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An eye into the aorta: the role of extracellular matrix genes

ZNF469 and PRDM5 previously associated with brittle cornea syndrome to the novel association with aortic and arterial aneurysmal diseases

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<td>Corresponding Author(s):</td>
<td>mohanakrishnan Sathyamoorthy</td>
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<tr>
<td>Corresponding Author' Email:</td>
<td><a href="mailto:m.sathyamoorthy@tcu.edu">m.sathyamoorthy@tcu.edu</a></td>
</tr>
<tr>
<td>Corresponding Author's Department:</td>
<td>Sathyamoorthy Laboratory, Department of Medicine</td>
</tr>
<tr>
<td>Corresponding Author's Institution:</td>
<td>Anne Burnett Marion School of Medicine at TCU</td>
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Review

An eye into the aorta: the role of extracellular matrix genes ZNF469 and PRDM5 previously associated with brittle cornea syndrome to the novel association with aortic and arterial aneurysmal diseases

Adam Wolf, MD1#, Peyton Moore, BS1#, Mohanakrishnan Sathyamoorthy, MD1,2

1Sathyamoorthy Laboratory, Department of Medicine, Anne Burnett Marion School of Medicine at TCU, Fort Worth, TX, 76123, USA.
2Consultants in Cardiovascular Medicine and Science – Fort Worth, 1121 5th Avenue, Suite 100, Fort Worth, TX, 76104, USA.

#Equal contributing first authors

Correspondence to: Dr. Mohanakrishnan Sathyamoorthy, MD, Department of Internal Medicine, Burnett School of Medicine, 4085 International Plaza, Fort Worth, TX 76109, USA. E-mail: m.sathyamoorthy@tcu.edu; ORCID: 0000-0003-1686-9264

Abstract

The extracellular matrix is a complex network of proteins and other molecules that are essential for the support, integrity, and structure of cells and tissues within the human body. The genes ZNF469 and PRDM5 each produce extracellular matrix-related proteins that, when mutated, have been shown to result in the development of brittle cornea syndrome. This dysfunction results from aberrant protein function resulting in extracellular matrix disruption. Our group recently identified and published the first known associations between mutations in these genes and aortic/arterial aneurysms and dissection diseases. This paper delineates the proposed effects of mutated ZNF469 and PRDM5 on various essential extracellular matrix components including various collagens, TGF-B, clusterin, thrombospondin, and HAPLN-1, and reviews our recent reports associating single-nucleotide variants in these genes’ development of aneurysmal and dissection diseases.

Keywords: ZNF469, PRDM5, extracellular matrix, aortic aneurysm, genetic basis of aortic disease, corneal diseases
INTRODUCTION

The extracellular matrix (ECM) is a network of proteins and other molecules that are essential for support, integrity, and structure for tissues and cells within the body. Though essential in various tissues, the ECM is particularly a critical player in vascular integrity. Through interactions with endothelial cells, the ECM provides scaffolding that is important for blood vessel development and stabilization. Arterial ECM is composed of various molecules including elastin, collagen, proteoglycans, and glycoproteins. Vascular ECM undergoes continuous remodeling consisting of protein synthesis and replacement of matrix proteins throughout the tunica intima, media, and adventitial layers. Errors in physiologic remodeling of the aortic wall are responsible for aortic aneurysm development, which is characterized by irreversible dilatation of the aortic lumen by greater than 50 percent of its original diameter. A genetic influence of thoracic aortic aneurysm (TAA) development is apparent, as approximately 30 genetic variations have been identified in patients with TAAs. Many of these known variations have a significant role in ECM development and maintenance, further suggesting a critical role between ECM regulation and TAA development.

The ECM is also a vital component of ocular development and integrity, as it provides a meshwork for cellular structure. Components of the ocular ECM include proteoglycans, collagen, elastin, laminin, fibronectin, fibrillin, and hyaluronic acid, among various other extracellular proteins. ECM is present within various compartments of the eye, particularly the stroma of the cornea. The corneal stroma is responsible for 90% of corneal thickness and has been identified as a densely packed, collagen-rich ECM. Defects in ECM genes and their subsequent regulators have resulted in ocular disease, particularly in syndromes related to the ECM-rich cornea.

