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# The paradoxical TGF-β vasculopathies

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## Abstract

Two new studies show that haploinsufficiency for *TGFB2* causes a familial syndrome of thoracic aortic aneurysms and dissections with other clinical features that overlap the Marfan, Loeys-Dietz spectrum of syndromes. Their finding of loss-of-function mutations in yet another transforming growth factor (TGF)-β pathway gene reinforces the seeming paradox of observed increases in the downstream TGF-β signaling pathway.

Thoracic aortic aneurysms (dilations) and dissections (tears) (TAADs) are a common cause of sudden death of young adults. Intracranial aneurysms and subarachnoid hemorrhage can also afflict seemingly healthy young adults, causing sudden mortality or severe morbidity. Such cardiovascular manifestations are often inherited in an autosomal dominant manner and can occur independently or as part of a syndrome, such as Marfan syndrome, Loeys-Dietz syndrome, arterial tortuosity syndrome and aneurysms-osteoarthritis syndrome. The aortic features of the Marfan, Loeys-Dietz (MLD) spectrum of disorders share clinical commonality in dilation of the aorta root, dysfunctional smooth muscle cells within the tunica media, with fragmentation and loss of elastic fibers, and excessive elaboration of extracellular matrix. Another common feature is paradoxical activation of the TGF-β signaling pathway in aortic lesions *in vivo*, despite the presence of what seem to be loss-of-function mutations in TGF-β signaling pathway components.

## *TGFB2* loss elevates TGF-β signaling

Two papers by Dianna Milewicz and colleagues and Bart Loeys and colleagues in this issue report that haploinsufficient loss-of-function mutations in a gene encoding TGF-β ligand, *TGFB2*, cause a novel syndromic form of TAAD[1,2]. This is an important finding because there has been considerable controversy surrounding the MLD syndromes, including discussion of whether decreased or elevated TGF-β signaling drives aortic aneurysm and other clinical manifestations[3]. The current finding is yet another example of loss of function of a TGF-β signaling component ultimately leading to a seemingly paradoxical increase in downstream signaling *in vivo*.

Two independent groups screened familial cases of thoracic aortic disease that could not be accounted for by mutations in the known causative genes *FBN1*, *TGFBR1*, *TGFBR2* and *SMAD3*. Boileau *et al.*[1] used 50K SNP genetic linkage analysis of two large families to map the locus to 1q41 around *TGFB2*, followed by exome sequencing of linked genes. Lindsay *et al.*[2] used a higher density (220K) SNP array analysis of severe probands from two families without previous genetic linkage analysis, and fortuitously found microdeletions

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(3.5 and 6.5 Mb) around *TGFB2*. Both groups found deleterious mutations in *TGFB2* that were also observed in affected family members but were not found in thousands of unrelated and unaffected individuals. Altogether, 12 independent mutations were identified, of which 8 were whole-gene deletions, frameshifts or nonsense mutations that are predicted to cause degradation of the cognate mRNA by nonsense-mediated decay, thereby indicating that the mutations cause loss of function. These mutations accounted for 1.5% (in ref. 1) and 25% (in ref. 2) of sampled familial cases of thoracic aortic disease that were not attributed to other known TAAD-causing genes. As with previous studies on what are known as 'TGF-β vasculopathies', despite causing genetic loss of function, mutations in both studies resulted in a paradoxical, although late, activation of the TGF-β signaling pathway, as shown by unequivocal elevation of the levels of phosphorylated SMAD2 and SMAD3 (SMAD2/3) in aortic lesions from *TGFB2*$^{+/-}$ persons and *Tgfb2*$^{+/-}$ mice, as well as by elevated ligand levels of either TGF-β1 (ref. 2) or TGF-β2 (ref. 1).

The canonical TGF-β signaling pathway[4] (Fig. 1) requires ligand binding to a heteromeric complex of type 1 and 2 serine/threonine kinase receptors. The TβRI receptor directly phosphorylates SMAD2/3, which then bind SMAD4 to accumulate in the nucleus where the complex transcriptionally activates many target genes, including the autoinductive *TGFB1* gene, the negative regulator *SMAD7* and the profibrotic factor *CTGF*. The three TGF-β ligands are synthesized as large latent forms that bind to extracellular matrix components, such as fibrillin, and are activated at the cell surface to release bioactive ligand, constituting a pivotal point for regulation of TGF-β bio-activity. However, TGF-β signaling is even more complicated. The TGF-β–TβRI-TβRII complex can also activate non-canonical signaling pathways, including ERK1 and ERK2 (ERK1/2) and TRAF6-TAK1-p38 MAPK pathways, which do not require the TβRI kinase to phosphorylate SMAD[5,6]. Moreover, TGF-β signaling output is strongly influenced by intersecting signaling pathways, including Ras, Notch and Wnt, and by crosstalk between ligands, receptors and SMADs of the TGF-β superfamily, such as activins, BMPs and myostatin[7].

