-free quantitative proteomic analysis. Among 376 identified proteins from insoluble pellets of aortic lysates, 37 proteins were above the identity threshold (Table S2). Many of these proteins are vital for extracellular matrix organization and elastic fiber assembly in these 37 proteins (Table). Interestingly, levels of fibulin-5 (0.61), elastin (0.82), fibrillin-1 (0.84), and lysyl oxidase homolog 1 (0.87) were decreased, whereas the levels of collagen α-1(I) (1.00), α-2(I) (1.09), and tubulin α-1A (1.00) were not altered in PpargC/− aorta.

At mRNA levels, Pparγ−/− aortas have significantly reduced expression of Eln, Fbn4, Fbn5, and a trend toward decreased Fbn1 expression (Figure 2I). In contrast, expression of Eln, Fbn4, Fbn5, and Mmp9 was not significantly changed in Pparγ+/− aorta (Figure S6). Taken together, these results suggest that severe PPARγ hypomorph itself, without advanced stimulations, reduces expression of elastic fiber components, which may also contribute to the structural change of mature elastic fibers.

Synergistic Effect of Ang II and PPARγ Deficiency on Elastic Fiber Disruption

To test whether Pparγ level may mark the aorta for its susceptibility to exogenous insult-induced injuries, we infused Ang II into mice at a moderate dose of 500 ng/kg per minute for 4 weeks. Ang II infusion significantly increased systolic BP in both WT and PpargC/− mice by ≈20 mm Hg (P<0.001 by 2-way ANOVA, Figure 3A; Figure S7) compared with untreated mice measured concurrently (Figure 2B). PPARγ genotype had a significant effect on elevation of systolic BP (P<0.01) without the interaction between Ang II and genotype. All Ang II–infused mice were fed a cholesterol-enriched western diet, but neither atherosclerotic lesions nor aneurysms developed in any of these mice. Elastin staining of Pparg+/+ thoracic aorta (TA) and AA showed organized, wavy and consistently thickened

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**Figure 2.** Elastic fiber fragmentation and expression of elastic fiber components in Pparγ−/− aorta. A, Immunoblot of peroxisome proliferator–activated receptor γ (PPARγ), cathepsin S (CTSS), and matrix metalloproteinase 9 (MMP-9) in the aorta (n=3 in each group). The relative intensities of the bands are indicated by densitometric quantification with wild-type. B, Telemetry and (C) tail-cuff systolic blood pressure (SBP) measurement in Pparγ+/+ and Pparγ−/− mice; n=5 (B) and 6 (C). D, Representative images of the elastic network and quantification of elastic fiber breaks in the longitudinal section of aorta. Arrows indicate breaks in the elastic fiber. Numbers of breaks of elastic layers per 10,000 μm² are shown in the right (n=5 in each group). E, mRNA levels of elastolytic enzymes in Pparγ−/− aorta are shown relative to the mean levels in Pparγ+/+ aorta as 1.0 (+/+ =7 and C/−=6). F, Immunohistochemical staining of MMP-9 in the longitudinal section of aorta. G, mRNA levels of inflammatory cytokines and macrophage markers relative to the mean levels in Pparγ−/− aorta as 1.0 (+/+ =20 and C/−=16). H, Immunoblot and quantification of extracellular matrix (ECM) components in the soluble fraction of aortic lysates (n=3 in each group). I, mRNA levels of ECM components (n=12–14 in each group). *P<0.05, **P<0.01, and ***P<0.001. Scale bars in D are 100 μm and in F are 50 μm. Lu indicates lumen.
elastin lamella arrangement. Although Pparg<sup>-/-</sup> TA and AA maintained relatively intact fiber structures, elastic lamellae in both of them became thinner, flattened, and lost the wavy feature (Figure 3B and 3C). Flattened elastic lamellae were also observed in Pparg<sup>-/-</sup> TA and AA. In addition, although the integrity of elastic lamellae was preserved in Pparg<sup>-/-</sup> TA, elastic lamellae in Pparg<sup>-/-</sup> AA exhibited severe destruction with extensive inconsistency in thickness and fragmentation into short, thin, and fragile segments. Quantitation revealed a marked reduction of elastic fiber consistency and an increase of breaks in the AA of Pparg<sup>-/-</sup> mice, but the fibers remained normal in Pparg<sup>-/-</sup> mice (Figure 3C; Table S3). Thus, our data suggest that 50% reduction in the PPARγ expression can still maintain the consistency of elastic fiber in response to a moderate dose of Ang II, but further reduction will affect structural integrity.

At the molecular level, we found that PPARγ deficiency in combination with Ang II infusion upregulated expressions of aortic TNF-α, IL-1β, monocyte chemoattractant protein-1 (Ccl2), and F4/80 (Adgre1; Figure 3D), as well as a series of elastin-degradation enzymes, including MMP-7, MMP-9, and cathepsin S (Ctss; Figure 3E). Gelatin zymography confirmed elevated MMP-9, but normal MMP-2, activity in the AA of Ang II–infused Pparg<sup>-/-</sup> mice (Figure 3F). However, MMP-9 activity remained comparable in the TA of Pparg<sup>-/-</sup> mice. Thus, reduction of PPARγ levels renders the aorta susceptible to Ang II–induced inflammation and elastic fiber disruption. In Ang II–induced mouse AAA, oxidative stress is critical in the regulation of pathogenic events. Accordingly, we examined expression of pro-oxidant (Cyba, Ncf1, and Ncf2) and antioxidant enzymes (Sod1 and Cat) in Pparg<sup>-/-</sup> and Pparg<sup>-/-</sup> aorta (Figure S8). Analysis of results with 2-way ANOVA showed that Ang II (500 ng/kg per minute) has significant effects on expression of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase components (Cyba, Ncf1, and Ncf2) and superoxide dismutase 1 (Sod1). However, no PPARγ genotype effect was found in the expression of Cyba, Ncf1, Ncf2, Sod1, and Cat (catalase). These results suggest that although oxidative stress is critical in Ang II–induced AAA, accelerated development of AAA seen in Pparg<sup>-/-</sup> mice is independent of oxidative stress.

