Manuscript Number: ATVB/2016/307909R1

Title: The role of microvascular tone and extracellular matrix contraction in the regulation of interstitial fluid: implications for aortic dissection

Authors: Ziad Mallat (University of Cambridge), Alain Tedgui (INSERM UMR-S 970), and Daniel Henrion (CNRS UMR 6214 - INSERM U1083)

The role of microvascular tone and extracellular matrix contraction in the regulation of interstitial fluid: implications for aortic dissection

Myogenic tone, interstitial fluid and aortic dissection

Ziad Mallat[1,2], Alain Tedgui[2], Daniel Henrion[3]

[1]Department of Medicine, Division of Cardiovascular Medicine,
University of Cambridge,
Cambridge, United Kingdom

[2]Institut National de la Santé et de la Recherche Médicale (Inserm) U970, Paris, France

[3]Inserm U1083, Centre National de la Recherche Scientifque (CNRS) UMR6214,
Laboratoire de Biologie Neurovasculaire et Mitochondriale Intégrée, Angers, France

Correspondence to:
Ziad Mallat, MD, PhD, Department of Medicine, Division of Cardiovascular Medicine, University of Cambridge, Cambridge, United Kingdom. E-mail:
zm255@medschl.cam.ac.uk

Keywords: aortic dissection; aneurysm; extracellular fluid; contraction; vasa vasorum.

Subject codes: Vascular Disease: Aneurysm; Aortic Dissection.

Word count: 4919.

Total number of Figures: 2.

Abstract

The pathophysiology of aortic dissection is poorly understood and its risk is resistant to medical treatment. Most studies have focused on a proposed pathogenic role of transforming growth factor (TGF)-β in Marfan disease and related Thoracic Aortic Aneurysms and Aortic Dissections (TAADs). However, clinical testing of this concept using angiotensin II type 1 receptor antagonists to block TGF-β signaling fell short of promise. Genetic mutations that predispose to TAADs affect components of the extracellular matrix (ECM) and proteins involved in cellular force generation. Thus, a role for dysfunctional mechanosensing in abnormal aortic wall remodeling is emerging. However, how abnormal mechanosensing leads to aortic dissection remains a mystery. Here, we review current knowledge about the regulation of interstitial fluid dynamics and myogenic tone, and propose that alteration in contractile force reduces vascular tone in the microcirculation (here, aortic vasa vasorum), and leads to elevations of blood flow, transmural pressure and fluid flux into the surrounding aortic media. Furthermore, reduced contractile force in medial smooth muscle cells coupled with alteration of structural components of the ECM limits ECM contraction, further promoting the formation of intramural edema, a critical step in the initiation of aortic dissection. The concept is supported by several pathophysiological and clinical observations. A direct implication of this concept is that drugs that lower blood pressure and limit interstitial fluid accumulation while preserving or increasing microvascular tone would limit the risk of dissection. In contrast, drugs that substantially lower microvascular tone would be ineffective, or may accelerate the disease and precipitate aortic dissection.

List of abbreviations:

ECM: Extracellular matrix
GAGs: Glycosaminoglycans
MT: Myogenic tone
Pif: Interstitial fluid pressure
SMC: Smooth muscle cell
TAAD: Thoracic Aortic Aneurysms and Aortic Dissections
TGF: Transforming growth factor
TRP: Transient receptor potential

Genetic mutations that predispose to TAADs affect structural components of the ECM, transmembrane structures involved in mechanical integrity, mechanosensing and signal transduction, or intracellular components of the cell contractile apparatus [1-3]. Concepts and studies are being developed to explain how such alterations may impact the response of the aorta to hemodynamic stress and drive abnormal aortic remodeling [4]. Here, we propose a focus shift towards the small vessels (arterioles and vasa vasorum) to explain, based on current knowledge about the (patho)physiology of blood flow regulation and fluid dynamics, how alterations in ECM and the cell contractile apparatus may instigate the frightening process of aortic dissection.

Microcirculatory vascular tone and the autoregulation of blood flow: the example of myogenic tone

Microcirculatory vascular tone is the main determinant of vascular resistance, and ensures tissue perfusion in response to changing metabolic demands. An increase in pressure induces a rapid and reversible vasoconstriction of small resistance vessels, an intrinsic property due to their ability to develop myogenic tone (MT)[5]. Besides protecting downstream capillaries from abrupt increases in blood pressure occurring in larger vessels, MT has a key role in the inward eutrophic vascular remodeling in response to a chronic rise in pressure.

Pressure induces deformation of cell membrane proteins such as stretch-activated ion channels; conformational changes in matrix and cytoskeletal proteins; activation of ECM and integrins, as well as activation of various cellular junctions (Figure 1). Vascular smooth muscle cell (SMC) depolarization and calcium entry are necessary for the development of pressure-induced contraction and myogenic tone. Although the nature of the channel(s) involved in MT development remains debated, canonical transient receptor potential (TRP) channels are strong candidates. Interactions of TRP Polycystin 1 and 2 (TRPP1 and TRPP2) with filamin A, an actin crosslinking protein, are critical for stretch-activated ion channel regulation and MT development[6]. Indirect evidence for a role of chloride channels in MT has also been suggested [7]. G-protein coupled receptors like the angiotensin type 1 receptor possess mechanosensitive properties, independent of ligand binding, and may activate TRPs[5, 8]. Recent studies also showed that interactions between SMCs and ECM are involved in MT. Pressure-induced deformation of ECM elements may activate integrins (e.g., β1, β3)[9], and induces cytoskeletal remodeling and contraction through modulation of stretch-activated ion channel/TRP and membrane depolarization[10]. Calcium sensitization of the contractile apparatus through RhoA/Rho-kinase and actomyosin crossbridge cycling allows force maintenance without high intracellular calcium concentration[5]. In addition to vascular SMCs, pericyte constriction in pre-capillaries involves similar transduction pathways and may play a significant role in the regulation of tissue perfusion[11].