There is a continuing interest in evaluating mutations in genes related to ECM development, maintenance, and physiologic remodeling, as several have shown to result in TAA phenotypes. Our group has recently identified that two genes, ZNF469 and PRDM5, previously only associated only with brittle cornea syndrome (BCS), are now associated with development of aortic and arterial aneurysmal diseases (TAAD).

This review provides an au courant association of known ECM regulatory genes ZNF469 and PRDM5 in the development of ECM-related diseases BCS and TAAD, establishing a likely connection between aneurysmal diseases and corneal ocular disease.
Brittle cornea syndrome (BCS)

Brittle cornea syndrome (BCS) is an autosomal recessive connective tissue disorder characterized by ocular and extra-ocular findings. Common ocular features of the disorder include thinning of the cornea, keratoconus, keratoglobus, and myopia. Corneal thinning, the hallmark of the disorder, increases the risk of corneal rupture in these patients with a potential for irreversible blindness. Corneal rupture is the most frequent presenting feature of BCS, often from spontaneous rupture or due to minor injury. Extra-ocular manifestations of BCS include hyperlaxity of the skin and joint hypermobility, two common findings in generalized connective tissue disorders.

Development of BCS is a result of a gene mutation in one of two genes: ZNF469 and PRDM5. Though the exact mechanism of ECM regulation is unknown for both PRDM5 and ZNF469, comparable down-regulation of ECM-associated genes were observed in mutants, suggesting a common pathway for both. Similar molecular changes in ECM genes and regulators with mutations in ZNF469 and PRDM5 results in phenotypes without significant variation from each other, further suggesting a common regulatory pathway.

While these genes have been directly attributed to the ocular manifestations of BCS, mutations in both have also resulted in extra-ocular symptoms. These symptoms, often characteristic of generalized connective tissue disorders, increase the suspicion of their involvement in other disease processes that ECM-related mutations are responsible for including aortopathy.

ZNF469

Zinc Finger Protein 469 is a collagen-related protein encoded by the poorly understood gene ZNF469, located on chromosome 16q24.2 (Figure 1). There is a shared homology of 30% between the ZNF469 protein with Clusterin and collagens I & III, strongly suggesting it plays a part in the production or regulation of collagen fibers. This gene is a member of the zinc-finger gene family, one of the most common motifs in
eukaryotes. Stanton et al. demonstrated that, through EMC disruption, ZNF-knockout mice experienced a significant decrease in biomechanical strength of the cornea. Certain mutations in ZNF469 have been identified as pathogenic for BCS, and novel mutations continue to be discovered.

Figure 1. Simplified ZNF469 gene structure, located on chromosome 16q24.2. Exons 1 & 2 are the primary exons noted in the literature as having mutations that are likely to be pathogenic, noting their coding role in the structure and function of ZNF469.

PRDM5

The PRDM5 gene is located on chromosome 4q27 and is a 23-exon protein coding gene responsible for production of the PRDM5 protein (Figure 2). The PRDM5 protein is recognized as a transcription factor of the PR-domain protein family, which contains a PR-domain and multiple zinc finger motifs. The functional transcription factor is involved in tumor suppression and regulation of extracellular matrix development in corneal and bone cells. PRDM5 as a transcription factor is crucial for both ECM development and maintenance in the cornea. Mutations in PRDM5 in humans have been associated with BCS development, with a disruption in its role as an ECM regulator being the suggested mechanism for pathogenesis. In subjects with mutations in PRDM5, downregulation of genes that encode molecules such as fibrillar collagens, connective tissue components, and various molecules involved in cellular migration and adhesion regulation were observed on microarray analysis of dermal fibroblasts. Mutation resulted in an increased corneal fragility and decreased corneal thickness.
**Figure 2:** Simplified gene structure of PRDM5 located on chromosome 4q27. The gene consists of a PR domain and multiple zinc finger motifs. Like other protein products created from PR domain containing genes, the PRDM5 protein is involved in transcriptional regulation.

Of the known ECM genetic mutations that are classically indicated in the development of TAAs, there are three major categories: collagen-disruptors, elastic fiber-disruptors, and transforming growth factor-beta (TGF-β)-disruptors. These are natural candidates, given that collagen and elastin are major constituents of the human aorta, while TGF-β signaling is a key player in function and maintenance of the aortic wall. The aorta, and specifically the thoracic segment, is under great pressure and as such the maintenance of function is of the utmost importance, and any compromise can have extreme consequence. To achieve that stability, it is important that each of the integral components of the aortic wall are functioning properly.

