# Hyperplastic Cellular Remodeling of the Media in Ascending Thoracic Aortic Aneurysms

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***Background***—Progressive medial degeneration and atrophy is thought to be a cause of ascending thoracic aortic aneurysms in the elderly. Extensive apoptosis of vascular smooth muscle cells (VSMCs) has been demonstrated in the media of abdominal aortic aneurysms. We investigated whether medial atrophy from loss of VSMCs occurs in primary ascending thoracic aortic aneurysms.

***Methods and Results***—Morphometric analysis of 28 nonaneurysmal ascending thoracic aortas and 29 ascending thoracic aortic aneurysms was performed by directly measuring the thickness of their vascular layers and by indirectly calculating the area of their vascular compartments. The cellular and matrix composition of the media was assessed at the structural, protein, and transcript levels. Despite thinning of the media secondary to vascular dilatation, there was an overall increase in the medial area of aneurysms. VSMC density was preserved, implying cellular hyperplasia as a result of the increased medial mass. There was decreased expression of matrix proteins, despite sustained synthesis of these molecules, which was associated with evidence of increased matrix degradation. The remodeling and expansion of the media was most evident in comparisons between nonaneurysmal aortas versus smaller aneurysms and did not evolve further in larger aneurysms.

***Conclusions***—The mechanisms for luminal enlargement in thoracic and abdominal aortic aneurysms differ significantly with regard to the survival of VSMCs and atrophy of the media but share common pathophysiology involving degeneration of the matrix. Hyperplastic cellular remodeling of the media in ascending thoracic aortic aneurysms may be an initial adaptive response to minimize increased wall stress resulting from vascular dilatation. **(*Circulation*. 2005; 112:1098-1105.)**

**Key Words:** aorta ■ aneurysm ■ remodeling ■ muscle, smooth



Aortic aneurysms are a relatively common cause of death because of rupture or dissection. Although aneurysms may form throughout the aorta, they most frequently occur in the abdominal aorta. Aneurysms of the ascending thoracic aorta are less common and are classified as those associated with degeneration of the arterial media that typically occur in the elderly and those secondary to other causes, such as congenital cardiovascular malformations, connective tissue disorders, and aortic dissection, which may also occur in younger patients.[1] The heterogeneous nature of aortic aneurysms is underscored by epidemiological, genetic, transcriptional, and histopathological distinctions between degenerative aneurysms of the thoracic and abdominal aortas.[2] Moreover, there are significant histological differences between ascending thoracic aortic aneurysms that present with or without aortic dissection.[1] Thus, the pathogenesis of aortic aneurysms may differ depending on the location and clinical presentation.

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The major histopathological features associated with ascending thoracic aortic aneurysms are abnormalities of the cellular and matrix constituents of the media, ie, vascular smooth muscle cells (VSMCs), elastin, collagen, and ground substance. Degenerative lesions in the media of ascending thoracic aortic aneurysms and dissections have been described for many years. Gsell[3] was the first to describe medial degeneration as initial focal loss of VSMCs followed later by disintegration of elastin and collagen. In contrast, Cellina[4] suggested that injury of elastic tissues was the primary lesion and occurred independently of the muscle lesions. An alternative theory, by their contemporary Erdheim,[5] that initial accumulation of ground substance induced subsequent loss of VSMCs and elastic fibers ("cystic medionecrosis") has been abandoned.[6] Modern terminology designates defective mural component(s) of aortic aneurysms as either VSMCs, elastic

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tissue, or both.[6] The finding that loss of VSMCs and elastin fragmentation are also seen in nondilated aging aortas, albeit to a lesser extent, argues that these lesions are not specific defects underlying aortic aneurysm formation or dissection but rather may result from medial injury and repair in response to normal hemodynamic forces.[7] Nonetheless, progressive medial degeneration and atrophy are generally thought to play an important role in the pathogenesis of ascending thoracic aortic aneurysms in the elderly.[1]

During the past 2 decades, there has been great progress in understanding the cellular and molecular mechanisms of pathological vascular dilatation. Clinical and basic research studies have demonstrated an imbalance between the synthesis and destruction of structural matrix proteins, particularly elastin and collagen, in the wall of aortic aneurysms. Degradation and loss of the elastic media is a consistent histopathological and biochemical feature of aneurysm specimens and is considered a central event in the development of aortic aneurysms.[8] Because the synthesis of elastin appears to be intact,[9,10] loss of elastic tissue is related to elastolytic proteinases produced by infiltrating leukocytes and vascular cells, such as matrix metalloproteinase (MMP)-2 and MMP-9.[11–13] The activity of these matrix-degrading enzymes is modulated by activators, eg, plasmin, and inhibitors, such as tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-2, within the aneurysm microenvironment.[11–14] In addition to proteolysis, a relative loss of elastin in aneurysms has also been attributed to dilution from discordant matrix production.[9,15] The outcome between increased synthesis and degradation of collagen in aortic aneurysms is more variable, resulting in decreased, preserved, or increased collagen content.[9,10,15] Matrix remodeling in aortic aneurysms is associated with pronounced VSMC depletion and medial atrophy.[16] The mode of VSMC death has been characterized as apoptosis and may be linked to inflammatory mediators.[17,18]

Most studies into the pathogenesis of aortic dilatation, including those cited above, have been in patients with abdominal aortic aneurysms.[8–18] In preparation for an investigation of the role of inflammatory mediators in the development of ascending thoracic aortic aneurysms, we initially examined which medial components were degraded in our aneurysm specimens compared with nonaneurysmal referent aortas. We thought it important to interpret the histomorphometric findings in a particular biopsy specimen, such as medial thickness and density of VSMCs, in the context of the overall size of that aneurysm, ie, medial area and total number of VSMCs.