Interstitial fluid pressure

Transcapillary fluid flux is also highly controlled by the level of interstitial fluid pressure (Pif) exerted on the outside of the capillaries. A reduction of Pif promotes and maintains

edema formation (Figure 2). Composition and functional properties of the ECM are major determinants of the level of Pif [12, 13]. Briefly, polyanionic glycosaminoglycans (GAGs) of the ECM are osmotically active, whereas collagens, elastic fibers and microfibrils play important roles in ECM contraction and oppose the decrease of Pif. In steady state, GAGs are under-hydrated. When fluid gains access to the interstitial space, the osmotically active GAGs absorb water molecules and swell, which tends to limit the increase of Pif due to an increase of interstitial volume. This phenomenon sustains edema formation. In that case, opposing contraction of the interstitium, driven by the physical properties of the microfibril/collagen network and force generation by its cellular constituents, plays a critical balancing effect and limits edema formation (reviewed in [12, 13]).

Genes associated with TAADs are involved in the regulation of microvascular tone and interstitial fluid pressure

Microvascular tone: Genetic mutations that predispose to TAADs affect components of the ECM and proteins involved in cellular force generation[1]. It is quite remarkable that many of those pathways are involved in the regulation of microvascular tone. This is the case of mutations altering pressure sensing (filamin A *FLNA*, and polycystins *PDK1* and *PKD2*), or mutations altering SMC contraction (myosin light chain kinase *MYLK*). Other pathways/genes involved in TAADs are expected to alter microvascular tone. This is the case of *ACTA2* (actin alpha 2, smooth muscle) and *MYH11* (myosin heavy chain 11, smooth muscle), which are involved in SMC contraction; gain-of-function mutation in *PRKG1* (protein kinase, cGMP-dependent, type I, involved in SMC relaxation), or mutations in *PLOD1* (procollagen-lysin, 2-oxoglutarate 5-dioxygenase 1, associated with severe muscle hypotonia). Mutations in *TGFB2*, *TGFBR1*, *TGFBR2* and *SMAD3*, which are associated with Loeys-Dietz syndrome, do not increase but rather limit TGF-β signaling in vitro[14]. In that regard, it is interesting to note that TGF-β activity regulates the arteriolar myogenic response[15], suggesting that reduced TGF-β signaling in Loeys-Dietz syndrome may impair microcirculatory tone. Other TAAD-related mutations affecting ECM components (e.g., COL3A1) have not been related directly to alteration of vascular tone. However, it is intriguing to note that COL3A1 may couple GPR56, an atypical G-protein coupled receptor, to the Gαq12/13 family of G proteins and may activate RhoA[16], a prominent pathway with essential role in the generation of vascular tone.

Interstitial fluid pressure: As summarized above, given the major roles of microfibril/collagen network in the regulation of Pif [12, 13], both direct (fibrillin-1 *FBN1* in Marfan syndrome, collagen type III A 1 *COL3A1* in Ehlers-Danlos syndrome, collagen type III A1 *COL1A*, etc.) and indirect (genes involved in TGF-β signaling) alterations of ECM components are expected to alter Pif. Depending on the context, both β1 and β3 integrins may modulate the generation and maintenance of Pif [17, 18]. Contraction of connective tissue is also modulated through force generation by its cellular components. Therefore, alteration in force generation (e.g., *MYLK*) may significantly impair Pif. Intriguingly, recent data indicate that TGF-β activity also regulates aldosterone levels, and sodium and water retention[19], suggesting that reduced TGF-β signaling in Loeys-

Dietz syndrome or inhibition of TGF-β signaling in Marfan, may both impair microvascular tone and promote interstitial fluid accumulation.

Alteration of microvascular tone and interstitial pressure as major determinants of aortic dissection

Based on this knowledge, we propose that a decrease of vascular tone (intrinsic or iatrogenic) upstream of or within the aortic vasa vasorum will lead to alteration of blood flow regulation and increased transmural pressure, which will promote the development of intra-parietal (adventitial and medial) edema (and potentially hemorrhage, dependent on the degree of alteration of microvessel integrity), which will instigate the process of aortic dissection. Any associated alteration of ECM that prevents an increase of Pif will further promote and sustain intramural edema. Additional alterations in draining lymphatics [20] (including reduced myogenic tone [21] or abnormal pulse transfer [22]) or in force components controlling transcapillary fluid flux [23, 24] will act as aggravating factors. Finally, alterations in endothelial cells and glycocalyx (as it may be the case at branching arterial sites or in areas of low shear stress) would alter vessel permeability and increase the porosity and hydration of the medial interstitium, particularly in response to increased intravascular pressure [25-28], and would further promote interstitial edema formation.

