

# ***FBNI* and TGF $\beta$ 1: Molecular mechanisms in the pathogenesis of thoracic aortic aneurysms and dissections.**

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## **Abstract**

Thoracic aortic aneurysms (TAAD) develop asymptotically until occurs aortic rupture or dissection often cause of morbidity. A high mortality is determined by TAAD and complications developing. 15,000 people die every year due to the complications of TAAD in USA. It takes 14th place according to the reasons of mortality among 55 years people and older.

The main risk factors for TAAD formation still remain under discussion. Hypertension, atherosclerosis, age, gender and eventually genetic predisposition are on the focus for the research. Only in certain cases it is caused by aortitis, atherosclerosis or inherited as a single gene mutation: in the fibrillin genes - Marfan syndrome, by inherited collagen mutations as in Ehler-Danlos syndrome, by mutations of the transforming growth factor-beta gene causing Loeys-Dietz syndrome or by actin gene mutations. Evidence has shown that *FBNI* mutations may predispose TAA in the absence of phenotypic characteristics of Marfan syndrome. Recently published data from the genome-wide association study (GWAS) identified novel associations of *FBNI* SNPs: namely, rs1036477, rs2118181, rs10519177, rs4774517, rs755251, with sporadic TAAD. These data extend knowledge on the molecular pathways leading to sporadic thoracic disease and connect it with a model of Marfan syndrome.

There are anatomical, histological and molecular findings discussed within pathogenesis of TAA development. *Fibrillin -1 (FBNI)*, transforming growth factor (TGF), matrix metalloproteases (MMP) are analyzed in the TAA molecular background and their importance for clinical determination and individualization are more often valuable. How *FBNI* or *TGFBR* mutations lead to the disease are not well understood at a molecular level, the proposed mechanisms trigger the alterations in calcium binding EGF-like domains and increased bioavailability of transforming growth factor. The purpose of this review is to concentrate the available research information with focus on TAA common development trying to find a key for early diagnostic points.

**Keywords:** Transforming growth factor, Bioavailability, Matrix metalloproteases (MMP), Thoracic aortic aneurysms (TAAs).

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## **Introduction**

Thoracic aortic aneurysms (TAAs) present a major group of diseases of cardiovascular pathology affecting 5.9:100,000 worldwide [1]. Aortic aneurysms develop asymptotically until occurs aortic rupture or dissection often cause of morbidity. The main risk factors for TAA formation still remain the same for all cardiovascular pathology, including hypertension, atherosclerosis, age, gender and eventually genetic predisposition, which is on the focus for ourdays researches [2].

TAA develop and expand as a result of the predominant destruction within remodeling process where the alterations weakening the vascular wall increase the risk of dissection and rupture [3,4]. The extracellular matrix (ECM) responsible for aortas wall resistance to the different blood pressure, vascular smooth muscle cells (VSMC) and endothelial cells involved to the different mechanisms of remodeling play the crucial role in the pathogenesis of TAA [5]. *Fibrillin -1 (FBNI)*, transforming growth factor (TGF), matrix metalloproteases (MMP) are analyzed in the TAA molecular background and their importance for clinical

determination and individualization are more often valuable. How *FBNI* or *TGFBR* mutations lead to the disease are not well understood at a molecular level, the proposed mechanisms trigger the alterations in calcium binding EGF-like domains and increased bioavailability of transforming growth factor [5-9].

## **Extracellular Matrix and Proteases**

The main roles of ECM destruction share various proteases: matrix metalloproteases (MMP), chymase, triptase, cathepsins, serine elastase from neutrophils, enzymes from tPA, uPA. This seems to be proved by Dobrin et al. [10,11]. His experiments *in vitro* showed that elastase and collagenase trigger vessel dilatation and rupture. Following the proteolysis studies a lot of researches' works were done on abdominal aortas, using reproducible animal models as well [12,13]. The proteolytic activity in tissues remains defendant from a balance between proteases and their inhibitors. Inhibitors of MMPs, tissue plasminogen activator (tPA) are more expressed in atherosclerotic diseases of aorta than in dilatative pathology of vessels, thus ECM destruction by decrease activity of inhibitors

in the wall of aortas may lead to their aneurysmatic issue [13]. The role of MMPs pathway has been described using gene and cell transfer to show high expression of TIMP and plasminogen inhibitor (PAI-1) in a remodeling process of aortas tissue [14].