**ECM GENES AND THORACIC AORTIC AND ANEURYSMAL DISSECTION DISORDERS**

**Pathogenic/Canonical**

**COL Genes**

In arterial vessels, the dominant types of collagen present are I (heterotrimer) and III (homotrimer) which encoded by COL1A1/2 and COL3A1. These collagens are chiefly responsible for the aortic walls tensile strength via the triple helix configuration. Though composition varies person-to-person, studies have shown that the concentration of collagen in the aortic wall generally increases significantly with age and comprises around ~25% of the thoracic aorta. COL4A1/2 produces collagen IV, which is less abundant overall but is prominent in the basement membranes of endothelial cells, and intimal/medial smooth muscle cells.
Mutations in COL1A1 and COL1A2, especially glycine-related missense mutations, are classically associated with osteogenesis imperfecta (OI), a heritable disorder characterized by bone fractures, hearing loss, and dental impairments. Though a rarer effect, there have been reports of OI family cohorts with aortopathy\textsuperscript{22,23}. There have also been reported cases of mutations replacing y-position arginine with glycine or cystine leading to aneurysmal disease\textsuperscript{24,25}. Mutations on COL3A1 are associated with vascular Ehlers-Danlos syndrome (vEDS) and have a wide array of devastating vascular consequences. Collagen IV has been identified as a protective agent against the development of abdominal aortic aneurysms (AAA) by Steffensen et al, who developed a murine model showing knockout mice with deficiency of COL4A1/2 correlated with AAA progression, in addition to a Danish human Cohort demonstrating that increasing Collagen IV degradation product levels correlate with progression of AAAs\textsuperscript{26}.

\textit{Transforming Growth Factor-Beta (TGF-\beta)}

TGF-\beta signaling plays a critical role in vasculature development and maintenance. Mutations in TGFBI/2 have been identified in several syndromic causes of thoracic aortic aneurysmal and dissections (TAADs) including Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and Shprintzen-Goldberg syndrome (SGS). MFS is classically associated with the pectus deformity, myopia, valvular regurgitation, arachnodactyly, and, due to cystic medial necrosis (CMN), aortic aneurysm. Dysregulation of TGF-\beta signaling due to mutations in fibrillin-1 (FBN1) is contributory CMN of the medial layer in the aortic wall, leading to aneurysm development\textsuperscript{27}. Functional FBN1 binds TGF-\beta ligands, and a loss of FBN1 is hypothesized to lead to an increase in bioavailable TGF-\beta. Dysregulated signaling due to an increase availability in TGF-\beta is partially responsible for aneurysm development\textsuperscript{28}.

Pathogenic mutations in TGF-\beta receptors have been found responsible for development of LDS, leading to aortic aneurysm development and an early age\textsuperscript{27}. Mutations in TGF-\beta receptor 1 (TGFRB1) and 2 (TGFRB2), have been associated with the development of LDS, a disease that shares many characteristics with MFS\textsuperscript{28}. Similarly, increased TGF-\beta signaling in SGS has negatively impacted aortic integrity. Mutations in the SKI proto-oncogene encoding a TGF-\beta repressor causing SGS is responsible for the increase in TGF-\beta signaling, leading to eventual aneurysm development\textsuperscript{27}. Though
TAA development is a common phenotype in SGS, aneurysm disease is commonly less aggressive than LDS\(^\text{28}\). Mutations in PRDM5 have resulted in downregulation of many important ECM components in fibroblasts, including TGFB2\(^\text{8}\).

**Emerging**

**ZNF469**

To date, we have identified nine patients with aneurysmal or dissection disease with SNV mutations in ZNF469, with many of these patients having one or more first degree relative with similar vascular phenotypes\(^\text{29}\). Our first report described a 72-year old female who developed chronic bilateral aortoiliac dissections and a subsequent rapidly enlarging aortic aneurysm that underwent operative management\(^\text{30}\). Clinical genotyping revealed three SNVs on Exon 2 of ZNF469. Our subsequent report of our entire cohort of nine patients was notable for a statistical difference in Exon localization of mutation and the age of onset/identification of disease in patients. Five patients had mutations in Exon 1, with an average age of 49 while the four patients with mutations in Exon 2 had an age of onset/identification of 68 years\(^\text{29}\). One of these patients had a pathogenic mutation in ZNF469 known to be casual for BCS, and though there have been no corneal manifestations of disease, the patient has fusiform ectasia of the aorta, hypermobility of joints, and a marfanoid habitus. The role of ZNF469 in ECM regulation is postulated by our group to be the cause of these TAAD phenotypes in our cohorts, with a molecular mechanism similar to the more well-established pathogenesis of BCS.