# Methods

## Clinical Data and Aortic Specimens

Research protocols were approved by the institutional human investigation committees and the New England Organ Bank, and informed consent was obtained. Clinical data and aortic specimens were collected during a 16-month period (November 2001 to February 2003) from patients with ascending thoracic aortic aneurysms (n=29) and patients with nonaneurysmal aortas undergoing coronary artery bypass surgery (n=10) or cardiac transplantation (n=3) and from cadaveric organ donors whose hearts were not procured for transplantation (n=15). Biopsies were obtained in the operating room from the anterior part of the ascending thoracic aorta within the pericardial reflection, which ensured a defined adventitial layer. Aortas from children and young adults (<35 years of age) or from adults with aneurysms secondary to other causes (such as Marfan syndrome or aortic dissection) were excluded from analysis. Full-thickness aortic specimens were formalin-fixed or frozen and stored at −80°C until analysis.

## Morphometry

Measurements of aortic transverse diameters were made in the operating room or from preoperative radiological studies. Wall thickness was determined from elastin van Gieson (EVG)–stained, transverse sections of aortic specimens under magnification using Image 1.62c software (Scion). Measurements of intimal, medial, and adventitial thickness were performed at 4 random points, and an average value was calculated. The areas of the vascular compartments were calculated from the transverse diameter of the aortas and the thickness of the vascular layers, assuming a circular cross-sectional shape of the aorta. Predicted ascending thoracic aortic diameter was calculated according to formulas derived from subjects in the Framingham Heart Study[19]: aortic diameter in men (cm)=17.39×[0.080×age (years)+2.403×height (m)+0.087×weight (kg)], and aortic diameter in women (cm)=13.61×[0.089×age (y)+3.807×height (m)+0.068×weight (kg)]. The predicted medial area in aneurysm patients was calculated from the predicted aortic diameter[19] and the nonlinear regression relationship of medial area to external diameter determined in our referent nonaneurysmal aortic specimens: medial area ($mm^2$)=74.97+5.55×[aortic diameter (cm)]$^2$.

## Histology and Immunohistochemistry

Paraffin-embedded sections were stained with hematoxylin and eosin, Sirius red, Alcian blue (at a pH of 1), and EVG using standard techniques and reagents. Alternatively, 5-$\mu$m frozen sections were labeled with anti–smooth muscle (SM) $\alpha$-actin (Dako). Binding of secondary antibodies (Jackson ImmunoResearch) was detected with peroxidase/3-amino ethyl carbazole kits (Vector). The density of VSMCs was determined by enumerating the nuclei surrounded by SM $\alpha$-actin$^+$ immunostaining within 3 random areas of the inner and outer media and calculating the mean number of cells per high-power field. Quantification of the extent of staining was performed with KS 300 Imaging System software (Zeiss). Specific color components of interest were isolated and quantified as the percent area of the total field that stained positive. The depth of medial elastin fragmentation was graded according to the following arbitrary scale: 0, no elastin fragmentation; 1, subintimal fragmentation; 2, fragmentation involving inner third of media; 3, fragmentation involving inner two thirds of media; and 4, full thickness medial fragmentation.

## Real-Time Quantitative RT-PCR

Total RNA from aortic specimens was isolated and DNAase-treated by use of the Nanoprep system (Stratagene) and quantified with the Ribogreen assay (Molecular Probes). The RNA underwent reverse transcription (RT) reactions with random hexamer primers according to the Multiscribe RT system protocol (Applied Biosystems). Real-time quantitative polymerase chain reaction (PCR) reactions were prepared with Taqman PCR reagents (Applied Biosystems) and primers designed according to their recommendations, including GAPDH, $\beta$-actin, SM $\alpha$-actin, collagen type 1 $\alpha$-1, collagen type 3 $\alpha$-1, elastin, chondroitin sulfate proteoglycan versican, biglycan, MMP-2, MMP-9, TIMP-1, and TIMP-2. Products were sequenced to ensure specificity. An iCycler and its system interface software were used to both run samples and analyze data (Bio-Rad Laboratories). Standard curves were constructed to determine the copy number of transcripts present in each sample. The expression level of each target was normalized as the copy number per GAPDH transcripts.

