# Contribution of Cellular Mechanisms in the Development of Thoracic Aortic Aneurysms

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**Abstract**
Thoracic aortic aneurysm (TAA) is a devastating vascular disease. TAA patients have dilated ascending aorta that eventually ruptures and leads to death. Treatment of TAA is limited to surgery only. The structural and morphological changes localized to the ascending region have intense effect on functioning of the aorta. Recent scientific studies have demonstrated that the underlying cause of TAA is a result of various alterations at the cellular level. Given that there is an absence of a direct pharmacological treatment for TAA, therefore a growing demand to determine the underlying mechanisms of TAA is utmost necessary to elucidate. Till date, a great progress has been made to diagnose and identify the risk factors of TAA, however a better understanding of the mechanisms that trigger the progression is needed in order to develop new therapeutic strategies. The current review compiles the recent highlights about the contributions of cellular mechanisms involved in the development of TAA.

**Keywords:** ascending aorta, cytoskeletal proteins, fibroblasts, smooth muscle cells, thoracic aortic aneurysms.

## Introduction

Aorta is the main artery carrying blood from the heart to the different organs of the body. The region of aorta that is closest to the heart is the ascending aorta present in the thoracic cavity (Figure 1). The nature of the tissue present in the ascending aorta is unique and making it prone to many pathological consequences. This region is subjected to aortic dilation, elastin fragmentation, dissection and rupture, ulcer formation or erosion of the vascular tissue and thrombus formation [1-3]. The triggering step for initiating these pathological conditions in the ascending aorta is not clearly defined. Around 45,000-47,000 deaths are reported every year from aortic diseases in United States alone and the incidence rate has increased over the decades[4 5]. This number may vary as many cases are undiagnosed and mistakenly classified as cardiac arrest.[4] The presence of ascending aorta in the thoracic cavity classifies this pathology as thoracic aortic aneurysms (TAA) (Figure 1). Many TAAs are asymptomatic and can occur at any age in both genders. So far the current treatment is limited to surgery [6-8]. Though with help of modern technology, less invasive clinical methods exist but are at their infant stages. Therefore, persistent needs are required to identify the elements that predispose to the disease and prevent the treatment from surgery to other alternative medicines. The current research is aimed at alternative medical interventions that can improve the damaged regions and restore vascularization of the aorta. To make this change, there is an immeasurable need to define the mechanisms involved in the development of TAA.

## Diagnosis and Risk Factors of TAA

TAA is generally detected during regular medical tests including chest X-ray and ultrasound of the thoracic cavity. The physician may further recommend for echocardiogram or computerized tomography or magnetic resonance angiography to further validate and detect the exact location of the damage in the

tissue. The later imaging techniques can detect the size and diameter of the aortic dilation. The range of the normal diameter of the ascending aorta is between 3-5-3.9 and if it expands more than 4 cm is considered as aneurysm and it can expand to 5.5 cm in diameter [9].

There have been various risk factors associated with TAA including age, hypertension, inflammation, smoking, atherosclerosis, dyslipidemia and age of the ascending aorta. The angle of bending of the ascending aorta has been also shown to be another risk factor for TAA [10]. Strenuous exercise or any kind of distress in the chest cavity or an injury during surgeries can also lead to the damage of the ascending aorta and causing TAA.

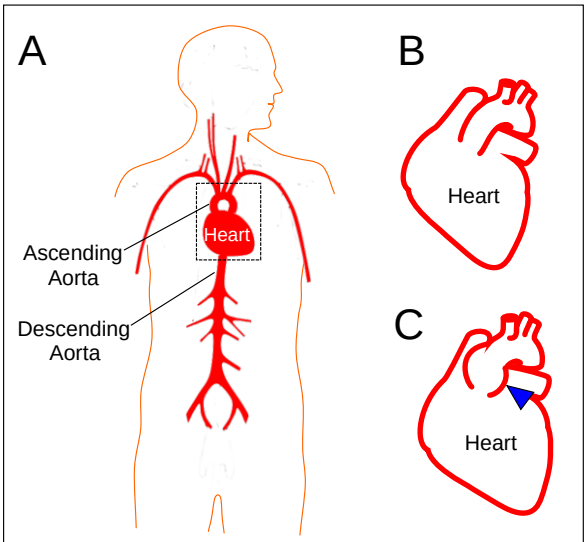


**Figure 1:** (**A**) Anatomy of the heart, ascending and descending aorta in the thoracic cavity. Boxed region is shown (**B**) of a normal heart and (**C**) a dilated ascending aorta of TAA heart (blue arrow).