Pathophysiological predictions of the model

Aortic dissection is believed to originate with a tear in the intima [29-31]. However, a true intimal rupture may be absent in a substantial percentage of aortic dissections.
Given that vasa vasorum only penetrate the outer third of the thoracic aortic media[32], the model presented here predicts that a large percentage if not most aortic dissections in humans will start by the formation of an intramural edema and a small tear in the outer third of the media. Recurrence and coalescence of small medial tears will eventually induce aortic medial delamination, which may culminate in the formation of a false channel if the tear reaches the intima.
This hypothesis is supported by the presence of leaky neovessels in the media of human TAADs[33] and by recent extensive histopathological analyses showing that most (20 out of 21) aortic tears originate in the outer third of the media alongside the vasa vasorum[34].

The model also provides an intriguing explanation for the detection of foci of GAGs in the outer media of diseased aortas, which are considered to induce significant stress concentrations and intra-lamellar Donnan swelling pressures [35, 36]. The mechanism behind increased detection of GAGs is unknown, but the current paradigm refers to increased GAG production [37, 38]. The model presented here suggests an additional or alternative mechanism whereby access of fluid to the interstitial space (secondary to alteration of microvascular tone and Pif) will inevitably induce swelling of the osmotically active and under-hydrated GAGs, leading to expansion of the vessel area occupied by GAGs. This would limit the increase of Pif (particularly in case of abnormal microfibril/collagen network) and would alter stress transfer within the aortic wall,

potentially initiating a vicious circle. It is also interesting to note that GAGs may modulate local generation of several active modulators of capillary leakage [39].
Since vasa vasorum are normally absent from the human abdominal aortic media [40], a further implication of the model is that medial dissection would be a rare event in the human abdominal aorta, a prediction supported by ample clinical observation. However, adventitial remodeling (with abnormal lymphatic drainage, inflammatory changes and neoangiogenesis) may become more prominent in the latter setting [41, 42]. This would also apply to aortas of small animals (e.g., rodents) where we propose that additional factors (e.g., hypertension [43], increased sodium/chloride and water reabsorption [43-45], and abnormal endothelial function [25, 26]) may combine together to ultimately increase interstitial fluid retention in adventitia and outer media, the major process that would instigate aortic dissection.

Clinical predictions and implications of the model

A potential power of the model is its suitability to pre-clinical and clinical testing.
A recent study showed that pressure-induced intramural delamination was associated with accumulation of interstitial fluid in aortic media of mice with disrupted TGF-β signaling, and could be prevented in part by pharmacologic improvement of SMC contractility ex vivo[46]. The results are consistent with the present hypothesis on the role of ECM contraction in limiting interstitial fluid retention and delamination. However, further studies are needed to examine whether the same results may apply to sub-vessel level SMC contraction in human thoracic aorta.

A major direct implication of the model relates to its potential to predict the effectiveness of medical strategies in patients with TAADs. According to the hypothesis presented here, drugs that lower blood pressure while preserving or increasing microvascular tone and Pif would limit the occurrence of aortic dissection. In contrast, drugs that further lower microvascular tone and Pif or promote interstitial fluid accumulation would be ineffective or may even precipitate aortic dissection.
As such, the model may support the effectiveness of beta-blockers in reducing aortic root dilatation in patients with Marfan syndrome [47, 48] and suggests that they could be useful in limiting vascular complications in other TAADs. Interestingly, a recent pilot trial reported a protective effect of beta-blockers in patients with Ehlers Danlos syndrome [49].
In contrast, the model predicts that calcium channel blockers, which substantially inhibit myogenic tone, would be ineffective or might accelerate disease progression and aortic dissection in TAADs. This hypothesis merits serious consideration. Interestingly, the results of a recent observational and retrospective study by Doyle et al. suggest that the use of calcium channel blockers in patients with Marfan syndrome and other forms of TAADs may be associated with increased risk of aortic dissection and need for surgery, in comparison with the use of other antihypertensive agents[50]. Following a series of mechanistic experimental studies in Marfan mice, Doyle et al. attributed the increased risk of aortic dissection to enhanced activation of protein kinase C beta pathway, and showed that the clinically available anti-hypertensive agent hydralazine both limited protein kinase C beta activation and normalized aortic growth in Marfan mice. This led the authors to consider hydralazine ‘an appealing alternative therapeutic strategy for

Marfan syndrome'[50]. In contrast, the model presented here does not support the use of hydralazine in the human setting of Marfan disease or other TAADs given the expected inhibitory effect of hydralazine on myogenic tone. Similarly, our model also suggests that the recent failure of losartan in Marfan patients [48, 51] may be due, at least in part, to the inhibitory effect of angiotensin type 1 receptor blockade on myogenic tone. Thus, we believe that modulation of blood pressure in TAAD patients should be considered in light of potential side effects that may result from excessive inhibition of myogenic tone. In the future, other pathways involved in maintenance of vascular integrity and regulation of vascular tone, e.g., selective sphingosine 1 phosphate signaling pathways [52-55], may be worth of consideration as potential adjunctive therapeutic targets.