## Statistical Analysis

Statistical analyses were performed using SAS version 8.2 statistical software (SAS Institute Inc). Two-sample *t* tests and 1-way ANOVA were used for mean comparisons between multiple groups. Pearson

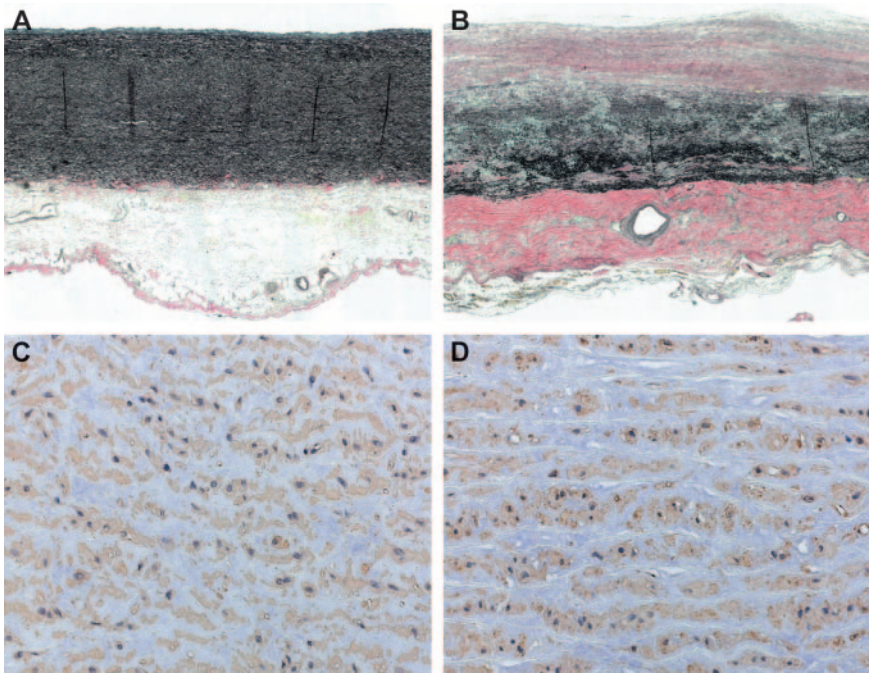


**Figure 1.** Histological appearance of ascending thoracic aortas. Nonaneurysmal aortas (A, C) and aortic aneurysms (B, D) were stained with EVG (A, B) or an antibody to SM $\alpha$-actin (C, D). Elastin is stained black by EVG and demarcates the media from the intima (above) and adventitia (below). Positive immunostaining is a brown color. Representative low-magnification (top) and high-magnification (bottom) photomicrographs of aortic transverse sections.

correlations, $\chi^2$ tests, and Fisher's exact tests were also performed to describe the association between different outcome variables. The Bonferroni correction was used to adjust for both multiple comparisons and correlated outcome variables. Two-tailed probability values of $P<0$ 0.01 were considered statistically significant for each test to ensure an overall study significance level of $P=0.05$.

## Results

### Aortic Specimens and Patient Characteristics

Biopsies were obtained intraoperatively from 28 nonaneurysmal, mid–ascending thoracic aortas and from both the maximally dilated (body) and least dilated (neck) portions of 29 fusiform ascending thoracic aortic aneurysms. The aneurysm body tissues were used for comparisons with the nonaneurysmal aortas. The aneurysm bodies were 1.4 to 2.9 times larger than the necks. The mean external diameter of the nonaneurysmal aortas was similar to the predicted aortic size based on anthropomorphic measurements ($3.1\pm0.5$ versus $3.34\pm0.2$ cm, respectively). In contrast, the observed external diameter of the aortic aneurysms greatly exceeded the expected aortic size ($6.0\pm0.8$ versus $3.32\pm0.2$ cm). The patients with nonaneurysmal and aneurysmal aortas did not differ for the major determinants of aortic size, ie, body surface area, weight, height, age, and sex (Data Supplement Table).

### Increased Medial Area of Ascending Thoracic Aortic Aneurysms

The width of the vascular wall layers was measured on cross sections of full-thickness aortic biopsies by computer-assisted video microscopy. The intima was thicker, the extent of the adventitia was similar, and the media was thinner in aortic aneurysms compared with nonaneurysmal aortas (Figures 1, A and B, and 2, A–C). However, the vascular wall layers are predicted to thin in response to vascular dilatation alone (in the absence of any atrophic vessel wall changes); eg, in an aorta that increases from 3 to 6 cm in external diameter, an initial hypothetical wall thickness of 1500 $\mu$m will decrease more than 50% to 721 $\mu$m if the aortic wall mass remains unchanged. Thus, the intimal mass must have greatly increased for the intimal thickness to expand, and the adventitial mass must have increased for the adventitial thickness to remain unchanged in aneurysms compared with nonaneurysmal aortas. Similarly, the medial mass may have actually increased, because the medial thickness of the aneurysms was only 24% less than that of the nonaneurysmal aortas ($1169\pm335$ versus $1532\pm283$ $\mu$m), despite a doubling in aortic diameter ($6.0\pm0.8$ versus $3.1\pm0.5$ cm). Moreover, the media of aneurysms thinned less than expected for the degree of aortic dilatation on the basis of predicted initial aortic size and medial thickness (data not shown).

We therefore calculated the medial area from the difference in areas circumscribed by the external and internal elastic laminae using the aortic diameter and wall thickness measurements. Surprisingly, the aneurysm medial areas were greater than those of nonaneurysmal aortas (Figure 2D). Two further comparisons of medial area were performed within the aneurysm group alone to avoid any confounding variables caused by differences in the 2 patient populations. First, the medial area of the body was greater than that of the neck in every aneurysm, although the anatomic site of the neck was occasionally within the aortic arch, where the vessel external diameter and medial thickness are expected to be less than that of the ascending aorta (Figure 2E). Second, the calculated medial area for 26 of 29 aneurysms was greater than that predicted from formulas of aortic size indexed by weight, height, age, and sex (Figure 2F). The results indicate that the vascular wall of ascending thoracic aortic aneurysms increased in mass, most prominently in the intima and adventitia, but also in the media.

