Expression of Peroxisome Proliferator-Activated Receptor-γ in Vascular Smooth Muscle Cells Is Upregulated in Cystic Medial Degeneration of Annuloaortic Ectasia in Marfan Syndrome

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Background—Cystic medial degeneration (CMD) is a histological abnormality common in annuloaortic ectasia (AAE) and aortic dissection with Marfan syndrome. Apoptosis and loss of vascular smooth muscle cells (VSMCs) is one of the features of CMD, but little is known about its pathogenesis. Peroxisome proliferator-activated receptor-γ (PPARγ), a transcription factor of the nuclear receptor superfamily, has been reported to show antiproliferative effects on VSMCs as well as anti-inflammatory effects on macrophages. PPARγ agonist has been recently reported to induce apoptosis of cultured VSMCs.

Methods—We examined the histopathology of nondissecting ascending aortas in AAE of Marfan patients (n=21) and control patients (n=6) at surgery. RT-PCR was performed to demonstrate expression of PPARγ in CMD. Localization of PPARγ was determined by double immunostaining using antibodies against PPARγ and cell-specific markers (ie, SMCs, macrophages, and T lymphocytes).

Results—PPARγ expression was upregulated in AAE samples but minimal in control samples by RT-PCR (P=0.07). Immunoreactivity against PPARγ in numerous nuclei of VSMCs was observed in CMD lesions. Severity of CMD correlated with positive immunoreactivity of PPARγ in medial VSMCs (P=0.03). No inflammatory cells (ie, macrophages or T lymphocytes) were detected in CMD lesions.

Conclusion—PPARγ expression is upregulated in SMCs of CMD without any inflammatory response. Activated PPARγ in VSMCs might be involved in the pathogenesis of CMD in Marfan’s aorta. Regulation of PPARγ might lead to clinical implication in protection against progression of AAE. (Circulation. 2002;106[suppl I]:I-259-I-263.)

Key Words: peroxisome proliferator-activated receptor-γ • vascular smooth muscle cell • Marfan syndrome

Cystic medial degeneration (CMD) is an important histological abnormality common in annuloaortic ectasia (AAE) with Marfan syndrome. The abnormality is also observed in congenital aortic disease,1 atherosclerosis, and aging.2 These aortic diseases develop as consequences of disruption of medial elastic layers in association with loss of vascular smooth muscle cells (VSMCs) and accumulation of proteoglycans.

Apoptosis and loss of VSMCs is one of the features of CMD,3,4 but little is known about its pathogenesis. It is widely accepted that apoptosis is a major mechanism for the control of cell number in developing and mature tissues under physiological and pathological conditions.5 Vascular remodeling in the process of angiogenesis and during vascular diseases is thought to involve apoptosis of VSMCs. Some reports have suggested that apoptosis may play an important role in the pathogenesis of aortic dilatation in patients with Marfan’s syndrome and in congenital aortic diseases.6

Peroxisome proliferator-activated receptor-γ (PPARγ), a transcription factor of the nuclear receptor superfamily, has been reported to show antiproliferative7,8 and antimigratory effects on VSMCs9 as well as anti-inflammatory effects on macrophages (Mφs).10 PPARγ agonist has been recently reported to induce apoptosis of cultured VSMCs11 and attenuate the development of intimal hyperplasia in animal models of balloon catheter–induced vascular injury.12

In this study, we examined whether expression of PPARγ in VSMCs is upregulated in CMD of AAE in Marfan syndrome. We demonstrate that aortic tissue samples obtained from Marfan patients express PPARγ in VSMCs, which correlates with the degree of CMD. We discuss its implication in Marfan’s aorta.

Methods

Subjects
We examined the histopathology of nondissecting ascending aortas in AAE of 21 Marfan patients compatible with the Gherent diagnostic criteria13 at Bentall’s operation (16 men and 5 women; age range,
21–42 years; mean age, 32.8 ± 7.4) and in 6 control patients with aortic regurgitation and mild aortic dilatation at aortic valve and graft replacement (all of 6 men, 36–56 years; mean age, 48.8 ± 11.0). All operations were done at Tokyo Women’s Medical University Hospital between 1995 and 2000. Range of maximum aortic diameter in Marfan patients was measured by chest computed tomography or magnetic resonance imaging between 45 and 90 mm (66.9 ± 13.2 mm); in control patients, between 40 and 57 mm (51.3 ± 7.9 mm). This study was approved by the institutional guidelines of Tokyo Women’s Medical University, and all patients gave oral informed consent.