Treatment with diuretics may limit the expansion of interstitial fluid and may constitute a therapeutic option. However, treatment with high-ceiling diuretics (e.g., bumetanide, furosemide) should be considered with caution, as they may reduce myogenic tone through inhibition of Na-K-Cl cotransporter (NKCC)1 [56]. Mineralocorticoid receptor antagonists (e.g., spironolactone, eplerenone) could also reduce NKCC1 activity and myogenic tone, although to a lower extent [57, 58]. This is supported by the fact that selective deletion of mineralocorticoid receptor SMCs does not alter myogenic tone in adult young mice [59]. An appropriate diuretic should therefore show a favorable profile between its beneficial effects on interstitial fluid accumulation and its potential deleterious impact on myogenic tone.
Other strategies to modulate Pif may be worth of investigation. This could be achieved through modulation of interstitial volume, direct modulation of synthesis/degradation and functionality of ECM components (e.g., GAGs, integrins) or through the targeting of 'inflammatory' mediators involved in edema formation (e.g., kinins, prostaglandins).

Finally, the model may have implications for the development and use of new diagnostic technologies. For example, imaging modalities that accurately detect interstitial edema may be of additive value in predicting aortic disease progression and susceptibility to dissection.

In conclusion, we propose that alterations of microvascular tone and interstitial pressure play a major role in the progression of TAADs and the initiation of aortic dissection. An effective therapeutic strategy should aim to limit hemodynamic stress on the large arterial conduit, reduce transcapillary fluid flux and interstitial fluid accumulation, while maintaining or increasing microvascular tone.

ACKNOWLEDGEMENTS

The authors would like to thank Bernard Lévy for helpful discussions.
We apologize for not being able to reference all primary evidence due to lack of space.

SOURCE OF FUNDING

British Heart Foundation, UK, and Institut National de la Santé et de la Recherche Médicale, France.

DISCLOSURES

None.

REFERENCES

1. Milewicz DM, Guo DC, Tran-Fadulu V, Lafont AL, Papke CL, Inamoto S, Kwartler CS, Pannu H. Genetic basis of thoracic aortic aneurysms and dissections: Focus on smooth muscle cell contractile dysfunction. *Annu Rev Genomics Hum Genet*. 2008;9:283-302.
2. Gillis E, Van Laer L, Loeys BL. Genetics of thoracic aortic aneurysm: At the crossroad of transforming growth factor-beta signaling and vascular smooth muscle cell contractility. *Circ Res*. 2013;113:327-340.
3. Jondeau G, Boileau C. Familial thoracic aortic aneurysms. *Curr Opin Cardiol*. 2014;29:492-498.
4. Humphrey JD, Milewicz DM, Tellides G, Schwartz MA. Cell biology. Dysfunctional mechanosensing in aneurysms. *Science*. 2014;344:477-479.
5. Kauffenstein G, Laher I, Matrougui K, Guerineau NC, Henrion D. Emerging role of g protein-coupled receptors in microvascular myogenic tone. *Cardiovasc Res*. 2012;95:223-232.
6. Sharif-Naeini R, Folgering JH, Bichet D, et al. Polycystin-1 and -2 dosage regulates pressure sensing. *Cell*. 2009;139:587-596.
7. Schubert R, Brayden JE. Stretch-activated cation channels and the myogenic response of small arteries. In: Kamkin A, Kiseleva I, eds. Mechanosensitivity in cells and tissues. Moscow; 2005.
8. Schleifenbaum J, Kassmann M, Szijarto IA, Hercule HC, Tano JY, Weinert S, Heidenreich M, Pathan AR, Anistan YM, Alenina N, Rusch NJ, Bader M, Jentsch TJ, Gollasch M. Stretch-activation of angiotensin ii type 1a receptors contributes to the myogenic response of mouse mesenteric and renal arteries. *Circ Res*. 2014;115:263-272.
9. Martinez-Lemus LA, Crow T, Davis MJ, Meininger GA. Alphavbeta3- and alpha5beta1-integrin blockade inhibits myogenic constriction of skeletal muscle resistance arterioles. *Am J Physiol Heart Circ Physiol* 2005;289:H322-9.
10. Hill MA, Meininger GA. Arteriolar vascular smooth muscle cells: Mechanotransducers in a complex environment. *Int J Biochem Cell Biol*. 2012;44:1505-1510.
11. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*. 2014;508:55-60.
12. Reed RK, Rubin K. Transcapillary exchange: Role and importance of the interstitial fluid pressure and the extracellular matrix. *Cardiovasc Res*. 2010;87:211-217.
13. Reed RK, Liden A, Rubin K. Edema and fluid dynamics in connective tissue remodelling. *J Mol Cell Cardiol*. 2010;48:518-523.
14. Gallo EM, Loch DC, Habashi JP, et al. Angiotensin ii-dependent tgf-beta signaling contributes to loeys-dietz syndrome vascular pathogenesis. *J Clin Invest*. 2014;124:448-460.
15. Litteri G, Carnevale D, D'Urso A, et al. Vascular smooth muscle emilin-1 is a regulator of arteriolar myogenic response and blood pressure. *Arterioscler Thromb vasc Biol*. 2012;32:2178-2184.