### Preserved VSMC Density in Ascending Thoracic Aortic Aneurysms

To determine whether the increase in aneurysm medial area was associated with changes in the cellular or matrix com-

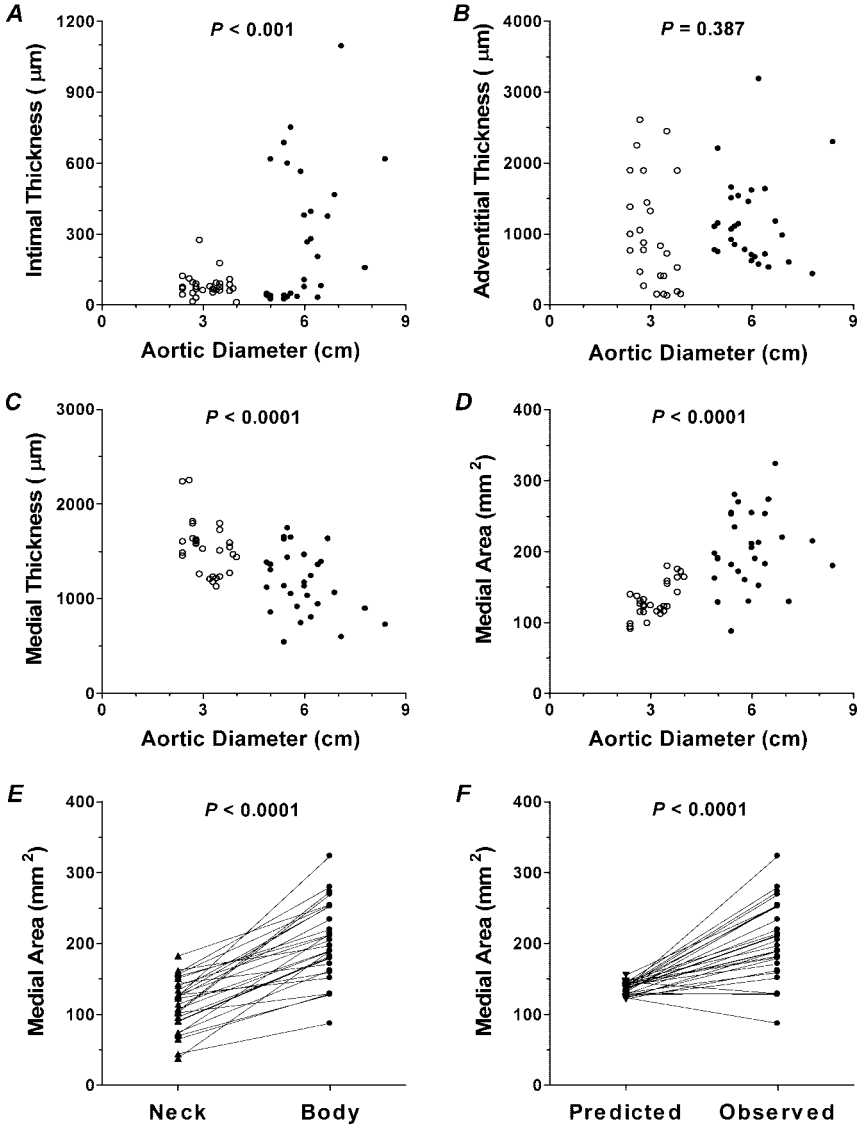


**Figure 2.** Remodeling of vascular compartments in ascending thoracic aortic aneurysms. The thickness of the intima (A), adventitia (B), and media (C) in nonaneurysmal aortas (open symbols) and aortic aneurysms (filled symbols) were measured directly under magnification. The medial area of nonaneurysmal aortas and aneurysm bodies (D) and necks (E) were calculated from measurements of the aortic diameter and wall thickness. Predicted medial areas (F) were based on predicted aortic size and medial area relationships from referent nonaneurysmal aortas. Data are individual measurements and in certain associations are paired (E, F). Comparisons between the groups were by *t* test, and the probability values are shown.

ponents of this vessel layer, we initially counted the number of VSMCs and measured the protein and RNA expression of the SM $\alpha$-actin contractile molecule. The number of SM $\alpha$-actin$^+$ cells per high-power field was similar in the media of aortic aneurysms compared with that of nonaneurysmal aortas (Figures 1, C and D, and 3A). The density of VSMCs was also alike in separate analyses of the inner and outer parts of the media, as well as the body and neck sections of aneurysms, compared with the nonaneurysmal referent specimens (data not shown). We were unable to count the number of medial lamellar units (musculoelastic fascicles) because of disorderly VSMC orientation and elastin fragmentation in a subgroup of aneurysms.

The expression of immunolabeled SM $\alpha$-actin protein, as determined by quantitative image analysis, was similar in sections from both aneurysmal and nonaneurysmal aortas (Figure 3B). In addition, the expression of SM $\alpha$-actin RNA by quantitative real-time RT-PCR was comparable in aneurysms and referent aortas (Figure 3C). Similar results were obtained when SM $\alpha$-actin transcripts were normalized to those of $\beta$-actin, another housekeeping gene, instead of GAPDH (data not shown).

