Ascending Aortic Proaneurysmal Genetic Mutations with Antiatherogenic Effects

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Abstract

Keywords

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- ▶ carotid intima-media thickness

Thoracic aortic aneurysms are common and are associated with a high morbidity and mortality. Despite this lethal diagnosis, there is an increasing body of evidence to suggest that the diagnosis of an aneurysm, specifically in the ascending thoracic aorta, may significantly reduce the risk of developing systemic atherosclerosis. Clinical observations in the operating room have shown pristine blood vessels in patients undergoing surgery for thoracic aortic aneurysms. There is now evidence that both the carotid intima-media thickness and arterial calcification, which are early and late signs of atherosclerosis respectively, are decreased in those with thoracic aortic aneurysms. These clinical studies are supported by molecular, genetic, and pharmacological evidence. Two principle mechanisms have been identified to explain the relationship of a proaneurysmal state conferring protection from atherosclerosis. These include an excess proteolytic balance of matrix metalloproteinase activity, leading to fragmentation of elastic lamellae and disordered collagen deposition. In addition, transforming growth factor β modulates vascular smooth muscle cells, extracellular matrix, and leukocytes. This confers protection from the initial plaque formation and, later provides stability to the plaque possibly through alteration of the types I and II transforming growth factor β receptor ratio. Furthermore, studies are now beginning to establish an important role for statins and estradiol in modulating these complex pathways. In the future, as our understanding of these complex mechanisms underlying aneurysmal protection against atherosclerosis increases, corresponding therapies may be developed to offer protection from atherosclerosis.

Thoracic aortic aneurysms (TAAs) are known as “silent killers” with no effective general screening strategy. The first symptom is frequently death or a major cardiovascular event such as aortic dissection or rupture.¹ Most recent estimations suggest that the prevalence rate of TAAs is 10.4 per 100,000 in the United States.² However, this is likely to be an underestimation of the true prevalence, as aneurysm-related deaths are commonly classified as “cardiac” and therefore not included in the aneurysm mortality figures. Population-based studies have now also shown that aortic aneurysms are

increasing in incidence.³ Even when identified before severe complications, the only effective intervention for advanced aneurysmal disease is major surgery, which has associated morbidity and mortality.⁴

However, there may be a silver lining to this devastating disease. There is now increasing evidence to suggest that the presence of a TAA may significantly reduce the risk of developing systemic atherosclerosis. Clinical observations have revealed that patients undergoing aortic root replacement for TAA have almost complete absence of any

atheromatous material in both the coronary arteries and aorta. Furthermore, the femoral artery, which is often cannulated during cardiopulmonary bypass, is usually soft and pliable in ascending aneurysm patients.⁵ This is consistent with findings of lower rates of atherosclerosis in type A dissections than type B dissections in a study analyzing 111 autopsy cases.⁶ In addition, patients with TAA have been identified as having a significantly lower incidence of coronary artery disease than those with abdominal aortic aneurysm (AAA).⁷ Genetic mutations rendering patients susceptible to TAA may therefore also offer protection from systemic atherosclerosis. This review focuses on pathology of nonsyndromic aneurysms of the ascending aorta. This is in contrast to descending TAAs and AAAs, which are typically associated with atheroma secondary to atherosclerotic risk

factors.^{8,9} Furthermore, we have also found that 9% of patients with TAA have an associated intracranial aneurysm, which is ninefold greater than the general population.¹⁰

Total Body Calcium Score

To explore the clinical observations of pristine femoral vessels found in the operating theater, we utilized a total body calcium score as a novel late measure of atherosclerosis.⁵ Achneck et al found that ascending TAA and type A dissections are both associated with reduced systemic atherosclerosis in non-Marfanoid individuals.⁵ The total body calcium score was based on the degree of calcification in the right coronary artery, left circumflex coronary artery, left anterior descending coronary artery, ascending aorta, aortic arch,

Fig. 1 Odds ratio of identifying calcification in six different areas of the coronary arteries and aorta. Whisker plots with 95% confidence interval. Reproduced with permission from Achneck et al.⁵ AAE, annuloaortic ectasia; Abd., abdominal aorta; Diss., dissection; LCA, left circumflex coronary artery; Thorax, thoracic aorta.

descending thoracic aorta, and abdominal aorta (total score out of 21 points). The degree of calcification was quantified by an expert radiologist using noncontrast CT scans. Patients in both the ascending TAA group (31 patients) and type A dissection group (33 patients) were found to have fewer calcifications in each of the coronary arteries and each segment of the aorta compared with a control group (86 patients) consisting of trauma patients requiring a CT scan (► Fig. 1). The patients in the type A dissection group had the strongest negative association with atherosclerosis with a calcium score 3.7 points less than the control group ($p < 0.0001$). The ascending TAA group had a calcification score almost 2 points lower than control group ($p < 0.03$). These findings were independent of all known atherosclerotic risk factors.⁵ However, atherosclerosis is a chronic process with calcium deposition occurring in the advanced stages, meaning that total body calcium score is likely to underestimate the true incidence of atherosclerosis.