**Atypical AAA With Severe SMA Dilation in High-Dose Ang II–Infused Pparg<sup>-/-</sup> Mice**

A higher dose of Ang II (1000 ng/kg per minute) infusion for 4 weeks is commonly used for aneurysm induction in rodents. At this dose, BPs of mice increased further as summarized in Figure S7. After 4 weeks of infusion, 1 of 11 Pparg<sup>+/+</sup> mice developed bulbous suprarenal aneurysm in the left side above the celiac artery branch (Figure S3). In a marked contrast, 8 of 11 (73%) Pparg<sup>-/-</sup> mice developed dilated suprarenal aneurysms (Figure 4A–4C). The aneurysms in Pparg<sup>-/-</sup> mice were, however, atypical and the enlargement was toward the ventral side and restricted to a narrow region involving roots of CA and SMA. Such profound SMA dilatation was not present in the aorta of Pparg<sup>-/-</sup> mice, and only 1 of 5 Pparg<sup>-/-</sup> mice developed similar but smaller suprarenal aneurysms compared with Pparg<sup>-/-</sup> mice. We monitored the time course of AAA in vivo by ultrasound on the mice 2 and 3 weeks post Ang II pump (1000 ng/kg per minute) implantation. The enlargement of the suprarenal aortic lumen of Pparg<sup>-/-</sup> mice was observed at 2 weeks (33%) and continuously increased at 3 weeks (66%). Suprarenal aortic lumen diameter was enlarged to 1.64±0.29 mm in Pparg<sup>-/-</sup> mice compared with 1.12±0.05 mm in Pparg<sup>-/-</sup> mice at the 3-week time point (Figure S9).

Histologically, upper portion of the aneurysm (Figure 4D) illustrates the dilatation at the branching point of CA in which elastic lamellae of the artery were severely destroyed, although their reminiscence clearly marks the continuity. Intimal thickening was characterized by the presence of both VSMCs and inflammatory cells, whereas adventitial hyperplasia was marked with extensive collagen deposition and inflammatory cells (Figure 4D). Similar abnormalities were observed near the ostium of SMA (Figure 4E). Neither signs of truncation of tunica media nor intramural hematoma, such as illustrated in the AAA of WT mice in Figure S3, were detected in any of the aneurysms of Pparg<sup>-/-</sup> mice.

Both the arterial lumen size and wall thickness of Pparg<sup>-/-</sup>-SMA were grossly increased (Figure S10A and S10B). Destruction of medial elastic lamellae (Elastin stain) and compensatory deposition of collagen (picrosirius red)
were found in the hypertrophic adventitia of \textit{Pparg} C/− SMA (Figure S10C). Immunohistochemical staining showed that the smooth muscle density was decreased in the medial layer. Interestingly, the dilated adventitial region contained predominantly fibroblasts (fibroblast activation protein [FAP]; Figure S10D), and showed a dramatic increase of Ki-67–positive signal (Figure S11), suggesting that these FAP-positive cells are highly proliferative. In addition, extensive macrophage infiltration (CD169) was evident in the outer layer of adventitia (Figure S10D). MMP-9 level was dramatically augmented in the medial to adventitial layer of \textit{Pparg} C/− SMA.

**No Change of Aneurysm Incidence by MMP Inhibition**

To examine whether MMP activity is crucial for the onset of \textit{Pparg} C/− AAA, a broad-spectrum MMP inhibitor doxycycline was administrated in Ang II–infused \textit{Pparg} C/− mice. Doxycycline (30 mg/kg per day) suppressed aortic MMP-9 activity in \textit{Pparg} C/− mice (Figure 5A). However, although doxycycline treatment attenuated SMA dilation and the diameter of aneurysmal dilatations in \textit{Pparg} C/− mice (Figure 5B and 5C), it did not reduce the severity and incidence of aneurysm (Figure 5D and 5E; \(P=0.65\) for incidence with \(\chi^2\) test, \(P=0.53\) even after including earlier 8/11). Moreover, doxycycline treatment did not ameliorate profound deterioration and fragile elastic lamellae in the lower AA of \textit{Pparg} C/− mice (Figure 5F), as illustrated by the parameters of AA elastic fiber impairments (Figure 5G). These results imply that the reduction of fiber component synthesis in \textit{Pparg} C/− aorta plays a key role in the initiation of AAA, the pathology of which is significantly enhanced by the increased MMP expression associated with inflammation.

**PPARγ Antagonism Inhibits Expression of Elastic Fiber Components in Fibroblasts**

In \textit{Pparg} C/− aorta, expression of both \textit{Eln} and \textit{Fbln5} was reduced even without Ang II infusion (Figure 2I). Because inflammation has been suggested to affect elastin expression,\(^{21}\) we next asked whether PPARγ antagonism or TNF-α elevation inhibits the expression of \textit{Eln} and \textit{Fbln5} in aortic smooth muscle cells and in fibroblasts. Treatments of rat and human aortic smooth muscle cells with neither GW9662 nor TNF-α altered the expression of \textit{Eln} and \textit{Fbln5} (Figure 6A; Figure S12). In a marked contrast, GW9662 significantly suppressed expression of \textit{Eln} and \textit{Fbln5} in mouse embryonic fibroblasts (MEFs; Figure 6B). TNF-α significantly suppressed \textit{Eln} expression and tended to decrease \textit{Fbln5} expression. PPARγ knockdown in MEFs reduced expression of both \textit{Eln} and \textit{Fbln5} (Figure 6C), indicating that low expression of PPARγ is sufficient to affect the expression of these genes in MEFs. Although PPARγ knockdown in human aortic adventitial fibroblasts significantly reduced expression of \textit{FBLN5}, it did not alter \textit{ELN} (Figure 6D). These results suggest that PPARγ also has a regulatory role in the expression of fibrillin-5 in human aortic adventitial fibroblasts.