Predisposition of MMP-2 and MMP-9 expression has been reported to be involved to the TAA development caused by ECM destruction [15,16]. Proteases origin, release and role for dilatation the aortic wall is recognized but the regulation process still remains under discussion. Cystic medial necrosis associated with TAA is definitely of non-inflammatory origin. Immunohistochemical staining with monoclonal antibodies against CD3C showed the significantly increased amount of T lymphocytes flattening the media of TAA [17]. Mononuclears as T lymphocytes and macrophages have been observed in various types of TAA within mixed etiologies [18].

This could prove that infiltration of T lymphocytes into TAA occurs through the vasa vasorum due to their excessive amount around the vasa vasorum within revascularization process [19,20]. Tang with colleagues found that Th1-type immune responses with activated CD4 and CD8 producing *interferon-γ* predominate in TAA suggesting the reason why the vessel wall is expanding [21].

Luminal thrombus occurred due to the atherosclerotic lesion in rare cases of TAA could collaborate to release proteases from polymorph nuclear neutrophils. This was detected by Sangiorgi et al. in type A dissections in humans. It has been found increased MMP-9 levels in plasma as early as 1 h after symptoms onset suggested that degranulation of polymorphonuclears release the proteases [17]. It is possible to speculate that the thrombus modulates the aortic size by trapping neutrophils with proteases release to the aortic wall.

### Smooth Muscle Cells

The results of affected SMC exposed to TAA development are multiple and complex. A medial degeneration in TAA is common not in aging process itself but was observed in patients with TAA. Histological investigations indicated that TAA has a greater medial area compare to healthy aorta. Collagen and elastin are significantly reduced in the wall. Paul et al. reported that SMC density is not reduced in TAA [21]. The changes in aortas wall parameters could be spotted due to SMC remodeling during dilatation process and proteolysis excluding the role of apoptosis when contractile type of myocytes turns to synthetic type [22]. The controversial findings were published by He et al. [18]. He used  $\alpha$ -actin staining method and noticed the normal aortas had significantly more  $\alpha$ -actin staining than pathological TAA. Another team with Zhu et al. investigating MYH11 gene in patients with familial TAA also observed the SMC reduction. Their works presumed the apoptosis might be the main reason of SMC changes in TAA wall despite they did not observe degraded DNA ladder in electrophoresis gel assay [16,21]. The increased knowledge of the genes affecting individually TAA syndrome could explain the differences in SMC density and apoptosis.

Experimental works with abdominal aortas showed that addition of SMC prevents aneurysmal formation, suspending expansion in an already dilated vessel [23,24]. This fact proposes to think the similar mechanism could be discovered for TAA despite the different structure between TAA and AAA (the medial

layer is thicker in thoracic aortas). As SMC supports vessel wall hypertrophy and contributes the healing process, when mechanical stimulation of these cells allows producing TGF-beta1, ECM and inhibitors of proteolysis, which all together increase aortas wall size [25]. The other way in contrast the macrophages respond to mechanical damage releasing proteases with effect on ECM and wall destruction during dissection formation. Both mechanisms: destruction and reconstruction or healing depends individually and perform in particular manner. The differences in aortic healing between dissection and aneurysm depend on a level of lesion but differences in patient homeostasis remains unclear [2].

### TGF-beta

The role of TGF-beta signaling pathways in ascending TAAs described in Marfan syndrome stimulated a particular interest within non syndromic features of TAA. TGF-beta is a family of soluble proteins, cytokines, including three TGF-beta isoforms that has been involved in various cellular processes: angiogenesis, proliferation, differentiation, apoptosis, wound healing and described in conjunction within modification of the ECM [3,26,27]. A lot of studies for its role in stimulating collagen production and regulation pathways involved in pathogenesis of fibrosis of the liver, heart and the lung [28,29]. The main sources of TGF harvest in the body are bone matrix and the  $\alpha$ -granules of platelets. Cells secrete TGF-beta in a biologically inactive, latent form bound to propeptide and called LAP (latency associated peptide). Use of transient acidification release TGF-beta from its non-covalent association with LAP. Other proteins such as proteoglycans, type IV collagen, and fibronectin bind TGF in non-covalent manner. The determination of TGF-beta in blood (free TGF-beta or associated with  $\alpha$ 2macroglobin- $\alpha$ 2M) has been involved in the diagnostics of cancer, immunological disorders, hematology and fibrotic diseases [30,31]. The classical TGF-beta or Smad mediated signaling pathway is important to induce ECM deposition as well as repressing ECM degradation (through TIMP-1, TIMP-3) [32,33].