**Histological Examination**
Fractured formaldehyde-fixed aortas of 21 AAE and 6 control patients were processed for light microscopy and immunohistochemistry. In addition to hematoxylin and eosin staining, Alcian-blue staining for acidic mucopolysaccharides deposition and loss of VSMCs were performed and scored on a scale of 1 to 4 points according to the severity of each finding (grading for MPS deposition: 1, minimal; 2, mild; 3, 1 cystic lesion; and 4, more than 2 cystic lesions; grading for elastic layer fragmentation: 1, minimal; 2, mild; 3, less than 1/2 medial layer; and 4, more than 1/2 medial layer; grading for fibrosis: 1, minimal; 2, mild; 3, focal fibrosis in less than 1/2 medial layer, and 4, focal fibrosis more than 1/2 medial layer). The total score was summated as the CMD score to assess the degree of CMD.

**Immunohistochemical Analysis**
Nuclear localization of PPARγ was determined by immunostaining with monoclonal antibody against PPARγ (Santa Cruz Biotechnology, Inc) by using an LSAB kit (Dako Japan Co Ltd.). VSMCs were identified by immunoreactivity for mature form of smooth muscle myosin heavy chain antibody (SM1; donated by Dr Ryozo Nagai, Tokyo University, Tokyo). CD68 (clone KP-1, Dako Co.) and CD45RO (clone UCHL-1, Dako Co.) were immunostained for detection of M6s and T lymphocytes, respectively. The reaction was visualized with diaminobenzidine, and nuclei were counterstained with hematoxylin. To determine the proportion of PPARγ-positive VSMCs for each sample, the total number of SM1-positive cells in the aortic media was counted for each section. Six fields per section were examined at X200 magnification, and measurements were performed independently by 2 investigators. Their observations were averaged. The investigators performing histological evaluation were blinded to the clinical data.

**Reverse Transcription Polymerase Chain Reaction**
Media of aortic tissues from 8 Marfan and 3 control patients were separated from both intima and adventitia to avoid contamination of endothelium and fatty tissue. Total RNA from these tissues was isolated by the single-step guanidinium thiocyanate-phenol-chloroform method by using RNAzol (TelTest, Friendswood, Tex). RT-PCR was performed to demonstrate expression of PPARγ in CMD. Two micrograms of total RNA was reverse-transcribed into cDNA with 1 U/mL of reverse transcriptase (SuperScript, Gibco-BRL, Gaithersburg, Md) at 37°C for 1 hour in standard buffer. The PCR reaction was carried out with Ready To Go PCR beading (Pharmacia Biotech). For the amplification of human PPARγ cDNA, 2 primers (5’-AAC TGG GAA ACT TGG GAG ATT CTC C-3’ and 5’-AAT AAT AAG GTG GAG ATG CAG GCT CC-3’) were used. The DNA fragments of human PPARγ cDNA (351 bp) and ms16 (a housekeeping gene as internal control: 103 bp) were amplified with the oligonucleotide primers as described previously. The PCR products were run on 2.0% agarose gels.

**Statistical Analysis**
Analyses were performed with the SAS System 6.12 (SAS Institute Inc., Cary, NC). The data were presented as frequency or means ± SEM. Chi-square test or Fisher’s exact probability test was applied for dichotomous data. Student’s t test or Welch’s t test was used for continuous data. The F test was used for homogeneity of variance testing. Spearman correlation coefficients (2-tailed) were used to evaluate the correlation between the CMD score and the percentage of PPARγ-positive VSMCs and between aortic diameter and relative PPARγ mRNA expression. Two-tailed P values

**Results**
**Light Microscopy and Immunohistochemistry in Marfan’s Aortas**
Typical CMD lesions with more than grade 3 MPS deposition and more than 7 points on total CMD score were observed in aortic media in 15 of 21 patients (Figure 1A and B). Intimal thickening was minimal, and there were no fatty streaks in any ascending aortas in Marfan and control patients. The majority of cell type was VSMC in media. No inflammatory cells (ie, macrophages or T lymphocytes) or foam cells were detected in CMD lesions. A few mast cells and macrophages were seen in the adventitia (Figure 2A-D).

**Expression of PPARγ in Marfan’s Aortas**
RT-PCR showed that PPARγ expression was upregulated in half of the AAE samples obtained from Marfan patients but minimal in 3 control samples (Figure 3A and B). Immu-
reactivity against PPARγ in numerous nuclei of medial VSMCs was observed in CMD lesions (Figure 1C). The percentage of PPARγ-positive VSMCs was significantly higher in Marfan’s aortas than control aortas (21.3±2.7% versus 3.3±0.8%; P<0.001; Figure 4A).

**Severity of CMD, Aortic Dilatation, and PPARγ Expression**

PPARγ expression estimated by RT-PCR showed association with maximum diameter of the ascending aorta (P=0.07; Figure 3A and B). The percentage of PPARγ-positive medial SMCs correlated with the severity of CMD (P=0.03) and with maximum aortic diameter (P=0.06; Figure 4B and C).