Searching for previously reported chromatin immunoprecipitation–sequencing data,\(^ {22}\) we found 3 potential PPARγ-binding sites in \textit{Fbln5}, but none in \textit{Eln}, \textit{Fbln4}, and \textit{Fbn1}. 

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**Figure 3.** Effects of moderate-dose angiotensin II (Ang II, 500 ng/kg per minute) infusion. A, Tail-cuff systolic blood pressure (SBP) measurement in Ang II–infused \textit{Pparg}+/+ and \textit{Pparg} C/− mice (n=9 in each group). B, Representative images of the elastic network in the thoracic aorta (TA) and abdominal aorta (AA). Scale bars are 100 μm. C, Parameters of elastic fiber integrity, including thickness, waviness, consistency, and breaks of elastic lamellae, in the AA (+/+ = 5, +/− = 3, and C/− = 7). \(*P<0.05\), **\(P<0.01\), and ***\(P<0.001\) by 1-way ANOVA with Tukey multiple comparison test. D and E, mRNA levels for inflammatory mediators and for elastolytic proteases, respectively, in the aorta. Data are expressed relative to the mean in \textit{Pparg} C/− aorta as 1.0 (n=5 in each group). \(*P<0.05\) and **\(P<0.01\) by Student t test. F, Gelatin zymography of protein lysate from TA and AA.
Chromatin immunoprecipitation–polymerase chain reaction in MEFs showed that PPARγ bound to Fbln5 after treatment of PPARγ agonist rosiglitazone, whereas GW9662 abrogates the binding of PPARγ to Fbln5 (Figure 6E), indicating that Fbln5 is the direct target of PPARγ. No interaction between PPARγ and Eln was identified.

Finally, expression of Mmp9 in rat aortic smooth muscle cells was induced 2- to 3-fold by GW9662 alone, and 2-fold by TNF-α (Figure 6F; P<0.001 for GW9662 and P<0.01 for TNF-α by 2-way ANOVA). In contrast, expression of Mmp9 in MEFs was induced 20-fold by TNF-α, but not affected by GW9662 (Figure 6G). Taken together, these results demonstrate a direct regulatory role of PPARγ in the expression of elastic fiber components, which takes place mainly in fibroblasts. Inflammation further contributes to elastic fiber deterioration in PpargC−/− aorta via the induction of Mmp9 in the vascular cells.

Discussion

Our study demonstrated the dosage effect of PPARγ on the elastic fiber integrity and that adequate PPARγ level is vital for production of elastic fiber components. Qualitatively, healthy elastic lamellae show wavy, curl features, and consistency in thickness, whereas poor ones become flattened and irregularly thickened. We observed a significant loss of waviness and a decrease of fiber thickness in moderate-dose Ang II–infused Pparg+/− aortas, despite they retained structural integrity without fiber fragmentation. PPARγ expression down to 25% further induces thickness inconsistency and fiber breaks in PpargC−/− aortas. Thus, a critical amount of PPARγ for maintaining normal elastic waviness is >50%, whereas the threshold level for structure deterioration is between 25% and 50%. These findings suggest that loss of elastic fiber waviness is a hallmark of early elastic fiber defect and likely attributable to PPARγ insufficiency.

Our liquid chromatography/mass spectrometer–based label-free quantitative proteomic analysis of mature elastic fiber components in the insoluble extracellular matrix fraction showed that fibulin-5, elastin, fibrillin-1 and lysyl oxidase homolog 1, all vital components for elastic fiber assembly, are downregulated in PpargC−/− aorta. During elastic fiber
assembly, elastin is transported to extracellular space, interacts with fibulin-5, and binds to fibrillin-1–formed microfibril scaffold. Lysyl oxidase and its gene family, including lysyl oxidase homolog 1, catalyzes cross-linking of elastin core and microfibril scaffold, forming the functional fiber unit.4,23 Both deficiencies in fibulin-5 and fibrillin-1 cause significant fragmentation and disorganization of elastic fibers, whereas lysyl oxidase homolog 1 deficiency leads to imprecise and lower cross-linking of elastic fibers.24 Thus, the reduction of these elastic fiber components suggests a direct regulatory role of PPARγ in elastic fiber production and assembly.

Infusion of Ang II raises BP in mice and a high-dose treatment provides an established model of AAA by hypertension.25 Independently, a decreased PPARγ expression in mice results in increased BP,18 and Pparg C/− mice consistently have 7 to 18 mm Hg higher basal as well as both moderate and high-dose Ang II–infused BP than WT mice. Thus, increased aortic wall stress likely contributes to higher AAA incidence of Pparg C/− mice treated with a high-dose Ang II. However, high-BP may not account for all the aspects of AAA development because Pparg C/− mice exhibit loss of elastic fiber integrity even without Ang II stimulation. Pparg C/− mice are more prone to develop aneurysms likely because of a combination of increased BP and weak elastic tissues. Supporting this, Kanematsu et al17,26 have previously shown that cotreatment of Ang II and β-aminopropionitrile, a lysyl oxidase inhibitor, markedly increased AAA incidence in mice, suggesting that induced degeneration of elastic lamellae enhances the aortic aneurysm incidence caused by hypertension.