In the classical TGF-beta signaling pathway all three isoforms dimerize and bind to a heteromeric receptor complex, both of them consisting of two types I and II receptors with serine-threonine kinase activity. Type II receptor activates type I receptor through transphosphorylation way [34,35]. The activated type I receptor phosphorylates a receptor- Smad (molecular signaling intermediates, named for their homologous in *Caenorhabditis elegans* (sma genes: SMA1, regulators of body size) and *Drosophilla* (mad genes; mothers against decapentaplegic(dpp)). The R-Smad interacts with co-Smad (Smad4) and this formed complex working as transcriptional regulator (activates or suppresses) passes the information to the nucleus inducing or repressing genes [36,37].

There are many speculations and hypothesis on other way of TGF-beta signaling- alternative or Smad independent within the researchers' studies. This alternative signaling can proceed with alternative key mediators in the classical way or signals spread directly without type I receptor involvement [37,38]. The lack of Smad and co-Smad activity and abnormal type I receptor; activation of R-Smad by other signaling mediators without direct interaction of TGF-beta receptors may also activate Smad independent signaling, which is not fully understood due its complexity and diversity [39,40].

The way of TGF-beta regulation is multiple and depends on different mechanisms: extracellular regulation of ligand availability, regulation at the transcriptional level by activators, repressors and terminators [41]. Also by multiple feedback and cross mechanisms that affect intracellular signal [42]. All these factors and their relationship to modify the response in signaling TGF-beta pathway are in Figure 1.

TGF-beta identified as cell growth regulator is valuable not only for its implication in matrix deposition, but it works in matrix degradation as mediator for structural events of the ECM. It was demonstrated *in vitro* by treating with TGF-beta and this resulted the production of type I and type II collagen, involved collagen gene expression [43,44]. These studies were important to explain the role of TGF-beta normal fibrogenesis. Later this signaling pathway was implicated for therapeutic target in pathological fibrosis [44,45]. It was observed that the overexpression of TGF-beta1 may stabilize the degeneration within ECM remodeling during aneurysm development. The study with rat abdominal aortic aneurysm when virus mediated overexpression was induced increased endogenous TGF-beta1 level and stabilized dilatation and decreased aortic wall degeneration.