Genetic factors are also responsible for the TAA formation. TAA can be syndromic and non-syndromic in nature. Marfan's syndrome (MS) is a result of genetic mutation in the fibrillin-1 gene[11 12]. MS is a connective tissue disorder that primarily occurs in the ascending aorta. It is also known as cystic medial degenerative disease. MS is an autosomal dominant disease with penetrance rate highly inconsistent. MS patients have loosely connected smooth muscle cells and microfibrils with extension of elastins [12 13]. MS patients also have defective collagen synthesis and deposition, reduced elastin fibres, increased expression of many extracellular matrix proteins and inflammation [14-16]. Visible signs of MS patients are thin and very tall build in structure, long arms, fingers, legs and toes with flexible joints. MS is recognized as a pathological condition with a progression of TAA. Another disorder linked to TAA is Loeys-Dietz Syndrome (LDS) [17 18]. This disease is due to genetic mutations in transforming growth factor **β**-receptor 1 and 2. Similar to MS syndrome, LDS is also a connective tissue disorder. Patients with LDS are characterized by skeletal and craniofacial abnormalities and are more prone to develop to TAA formation. Ehlers-Danlos syndrome (EDS) is also coupled with TAA [19 20]. This is also an autosomal dominant disorder. This vascular disease hits due to malfunctioning in pro-collagen production. Mutations in the Col3A1 gene have been reported in these patients. In other cases, TAA has also been observed in patients with Turner syndrome [21], Noonan Syndrome [22] and polycystic kidney disease [23]. The above-mentioned genetic diseases of TAA are syndromic. Non-syndromic TAA are also reported which include thoracic aortic aneurysms and dissections and familial aortic dissections [24 25]. Sporadic TAA are described also but they are rare and occur in isolated cases. Sporadic cases are due to autoimmune defects, inflammatory, Takayasu arteritis, and rheumatoid arthritis. Several diagnostic and risk factors have been identified and connected them with TAA. Recent studies have made a great progress in demonstrating a link between the risk factors and pathophysiological changes occurring in ascending aorta, however, each individual case is completely different from the other. There is more need to understand in depth about the mechanisms responsible for the development of TAA.

**Characterization of TAA**

TAA is a life-threatening disorder that has a huge impact on the lifestyle of an individual once being diagnosed. TAA is a complex and

heterogeneous in nature that is localized to the ascending aortic region mainly but rare cases are also seen in the descending aorta (Figure 1). In general, ascending aorta is comprised of three main layers: intima, media and the adventitia. All these layers are separated by elastic fibers and laminae. The cellular composition of intimal and medial layers is endothelial and smooth muscle cells, respectively (Figure 2). Adventitia is heterogeneous in composition, having vast array of cells; fibroblasts, macrophages, nerve cells, adipose, immune cells in collagen rich in extracellular matrix (Figure 2) [26 27]. TAA is characterized by luminal enlargement through the whole of the ascending aorta (Figure 1). TAA is a progressive disease, where the aortic intima and medial layers are torn, which allows the entry of blood cells into the media and causes the split of medial layer and that opens up to the development of another channel known as false lumen. At this stage, medial changes lead to elastin fragmentation and medial thickening that profoundly affects the blood flow in the aorta [28]. Further, this region is prone to rupture in patients with continuous aneurysm expansion and aortic wall weakening. The contribution of each cellular component of the ascending aortic wall in TAA is discussed in the following section.

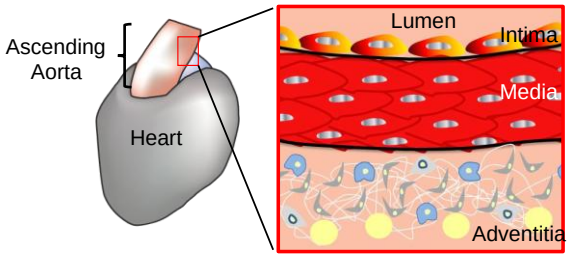


**Figure 2:** Schematic representation of ascending aortic vessel wall. Intima and media comprise of ECs and SMCs, respectively. Adventitia comprises of fibroblasts (grey), adipose (yellow), macrophages (blue) and immune cells (light blue) in a cell matrix (white mesh lines).