The morphological features of the Ang II–induced AAA in Pparg C/− mice, however, differ in some specific manners from those typically described on AAA in rodents including those described by Kanematsu et al.17,26 First, no medial rupture was present in Pparg C/− aorta, despite increased fragmentation of elastic lamellae. Perhaps enhanced fibroblast proliferation and collagen synthesis in the adventitia acts to protect the wall against rupture dampening the stress in the media. Second, aortic dilatation in Pparg C/− mice seems to be initiated near the branching of CA and SMA, and involves the severe dilatation and adventitial fibrosis of these arteries. The turbulence of blood flow is likely to be high at this location because CA and SMA are the major branches of AA. Another consideration is that VSMCs of CA and SMA are mesothelium origin,27 whereas aortic VSMCs are dorsal somite origin.28 Being composed of 2 different types of embryonic cells may render the ostia more susceptible to injury. In addition, PPARγ dependency in the cells of 2 different origins may also differ. Finally, a recent report by Davis et al29 addressed Ang II–induced SMA aneurysm in mice lacking low-density lipoprotein receptor–related protein 1 (LRP1), specifically in smooth muscle cell–specific LRP1−/−. These mice showed dilated SMA with severe adventitial and intimal thickenings similar to what we observed in Pparg C/− mice. The difference was that the dilatation in smooth muscle cell–specific LRP1−/− mice seems to be restricted to SMA, whereas the dilatation in Pparg C/− mice also involves CA and aorta. We note that there is a potential LRP1/PPARγ interaction,30 and the LRP1 gene has a PPARγ-binding site.31

Immediate backup by de novo generation of components for fiber production and assembly is a critical process
in forming a functional fiber unit during AAA development. However, overwhelming degradation and massive destruction of elastic fiber can mask the underlying defects of elastic fiber production. For instance, the upregulation of elastolytic MMP-9 and cathepsin S was profound in Ang II–infused PpargC/− aortas. Doxycycline administration before AAA induction has been shown to reduce the incidence and dilation of AAA efficiently through inhibiting MMP in elastase-induced WT or hypercholesterolemic Ang II–infused mice.32,33 However, doxycycline did not regress or prevent the progression of established Ang II–induced AAAs.34 Our study showed that doxycycline pretreatment attenuated aneurysmal dilation, but it did not reduce the incidence of AAA in PpargC/− mice. Importantly, doxycycline did not ameliorate profound deterioration of elastic lamellae in PpargC/− AA. Thus, attenuation of AAA incidence in the aorta by doxycycline seems to require intact elastogenesis machinery, whereas it is not effective in the elastogenesis-defective aorta. Although proteases other than MMPs mediating this profound destruction cannot be excluded, the negative effect of doxycycline on PpargC/− AAA is likely because of pre-exited defects in elastic fiber integrity and elastogenesis. The controversial effects of doxycycline in attenuating AAA growth in clinical studies35,36 could also be related to the individual variations in elastogenesis machinery.

The role of VSMCs in vascular wall integrity and the significance of VSMC loss in AAA development have been well documented.37 A loss of PPARγ in VSMCs has been shown to promote aortic dilatation and elastin degradation in CaCl2-induced AAA, indicating a critical role of PPARγ in attenuation of inflammation and elastic fiber degradation in VSMCs during AAA. In contrast, fibroblast, another actively participant in the structural remodeling of AAA, has not gained much attention to date. Despite that the lack of cell selectivity in PpargC/− model is a limitation in dissecting the cell-specific role of PPARγ level, our current results strongly suggest an important role of fibroblast PPARγ in AAA development. For example, we found that one of the predominant cell types in both human and mouse AAA lesions expressing high-level of PPARγ had fibroblast-like features. In addition, a fibroblast marker, FAP, was substantially elevated in the dilated adventitia of PpargC/− aneurysm. FAP is expressed by activated (highly proliferative) fibroblasts in epithelial tumor stroma, arthritis, and wound healing.38 Although we cannot determine the origin of these FAP-positive cells, a dramatic increase of Ki-67–positive signal in the dilated adventitia containing predominantly FAP-positive cells at least suggest that they are highly proliferative (Figure S11). Taken together, our observations that PpargC/− fibroblasts are actively contributing to
collagen deposition and enlargement of aneurysm adventitia suggest that the regulatory role of PPARγ in fibroblasts has a direct impact on the overall vessel integrity in AAA.

In our in vitro study showed that PPARγ inhibition in MEFs, but not in VSMCs, dramatically attenuated expression of Eln and Fbln5. Chromatin immunoprecipitation analysis in MEFs further confirmed a direct interaction between PPARγ and Fbln5. This PPARγ-interacting region is located in the introns of Fbln5, and a similar indirect regulation by PPARγ has been reported.99 FBLN5 expression was also reduced in the PPARγ-knockdowned human aortic adventitial fibroblasts, albeit to a lesser degree. It is worth noting that the effects of PPARγ knockdown on expression of Eln differ between MEFs and human aortic adventitial fibroblasts. Although species variation cannot be ignored, the response of embryonic fibroblasts may differ from that of adult primary vascular fibroblasts. Elastogenesis is restricted to a short period of embryonic and neonatal stages, and by the postnatal day 14, right after the deposition of elastin and assembly into extracellular fibers, the synthesis and production of elastic fibers are shut down rapidly. However, in the pathological condition, such as AAA, adventitial fibroblasts encounter numerous stimulants, such as Ang II and inflammatory cytokines. These stimulated fibroblasts may have a distinct regulatory program on elastic fiber components from fully differentiated, quiescent cells. Because we did not detect a direct interaction between PPARγ and Eln, the downregulation of Eln by PPARγ antagonism in MEF may be mediated through an indirect mechanism. Although the detailed regulation of Fbln5 and Eln expression by PPARγ requires further investigation, PPARγ may be considered as a potential target for regulating elastogenesis in AAA therapy.

**Perspectives**

Our study of mice with varying Pparg expression indicates that quantitative variants causing decreased Pparg expression are a risk factor for AAA. Without advanced stimulations, >25% of normal PPARγ level is necessary to maintain elastic fiber components in the aorta and fibroblasts are vital for this action. Thus, this study highlights the importance of adequate PPARγ level in AAA treatment through orchestrating proper elastogenesis and preserving elastic fiber integrity. Importantly, new pharmacological interventions targeting PPARγ should be revisited in AAA therapy.