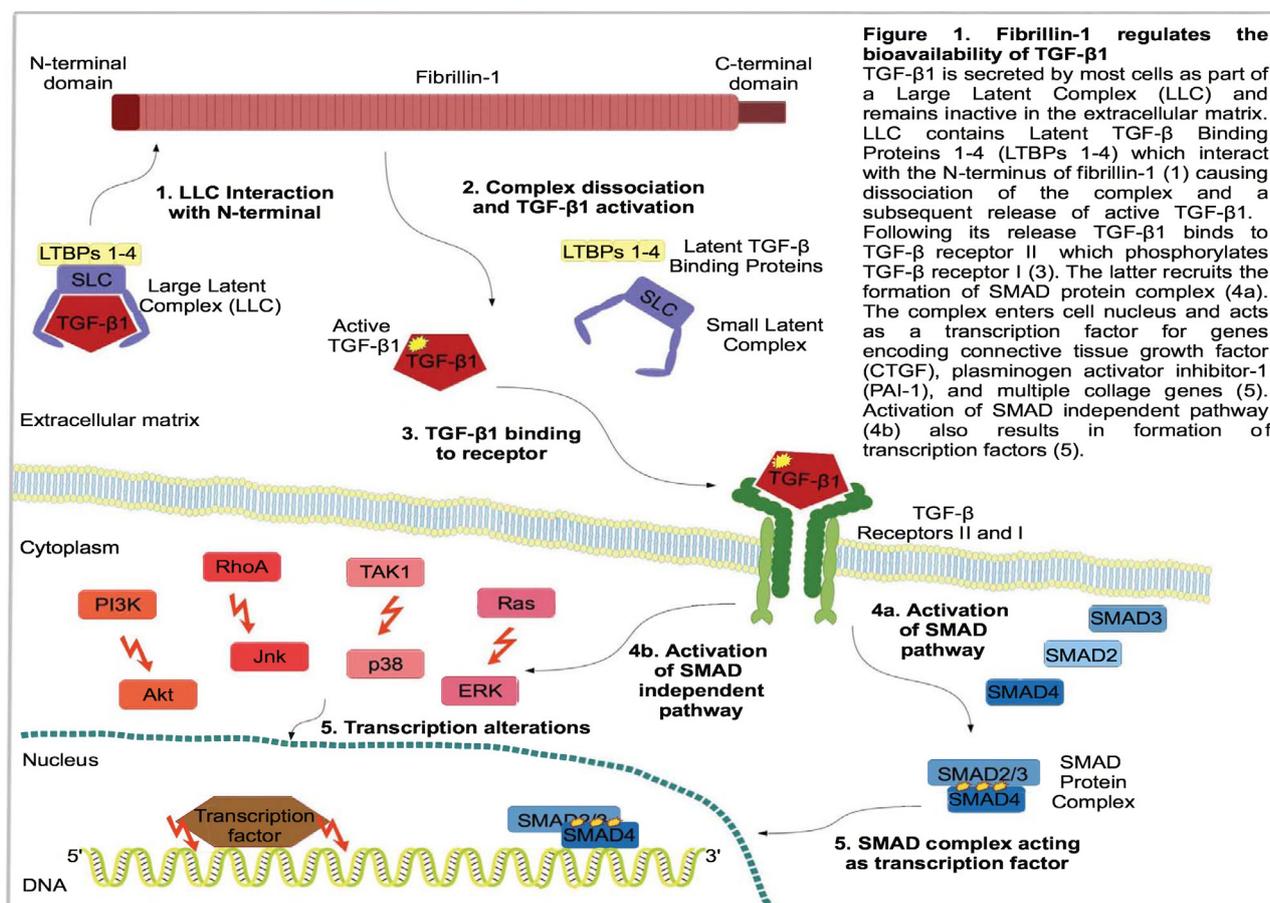
TGF-beta signaling regulates vascular matrix proteins while the abnormalities in its pathway may cause harm to normal

vascular structure and function. There are known several vascular disorders in conjunction with alterations within TGF-beta pathway, including aortic aneurysm syndromes, primary pulmonary fibrosis and atherosclerosis [46-48].

## TGF-beta and Aneurysm Development

There are two major groups of published investigations: first of them demonstrated different mutations of TGF-beta type I and II receptors within the kinase domain, another group stated that the TAA development is associated with increased proteolysis of ECM due to increased activity of TGF-beta.

Denton et al. with his works *in vitro* developed transgenic mouse with control of the COL1A2 promoter expressed a fibroblast restricted -kinase deficient TGF-beta RII. They found that (due to overexpression of the mutant receptor) the transgene works with dominant-negative effect on mouse and performs fibroblast specific suppression of TGF-beta signaling developing paradoxical results- the mice appeared with dermal and pulmonary fibrosis. The authors explained that the dominant-negative TGF-beta receptor may increase TGF-beta signaling in functional complexes by interactions of ligands and the presence of the mutant TGF-beta RII may change the orientation of wild type receptors with simplest way for signaling. The increased TGF-beta signaling may be



**Figure 1: Fibrillin-1 regulates the bioavailability of TGF- $\beta$ 1.** TGF- $\beta$ 1 is secreted by most cells as part of a Large Latent Complex (LLC) and remains inactive in the extracellular matrix. LLC contains Latent TGF- $\beta$  Binding Proteins 1-4 (LTBPs 1-4) which interact with the N-terminus of fibrillin-1 (1) causing dissociation of the complex and a subsequent release of active TGF- $\beta$ 1. Following its release TGF- $\beta$ 1 binds to TGF- $\beta$  receptor II which phosphorylates TGF- $\beta$  receptor I (3). The latter recruits the formation of SMAD protein complex (4a). The complex enters cell nucleus and acts as a transcription factor for genes encoding connective tissue growth factor (CTGF), plasminogen activator inhibitor-1 (PAI-1), and multiple collagen genes (5). Activation of SMAD independent pathway (4b) also results in formation of transcription factors (5).

associated with heterozygous TGF-beta receptor mutations which can work accessory [48].