**Cellular mechanisms involved in TAA**

Recent scientific studies have improved our understanding of TAA, which holds a promising potential of developing into new therapeutic strategies for treating this pathology. Ascending aorta present in the thoracic cavity consists of vast array of cellular components that maintain and regulate the proper functioning of the tissue. Any alterations can further change the course of work done by each specific cell type. This leads to morphological and structural variations in the tissue. Different compartments of the aorta are smooth muscle cells; fibroblast cells and several cytoskeletal members are associated with TAA formation. Briefly, the role of each cellular compartment involved in the progression of TAA is highlighted and discussed below.

***Smooth muscle cells:*** Medial layer of the aorta is primarily comprised of vascular smooth muscle cells separated by elastin fibers. Smooth muscle cells are highly plastic in nature and switch between different phenotype conditions [29 30]. One form is synthetic or proliferative phase and another is the highly contractile and completely differentiated or a mature smooth muscle cell. This switching is transient and reversible depending on the environment around smooth muscle cells. The contraction of smooth muscle cells is due to the presence of actin and myosin complexes, which are connected to the cell membrane via filamin A and actin proteins. The main function of smooth muscle cells is to maintain the cell shape, alignment and migration. Vascular smooth muscle cells also regulate the functions of cytoskeleton proteins [31]. Studies have shown that smooth muscle cells interact with the neighboring cellular compartments including the collagen, elastin, fibrillin and fibrullin. These components basically belong to the family of extracellular matrix proteins. Smooth muscle cells interact with these elements through the receptor signaling especially the integrin, G-coupled and discoidin receptors [32-34]. Variations occurring in the proteins regulated by smooth muscle cells of ascending aorta are responsible for the development of TAA. Since this disrupts the shape and alignment of the smooth muscle cells. The downstream effect of this leads to abrupt signaling and synthesis of different proteins by smooth muscle cells. In TAA patients, smooth muscle cells undergo apoptosis and the fragmentation of elastin fibers [35]. Mutations

have been reported in actin assembly induction protein, β-myosin heavy chain and filamin A genes that are encoded by smooth muscle cells and linked to TAA and EDS [36-38].

***Fibroblasts:*** Fibroblast is the main cellular element of the aortic adventitia. Recent studies have shown that fibroblasts are involved in inflammation, remodeling of the aortic tissue and thoracic aortic aneurysm and dissection [39-41]. Alterations in elastin fibers and smooth muscle cells are accompanied with the changes in function of fibroblasts. Fibroblasts have a tendency to change its phenotype into myofibroblasts in response to any damage. Myofibroblasts are easily distinguishable from fibroblasts as they stained also with smooth muscle cell markers such α-actin and myosin heavy chain. In human samples with TAA, have demonstrated altered expression of myofibroblasts markers such as fibroblast specific protein-1 [42 43]. In animal model of TAA, the abundance of myofibroblasts was significantly increased in the aortic tissue [41]. Fibroblasts and fibroblasts-derived cells, myofibroblasts could be promising potential cell types to re-vascularize the aorta.

***Collagen:*** Media, adventitia and basement membrane of the ascending aorta is rich in collagens. The main function of collagen is give tensile strength and stiffness to the aortic vessel wall. There are different types of collagen such as Type I, II, III and IV. All these forms of collagen genes are expressed in the aortic wall however; Type I and III are relatively higher in this tissue. Collagen also regulates several signaling pathways that determine adhesion and proliferation of cells [44 45]. Genetic variations in the collagens contribute to the development of TAA formation. As mentioned earlier, mutations in Col3A1 leads to EDS [19]. In addition, mutations in Col1A1, Col1A2 Col4A1 and Col4A5 genes are also associated with aneurysm formation [46-48]. During TAA, Type I and III are increased subsequently leading to collagen deposition and elastic fragmentation and weakening. Later this leads to stiffness of the vessel and augmentation of aorta to dissect and rupture [49].

***Elastin:*** Elastin is a very critical component of the aorta. It is maintains the structure of the aortic wall. Elastin responds to any external stimuli including mechanical or chemical and also plays an important role regulates signaling between cells. Elastin interacts with smooth muscle cells of the media, extracellular components of the adventitia and also with endothelial cells in the intima to maintain the structure and organization within the wall [50]. Elastin confines the migration and proliferation of the cells [51]. One of the most common features of TAA is elastin fragmentation [2]. Deficiency of elastin in mice leads to death due to deformed smooth muscle cells [52].

