abstract

Thoracic aortic aneurysm (TAA) is a genetically mediated disease with variable age of onset. In the pediatric age range, nonsyndromic TAA frequently has a milder course than syndromic forms of TAA, such as Marfan syndrome or Loeys-Dietz syndrome. Herein, we describe 17-year-old identical twin brothers with severe progressive TAA due to a novel de novo ACTA2 mutation. Interestingly, both boys were diagnosed at age 11 with congenital mydriasis, a recently recognized manifestation of some ACTA2 mutations due to smooth muscle dysfunction. One of the brothers presented with acute-onset lower back pain that was identified as dissection of an abdominal aortic aneurysm. Imaging of the chest at this time showed severe fusiform TAA. Cardiac imaging in his twin showed similar TAA, but no abdominal aortic aneurysm. Both brothers underwent valve-sparing aortic root replacement, but have had progressive aortic disease with recurrent dissection requiring multiple surgeries. This case emphasizes the importance of identifying physical stigmata of smooth muscle dysfunction, such as mydriasis, as potential markers for associated aortopathy and vascular diseases. *Pediatrics* 2014;134:e1218–e1223

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KEY WORDS

aortic aneurysm, ACTA2, mydriasis, aortopathy, genetics, natural history

ABBREVIATIONS

AAA—abdominal aortic aneurysm
AAAD—abdominal aortic aneurysm and dissection
SMA—smooth muscle actin
TAA—thoracic aortic aneurysm
TAAD—thoracic aortic aneurysm and dissection
TGFβ—transforming growth factor β

Drs Ware and Hinton provided clinical care for the patients, conceptualized and designed the study, and drafted the initial manuscript; Ms Shikany provided clinical care for the patients, coordinated tissue and data collection, and critically reviewed the manuscript; Drs Landis and James coordinated, assembled, and interpreted cardiac imaging, and reviewed the manuscript; and all authors approved the final manuscript as submitted.

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Thoracic aortic aneurysm (TAA) is a subclinical disease that can be associated with aortic dissection and sudden death. TAA may occur as a nonsyndromic condition or in conjunction with inherited connective tissue disorders, such as Marfan syndrome or the more severe Loes-Dietz syndrome. The genetic basis of nonsyndromic familial TAA is identifiable in ~20% of cases with up to 14% attributable to smooth muscle actin (SMA) mutations (ACTA2). In addition, ACTA2 mutations have been associated with diverse vasculopathies, including cerebrovascular anomalies such as moyamoya disease and arterial occlusive diseases, for example, coronary artery disease. Some mutations have been associated with progressive disease, but the natural history and underlying pathogenesis of nonsyndromic TAA remains incompletely understood.

CASE REPORT

We identified 17-year-old identical twin brothers with severe fusiform TAA, recurrent aortic dissections, and additional diverse vascular abnormalities that were attributed to a mutation in ACTA2. The clinical course was remarkable because the twins experienced dissections at the same time. Patient characteristics and phenotypic details are shown in Table 1. Twin 1 (family member II-2 on pedigree, Fig 1A) presented with abdominal and back pain leading to an unexpected diagnosis by computed tomography scan of abdominal aortic aneurysm and dissection (AAAD) originating near the diaphragm and extending 6 cm superior to the mesenteric and renal arteries. The AAAD was determined to be stable and elective repair was deferred. Eye findings were notable for congenital mydriasis (dilation of the pupils) that was first appreciated at age