**Acknowledgments**

We thank Dr Shaw-Jenq Tsai at Department of Physiology of National Cheng Kung University for critical suggestions and Yu-Tzu Chang for technical assistance.

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**Disclosures**

None.

**References**

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Peroxisome Proliferator–Activated Receptor γ Level Contributes to Structural Integrity and Component Production of Elastic Fibers in the Aorta

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Supplemental Methods

Mice

Generation of mice carrying the modified \textit{Pparg} locus has been described.\textsuperscript{1} \textit{Pparg}^{C/C}, \textit{Pparg}^{C/+}, and \textit{Pparg}^{+/+} mice were littermates of F2 generation from a cross between 129S6 and C57BL/6J F1 heterozygotes. The \textit{Pparg}-C allele was subsequently placed on a C57BL/6J background by backcrossing for eight generations. \textit{Pparg}^{+/+}, \textit{Pparg}^{-/-} and \textit{Pparg}^{C/-} mice were F1 littermates from the mating of \textit{Pparg}^{C/+} mice on a C57BL/6J background with \textit{Pparg}^{+/+} mice on a 129S6 background (kindly provided by Dr. Ronald Evans at the Salk Institute).\textsuperscript{2}

For Ang II infusion, anesthetized mice received subcutaneous implantation of ALZET osmotic minipumps (1004; DURECT Corporation), delivering angiotensin II (A9525; Sigma-Aldrich) at 500 or 1000 ng/kg per minute for 4 weeks. All Ang II-infused mice were fed a western diet (D12079B; Research Diets). Doxycycline hyclate (D9891; Sigma-Aldrich) was administrated in drinking water, covered with aluminum foil, at a dose of 30 mg/kg per day and prepared fresh every other day. Mice were anesthetized by intraperitoneal injection of zoletil-Rompun mixture (1 ml Zoletil\textsuperscript{®} (50mg/ml) + 0.1 ml Rompun\textsuperscript{®} + 3.9 ml normal saline) \cdot 0.1 ml mixture per 20 g mouse body weight. Mice were housed in a specific pathogen-free barrier facility with the humidity and temperature controlled. All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of National Cheng Kung University.

Measurements of Blood Pressure

Blood pressure was measured in conscious mice by using the tail cuff blood pressure measurement system (BP-2000; Visitech Systems). The mice were training for 2 days. Blood pressures were measured 20 times per day for 3 consecutive days. The results were calculated as the average from three trials of five to ten measurements each day for 3 consecutive days. For continuous BP recording, a telemetric device (PA-C20; Data Sciences International) was implanted via the left carotid artery into the aorta. Continuous BP results were recorded every 5 minutes for 14 days beginning 10 days after surgery to allow mice to recover normal diurnal rhythms.

Immunohistochemical Staining

Paraffin-embedded sections (5 \textmu m) were deparaffinized and rehydrated. Antigen retrieval was conducted by boiling sections in 10 mmol/L sodium citrate buffer at pH 6.0 for 10 min. Sections were incubated with primary antibodies against PPAR\textgamma\ (sc-7196; Santa Cruz Biotechnology), MMP-9 (AB19016; Millipore), SMactin (A2547; Sigma-Aldrich), FAP (ab53066; Abcam), MOMA-1 (GTX42355; GeneTex), CD68 (M081401; Dako) and Ki-67 (ab15580; Abcam). Secondary antibody staining (VECTASTAIN ABC kit; Vector
Laboratories) was performed. Slides were developed using 3, 3′-diaminobenzidine substrate-chromogen solution (K3468; Dako) and counterstained with hematoxylin. The specificity of PPARγ antibody was confirmed by negative control procedures, including no primary antibody control, primary antibody isotype control, and no antigen retrieval, which gave consistently negative results (Figure S1).

**Consistency of Elastic Lamella**

Consistency of elastic lamella was determined by the ratio of the length of elastic fibers with constant thickness to the length of the medial area section. The number of elastin breaks was counted and divided by the medial area.

**RNA Analysis**

Aortic tissues were stored in RNAlater (Ambion). Total RNA was extracted with REzol (Protech Technology Enterprise). Level of mRNA was analyzed with SYBR green-based real-time quantitative RT-PCR assays (StepOne; Applied Biosystems), with β-actin as the reference gene in each reaction. Sequences of the primers used for real-time PCR assays are shown in Table S5.

**Immunoblot Analysis**

Twenty micrograms of total proteins were subjected to electrophoresis, transferred to PVDF membranes, and probed with antibodies against tropoelastin (CL55041AP; Cedarlane), collagen type I (234167; Calbiochem), fibulin-4 (36475; Epitomics), fibulin-5 (12188-1-AP; Protein Tech), MMP-9 (AB19016; Millipore), PPARγ (2443; Cell signaling), PPARγ P-Ser112 (ab60953; Abcam), Cathepsin S (ab18822; Abcam) and α-tubulin (T5168; Sigma-Aldrich). Immunoreactive detection was performed with a chemiluminescent detection system (RPN2106; GE Healthcare).

**Proteomics Analysis**

The insoluble pellets of homogenized aortas (100 µg for each aorta) were used for in-solution trypsin digestion. The LC-MS/MS (HCT ultra ion trap MS; Bruker) was performed with a HPLC system (UltiMate 3000; Dionex). The sample was injected into a trap column (Acclaim PepMap100, 164199; Dionex). The trapped analyses were separated by an analytical column (Acclaim PepMap100, 164261; Dionex). Three MS only and 1 autoMSMS runs were performed for each sample. The LC-MS/MS spectra were analyzed for peptide identification by Swissport (release 56.1) database with the search engine MASCOT (version 2.2.07). The relative protein abundance based on a label free quantitative analysis was expressed as the ratio of $Pparg^{Ccr}$ to $Pparg^{+/+}$ by ProteinScape 3.0 (Bruker Daltonics).
**Gelatin Zymography**

Aortic lysates were subjected to electrophoresis on 8% SDS-PAGE co-polymerized with 1% gelatin as the substrate. The gel was incubated at room temperature for 1 h in renaturing buffer, and incubated at 37°C for 24 h in developing buffer. The gels were stained with 0.05% Coomassie Brilliant Blue, and then destained with destain buffer.