***Microfibrils:*** Microfibrils are component of elastin fibers that give strength to the aorta [53]. Elastin microfibrils interface located (EMILIN) proteins are present in extracellular matrix. There are different EMILINs identified; EMILIN 1, 2 and 3, out of which EMILIN 1 is learned the most and minimum known is about EMILIN 3. EMILIN 1 -/- deficiency in mice has demonstrated decrease in aortic aneurysm formation. Other characteristics of EMILIN 1 -/- mice are irregular collaboration among smooth muscle cells and elastic fibers [54]. Absence of EMILIN 2-/- is associated with the increased risk of developing cardiovascular diseases [55]. EMILIN is able to bind to the fibulins and elastins. Future experiments are required to define the exact role of EMILINs in TAA.

***Fibrillin:*** Microfibrils are comprised of fibrillins. There are two isotypes of fibrillins identified as fibrillin 1 and 2. Fibrillin 3 is also identified but is not studied much. Fibrillins interact with collagen and other members of extracellular matrix. The function of fibrillins is to provide strength to any tissue. Fibrillin 1 isoform is expressed throughout life and participate in regulating receptor signaling including integrin and proteoglycans. Mice with fibrillin 1 deficiency have been established as an excellent model of MS [56-58].

Genetic mutation in fibrillin 1 leads to C to G change at 1039 nucleotide position that is susceptible and predisposes to MS, a form of TAA. Fibrillin 1 mutations lead to hardening of the aortic wall, increase in transforming growth factor **β**1 expression, inflammation, elastin degradation and extracellular matrix up-regulation [56]. Fibrillin 2 is primarily expressed during the embryonic phase and is responsible for the development of aorta [59]. Unlike fibrllin 1 -/- mice, the absence of fibrillin 2 has no effect on the development of aortic aneurysm.

***Fibulin:*** Fibulin is one of the primary members of extracellular matrix family. The main function of fibulin is in the structural organization of the aortic wall. Fibulin 5 functions as an important role in the development of lamellar structure by connecting elastin and smooth muscle cells [60]. Studies have shown that mutations in fibulin 4 and 5 are related to aortic aneurysms in humans [61 62]. Deficiency of fibulin 4 and 5 in mice has resulted in enlargement of ascending aorta. It has demonstrated that aortic tortuosity, slack skin and disordered elastin fibers [63 64]. The other forms of this family are fibulin 1, 2 and 3 that have no consequences on aortic aneurysms.

**Other Mediators of TAA**

Apart from cellular mechanisms involved in TAA, there are biochemical-signaling pathways that are associated with this etiology. Briefly these are inflammatory, oxidative, extracellular matrix proteins, transforming growth factor-**β** and angiotensin II signaling pathways. Researchers have also focused on these pathways and its effect on downstream targets. Any variations in the expression of the genes involved in these pathways have been demonstrated to be associated in the development of TAA. In addition, there are other mediators regulating the TAA formation such as mechanical pathways. Sheer wall stress and radial strain on the aortic wall play a very critical role in the blood flow through the aorta. Any malfunctioning of a mechanical signal can implicate on the structure and function of resident cells of the aorta.

**Treatment and drugs**

Till date, there is no direct pharmacological therapy for treating TAA. However, the results of animal model studies have demonstrated encouraging role of few drugs in treating TAA. Angiotensin II receptor blocker (ARBs) are commonly prescribed to hypertensive patients. In mice model of TAA, fibrillin 1 -/- mice, losartan, an ARB has demonstrated to attenuate diameter of aorta and enhanced the structure of the aorta [56]. Similarly, **β-**adrenoreceptor blocker also showed similar results as of ARB. Another assuring inhibitor is doxycyclin, a matrix metalloproteinase (MMP) inhibitor showed in the same model, reduction of extracellular matrix proteins in particular MMP2 and MMP9 [65 66]. Finally, Habashi et al also showed that giving a neutralizing antibody against transforming growth factor **β**1 into a TAA animal model restored the aortic wall structure [56]. Based on these excellent observations in animal model studies, some of these drugs have entered into clinical trails [67 68].

**Conclusion**

TAA is a very complicated disorder with a huge variation among each case. However, past few years of research with the help of modern techniques and technologies has visualized the discovery of many new results that have provided precious information about the pathology of TAA. These findings have laid the foundation in a new direction for the development of novel treatment strategies in treating TAA. However, unceasing research in this field is the need of the hour to elucidate and complete the puzzle of understanding TAA. This knowledge will definitely lead to more pharmacological therapies that are more specific for each individual. In future, there will be more clinical insights for curing TAA.

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**Conflict of Interest**

The authors have no conflict of interest.

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