To investigate whether the growth or survival of VSMCs was altered, we assessed the expression of proliferation (Ki67 immunostaining) and apoptosis (TUNEL staining) markers in the media. We found evidence of both markers but could not determine a proliferative or death index for VSMCs, because many of the dividing or dying cells appeared to be infiltrating leukocytes (data not shown). Our cell-counting data demonstrate that the density of VSMCs was preserved in ascending thoracic aortic aneurysms, which suggests that the total number of VSMCs must have expanded because of the increase in aneurysm medial area.

## Decreased Matrix Expression in Ascending Thoracic Aortic Aneurysms

The expression of matrix proteins was then determined by quantitative image analysis of Sirius red, black component of EVG, and Alcian blue staining, which largely label collagen, elastin, and sulfated forms of ground substance (such as

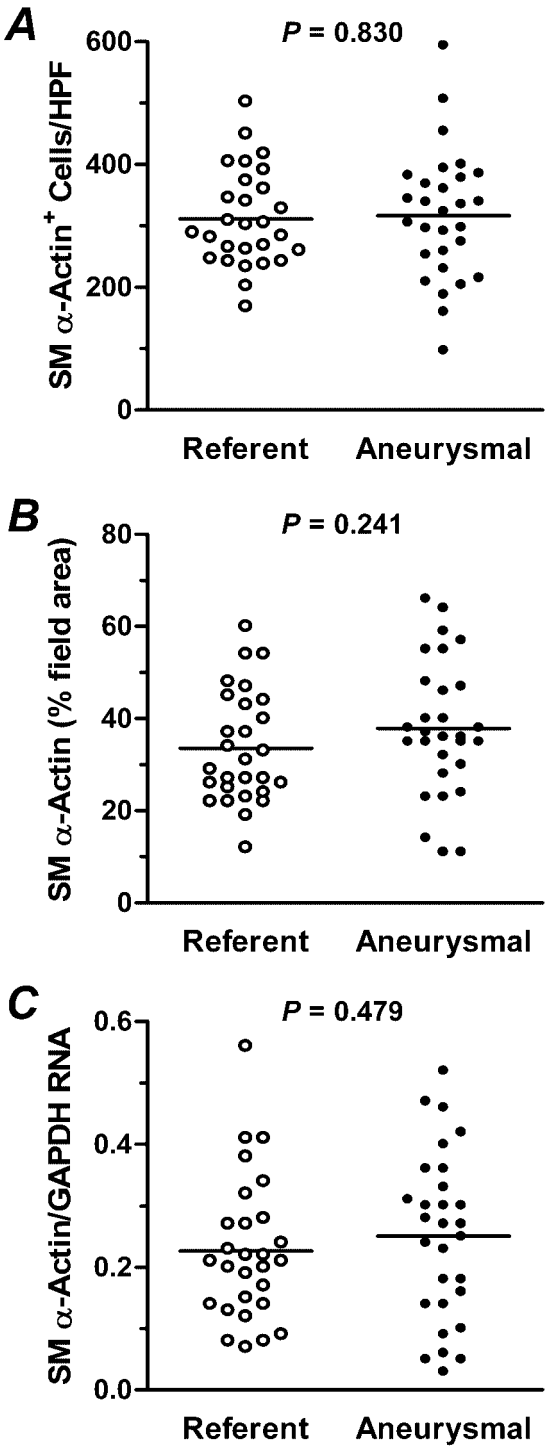


**Figure 3.** Ascending thoracic aortic aneurysms have preserved VSMC density and expression of SM α-actin. The number of SM α-actin$^+$ cells were counted as the average in 3 high-power fields (HPF) of the media (A). The extent of SM α-actin$^+$ immunolabeling was determined by quantitative image analysis and expressed as the percentage area of the total field staining positive (B). SM α-actin transcripts were quantified by real-time RT-PCR (C). Data are individual measurements in referent nonaneurysmal aortas (open symbols) and aortic aneurysms (closed symbols) with means (horizontal bars). Comparisons between the nonaneurysmal versus aneurysm groups were by *t* test, and the probability values are shown.

versican and biglycan), respectively. The extent of staining for collagen and elastin, but not for ground substance, was significantly less in aneurysms compared with nonaneurysmal aortas (Figure 4A). The changes in collagen expression largely reflected diminished Sirius red staining within the inner media, whereas the decreased expression of elastin was equally evident in both the inner and outer media (data not shown). The 18% and 45% decreases in collagen and elastin expression, respectively, indicate at least a relative loss of medial matrix protein density, although the absolute total content of collagen and elastin may have actually increased, because the medial area of aneurysms was 55% larger than that of nonaneurysmal aortas.

Because the amount of matrix protein in the media is a balance between the synthesis and degradation of these molecules by vascular cells and infiltrating leukocytes, we assessed the transcript expression of certain matrix molecules and regulatory enzymes by quantitative real-time RT-PCR (Figure 4, B–D). There was no difference in types I and III collagen, elastin, and biglycan transcript expression in aneurysmal versus nonaneurysmal aortic tissues, consistent with our observation of preserved VSMC density in the media of aneurysms. Modestly increased expression of versican RNA may explain the conserved Alcian blue staining within the media of aneurysms. There was also a trend toward increased MMP-9 RNA, but not MMP-2, TIMP-1, or TIMP-2 transcripts, in aneurysm specimens compared with nonaneurysmal aortas. The transcript analysis is suggestive that the decreased expression of matrix proteins in the aneurysm media may be because of an increased breakdown rather than a decreased synthesis of these molecules.