16. Luo R, Jeong SJ, Jin Z, Strokes N, Li S, Piao X. G protein-coupled receptor 56 and collagen iii, a receptor-ligand pair, regulates cortical development and lamination. *Proc Natl Acad Sci U S A*. 2011;108:12925-12930.
17. Reed RK, Rubin K, Wiig H, Rodt SA. Blockade of beta 1-integrins in skin causes edema through lowering of interstitial fluid pressure. *Circ Res*. 1992;71:978-983.
18. Liden A, Berg A, Nedrebo T, Reed RK, Rubin K. Platelet-derived growth factor BB-mediated normalization of dermal interstitial fluid pressure after mast cell degranulation depends on beta3 but not beta1 integrins. *Circ Res*. 2006;98:635-641.
19. Kakoki M, Pochynyuk OM, Hathaway CM, et al. Primary aldosteronism and impaired natriuresis in mice underexpressing tgfbeta1. *Proc Natl Acad Sci U S A*. 2013;110:5600-5605.
20. Machnik A, Neuhofer W, Jantsch J, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*. 2009;15:545-552.
21. Scallan JP, Wolpers JH, Davis MJ. Constriction of isolated collecting lymphatic vessels in response to acute increases in downstream pressure. *J Physiol*. 2013;591:443-459.
22. Parsons RJ, McMaster PD. The effect of the pulse upon the formation and flow of lymph. *J Exp Med*. 1938;68:353-376.
23. Guyton AC, Granger HJ, Taylor AE. Interstitial fluid pressure. *Physiol Rev*. 1971;51:527-563.
24. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev*. 1993;73:1-78.
25. Tedgui A, Lever MJ. Filtration through damaged and undamaged rabbit thoracic aorta. *Am. J. Physiol.* 1984;247:H784-H791.
26. Tedgui A, Lever MJ. Effect of pressure and intimal damage on ^{131}I-albumin and ^{14}C-sucrose spaces in aorta. *Am J Physiol*. 1987;253:H1530-H1539.
27. Levick JR, Michel CC. Microvascular fluid exchange and the revised starling principle. *Cardiovasc Res*. 2010;87:198-210.
28. Curry FE, Adamson RH. Endothelial glycocalyx: Permeability barrier and mechanosensor. *Ann Biomed Eng*. 2012;40:828-839.
29. O'Gara PT, DeSanctis RW. Acute aortic dissection and its variants. Toward a common diagnostic and therapeutic approach. *Circulation*. 1995;92:1376-1378.
30. Golledge J, Eagle KA. Acute aortic dissection. *Lancet*. 2008;372:55-66.
31. Erbel R, Aboyans V, Boileau C, et al. 2014 ESC guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The task force for the diagnosis and treatment of aortic diseases of the European Society of Cardiology (ESC). *Eur Heart J*. 2014;35:2873-2926.
32. Wolinsky H, Glagov S. Nature of species differences in the medial distribution of aortic vasa vasorum in mammals. *Circ Res*. 1967;20:409-421.
33. Kessler K, Borges LF, Ho-Tin-Noe B, Jondeau G, Michel JB, Vranckx R. Angiogenesis and remodelling in human thoracic aortic aneurysms. *Cardiovasc Res*. 2014;104:147-159.

34. Osada H, Kyogoku M, Ishidou M, Morishima M, Nakajima H. Aortic dissection in the outer third of the media: What is the role of the vasa vasorum in the triggering process? *Eur Cardiothorac Surg*. 2013;43:e82-88.
35. Roccabianca S, Ateshian GA, Humphrey JD. Biomechanical roles of medial pooling of glycosaminoglycans in thoracic aortic dissection. *Biomech Model Mechanobiol*. 2014;13:13-25.
36. Sorrentino TA, Fourman L, Ferruzzi J, Miller KS, Humphrey J, Roccabianca S. Local versus global mechanical effects of glycosaminoglycans in carotid arteries. *J Biomech Eng*. 2015;137:041008. doi: 10.1115/1.4029303.
37. Humphrey JD. Possible mechanical roles of glycosaminoglycans in thoracic aortic dissection and associations with dysregulated transforming growth factor-beta. *J Vasc Res*. 2013;50:1-10.
38. Bellini C, Wang S, Milewicz DM, Humphrey JD. MYH11(R247C/R247C) mutations increase thoracic aorta vulnerability to intramural damage despite a general biomechanical adaptivity. *J Biomech*. 2015;48:113-121.
39. Renne T, Schuh K, Muller-Esterl W. Local bradykinin formation is controlled by glycosaminoglycans. *J Immunol*. 2005;175:3377-3385.
40. Wolinsky H, Glagov S. Comparison of abdominal and thoracic aortic medial structure in mammals. Deviation of man for usual pattern. *Circ Res*. 1969;25:677-686.
41. Michel JB, Martin-Ventura JL, Egido J, Sakalihasan N, Treska V, Lindholt J, Allaire E, Thorsteinsdottir U, Cockerill G, Swedenborg J. Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. *Cardiovasc Res*. 2011;90:18-27.
42. Sano M, Sasaki T, Hirakawa S, Sakabe J, Ogawa M, Baba S, Zaima N, Tanaka H, Inuzuka K, Yamamoto N, Setou M, Sato K, Konno H, Unno N. Lymphangiogenesis and angiogenesis in abdominal aortic aneurysm. *PLoS ONE*. 2014;9:e89830.
43. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein e-deficient mice. *J Clin Invest*. 2000;105:1605-1612.
44. Liu S, Xie Z, Daugherty A, Cassis LA, Pearson KJ, Gong MC, Guo Z. Mineralocorticoid receptor agonists induce mouse aortic aneurysm formation and rupture in the presence of high salt. *Arterioscler Thromb Vasc Biol*. 2013;33:1568-1579.
45. Nishijo N, Sugiyama F, Kimoto K, Taniguchi K, Murakami K, Suzuki S, Fukamizu A, Yagami K. Salt-sensitive aortic aneurysm and rupture in hypertensive transgenic mice that overproduce angiotensin II. *Lab Invest*. 1998;78:1059-1066.
46. Ferruzzi J, Murtada SI, Li G, Jiao Y, Uman S, Ting MY, Tellides G, Humphrey JD. Pharmacologically improved contractility protects against aortic dissection in mice with disrupted transforming growth factor-beta signaling despite compromised extracellular matrix properties. *Arterioscler Thromb Vasc Biol*. 2016;36:919-927.
47. Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in marfan's syndrome. *N Engl J Med*. 1994;330:1335-1341.
48. Lacro RV, Dietz HC, Sleeper LA, et al. Atenolol versus losartan in children and