Carotid Intima-Media Thickness

Carotid artery intima-media thickness (CIMT) is an important alternative measure of atherosclerosis. It is an easily determined, objective measure that can identify atherosclerosis at an earlier stage than total body calcium score.^{5,11} Our group, investigated CIMT in 59 patients with known ascending aortic aneurysms, compared against 29 controls. We found that

patients with ascending aortic aneurysms had a 0.131 mm lower CIMT value than controls ($p = 0.0002$) (► Fig. 2). Patients with aneurysms had CIMT values of 0.50 ± 0.13 versus 0.60 ± 0.11 mm in the control group, independent of risk factors for atherosclerosis.¹² This suggests that proaneurysmal genetic mutations may offer protection from atherosclerosis. This is important because a meta-analysis of eight papers found that a 0.1 mm reduction in CIMT is consistent with a 10 to 15% decreased risk of myocardial infarction (MI) and 13 to 18% decreased risk of stroke.¹³ However, the true protective link between aneurysms and a reduced risk of atherosclerosis is likely to be underestimated in this study. There were significant cardiovascular risk factor discrepancies between the study groups, which may have reduced the magnitude of the observed association. For example, there was a higher proportion of men in the aneurysm group (75 vs. 21%; $p < 0.01$), who are known, on average, to have a 0.046 mm greater CIMT than females.¹² Furthermore, there was a trend toward a higher incidence of hypertension and hyperlipidemia in the aneurysm group.¹² The remarkable consistency between early indicators (CIMT) and late indicators (total body calcium score) of atherosclerosis provides evidence that the ascending aneurysm offers real protection from atherosclerosis (► Fig. 3).^{5,12}

A larger prospective cohort study of 904 patients (502 with aneurysmal disease and 402 with occlusive arterial disease) used CIMT as an outcome measure and had similar findings.¹⁴

Fig. 2 Representative ultrasound comparison of carotid intima-media thickness score between a patient with an ascending thoracic aortic aneurysm and a control. (a) Ultrasound of typically thin and clean distal portion of the right common carotid artery from a patient with an ascending thoracic aortic aneurysm. (b) Enlarged section of image (a). The magenta line indicates the media-adventitia interface and the green line the lumen-intima interface. Carotid intima-media thickness = 0.35 mm. (c) Ultrasound image of typically thicker distal portion of the right common carotid artery from a control patient. (d) Enlarged section of image (c). Carotid intima-media thickness = 0.82 mm. Reproduced with permission from Hung et al.¹²

Fig. 3 Comparison of (a) overall carotid intima-media thickness scores and (b) overall total body calcium scores relative to the control group. Age is depicted as a 10-year interval. BMI is depicted as a 10-unit interval. * $p < 0.5$ and ** $p < 0.01$ were statistically significant. Reproduced with permission from Hung et al.¹² and Achneck et al.⁵ BMI, body mass index; DM, diabetes mellitus; smoke, smoking; dis., dissection; Dyslip., dyslipidemia; FH, family history; IMT, intima-media thickness; HTN, hypertension; G, male gender.

The authors found that aneurysmal patients had a CIMT 0.15 mm lower (95% confidence interval, 0.10–0.20; $p < 0.001$) than occlusive arterial disease patients.¹⁴ However, interpretation of this study is limited because a subgroup analysis was not performed of patients with thoracic and AAAs, which are known to exhibit markedly different pathophysiology.^{8,9}

Myocardial Infarction

Taking this analysis one step further, we studied the relationship between ascending TAAs and the prevalence of MI.¹⁵ Our observational study compared the prevalence of MI in 487 patients who had surgical repair for ascending TAA against 500 control patients. The mean age, gender distribution, and atherosclerosis risk factors' profile were not significantly different between patients from the TAA group and the control group. The analysis showed a 50% reduction in the prevalence of MI in the TAA group ($p < 0.001$) (►Fig. 4). The odds ratio of a patient with an ascending TAA having an MI was near zero at 0.05, more than 20 times less than a patient with a known risk factor for MI. There were also significantly fewer patients with coronary artery disease in the TAA group (61 patients) compared with the control group (140 patients) ($p < 0.001$).¹⁵ This strengthens the evidence that ascending TAAs offer protection against atherosclerosis.