**Ultrasound Imaging**

Ultrasound imaging was performed using a high-resolution ultrasound imaging system (Vevo 770; VisualSonics) with 40 MHz frequency real-time microvisualization scanhead (RMV 704). Mice were placed in a supine position on a heating pad under isoflurane anesthesia and depilated with hair removal cream. The 2D imaging in B-mode was used to localize the suprarenal abdominal aorta and the cross-sectional view was recorded to measure the diameter of aortic lumen.

**Cell Culture**

Human aortic smooth muscle cells (HASMCs, C-007-5C; Life Technologies) and human aortic adventitial fibroblasts (HAoAFs, CC-7014; Lonza) were derived from thoracic aorta. The culture protocols of human cells were followed the manufacturer’s instructions. Rat aortic smooth muscle cells (ASMCs) and mouse embryonic fibroblasts (MEFs) were grown in DMEM containing penicillin/streptomycin supplemented with 10% FBS. Cells were treated with various stimuli for 24 hours and collected for mRNA analysis. For small interfering RNA (siRNA) knockdown, a final concentration of 40 nmol/L siRNA against PPARγ (s72013; Ambion) and scrambled control siRNA were transfected into MEFs by Lipofectamine 3000 (Life Technologies) and incubated for 3 days.

**Chromatin Immunoprecipitation (ChIP) Assay**

The procedure for ChIP was described previously.\(^3\) In brief, the interaction between protein and DNA was fixed by using 1% formaldehyde for 10 minutes. Cells were harvested and sonicated to fragment DNA (average size of 200–500 bp). PPARγ antibody (ab41928; Abcam) was used to immunoprecipitate the PPARγ protein and DNA complexes. Potential PPARγ binding sites were amplified by specific primers (Table S5) after reversing cross-linking.
References


Table S1. Aortic geometry of Pparg<sup>+/+</sup> and Pparg<sup>C/-</sup> mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>+/+ (n=5)</th>
<th>C/- (n=5)</th>
<th>P-value</th>
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<td>Media thickness (μm)</td>
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<td>41.53 ± 3.71</td>
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<tr>
<td>Lumen CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.34 ± 0.05</td>
<td>0.33 ± 0.06</td>
<td>0.89</td>
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<tr>
<td>Media CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
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<tr>
<td>Lumen / media ratio</td>
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<td>3.54 ± 0.52</td>
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<td>Perimeter (mm)</td>
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<td>2.43 ± 0.14</td>
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</table>

CSA: cross-section area. P-value is resulted from Student’s t test.
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<tr>
<th>Protein description</th>
<th>Ratio (C- : +/+)</th>
<th>Quantifiable peptides</th>
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<td>Creatine kinase B-type</td>
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<td>3</td>
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Table S2. Protein identified by a LC/MS-based label-free quantitative proteomic analysis in the insoluble fraction of aortic lysate.
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Identified peptides: number of peptides with MASCOT individual ion score of >20. Quantifiable peptides: number of Identified peptides with quantitation ratio (C/- versus +/+). N=5 in each group.
Table S3. Aortic geometry of the mice infused with 500 ng/kg per minute of Ang II and fed with a western diet

<table>
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<tr>
<th>Parameters</th>
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<th>+/- (n=3)</th>
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<tr>
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<td>33.13 ± 3.24</td>
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<tr>
<td></td>
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<td>32.33 ± 1.94</td>
<td>32.48 ± 1.55</td>
<td>0.89</td>
</tr>
<tr>
<td>Elastic fiber content (arbitrary unit)</td>
<td>TA 1.67 ± 0.24</td>
<td>1.67 ± 0.17</td>
<td>1.36 ± 0.11</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>AA 1.48 ± 0.30</td>
<td>0.79 ± 0.06</td>
<td>1.14 ± 0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>Mean distance between elastic lamellae</td>
<td>TA 6.75 ± 0.21</td>
<td>6.25 ± 0.58</td>
<td>7.25 ± 0.48</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>AA 7.40 ± 0.23</td>
<td>7.49 ± 0.68</td>
<td>7.26 ± 0.40</td>
<td>0.93</td>
</tr>
<tr>
<td>Number of elastic lamellae</td>
<td>TA 5.45 ± 0.20</td>
<td>5.31 ± 0.11</td>
<td>5.12 ± 0.07</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>AA 4.53 ± 0.14</td>
<td>4.38 ± 0.15</td>
<td>4.48 ± 0.20</td>
<td>0.89</td>
</tr>
</tbody>
</table>