young adults with marfan's syndrome. *N Engl J Med*. 2014;371:2061-2071.
49. Ong KT, Perdu J, De Backer J, et al. Effect of celiprolol on prevention of cardiovascular events in vascular ehlers-danlos syndrome: A prospective randomised, open, blinded-endpoints trial. *Lancet*. 2010;376:1476-1484.
50. Doyle JJ, Doyle AJ, Wilson NK, et al. A deleterious gene-by-environment interaction imposed by calcium channel blockers in marfan syndrome. *eLife*. 2015;4. pii: e08648. doi: 10.7554/eLife.08648.
51. Milleron O, Arnoult F, Ropers J, et al. Marfan sartan: A randomized, double-blind, placebo-controlled trial. *Eur Heart J*. 2015;36:2160-2166.
52. Bolz SS, Vogel L, Sollinger D, Derwand R, Boer C, Pitson SM, Spiegel S, Pohl U. Sphingosine kinase modulates microvascular tone and myogenic responses through activation of RhoA/Rho kinase. *Circulation*. 2003;108:342-347.
53. Ohmori T, Yatomi Y, Osada M, Kazama F, Takafuta T, Ikeda H, Ozaki Y. Sphingosine 1-phosphate induces contraction of coronary artery smooth muscle cells via S1P2. *Cardiovasc Res*. 2003;58:170-177.
54. Murakami A, Takasugi H, Ohnuma S, Koide Y, Sakurai A, Takeda S, Hasegawa T, Sasamori J, Konno T, Hayashi K, Watanabe Y, Mori K, Sato Y, Takahashi A, Mochizuki N, Takakura N. Sphingosine 1-phosphate (S1P) regulates vascular contraction via S1P3 receptor: investigation based on a new S1P3 receptor antagonist. M*ol Pharmacol*. 2010;77:704-713.
55. Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, Pham TH, Wong JS, Pappu R, Coughlin SR. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J Clin Invest*. 2009;119:1871-1879.
56. Orlov SN, Koltsova SV, Tremblay J, Baskakov MB, Hamet P. NKCC1 and hypertension: Role in the regulation of vascular smooth muscle contractions and myogenic tone. *Ann Med*. 2012;44 Suppl 1:S111-118.
57. Jiang G, Cobbs S, Klein JD, O'Neill WC. Aldosterone regulates the Na-K-2Cl cotransporter in vascular smooth muscle. Hypertension 2003;41:1131-5.
58. Pires PW, Jackson WF, Dorrance AM. Regulation of myogenic tone and structure of parenchymal arterioles by hypertension and the mineralocorticoid receptor. *Am J Physiol Heart Circ Physiol*. 2015;309:H127-H136.
59. McCurley A, Pires PW, Bender SB, Aronovitz M, Zhao MJ, Metzger D, Chambon P, Hill MA, Dorrance AM, Mendelsohn ME, Jaffe IZ. Direct regulation of blood pressure by smooth muscle cell mineralocorticoid receptors. *Nat Med*. 2012;18:1429-1433.