## Unraveling the TGF-β paradox

How do loss-of-function mutations paradoxically activate the downstream signaling pathway, thus leading to tunica media degeneration and fibrosis? The simplest explanation is overshoot of a negative feedback loop that is transmitted through the remaining wild-type allele and/or, in the case of TGF-β2 loss, by its paralogous ligand, TGF-β1 (Fig. 1). However, this model would predict a more rapid imbalance in SMAD2/3 signaling rather than the protracted period required before this paradox is observed (at >4 months of age in *Tgfb2*$^{+/-}$ mice). It is tempting to suggest involvement of a precipitating inflammatory component influenced by *TGFB2* haploinsufficiency that initiates a stress response resulting in excessive aortic TGF-β1 and angiotensin II (Ang II) activity. TGF-β2 was originally identified as an immune suppressor[8] and is involved in the development of tolerance in antigen-presenting cells[9]. Together with *TGFBR2* and *SMAD3*, *TGFB2* has also been implicated in Kawasaki disease, an inflammatory condition that can result in aortic aneurysms[10]. Although MLD syndromes are not considered to be inflammatory, recent clinical studies suggest that inflammatory cells may contribute to the pathogenesis of thoracic aortic aneurysms[11]. Notably, both TGF-β1 and Ang II are profibrotic factors activated by stress responses and reactive oxygen species, and they are reciprocally activated by each other[12,13]. Moreover, the Ang II receptor, AT1, can initiate rapid TGF-β–independent phosphorylation of SMAD3 (refs. 12,13) that might contribute to promiscuous signaling by phosphorylated SMAD despite TGF-β receptor insufficiency.

Clearly, inflammation is not the entire story and could not account for the widespread congenital phenotypes outside of the cardiovascular system. Understanding how the

rewiring of the TGF-β signaling pathway in MLD spectrum disorders leads to overactive phosphorylated SMAD2/3 is therefore mechanistically important and is not only of academic interest. Several pharmacological inhibitors that target Alk4, Alk5 (TβRI) and Alk7 (Alk4/5/7) kinases with similar affinity are under clinical development[14] and might be useful for therapy. If higher levels of phosphorylated SMAD2/3 result from hyperactivation of the canonical TGF-β signaling pathway, pharmacological Alk4/5/7 inhibition or antibodies against TGF-β1 might effectively normalize this perturbation. In contrast, SMAD phosphorylation by p38 or ERK1/2 would be resilient to pharmacological Alk4/5/7 inhibition. An alternative mechanism of activation of phosphorylated SMAD2/3 via enhanced myostatin- activin-Alk4 signaling may be rectified by pharmacological Alk4/5/7 inhibition but not by antibodies targeting TGF-β. Finally, if promiscuous activation of SMAD2/3 is entirely driven by AT1 (refs. 12,13), neither drug class would be effective, but AT1 inhibitors, such as losartan, would be[15]. There is still much work to be done in elucidating how TGF-β signaling pathways are rewired. But, in the meantime, clinical geneticists will be encouraged by the discovery of a novel diagnostic tool for TAAD.

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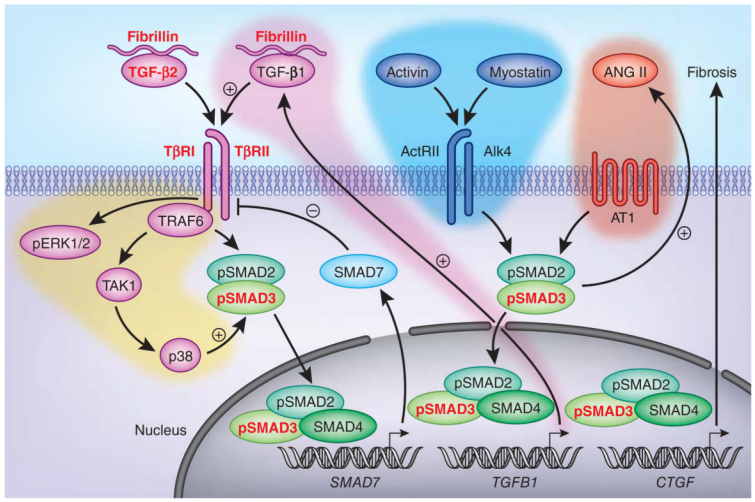


**Figure 1.**

Canonical and non-canonical TGF-β signaling pathways. This diagram shows possible routes of SMAD2 phosphorylation (pSMAD2) independent of the SMAD kinase activity of TβRI, including by TRAF6-TAK1-p38 (yellow), through Alk4 kinase activated by activin and/or myostatin (blue), by direct phosphorylation by AT1 (orange) or through excessive autocrine production of the paralogous ligand TGF-β1 (purple). Molecules in red type (fibrillin, TGF-β2, TβRII, TβRI and SMAD3) are altered by mutation in MLD syndromes. Note that TGF-β1 and Ang II are stress-response proteins, and both are known inducers of fibrosis that are reciprocally activated by each other[12,13]. pSMAD3, phosphorylated SMAD3.