Matrix Metalloproteinases

There is a growing body of evidence to suggest that matrix metalloproteinases (MMPs) play a fundamental role in both aneurysmal and atherosclerotic development. MMPs are known to selectively degrade components of the extracellular matrix (ECM). Tissue inhibitors of MMPs (TIMPs) are important regulators of MMP activity, acting to oppose arterial remodeling and prevent intimal thickening.^{16,17} Both MMPs and TIMPs can be produced constitutively in cells of the vasculature.^{18,19} Characteristic findings in TAAs include ECM degradation leading to fragmentation of elastic lamellae

within the tunica media, ineffective elastogenesis, and disordered collagen deposition.^{20–22}

An established link is recognized between the imbalance of MMP and TIMP activity to the pathophysiology of TAA and atherosclerosis (►Table 1).^{23–25} Work using murine atherosclerotic models with single or combined apolipoprotein E (ApoE) and TIMP-1 deficiency found statistically significant ($p < 0.001$) increases in atherosclerotic lesions throughout the thoracic aorta in mice wild type for TIMP-1. Interestingly, aneurysms were less common in the mice wild type for TIMP-1. Zymographic analysis of aortic extracts revealed higher levels of MMP-2 in TIMP-1-deficient mice. This work indicated, in murine models, that overexpression of TIMP-1 was protective against TAA formation and rupture.²⁶ MMP-1 may also protect against plaque progression; ApoE knockout mice overexpressing MMP-1 in macrophages have significantly reduced atherosclerotic lesion size, lipid deposition, and collagen content.²⁷ Furthermore, Silence et al found that in

Fig. 4 Prevalence of CAD and MI are both significantly lower in patients with ascending thoracic aortic aneurysm versus control. Reproduced with permission from Chau et al.¹⁵ CAD, coronary artery disease; MI, myocardial infarction.

Table 1 A list of suggested MMPs/TIMPs implicated in cardiovascular pathology with relative associations with atherosclerosis and aneurysmal development^a

MMPs/TIMPs	Aneurysmal		Atherosclerotic	
	Proaneurysmal	Antianeurysmal	Proatherosclerotic	Antiatherosclerotic
MMP-1 (interstitial collagenase)	H: AA ⁶⁹		H: CoA, ⁷⁰ H: CaA ⁷¹	
MMP-2 (72 kDa gelatinase, gelatinase A)	NH: AA, ⁷² TA ⁷³	H: TA, ³³ TA ⁷⁴	H: TA, ³³ H: CaA ⁷¹	H: CoA ⁷⁰
MMP-3 (stromelysin-1)	NH: TA ²⁸		H: CoA ⁷⁰	NH: TA, ²⁸ BCA ²³
MMP-7 (matrilysin)	H: AA ⁷⁵			
MMP-8	H: AA ⁷⁶		H: CaA, ⁷¹ H: CoA ⁷⁶	
MMP-9 (92 kDa, gelatinase, gelatinase B)	NH: AA, ⁷² TA, ⁷⁷ TA ²⁵		H: TA, ³³ CoA, ⁷⁰ CoA, ⁷¹ CeA ⁷⁸	NH: BCA ²³
MMP-10			H: CaA ⁷¹	
MMP-12 (matrilysin-2)	H: AA ⁶⁹		NH: BCA, ²³ H: CaA ⁷¹	
MMP-13 (interstitial collagenase-3)	H: TA ⁷⁹		NH: TA ⁸⁰	
MMP-14 (Membrane type 1-MMP)	NH: TA, ⁷⁴ H: CeA ⁸¹	H: AA ⁶⁹	H: CaA ⁷¹	
TIMP-1	H: AA, ⁶⁹ TA ²⁵	NH: TA, ²⁶ TA ²⁴	NH: TA, ²⁶ TA ³³	
TIMP-2	NH: TA, ⁷⁴ H: TA ²⁵	H: AA ⁶⁹	H: TA ³³	
TIMP-3	H: AA ⁶⁹		H: CaA ⁷¹	

Abbreviations: AA, abdominal aorta; BCA, brachiocephalic artery; CaA, carotid artery; CeA, cerebral artery; CoA, coronary artery; H, human study; MMP, matrix metalloproteinase; NH, nonhuman study; TA, thoracic aorta; TIMP, tissue inhibitors of matrix metalloproteinase.