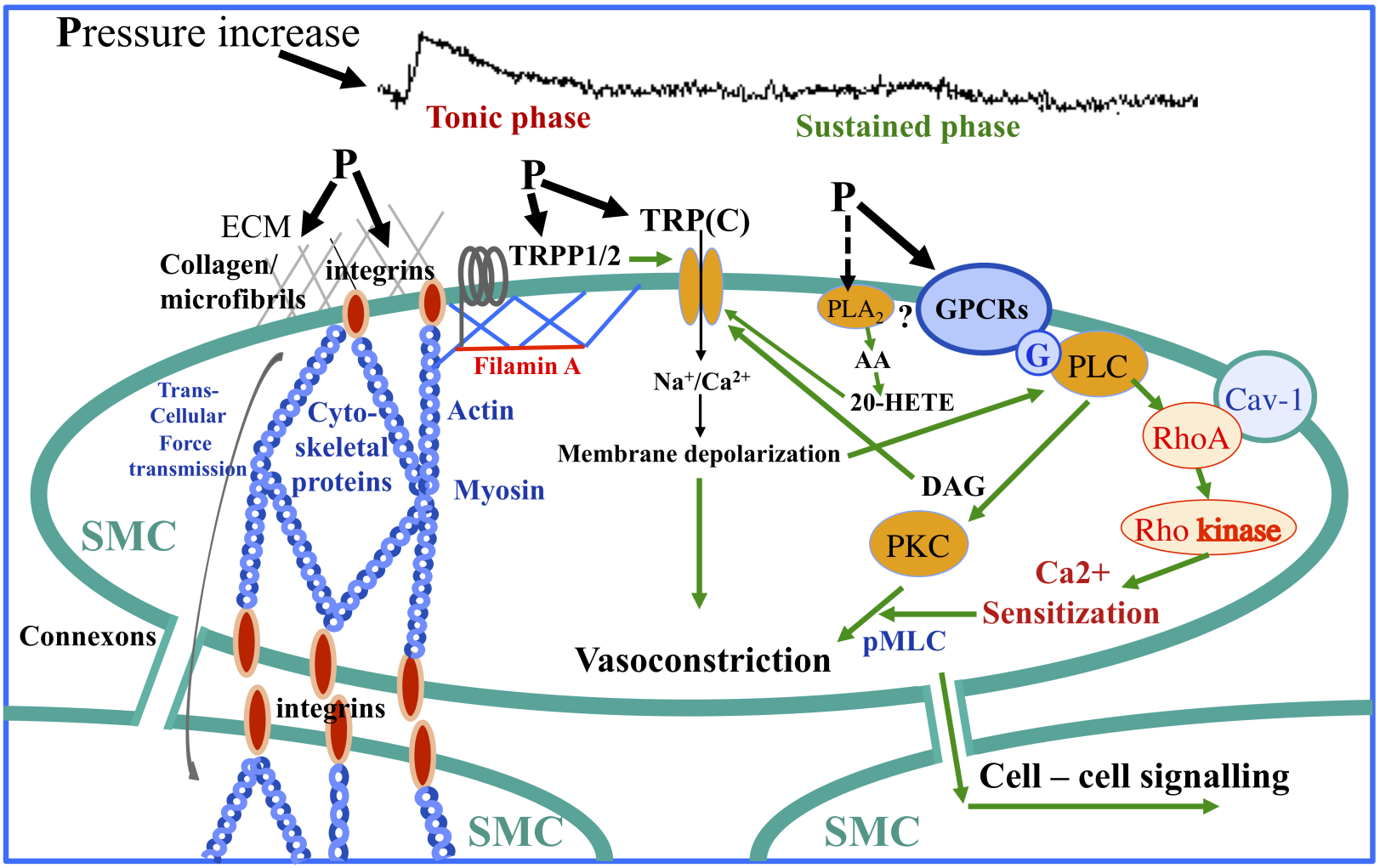
HIGHLIGHTS:

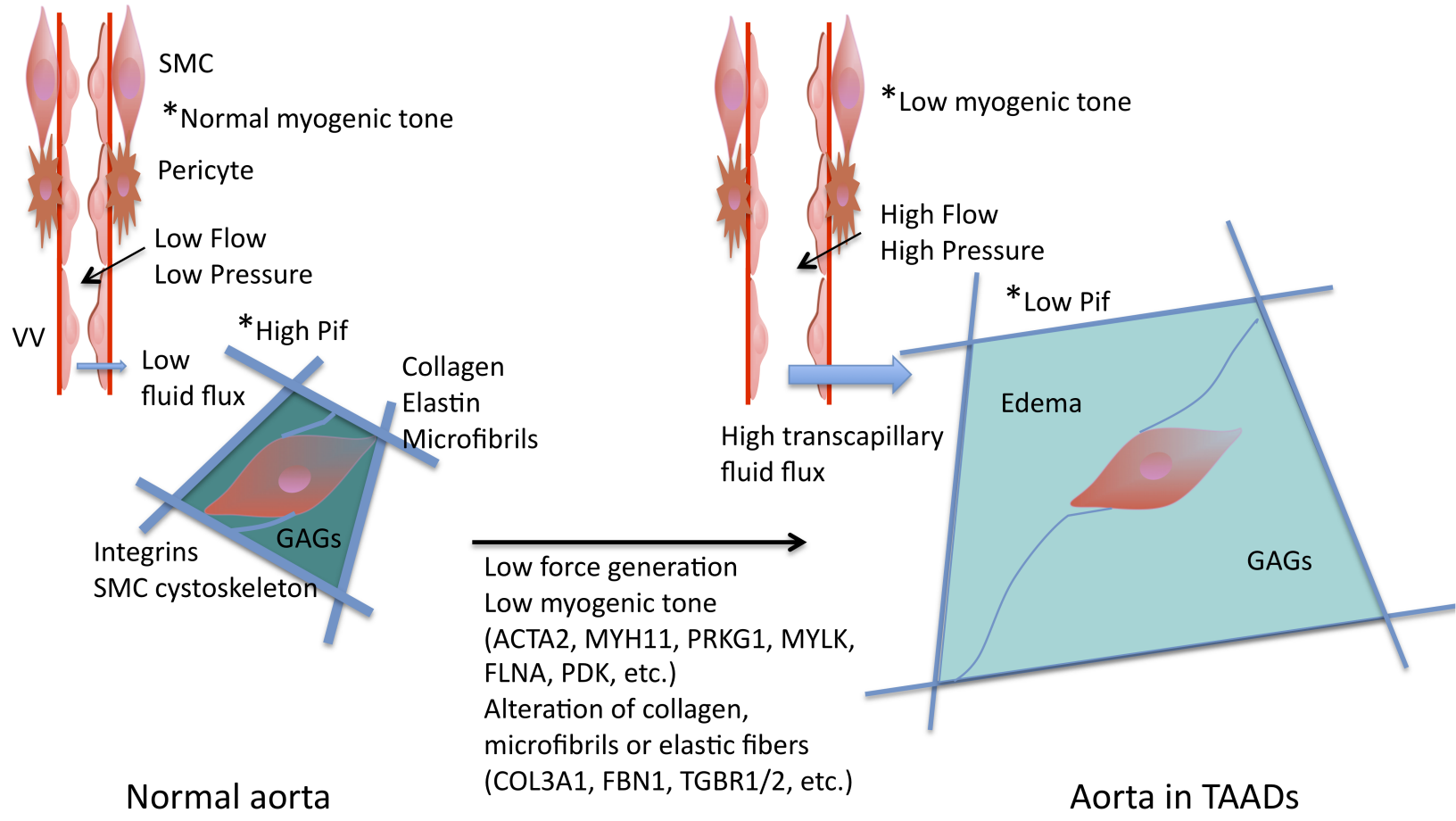
- Genetic mutations that predispose to thoracic aortic aneurysms and aortic dissections affect components of the extracellular matrix and proteins involved in cellular force generation.
- We propose that those alterations lead to reduced myogenic tone in vasa vasorum and defective regulation of interstitial fluid pressure in the aortic wall
- Those events create increased accumulation of interstitial fluid, and induce intra-lamellar swelling pressures and significant stress concentrations around foci of glycosaminoglycans, which may promote aortic wall delamination and dissection
- Strategies that limit hemodynamic stress on the aortic wall, reduce transcapillary fluid flux and interstitial fluid accumulation, while maintaining or increasing myogenic tone, should be effective in the prevention of aortic dissection.