TGF-beta signaling increase by kinase deficient receptors alters receptor stability. This process is regulated by receptor interactions with accessory proteins that interacts TGF-beta signaling. Guglielmo et al. stated that TGFBR2 mutations in TAA syndromes may facilitate interactions with SARA (Smad anchor for receptor activation) or decrease relation with Smad7, subsequently resulting prolongation of TGF-beta signal transmission [49].

There is the possibility that TGF-beta receptor complexes, incorporating kinase deficient receptors with lack of direct signal, able to form signaling platforms with interactions among signaling components. The stimulation of human mesangial cells with TGF-beta resulted collagen gene COL1A2 expression, which was dependent on kinase (P13K) activity. With use of P13K inhibitors and mutants of Smad3 was concluded that the binding of TGF-beta allows Smad3 mediated collagen gene expression with no dependency on phosphorylation by TGF-beta RI. Thus, TGF-beta RII mutations, which are able to bind TGF-beta, could induce alternatively signaling without receptor kinase activity [50,51].

As mentioned previously increased TGF-beta activity is related with TAA formation inducing matrix deposition, regulating its degradation through MMP activity. TGF-beta induced ECM degradation may take place with or without Smad mediated pathways [52,53]. TGF-beta role within the aneurysm syndromes has been described in Marfan (MFS) context, where the dilated aorta is very common among other genetically determined features [54].

Despite the hypothesis and all proposals explaining the cellular response to TGF-beta the mechanisms “how it works” are still unclear. There are different cells in aortas tissue and as known during the studies, the different type of cells reacts differentially. Thus, the complex of various mechanisms and reactions should take place in aortas aneurysms development. (See the Chapters “Extracellular matrix and proteases” and “Smooth muscle cells”).

### **Fibrillin-1 and Aneurysms**

The *FBNI* gene is very large, app 200 kb in size and its coding sequence divided into 65 exons, located on chromosome 15q-21.1 [55,56]. It encodes fibrillin-1 protein, which is large enough – 350 kDa. Fibrillin-1 protein consists of epidermal growth factor domains and a small number of TGF-beta1 domains [57-70].

Genetic predisposition in the etiology of thoracic aortic aneurysm and dissection has a very high value. Thoracic aortic disease is inherited in an autosomal dominant manner with or without syndromic features. There are known syndromes as Marfan (MFS), Loeys-Dietz, Weill-Marchesani which are associated with various mutations in *FBNI* gene. The absence of phenotypic characteristics may be not enough evaluated with the potential risk to develop aneurysm and/or dissection bearing in mind that *FBNI* mutations may have asymptomatic features and genetic determination can predispose the risk [5].

LeMaire et al. performed a multistage genome-wide association

study on a spectrum of sporadic thoracic aneurysm and dissection (STAAD) to identify the single nucleotide polymorphisms (SNPs) associated with *FBNI*. This study was performed on 327 628 SNPs for 765 patients with sporadic thoracic aneurysm and/or dissection and compared with healthy by cardiovascular point more than 4000 individuals. None of 765 patients have had a history of Marfan syndrome or other familial inheritance. Five SNPs among all of them were identified with genome-wide significance ( $p < 5 \times 10^{-8}$ ). All five SNPs (rs1051977, rs4774517, rs755251, rs1036477, rs2118181) fall into an area of linkage disequilibrium app 305kb in size, encompassing the entire *FBNI* gene. An important finding was a very strong association of rs10519177 with dissections of aortic aneurysms (OR=1.8, with  $p=1.2 \times 10^{-8}$ ) [70]. Others two studies with *FBNI* SNPs and TAAD association were performed by Iakubova with colleagues at Yale University [71] and by Lesauskaite leading her team at Lithuanian University of Health Sciences [72]. Quite valuable findings were established within the latter study: patients with ascending aortic aneurysm had higher minor allele frequency in *FBNI* SNPs *rs755251s*, *rs10519177*, and *rs4774517*, as compared to the reference group ( $p=0.003$ ). Minor alleles of all *FBNI* SNPs studied were more frequent ( $p \leq 0.0005$ ) among patients with Stanford A dissection as compared to the reference group subjects [72]. The studies demonstrate the association of several polymorphisms, encompassing the *FBNI* gene with sporadic TAAD in the absence of syndromes or etiology of familial history of TAAD. The future experiments and growing knowledge of *FBNI* mutations may be valuable to diagnose and determine the risk of TAAD.