^aPlease note that studies are not completely consistent, especially vis-à-vis pro- or antianeurysmal or atherosclerotic effects. Note also that the increased proteolytic state in TAA disease involves increased MMP activity and decreased TIMP activity (increased MMP/TIMP ratio). Thus, the thesis of our discussion is that proaneurysmal and antiatherogenic potential would vary directly with MMP activity and inversely with TIMP activity.

Note: Blue, MMPs that may be implicated in generating a proaneurysmal and antiatherosclerotic phenotype. Orange denotes MMPs/TIMPs that may be implicated in generating a proaneurysmal and atherosclerotic phenotype.

ApoE-deficient mice, MMP-3 is important for plaque stabilization, potentially by digesting ECM, and is proaneurysmal by degrading the elastic lamina propria.²⁸ One limitation of these in vivo molecular studies is that they do not address how a thin, nonocclusive fibrous cap can lead to atheroma rupture.²⁹

The aorta is heterogeneous in origin and consequently pathological processes have characteristic regional patterns. This, in part, can be explained by differences in the embryologic origin of different parts of the aorta, with the ascending aorta thought to develop from the neural crest, while the descending and abdominal aorta originate from the mesoderm.^{30–32} Therefore, we hypothesize that ascending and descending aortic aneurysms are really two different diseases, with the ligamentum arteriosum being the dividing point (► **Fig. 5**).⁹ Schmoker et al studied the following two distinct histological types of TAA in human samples: atherosclerotic (found in the descending aorta) and nonatherosclerotic (found in the ascending aorta).³³ They found increased MMP-2 and TIMP-1 and TIMP-2 expressions in ascending compared with descending TAA samples which may explain the variation in atherosclerosis in these two distinct parts of the thoracic aorta.³³ Furthermore, our group recognized that MMP-2 and MMP-9 are important in the pathological process of aortic dissections and aneurysms but that expression is significantly increased in those with a dissection.²⁵

It is well recognized that patients with bicuspid aortic valves are strongly associated with TAA development.³⁴ Contemporary opinion suggests that this is a chronic active process of combined tissue remodeling and inflammation.³⁵ Studies of the human thoracic aorta have suggested that bicuspid aortic valves have increased expression of MMP-1, MMP-2, MMP-7, and possibly MMP-9.^{36–40} Furthermore, evidence suggests that MMP/TIMP balance is altered in bicuspid aortic valves depending on where the aortic valve leaflets fuse.⁴¹

There has been investigation into MMPs using genetic studies. This has revealed that polymorphisms in the human MMP-3 promoter are associated with angiographically documented, rapid progression of coronary atherosclerosis.⁴² Furthermore, MMP-3 6A/6A polymorphisms have been found to be protective in human thoracic aortic samples from aneurysmal pathology.⁴³ A common site of atherosclerosis, the carotid artery, shows polymorphisms in the MMP-3 6A/6A and MMP-1 2G/2G to be independent risk factors for internal carotid artery stenosis.^{44–48} This evidence has been translated into human observational studies, further suggesting links between MMP expression and atherogenesis.^{49,50}

A breakdown of the equilibrium between MMPs and TIMPs could be key to aneurysmal and atheromatous development. MMP inhibitors may therefore be an important pharmacological intervention in the future, to counteract the process of aneurysmal development.⁵¹

Fig. 5 Aortic aneurysm is really two diseases: ascending/arch disease differs markedly from descending/abdominal disease. Reprinted with permission from Eleftheriades and Farkas.⁹

Transforming Growth Factor β

It is well established that the 3 isoforms of transforming growth factor β (TGF- β) (TGF- β 1, TGF- β 2, and TGF- β 3) are upregulated in patients with known TAAs.¹⁷ Evidence is now mounting to suggest that TGF- β may also offer protection from atherosclerosis. TGF- β is currently understood to exert its protective effects in two principle ways. First, it involves the maintenance of normal blood vessel structure to prevent fatty streak lesion formation. This is achieved by TGF- β suppression of vascular smooth muscle cell proliferation and migration^{52,53} and increased expression of the principle ECM proteins, collagen, and fibronectin.⁵⁴