During our microscopic examination of the aortic tissues, we gained the impression that elastin fragmentation progressed within the media from the internal toward the external elastic lamina with increasing size of the aneurysms. We therefore graded medial elastin fragmentation according to an arbitrary scale along an abluminal direction. Aneurysm specimens had greater medial elastin fragmentation extending further into the media compared with nonaneurysmal aortas (grades $2.2\pm0.3$ versus $0.5\pm0.1$, $P<0.0001$), confirming structural abnormalities in addition to quantitative changes of the extracellular matrix in this disease process.

## Progression of Remodeling in Ascending Thoracic Aortic Aneurysms

We further analyzed our results within smaller (<6.0 cm, n=15) and larger (≥6.0 cm, n=14) aneurysms to assess the evolution of remodeling changes during aneurysm development (Table). There were relatively few differences between the smaller and larger aneurysms, implying that the adaptive changes of the media that occurred between nonaneurysmal aortas and smaller aneurysms did not persist with further growth of the aneurysms. Interestingly, there was a trend for increased VSMC markers in the media of smaller aneurysms, which was associated with a robust increase in medial area for this subgroup. Further comparisons showed an increase in SM α-actin immunostaining of the inner, but not outer, media of smaller aneurysms compared with nonaneurysmal aortas (40.3% versus 27.6% of field area, $P=0.004$), suggesting that

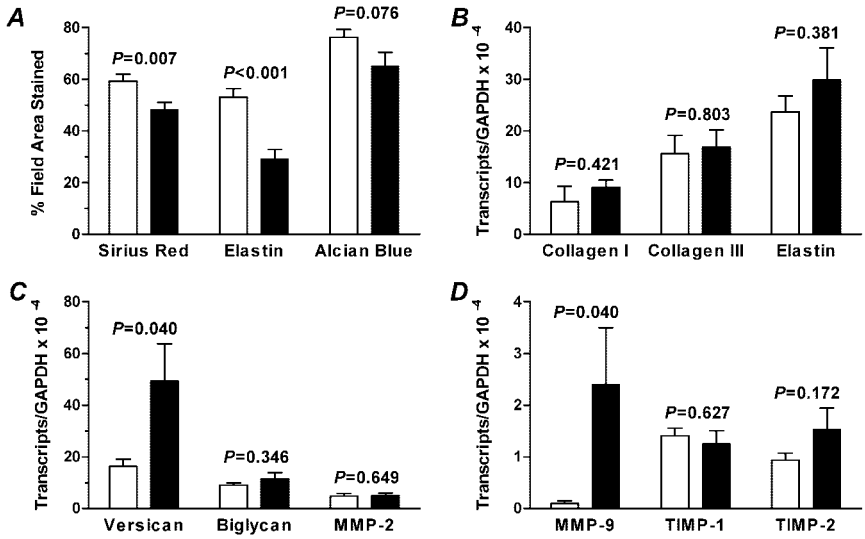


**Figure 4.** Decreased expression of matrix proteins in ascending thoracic aortic aneurysms. The extent of Sirius red, black component of EVG, and Alcian blue staining in the media of nonaneurysmal aortas (open bars) and aortic aneurysms (filled bars) was determined by quantitative image analysis and expressed as the percentage area of the total field staining positive (A). Transcripts for matrix components and regulatory enzymes were quantified by real-time RT-PCR (B–D). Data are mean±SEM. Comparisons between the nonaneurysmal versus aneurysm groups were by *t* test, and the probability values are shown.

remodeling changes and stimuli predominated in the inner part of the aorta. Decreased expression of TIMP-1 transcripts in the smaller aneurysms may have contributed to early remodeling before the increased expression of MMP-9 RNA in the larger aneurysms. The larger aneurysms had a greater depth of elastin fragmentation than the smaller aneurysms and the nonaneurysmal aortas.

Finally, we tested for covariation between aortic size and the other continuous variables of patient characteristics, aortic dimensions, and vascular properties that were measured in the preceding analyses. The strongest correlations were between external aortic diameter and intima thickness ($r=0.50$, $P<0.0001$), medial thickness ($r=-0.60$, $P<0.0001$), intimal area ($r=0.60$, $P<0.0001$), medial area ($r=0.67$, $P<0.0001$), adventitial area ($r=0.55$, $P<0.0001$), elastin staining of media ($r=-0.56$, $P<0.0001$), and MMP-9 RNA expression ($r=0.41$, $P=0.0015$). These results confirm that the vessel wall, including the medial compartment, progressively increased in mass with aortic aneurysm enlargement and that elastin loss was a consistent feature in the development of aortic aneurysms associated with an accumulation of MMP transcripts.

## Discussion

This work provides the following insights into the pathophysiology of ascending aortic aneurysms. (1) Although the media becomes thinner as an aneurysm develops, because of the larger aortic diameter, the actual mass of media is increased, not decreased, as previously speculated. (2) The density of medial VSMCs is preserved in ascending thoracic aortic aneurysms, in contradistinction to abdominal aortic aneurysms. (3) Increased destruction, most likely via MMP-9, and not decreased synthetic activity, underlies the impaired presence of matrix proteins in the aneurysmal aortic wall.