**Downstream targets of ZNF469**

**COL1A1, COL3A1, and COL4A1**

Given their significant homology, ZNF469 is predicted to act as a regulator of collagen fiber production. Current research shows that mutations in ZNF469 can disrupt collagen assembly in multiple ways, including collagen receptor dysfunction, decreased collagen IV production via COL4A1 downregulation, and collagen fibril thinning\(^\text{6,8,31}\). *Burkitt Wright, et al.* demonstrated that both ZNF469 and PRDM4 knockout models resulted in a dysregulated/absent type 3 collagen matrix, implying a regulatory role of these genes in the transcription of COL3A1\(^\text{8}\). In 2012, *Al-owain et al.* reported on a large family that had cardinal ocular symptoms of BCS and tested positive for a novel
mutation in exon 2 of ZNF469, and the team noted that the family also possessed EDS phenotypes of severe kyphoscoliosis and joint hypermobility\textsuperscript{32}. In addition to EDS-like phenotypes, Rolvien et al. discovered novel ZNF469 mutations in two siblings that had mild BCS that possessed both EDS-like and OI-like phenotypes with blue sclerae, sensorineural and conductive hearing loss, and joint hypermobility\textsuperscript{33}. These cases of familial mutations in ZNF469 highlight the overlap between BCS and other collagen related disorders.

\textit{Clusterin}

Clusterin, also known as Apolipoprotein J, is a diversely functioning glycoprotein that has been implicated in the development of many diseases ranging from cerebral amyloid angiopathy to cancer. This chaperone protein aids in the clearance of ECM debris and degradation products and helps to facilitate cell-matrix functions\textsuperscript{34-36}. It has been shown to play an important role in vascular smooth muscle cell (VSMC) differentiation and nodule formation\textsuperscript{37}. In a mouse model, Shirasawa et al showed that clusterin-deficient mice experienced a significant reduction in neointimal hyperplasia after induced vascular insult through VSMC cell-cycle arrest and blunting of VSMC proliferation\textsuperscript{38}. The elucidation of the impact of ZNF469 mutation on clusterin and predisposition to TAA development warrants further investigation.

\textit{Thrombospondin-1}

TMBSP1 is an anti-angiogenic glycoprotein known to interact with many ECM components, including elastin\textsuperscript{39,40}. In addition to elastin, it was recently discovered by Rosini et al. that TMBSP1 plays a direct role in collagen homeostasis when they showed that TMBSP1 knockout mice displayed a significant reduction in the crosslinking of collagen and subsequent increased conversion of prolysyl oxidase to its active state\textsuperscript{41}. They also found that intact TMBSP1 display the propensity to bind to precursor collagen, blocking the binding of active lysis oxidase, demonstrating a major role for TMBSP1 in the homeostasis of collagen. The anti-angiogenic effects of TMBSP1 involves upregulation of pro-apoptotic endothelial cell pathways and vascular endothelial growth factor (VEGF) antagonizing. Yamashiro et al demonstrated via murine models that negative alterations in TMBSP1 signaling was key in the development of TAAAs via disruption of elastin and cytoskeleton remodeling\textsuperscript{42}. 
Rhobach et al. demonstrated that mutations in ZNF469 and PRDM5 led to significantly decreased production of TMBSP1, demonstrating the regulatory role of ZNF469 and PRDM5\(^6\). A recent study by Saddic et al. demonstrated that when compared to controls, thoracic aortas experiencing aneurysm and dissection had significantly increased levels of TMBSP1, suggesting it may serve as a biomarker of aortic disease\(^43\). In patients with ZNF469 mutation, interactions between ZNF469 and TMBSP1 may play an important role in the development of TAA given their relationship with collagens and elastin.