<table>
<thead>
<tr>
<th>Table 1: Genotype and Phenotype of Identical Twins with TAAD</th>
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<tr>
<td><strong>Twin 1 (Proband, II-2)</strong></td>
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<tr>
<td>Genotype</td>
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<tr>
<td>Normal genetic testing for TAA</td>
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<tr>
<td>Normal genetic testing for thrombophilia</td>
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<tr>
<td>PAI-1 homozygous (4G/4G)</td>
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<tr>
<td>TAA</td>
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<tr>
<td>AAA</td>
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<td>Aortic valve annulus</td>
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<td>Aortic root*</td>
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<td>Sinotubular junction</td>
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<td>Ascending aorta</td>
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<tr>
<td>Aortic valve morphology</td>
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<td>Aortic insufficiency</td>
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<td>Aortic dissection</td>
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<td>2: Type A dissection</td>
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<tr>
<td>Mitral valve</td>
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<td>Systemic hypertension</td>
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<td>LVEDD</td>
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<td>Preoperative LVEF</td>
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<td>Head/neck imaging</td>
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<td>Beighton score</td>
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<td>Marfan systemic score</td>
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<td>Mydriasis</td>
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<td>Iris flocculi</td>
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<td>Bladder dysfunction</td>
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<td>But hyperplasia</td>
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<tr>
<td>Livedo reticularis</td>
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<tr>
<td>Surgery</td>
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<tr>
<td>2: Thoracoabdominal aorta graft placement</td>
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<td>Thrombosis</td>
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BP, blood pressure; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; N/A, not applicable.

* The aortic root dimension is measured at the level of the sinuses of Valsalva.
11 years (Fig 1C). Screening echocardiograms in both twins showed severe dilation of the proximal thoracic aorta, involving both the aortic root at the level of the sinuses of Valsalva and the ascending aorta (Fig 1 D and E). Further imaging revealed that both brothers had marked aneurysmal dilation of the internal carotid arteries and nonspecific intracranial vascular anomalies. Cardiac screening in first-degree relatives was normal. Molecular testing identified a de novo ACTA2 p.Lys328Asn heterozygous mutation in the proband (Fig 1B). Testing of FBN1, TGFBR1, TGFBR2, and MYH11 was normal and identical twin status was confirmed by zygosity testing.

Twin 2 presented with chest and back pain that was due to type A thoracic aortic aneurysm and dissection (TAAD). This dissection originated in the aortic root and extended 8 cm into the transverse aorta. The dissection was repaired with a valve-sparing aortic root replacement (David procedure) that also required replacement of the ascending and transverse aorta. Interestingly, postoperative complications included deep vein thromboses in both twins, and a pulmonary embolus requiring filter placement in the inferior vena cava in twin 2. Six months later, twin 1 underwent stent placement in the right carotid artery because of arterial stenosis, highlighting the copresence of aneurysmal and occlusive vascular disease. Three months after the stent was placed, there was a new type B TAAD in twin 1 that extended distally, connecting with the unrepaired AAAD. Subsequently, the distal aortic arch and thoracoabdominal aorta were replaced with Dacron grafts. Twin 1 experienced a spinal cord infarct after this surgery, resulting in lower extremity paralysis. Twin 2 suffered an ischemic stroke nearly 1 year after surgery.

Clinical pathology reported classic findings associated with TAAD and AAAD in the specimens retrieved from twin 1, including elastic fiber fragmentation, smooth muscle cell disarray, and adventitial fibrosis. Interestingly, there was no endothelial disruption or inflammatory infiltrate noted in the thoracic specimen, and the vasa vasorum was described as grossly normal. Additional research experiments demonstrated discrete areas of increased or decreased expression of SMA, the protein encoded by ACTA2, despite an apparently normal overall content. This irregular expression was observed in both thoracic and abdominal aorta tissue types, but the extent of areas characterized by misexpression and the smooth muscle disarray were qualitatively more pronounced in the TAA tissue (Fig 2). Given the known inflammatory component of AAA, we examined immunohistopathologic markers of inflammation in both AAA and TAA in the same genotype-positive individual (family member II-2, see pedigree in Fig 1A). Interestingly, CD-45, a marker of leukocytes and differentiated hematopoietic cells, was increased in clusters of cells in both the media and adventitia of the AAA, and was present only in the adventitia layer of the TAA. CD-68, a macrophage marker that is normally absent from aorta, was weakly present in the TAA adventitia, and strongly present in the AAA media and adventitia.