The second protective effect exerted by TGF- β involves maintenance of stable atherosclerotic plaques once they have formed. Stable plaques in human coronary arteries have been shown to have higher levels of TGF- β 1 than unstable plaques.⁵⁵ This suggests that TGF- β 1 may reduce the risk of plaque destabilization. This stabilizing role is validated by

another study of patients undergoing carotid endarterectomy, with plaque TGF- β 1 mRNA levels up to threefold higher in asymptomatic plaques.⁵⁶ Raised expression of TGF- β 1 may achieve plaque stability by modulating lymphocytes and macrophages.⁵⁶ Disruption of TGF- β 1 signaling in T lymphocytes results in increased lesion size and the development of an unstable phenotype in humans.⁵⁷ TGF- β has also been shown to inhibit the deleterious transformation of monocytes to macrophages and the production of macrophage foam cells.^{58,59} In addition, TGF- β also maintains plaque stability through promoting ECM production in the fibrous cap. Administering ApoE-deficient mice with neutralizing anti-TGF- β antibody reduces collagen levels and accelerates atherosclerotic lesion development.⁶⁰ These two antiatherogenic effects of TGF- β may be explained by alterations in the types I and II TGF- β receptors.⁶⁰ Healthy vessels have a high ratio of type II:I receptors, however, a transient fall in TGF- β expression causes the type II:I receptor ratio to lower and plaques to form.^{61,62} In summary, the loss of TGF- β leads to leukocyte-rich, matrix-poor plaques at risk of rupture.

Genetic studies also support the antiatherogenic effect of TGF- β with three studies from Europe, Japan, and Iran findings that several polymorphisms that decrease TGF- β levels are associated with acute MI.^{63–65}

The implications of TGF- β in the process of atherogenesis have stimulated investigation into new pharmacological therapeutic strategies. Statin therapy has been shown to increase expression of TGF- β and TGF- β type II receptor.^{61,66} Another study showed that ApoE/low-density lipoprotein receptor double knockout mice administered with atorvastatin had a reduction in the degree of atherosclerosis. This indicates a nonlipid lowering effect of atorvastatin which was consistent with an increase in vessel wall SMAD1 + 2 proteins which are part of the TGF- β cascade and endoglin, which is an auxiliary receptor of TGF- β signaling.⁶⁷ Estradiol has also been implicated in TGF- β synthesis. A study using ApoE knockout mice found that estradiol significantly increased TGF- β levels in the fatty streaks of blood vessels.⁶⁸ This effect was abolished when anti-TGF- β antibodies were applied, and crucially led to a significant increase in lymphocyte infiltration, a key role in the process of atherogenesis.⁵⁶

Conclusion

Undetected TAAs have long been associated with very poor clinical outcomes. However, our observations in the operating room suggest that patients with TAAs have pristine blood vessels. This has been confirmed with total body calcium score and CIMT measurements as well as clinical observation of the occurrence of MIs in patients with TAA, all of which indicate that TAAs may offer protection from systemic atherosclerosis. The mechanism by which this occurs may involve MMP's and the TGF- β pathway, an association which has been validated using a combination of molecular, genetic, and pharmacological investigations.

There are however many gaps in our current understanding of TAA as a protective mechanism for atherosclerosis formation. Murine models provide valuable information

regarding the molecular pathways involved in atherosclerotic and aneurysmal development however careful interpretation of these models must be made to ensure the correct human correlation. It remains to be established how local dilatations of the thoracic aorta offer protection against systemic atherosclerosis. Currently, we need to further understand the natural history of these modulatory pathways to accurately determine how this changes in disease. Also, it remains to be established whether the protection provided by TAA extends across all age groups, as the total body calcium score, CIMT, and MI studies all had an approximate average age of 60 years. The aorta is a heterogeneous vascular structure, composed of segments of different embryological origins, offering additional complexities in understanding the underlying mechanisms.

As our understanding of the underlying mechanisms develops, therapeutic strategies to protect against atherosclerosis may be possible (with this review highlighting the potential for drugs such as statins and estradiol to combat cardiovascular disease).

It is important to note that within the literature there is conflicting evidence regarding the true molecular pathways underpinning atherosclerosis and aneurysm development. This highlights the complexity of the pathways and mechanisms associated with atherosclerosis and the need of additional clarification. The diagnosis of TAA, which has such a bleak outlook for the patient, now appears to have a silver lining in the form of protection from atherosclerosis.

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