FIGURE LEGENDS

Figure 1. Summary of the mechanism of myogenic tone with a focus on pathways associated with TAADs.
In response to an increase in blood pressure (**P**), a resistance artery contracts in response to the activation of cationic influx through stretch-activated channels such as transient potential (TRP) channels, possibly TRPC. Pressure may activate several pathways, which may act simultaneously and/or synergistically. Beside the direct activation of TRP channels by pressure, the interaction of polycystin 1 and 2 (TRPP 1 and 2) with filamin A and the actin cytoskeleton may induce TRP opening. TRP activation leads to Na^+ and Ca^{2+} entry, membrane depolarization and contraction. Deformation of the extracellular matrix (ECM) by pressure may be transmitted to the integrins and the cytoskeleton and transmitted to the surrounding smooth muscle cells (SMC). Alternatively, pressure may also activate G protein-coupled receptor such as AT1R and subsequently stimulate TRP either directly or through phospholipase C (PLC) activation and diacylglycerol (DAG) production. DAG activates protein kinase C and SMC contraction. Phospholipase A2 (PLA2) has also been shown to play a role through the synthesis of 20-HETE from arachidonic acid (AA). Finally, activation of RhoA in association with caveolin-1 (Cav-1) and of Rho-kinase allows sensitization of the contractile apparatus to calcium with modulation of myosin light chain phosphorylation (pMLC) as a critical downstream event.

Figure 2. Microcirculatory abnormalities and formation of intra-parietal edema at the origin of aortic dissection. Myogenic tone in vascular smooth muscle cells (SMC) and pericytes of small vessels and pre-capillaries limits the increase of flow and pressure in the vasa vasorum (VV). Collagen, elastic fibers and microfibrils of the connective tissue and their interactions with the medial SMC are critical for connective tissue 'contraction' and are important to maintain high interstitial fluid pressure (Pif). Genes/pathways associated with TAADs are involved in the generation of myogenic tone and Pif. We propose that alterations of microvascular tone and interstitial pressure generation would induce interstitial edema and swelling of the osmotically active glycosaminoglycans (GAGs), particularly in the presence of altered connective tissue. This would alter stress transfer and generate significant heterogeneities in stress concentration within the aortic wall, potentially initiating a vicious circle. Multiple contiguous foci of intra-parietal edema will eventually coalesce, leading to intra-parietal dissection. Alterations in lymphatic drainage (not depicted) may further aggravate interstitial edema and impact on vascular function (see main text). Parts of the scheme is adapted from reference 13.


Pressure increase
Tonic phase
Sustained phase
P
P
P
ECM
Collagen/
microfibrils
integrins
TRPP1/2
TRP(C)
Filamin A
PLA2
?
GPCRs
G
PLC
AA
20-HETE
Na+/Ca2+
Membrane depolarization
DAG
Cav-1
RhoA
Rho kinase
PKC
Ca2+
Sensitization
pMLC
Vasoconstriction
Trans-
Cellular
Force
transmission
Cyto-
skeletal
proteins
Actin
Myosin
SMC
Connexons
integrins
SMC
SMC
Cell – cell signalling


SMC
*Normal myogenic tone
Pericyte
Low Flow
Low Pressure
VV
*High Pif
Low
fluid flux
Collagen
Elastin
Microfibrils
GAGs
Integrins
SMC cystoskeleton
*Low myogenic tone
High Flow
High Pressure
*Low Pif
Edema
High transcapillary
fluid flux
GAGs
Low force generation
Low myogenic tone
(ACTA2, MYH11, PRKG1, MYLK,
FLNA, PDK, etc.)
Alteration of collagen,
microfibrils or elastic fibers
(COL3A1, FBN1, TGBR1/2, etc.)
Normal aorta
Aorta in TAADs