TA: thoracic aorta. AA: abdominal aorta. Elastic fiber content was quantified by the ratio of elastic fiber staining area to the medial area. Elastic fiber staining area was selected manually by a specific RGB threshold and quantified by HistoQuest software (TissueGnostics). P-value is resulted from one-way ANOVA analysis between three groups.
Table S4. Aortic geometry of the mice infused with 1000 ng/kg per minute of Ang II and fed with a western diet.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>+/+ (n=3)</th>
<th>C/- (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial thickness (μm)</td>
<td>TA 76.27 ± 16.99</td>
<td>61.78 ± 3.35</td>
</tr>
<tr>
<td></td>
<td>AA 82.98 ± 17.33</td>
<td>55.42 ± 10.65</td>
</tr>
<tr>
<td>Elastic fiber content (arbitrary unit)</td>
<td>TA 0.76 ± 0.07</td>
<td>1.10 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>AA 0.92 ± 0.22</td>
<td>1.04 ± 0.24</td>
</tr>
<tr>
<td>Elastic fiber breaks (Breaks per 10000μm²)</td>
<td>TA 0.56 ± 0.13</td>
<td>1.22 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>AA 1.06 ± 0.40</td>
<td>4.06 ± 1.07 *</td>
</tr>
<tr>
<td>Mean distance between elastic lamellae</td>
<td>TA 13.83 ± 2.73</td>
<td>12.69 ± 0.90</td>
</tr>
<tr>
<td></td>
<td>AA 16.57 ± 1.97</td>
<td>11.73 ± 2.34</td>
</tr>
<tr>
<td>Number of elastic lamellae</td>
<td>TA 5.51 ± 0.13</td>
<td>4.94 ± 0.12 *</td>
</tr>
<tr>
<td></td>
<td>AA 4.92 ± 0.51</td>
<td>4.77 ± 0.88</td>
</tr>
</tbody>
</table>