**Discussion**

Previous studies have demonstrated evidence that PPARγ might influence macrophage-dependent events in the development of atherosclerosis associated with inflammation.16,17 PPARγ was expressed not only in normal human monocytes but also in foam cells and atherosclerotic plaques. Natural and synthetic PPARγ ligands were demonstrated to inhibit expression of proinflammatory genes and to have an anti-atherogenic effect in the vessel wall.16 Several reports have shown that PPARγ is expressed in cultured VSMCs,18,8 and in rat neointima after balloon injury.19 This study provides the first documentation of the expression of PPARγ in aneurysms of Marfan’s aorta with pathological CMD. Upregulation of PPARγ expression is associated with severity of CMD and with increase of aortic diameter, suggesting that PPARγ expression in medial VSMCs might be based on pathogenesis of CMD and disease progression of Marfan’s aorta. Moreover, immunohistochemical analysis of cell components revealed that there was no inflammatory cell in CMD lesion. PPARγ expression in the CMD of Marfan’s aorta was not associated with inflammation, which is unique pathology.

What is the pathophysiological role of PPARγ in aortic aneurysm with Marfan syndrome? Dedifferentiation of VSMCs is an important phenotypic change during the progression of atherosclerosis, restenosis,20 and aneurysm formation.21,22 Bunton et al have previously reported that fibrillin-1-deficient mouse, a mouse model of Marfan syndrome, showed phenotypic alteration of medial VSMCs preceding elastolysis.23 Segura et al have shown expression of matrix metalloproteinases in VSMCs in thoracic aortic aneurysms with Marfan syndrome that causes fragmentation of medial elastic layers.24 Elastolysis induced by matrix metalloproteinases is one important feature in pathology of Marfan patients. These investigators suggest phenotypic change of medial VSMCs to the activated phenotype might have a pivotal role in aorta of Marfan syndrome. Upregulation of PPARγ in VSMCs might thus be involved in the pathogenesis of CMD via the activated phenotype of medial SMCs in Marfan’s aortas. PPARγ agonists such as troglitazone and pioglitazone inhibit proliferation and matrix metalloproteinase expression in cultured VSMCs.8 PPARγ agonists also inhibit intimal VSMC growth in the balloon-injury animal model.25 Although the pathophysiological significance of PPARγ expression in VSMCs of Marfan’s aorta without any inflammatory

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**Figure 3.** A, RT-PCR showed that PPARγ expression (351 bp) is upregulated in AAE samples obtained from Marfan patients but minimal in control samples. B, Relative PPARγ mRNA expression levels compared with ms16 mRNA. Upregulation of PPARγ expression is associated with increase of aortic diameter (R=0.63; P=0.07). The data points for control patients are shown as square boxes.
response is unclear, we speculate that PPAR-γ may act to counterbalance activation and proliferation of VSMCs.

We have previously reported acceleration of VSMC apoptosis in Marfan’s aorta\(^1\) and that this might be involved in the progression of pathogenesis in aortic dilatation. Aizawa et al have shown that the PPAR-γ agonist pioglitazone induces apoptosis in cultured VSMCs in a nitric oxide-dependent manner by cytokine activation, and reduces intimal hyperplasia in a rat balloon-injured model by enhancing VSMC apoptosis.\(^12\) Although evidence for a direct causal relationship between PPAR-γ and its ligand was not demonstrated, pioglitazone was a potent inducer of apoptosis in vascular lesions. In aortic aneurysm with Marfan syndrome, VSMC apoptosis may play a substantial role in progression of aortic disease and formation of CMD via PPAR-γ activation.

One question that remains is whether PPAR-γ is a primary or secondary component of CMD formation in Marfan syndrome. CMD is also observed in congenital aortic disease,\(^1\) atherosclerosis, and aging.\(^2\) We examined aortas in the size-matched non-Marfan’s AAE patients, ie, coarctation of aorta and atherosclerosis at graft operation. We could find CMD in these patients. PPAR-γ expression could be demonstrated in these patients by immunohistochemical and RT-PCR methods (data not shown). So we suppose that the expression of PPAR-γ is associated with aneurysm formation with CMD because of various causes and not unique phenomena in Marfan’s syndrome. Further studies are required to characterize the importance of PPAR-γ within CMD. If this is clarified, regulation of PPAR-γ might lead to the clinical implication of protection against progression in AAE with Marfan’s syndrome.

**Conclusion**

Expression of PPAR-γ was upregulated in VSMCs of Marfan’s aortas with CMD and associated with severity of CMD and increasing aortic diameter. Our results suggest that PPAR-γ expression might reflect pathogenesis of CMD and disease progression of Marfan’s aorta without any inflammatory response.

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