We find medial expansion and preserved VSMC density in ascending thoracic aortic aneurysms, in contrast to the marked medial atrophy and extensive loss of VSMCs that occurs in abdominal aortic aneurysms.[16–18] The lack of apoptosis and preserved density of VSMCs in ascending thoracic aortic aneurysms has been observed previously.[20] A similar disparity in medial remodeling was found in atherosclerosis of the aorta, in which the media underlying intimal plaques was conserved in the thoracic aorta but was atrophic in the abdominal aorta.[21] The resistance of thoracic aorta VSMCs to pathological attrition is not universal, because medial atrophy and necrosis has been described in the histology of thoracic aortic aneurysms secondary to congenital malformations,[22,23] Marfan syndrome,[1,23] and aortic dissection,[1,24] although measures of medial mass or VSMC density were not determined in these studies. Because we excluded cases of ascending thoracic aortic aneurysms secondary to Marfan syndrome and aortic dissection, we may have selected for a subgroup of patients without medial atrophy. However, our study patients should be comparable to those with abdominal aortic aneurysms, because Marfan syndrome and aortic dissection are rare in the latter population.

The reasons for the difference in VSMC survival between diseases of the thoracic and abdominal aorta are not known. Possible explanations for different thresholds to pathological apoptotic stimuli and/or compensatory proliferative signals include differences in the embryology and structure between the proximal and distal aorta. VSMCs in the ascending thoracic aorta are descended from ectodermal neural crest precursors, whereas those in the abdominal aorta arise from local mesenchymal cells of mesodermal origin and respond differently to cytokines and growth factors.[25] The media of the thoracic, but not abdominal, aorta contains vasa vasorum[26] and thus may be less prone to ischemic injury, particularly in association with a thickened intima acting as a barrier to luminal diffusion. The thoracic aorta has a greater expression of collagen and elastin than the abdominal aorta.[27] Finally, each lamellar unit in the thoracic aorta supports a lesser tensile strength of approximately 2000 dynes/cm$^2$ compared with 3000 dynes/cm$^2$ in the abdominal aorta.[28]

Aside from the difference in cellular remodeling of the media, there are similarities between the pathological findings of thoracic and abdominal aortic aneurysms with regard to matrix degeneration. We found loss of matrix protein expression, despite sustained synthesis of these molecules,

**Remodeling in Smaller (<6 cm) and Larger (≥6 cm) Ascending Thoracic Aortic Aneurysms**

| | Nonaneurysmal (n=28) | Smaller Aneurysms (n=15) | Larger Aneurysms (n=14) |
|---|---|---|---|
| Aortic dimensions | | | |
| External diameter, cm | 3.1±0.1 | 5.4±0.1* | 6.6±0.2*† |
| Intimal thickness, μm | 79±9 | 237±77* | 322±75* |
| Medial thickness, μm | 1532±53 | 1232±94* | 1101±80* |
| Adventitial thickness, μm | 1000±139 | 1193±105 | 1118±214 |
| Wall thickness, μm | 2611±174 | 2662±154 | 2541±251 |
| Medial area, $mm^2$ | 130±5 | 192±15* | 214±13* |
| VSMC characteristics | | | |
| SM α-actin$^+$ cells/high-power field | 312±15 | 329±28 | 304±28 |
| SM α-actin$^+$ area, % | 33.6±2.3 | 41.1±3.7 | 34.2±3.9 |
| SM α-actin/GAPDH RNA | 0.23±0.02 | 0.28±0.03 | 0.21±0.04 |
| Matrix characteristics | | | |
| Sirius red$^+$ area, % | 59.2±2.7 | 49.4±3.8 | 47.1±4.2 |
| Elastin$^+$ area, % | 53.1±3.3 | 34.3±4.7* | 23.8±5.5* |
| Alcian blue$^+$ area, % | 76.3±3.0 | 70.8±6.4 | 59.1±8.5 |
| COL I/GAPDH RNA $\times10^{-4}$ | 6.3±3.0 | 6.6±1.2 | 11.8±2.6 |
| COL III/GAPDH RNA $\times10^{-4}$ | 15.6±3.5 | 16.0±4.7 | 17.7±5.0 |
| Elastin/GAPDH RNA $\times10^{-4}$ | 23.7±3.1 | 34.1±11.3 | 25.4±4.3 |
| Versican/GAPDH RNA $\times10^{-4}$ | 16.3±3.0 | 18.9±1.6 | 49.3±14.5* |
| Biglycan/GAPDH RNA $\times10^{-4}$ | 9.1±0.8 | 9.4±1.0 | 11.7±2.3 |
| MMP-2/GAPDH RNA $\times10^{-4}$ | 4.9±1.0 | 3.8±0.5 | 5.0±1.0 |
| MMP-9/GAPDH RNA $\times10^{-4}$ | 0.1±0.0 | 1.3±0.7 | 3.6±2.1* |
| TIMP-1/GAPDH RNA $\times10^{-4}$ | 1.4±0.2 | 0.7±0.2* | 1.9±0.5 |
| TIMP-2/GAPDH RNA $\times10^{-4}$ | 0.9±0.1 | 1.1±0.4 | 2.0±0.7 |
| Elastin fragmentation, % grade 0/1/2/3/4 | 50/50/0/0/0 | 13/47/20/7/13* | 7/14/0/29/50*† |

Data are mean±SEM or %. Comparisons were by ANOVA for the continuous variables or by Fisher's exact test for elastin fragmentation grade.