TA: thoracic aorta. AA: abdominal aorta. *P-value is resulted from Student's t test. *P < 0.05 compared with Pparg +/+ mice.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Amplicon</th>
</tr>
</thead>
</table>
| **Mmp2** | Forward: CCC CGA TGC TGA TAC TGA  
Reverse: CTG TCC GCC AAA TAA ACC | 152 bp |
| **Mmp9** | Forward: CCT GGA ACT CAC ACG ACA TCT TC  
Reverse: TGG AAA CTC ACA CGC CAG AA | 82 bp |
| **Mmp7** | Forward: GCA GGC ATT CAG AAG TTATAT G  
Reverse: ACC CAT CCA CAG CAC AAG | 168 bp |
| **Mmp12** | Forward: CCC ACT TCG CCA AAA GGT TT  
Reverse: CAT GAG CTC CTG CCT CAC ATC | 73 bp |
| **Ctss** | Forward: GGT TGG CTATGG GAC TCT TG  
Reverse: GCAATT CCG CAG TGA TTT TT | 123 bp |
| **Timp1** | Forward: ATT CAA GGC TGT GGG AAA TG  
Reverse: CTC AGA GTACGC CAG GGAAC | 183 bp |
| **Timp2** | Forward: CAC GCT TAG CAT CAC CCA  
Reverse: TGA CCC AGT CCA TCC AGA G | 134 bp |
| **Tnf** | Forward: CAT CTT CTC AAA ATT CGA GTG ACA A  
Reverse: TGG GAG TAG ACA AGG TAC AAC CC | 175 bp |
| **Il1b** | Forward: GCA ACT GTT CCT GAA CTC AAC T  
Reverse: ATC TTT TGG GGT CCG TCA AT | 89 bp |
| **Ccl2** | Forward: CCC ACT CAC CTG CTG CTACT  
Reverse: TCT GGA CCC ATT CCT TCT TG | 164 bp |
| **Adgre1** | Forward: CTT TGG CTATGG GCT TCC AGT C  
Reverse: GCAAGG AGG ACA GAG TTT ATC GTG | 165 bp |
| **Eln** | Forward: CTG CCA AAG CTG CCA AAT AC  
Reverse: CTC CAG CTC CAA CAC CAT AG | 99 bp |
| **Fbln4** | Forward: GGG TTATTT GTG TCT GCC TCG  
Reverse: TGG TAG GAG CCA GGAAGG TT | 218 bp |
| **Fbln5** | Forward: TCC AAC TAC CCC ACG ATT TCAAG  
Reverse: GGC AGT AAC CAT AGC GAC ATT C | 229 bp |
| **Fbn1** | Forward: TCA TCG GAG GCT ATAGGT GTA GCT  
Reverse: CAC TCA GGC ACT GTT TTT CAT C | 90 bp |
| **Col1a1** | Forward: TCA GAG GCG AAG GCA ACA GTC  
Reverse: GCA GGC GGG AGG TCT TGG | 120 bp |
<p>| <strong>Col3a1</strong> | Forward: GAC AGA TTC TGG TGC AGA GA  | 107 bp |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lox</strong></td>
<td>GTC ACC AAC ATT ACC ACA GCA</td>
<td>CAT CAA CGA CAT CTT CAG GAA T</td>
</tr>
<tr>
<td><strong>Loxl1</strong></td>
<td>CTA TGC CTG CAC CTC TCA CA</td>
<td>GTA GTT CCC AGG CTG CAC AT</td>
</tr>
<tr>
<td><strong>Eln chip</strong></td>
<td>CCT CTA TGC CCT GTA CTG TAT AAG A</td>
<td>AGG CTT TAA GGA ATG TGA AGT GTA G</td>
</tr>
<tr>
<td><strong>Fbln5 chip</strong></td>
<td>GAT GCA CAA ATA CCC GCC AT</td>
<td>GTA GTT GCC CAC CTC TCA</td>
</tr>
<tr>
<td><strong>Pparg</strong></td>
<td>CAT AAA GTC CTT CCC GCT GA</td>
<td>CAT GTC GTA GAT GAC AAA TGG</td>
</tr>
<tr>
<td><strong>rat Eln</strong></td>
<td>CTG CAT CCA AAG CTG CTAAA</td>
<td>CCT GCT ACT CCA CCA GGA AC</td>
</tr>
<tr>
<td><strong>rat Fbln5</strong></td>
<td>GGA CCA GCC ATT CAC CAT CTT</td>
<td>GTC GTT GCT TGC ATC TGG AA</td>
</tr>
<tr>
<td><strong>rat Mmp9</strong></td>
<td>TGG ATC CCC AGA GCG TTA CT</td>
<td>AAT AGG CCT TGT CTT GGT AGT GAAA</td>
</tr>
<tr>
<td><strong>human PPARG</strong></td>
<td>GAG CCC AAG TTT GAG TTT GC</td>
<td>CAT AAC ATC CAG GAC TCAA ATC C</td>
</tr>
<tr>
<td><strong>human ELN</strong></td>
<td>GGA GGA CTC GGA GTC GGA G</td>
<td>CCA GCA GCA CCG TAT TTAGCT</td>
</tr>
<tr>
<td><strong>human FBLN5</strong></td>
<td>GGA TCA GTG ATAACC GCT GTA TGT</td>
<td>TGT CCC GGT ACAAGA TGG TAAAG</td>
</tr>
</tbody>
</table>
Figure S1. Negative controls of immunohistochemical staining of PPARγ in normal human abdominal aorta (A and B) and human AAA (C-H). The controls were no primary antibody (A-D), primary antibody isotype control (E and F) and no antigen retrieval (G and H) followed by incubation with secondary antibodies and detection reagents. Scale bars are 100 μm.
**Figure S2.** Immunohistochemical staining of CD68 in human AAA. A small portion of CD68-positive cells were found in the adventitia of human AAA. Some CD68-positive cells with larger cytoplasm and irregular shape are macrophages (left picture, open arrow). Other CD68-positive cells with smaller size and lightly stained nuclei are likely monocytes (right picture, open arrowhead).
Figure S3. Ang II-induced dissecting AAA in a wild-type mouse treated at 1000 ng/kg per minute for 4 weeks. A, Gross view of aneurysm characterized by the bulge of upper portion of the suprarenal aorta. Verhoeff’s stain of the section (B) from the upper portion shows intact tunica media and with large but organized intramural hematoma that is enclosed within relatively intact adventitia shown by the collagen in (C) (polarized view of picrosirius red stained section). Section (D) shows torn tunica media and severely dilated false lumen that are hold by the organized hematoma and adventitia. Thickening of adventitia is highlighted by the extensive collagen synthesis in (E). Collagen in the organized hemostatic plugs in (C) and (E) are very limited. Scale bars are 1000 μm.
Figure S4. A, mRNA level of PPARγ in the Pparg<sup>C/-</sup> aorta is 40% normal. *P<0.05 compared with Pparg<sup>+/-</sup> mice. B, Immunohistochemical staining of PPARγ in Pparg<sup>+/-</sup> and Pparg<sup>C/-</sup> aorta, confirming the reduction of PPARγ protein in the Pparg<sup>C/-</sup> aorta. C, H&E staining of the cross-section of aorta indicates normal vascular dimension of the Pparg<sup>C/-</sup> aorta. Scale bars are 100 μm.
Figure S5. Ser82 phosphorylation of PPARγ in aorta was determined by immunoblotting using antibodies against P-Ser82 PPARγ and PPARγ. The relative ratio of P-Ser82 PPARγ to total PPARγ was significantly higher (1.8X, P<0.05) in PpargC/- aorta than in Pparg+/+ aorta, while the relative ratio of P-Ser82 PPARγ to β-actin did not differ.
Figure S6. Expression of ECM components and MMP-9 in Pparg<sup>+/−</sup> aorta. Data are expressed relative to the mean in Pparg<sup>+/+</sup> aorta as 1.0 (<sup>+/+</sup>=5, <sup>+/−</sup>=5). *P<0.05 compared with Pparg<sup>+/+</sup> mice.
Figure S7. Tail-cuff BP measurements in mice infused with Ang II (0, 500, or 1000 ng/kg per minute) and fed with a western diet for 4 weeks. SBP: systolic BP. DBP: diastolic BP. MBP: mean BP. n=6, 9 and 4 in each genotype of Ang II 0, 500, 1000 ng/kg/per minute groups, respectively.
Figure S8. Expression of (A) pro-oxidant and (B) anti-oxidant enzymes in Pparg<sup>C/-</sup> and Pparg<sup>++/+</sup>aorta without and with Ang II (500 ng/kg per minute) infusion for 4 weeks. Data are expressed relative to the mean in untreated Pparg<sup>++/+</sup> aorta as 1.0 (n=5~7 in each genotype). Two-way ANOVA analysis showed that treatment of Ang II had significant effect on expression of Cyba (P<0.001), Ncf1 (P<0.001), Ncf2 (P=0.078), Cat (P<0.01) and Sod1 (P<0.001). However, neither PPAR<sub>γ</sub> genotype effect nor interaction between Ang II and genotype was found in expression of all these genes.
Figure S9. Time course of AAA development in Ang II-infused Pparg<sup>C/-</sup> mice. A, Representative photographs of AAA and (B) suprarenal aortic lumen diameter measured by ultrasound imaging in the mice of 2 and 3 weeks post-Ang II (1000 ng/kg per minute) infusion. n=3 in each group.
Figure S10. Histological characteristics of the Ang II-infused PparγC/− SMA aneurysm. A and B, Cross-section and quantification of SMA lumen area. *P<0.05. C, Elastin and collagen (picrosirius red) staining of SMA. D, Immunohistochemistry staining for smooth muscle cells (SMαctin), fibroblasts (FAP), macrophages (CD169) and MMP-9 in SMA. Lu: lumen. M: media. Scale bars in (B) are 1000 μm and in (C and D) are 200 μm.
Figure S11. Immunohistochemical staining of Ki-67 in dilated PpargC/- SMA and Pparg+/+ AA after Ang II (1000 ng/kg per minute) infusion for 4 weeks. The dilated adventitia of PpargC/- SMA shows a dramatic increase of Ki67-positive signal. Lu: lumen; Med: media; Adv: adventitia. Scale bars are 100 μm.
**Figure S12.** Expression of *ELN* and *FBLN5* after treatment with GW9662 or TNF-α in human aortic smooth muscle cells. Data are expressed relative to the mean in untreated groups as 1.0 (n=3 in each group).