**PRDM5**

Mutations in PRDM5 have shown to result in downregulation of important ECM components including fibrillar collagens (e.g., COL4A1), connective tissue components (e.g., HAPLN1, TMBSP1), and cell migratory and adhesion regulators (e.g., EDIL3 and TGFB2) in dermal fibroblasts\(^6,8\). Involvement in ECM regulation and maintenance has increased suspicion for a potential role in other diseases that are a result of mutations in ECM-related genes, including TAA.

Though prior findings related to PRDM5 have been isolated to development of BCS, our recently submitted report demonstrated the first association between single nucleotide variants (SNVs) in PRDM5 and TAA development\(^44\). Notably, two patients with SNVs in PRDM5 presented with the presence of a TAA. Two mutations, a p.R83H (c.248G>A) and p.E129A (c.386A>C), were discovered in the patient group\(^44\). The effects of these mutations on downstream targets and tissue level effects have yet to be demonstrated. However, this represents the first association between PRDM5 and aortopathy and along with ZNF469, serves as another connection between ECM-related ocular disease and development of aortopathy such as TAAD.

**Downstream targets of PRDM5**

**HAPLN1**

Hyaluronan and proteoglycan link protein 1 (HAPLN1) is a protein encoded by the gene HAPLN1 with diverse function and is thought to be a structural component of the EMC and colocalizes with collagen in the ECM\(^45\). Burkitt Wright et al demonstrated that when a loss-of-function mutation was induced in PRDM5, resulting levels of HAPLN1 were decreased nearly 30-fold, suggesting a regulatory role of PRDM5 in HAPLN1 expression\(^8\). HAPLN1 is thought to play a role in acute TAA and dissection
disease, as samples from such patients contained significantly increased HAPLN1 concentration when compared not only to other tissues such as fat, skeletal muscle, and the left atrium, but also to samples of the aorta in coronary bypass grafting patients. HAPLN1’s role in the development of aortopathy has yet to be elucidated but given its presence in disease aortas and relationship to PRDM5, collagens, and the ECM structure, it is a target gene that deserves attention in the pathogenesis of TAAs.

**TGFB2**

Mutations in PRDM5 have resulted in downregulation of many important ECM components in dermal fibroblasts, including TGFB2. PRDM5, a known player in BCS development, has recently been associated with TAA development in two subjects. In addition to its association with LDS, TGFB2 mutation, reported by Boileau et al., has been reported as a driver of familial TAA in two large family cohorts. The proposed mechanism of a haploinsufficiency of the TGFB2 gene via frameshift and nonsense mutations cause a lack of circulating cellular TGF-β2 as a driver for development of TAA disease. Though the mechanism behind PRDM5 mutation and subsequent aneurysm development is currently unknown, dysregulation in TGF-β signaling is an intriguing and potential mechanism due to its known role in aneurysm development.

**CONCLUSION**

The ECM is the core structural support network of so many tissues, including the eye and arterial vessels. Our work related to ZNF459 and PRDM5 now provides a link between the eye and the aorta in terms of structure and function of the ECM and subsequent clinical consequences of their dysfunction in these previously unrelated genes. This paper highlights the phenotypic overlap between multiple syndromic diseases that were once thought to be independent, caused by mutations previously not recognized as causal for aneurysmal and dissection disease. The driving force in this overlap is our hypothesis that downstream effects of mutated ZNF469 and PRDM5 genes on various collagens, clusterin, TMBSP1, TGF-B, and even further downstream effects on VSMC, elastin, and VEGF result in aberrant ECM proteins (Figure 3). These aberrant proteins have the potential to disrupt many processes of the ECM and, for the same reason that they cause BCS, have the potential to cause TAA, and have phenotypic overlap with other disorders like EDS and OI. We anticipate this work will
lead to further mechanistic study of how alterations in these genes lead to proteomic manifestations that disrupt the ECM leading to these pathobiologies.

**Figure 3.** A representation of the relationship and overlap between ZNF469 and PRDM5 and their downstream targets, highlighting the extensive network of ECM proteins that are potentially affected by mutations in the each.

**DECLARATIONS**

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**Authors’ contributions**
Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Wolf, A., Moore, P., Sathyamoorthy, M. Performed data acquisition, as well as provided administrative, technical, and material support: Sathyamoorthy, M., Wolf, A., Moore, P.

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All authors declared that there are no conflicts of interest.

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TCU IRB#2022-106 for ZNF469 data, individual subject consent for PRDM5.

**Consent for publication**

Obtained from all subjects and on file at CCMS-FW

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