*$P<0.01$ aneurysms vs nonaneurysmal aortas; †$P<0.01$ larger aneurysms vs smaller aneurysms.

associated with a trend toward increased expression of MMP-9 transcripts. However, expression of matrix transcripts may have derived from the thickened intima, and not the media, of aortic aneurysms. We did not find convincing evidence for a switch in VSMCs from contractile to synthetic phenotypes, because the transcript expression of the major contractile and matrix molecules remained largely unchanged. Fragmentation of elastin extended from the inner to outer media with increasing size of the aneurysms. Loss of elastin is a constant feature of aortic aneurysms,[8] with greater degradation of elastin in the inner aortic wall.[9] Because disruption of elastin lamellae also predominates in the inner media of nondilated aging aortas, this finding may reflect injury from hemodynamic forces rather than representing a specific factor involved in the development of aortic aneurysms.[7] Although we measured only MMP-9 transcripts in this study, increased expression of MMP-9 protein and enzyme activity has been reported in abdominal and thoracic aortic aneurysms.[12,13,20] The elastolytic and collagenolytic properties of MMP-9 may be at least partially responsible for the loss of elastin and collagen in aortic aneurysms.

The wall thickness of abdominal aortic aneurysms is preserved or increased despite luminal dilatation.[9,13,16] Vascular remodeling results in a considerable gain of wall mass, and the dry weight and protein content of abdominal aortic aneurysms is approximately 4-fold higher than that of equal lengths of normal-size aortas.[15] The predicted thinning of the arterial wall from stretching alone does not occur, because a 167% increase in intimal thickness and a 19% increase in adventitial thickness compensate for an 83% decrease in medial thickness in abdominal aortic aneurysms compared with reference aortas.[16] We found similar changes in the intimal and adventitial thickness of ascending thoracic aortic aneurysms, except that the medial thinning was far less pronounced than that reported in abdominal aortic aneurysms and much less than predicted from vascular dilatation and consequent stretching of the arterial wall. The morphometry results in this study cannot be considered absolute measurements of aortic wall thickness and area, because the specimens were not perfusion-fixed at physiological blood pressure and thus do not take into account the shrinkage artifact that occurs with conventional specimen processing.[28] Moreover, our calculations may be affected by the limited number of measurement points and by possible noncircular shapes of the aorta. Validation of our results requires progress in noninvasive imaging of the aorta whereby the medial compartment can be clearly delineated and accurately assessed. Our histomorphometric analysis with regard to the cellularity and the mass of the media in ascending thoracic aortic aneurysms is also limited by a lack of mechanistic insight into the balance between cellular proliferation and death of VSMCs. It is important to note that an analysis of the expression of medial cellular and matrix constituents alone may greatly underestimate the functional disturbances of the arterial wall because of structural disorganization. Maintenance of aneurysm wall thickness partially compensates for the increased wall stress resulting from increased lumen size,[9] and the expanded intima contains appreciable quantities of connective tissue, which may contribute to the tensile support of the artery wall.[29]

The mechanisms for the remodeling of the aneurysm wall are unknown, and it is not possible to dissect cause from effect in descriptive clinical studies. The medial remodeling was most evident in comparisons between nonaneurysmal aortas versus smaller aneurysms and did not evolve further in larger aneurysms, suggesting that the cellular hyperplasia of the media may have been an initial adaptive response to increased wall stress resulting from vascular dilatation. VSMCs are known to respond to mechanical strain by increased matrix synthesis and cellular proliferation.[30,31] Our observations are consistent with a model of dynamic medial remodeling of ascending thoracic aortic aneurysms rather than medial degeneration and atrophy alone. An alternative interpretation of our findings is that the intact media and viable VSMCs are required for aneurysm formation in the ascending thoracic aorta, ie, the aorta may grow in size because of intrinsic vascular wall remodeling (eg, in response to inflammatory stimuli) in addition to passively enlarging

and eventually rupturing in response to hemodynamic forces. VSMCs are potential mediators of vascular remodeling, because they synthesize matrix proteins and secrete MMPs and modulators of their activities.[11–14] Interestingly, progressive aortic root dilatation in adults with congenital heart disease may result from intrinsic properties of the aortic wall independently of hemodynamic factors.[32] Moreover, ascending thoracic aortic allografts in nonimmunosuppressed recipients function satisfactorily as valved conduits without significant aneurysmal enlargement, despite the absence of viable VSMCs.[33]

Contrary to the paradigm of medial atrophy and VSMC death in abdominal aortic aneurysm development, we show an increased medial area and a preserved density of VSMCs in ascending thoracic aortic aneurysms. Loss of VSMCs is not necessary for the development of aortic aneurysms. Possible medical interventions to prevent VSMC apoptosis in abdominal aortic aneurysms may not be effective in the treatment of ascending thoracic aortic aneurysms. The present study supports the concept that interactions between mechanical and biological factors lead to pathological vascular remodeling of the arterial wall in aortic aneurysms.

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