### **FBNI and TGF-beta**

Fibrillin-1 works as a major structural component of ECM microfibrils and also regulates TGF-beta1 activity [57] (See Figure 1).

In the wild type population fibrillin-1 acts regulating the activation of TGF-beta, while abnormal fibrillin-1 with deficient functionality causes excessive amounts of active TGF-beta to be released from the matrix. It was demonstrated in mice with deficiency of fibrillin-1 and that aortic aneurysm in these mice can be prevented by administration of TGF-beta neutralizing antibodies [62,63].

The angiotensin pathway provides another way to target TGF-beta. Angiotensin II is a vasoconstrictor that signals through angiotensin II type I receptor and has been known in animal experiments by Everett et al. in 1994 [64]. Also angiotensin II activates trombospondin -1, which is an activator of TGF-beta signaling. The medicaments, which can block angiotensin receptor (Losartan- AT1 receptor antagonist) usually use for hypertension treatment can decrease TGF-beta signaling and its activity in plasma.

Brooke et al. reported that losartan was effective in within TGF-beta control in Marfan syndrome patients. This study highlighted the importance of cytokine activation through ECM and may be valuable in other conditions where TGF-beta is increased (organ fibrosis, cancer progression and others) [65]. The angiotensin receptor blocator Losartan is effective to reduce the thoracic aneurysm formation in Marfan syndrome [64].

The same study of Brooke et al. [65] demonstrated a significant

decrease in the aortic root enlargement in population of aortic disease with several etiologies with administration of ARB. The very interesting finding was performed by Chung and coworkers with their experiments on mice [66]. They showed that long term doxycycline administration together with atenolol (beta blocker) may prevent thoracic aortic aneurysm formation in Marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9 by multiple actions. As previous researches have been focused on doxycycline effect within abdominal aneurysms and was showed the suppression of aneurysmatic expansion due to the proteases inhibition [66-68].

There are controversial findings with TGF-beta1 concentration measurement levels, within different populations published since 2013. The scientist group from Japan stated that circulating TGF-beta1 is not a diagnostic and therapeutic marker for MFS patients because they didn't find any statistical difference between MFS patients and controls by measuring TGF-beta concentration in plasma [69]. Another study provided by The Netherlands scientists stated an opposite opinion that elevated TGF-beta1 level in MFS patients is correlated with larger aortic root diameters and may predict cardiovascular events serving as prognostic biomarker [70]. A researchers' team from Lithuania [73] measured the TGF beta1 concentration for sporadic TAAD and found to be a valuable marker, especially for dissections. They found the association of the TGF beta1 concentration with different genotypes of *FBN1* SNPs [74]. The main reason why such different results among studies could explore might be a genetic difference, the presence of a mutation in TGF-beta receptor genes rather than in the *FBN1* gene. That is to say, further and wider analysis is needed by mentioned TGF-beta and *FBN1* interaction mechanism to assess.

## Conclusions

Over 600 *FBN1* mutations have been discovered in connection with Marfan syndrome and eventually this list continues to grow. The genetic findings may be the basic diagnostic background to determine the risk of non syndromic TAAD. However, there are many questions requiring explanation or discovery in pathogenesis of TAAD development. First, the interactions of *FBN1* and TGF-beta whether the fibrillin-LTBP junction is needed to protect the LLC from proteolytic activation or whether *FBN1* functions more directly in controlling assembly or stability of latent TGF-beta complexes. Second, the balance between the need for TGF-beta in normal development and suppression of its activity in the treatment of disease must be evaluated in future works. Third, aortic diseases are heterogeneous along the whole vessel, different determinants and contribution of SMC, inflammation and thrombus formation, a failure of reconstruction and all these matters according to the different embryonic origin of abdominal and thoracic parts. Thus, future works to be done on namely different cellular and molecular mechanisms, diameter and length increase, rupture and dissection. The clinical application of the genetic studies on *FBN1* polymorphisms must have a wide approach in practice and complexity of multiple studies.

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