Transforming growth factor $\beta$ (TGF$\beta$)

**FIGURE 1**
Genetically triggered thoracic and abdominal aortic dissection. Pedigree analysis demonstrates aortopathy affecting the twin brothers only (A). The pherogram demonstrates a de novo heterozygous mutation in ACTA2 (arrow, B) that was found in each of the brothers, but not the parents. Interestingly, mydriasis was an early subclinical manifestation of smooth muscle dysfunction associated with ACTA2-associated vasculopathy in both brothers (C). Parasternal long-axis echocardiographic images show marked dilation of the proximal thoracic aorta in both brothers (D, E). The extent of the thoracic dissection (arrow) is shown by a 3-dimensional reconstruction of a chest computed tomography scan (F), and the location of the abdominal dissection is also shown (arrow, G). Ao, aorta; LV, left ventricle.
signaling was also examined. Canonical (p-Smad2/3) and noncanonical (p-Erk1/2) signaling was present in unaffected control tissue in all 3 layers (intima, media, adventitia) of the aorta (data not shown). In comparison with unaffected aorta tissue (control), both p-Smad2/3 and p-Erk1/2 signaling were markedly increased in affected thoracic and abdominal aorta tissue with disproportionately stronger expression observed in the adventitia layer. In addition, the endothelium of the TAA sample was grossly intact (inset, Fig 2B). Taken together, these findings indicate that individuals with ACTA2 mutation demonstrate severely progressive aneurysmal disease that is characterized by smooth muscle and TGFβ signaling abnormalities in conjunction with a potential role for inflammation in the thoracic aorta.

DISCUSSION

Mutations in ACTA2, the gene encoding the smooth muscle–specific isoform of α-actin, account for 14% to 20% of familial TAA cases. The penetrance of disease in individuals with mutations is approximately 50%, which is lower than the penetrance of other genes causing TAA and much lower than TAA in Marfan or Loey-Dietz syndromes. The estimated median survival of 67 years has suggested that ACTA2 mutations result in premature death in a subset of cases but less severe disease than some of the syndromic forms of TAA that are well-recognized causes of aneurysmal disease in the pediatric population. However, in the original report of ACTA2 mutations as causes of TAA, 4 (8%) of 52 individuals with TAA were adolescents at presentation, and 2 of these 4 died of dissection, indicating the potential for severe disease in childhood.4 In this context, congenital mydriasis may highlight smooth muscle dysfunction and prompt further investigation of subclinical vasculopathy.7,8

Recently, genotype-phenotype correlations have begun to emerge for ACTA2 mutations. The p.Arg179His mutation has been associated with global smooth muscle dysfunction and diverse vasculopathies, including congenital mydriasis, hypotonic bladder, malrotation, gut hypoperistalsis, pulmonary hypertension, patent ductus arteriosus, and central nervous system anomalies.9–12 Cerebrovascular occlusive disease is a common complication associated with ACTA2 mutations. Analysis of mutations that have been identified in more than 15 individuals indicate that p.Arg258Cys and p.Arg258His mutations confer a 6.5-fold increased risk of stroke as compared with other mutations, but are not associated with coronary artery disease. In contrast, the p.Arg118Gln and p.Arg149Cys mutations are commonly associated with coronary artery disease, but rarely with stroke.13 The basis for these genotype-phenotype correlations appears to lie
in the differential effects on actin behavior, with allele-specific effects on ATP binding/hydrolysis, actin-myosin interaction, or assembly or stability of actin filaments. The mechanism of action of the novel p.Lys328Asn missense mutation identified in these cases is unclear, but SMA staining did not indicate a substantial decrease in actin filaments, which has been identified with mutations that decrease F actin stability, such as p.Arg118Gln.

Some vascular disease resulting from ACTA2 mutations is associated with thrombosis, and this may reflect a secondary effect of vessel occlusion or an intrinsic defect in clotting. Both twins had deep vein thrombosis of unclear etiology, possibly attributed entirely to immobility after surgery. However, in light of twin 1’s preoperative history of venous thromboembolitis and normal thrombophilia evaluation, these recurrent events of venous thrombosis may represent enhanced genetic susceptibility to this type of complication. Although vascular occlusive disease with ACTA2 mutations has been extensively described in the arterial system, vascular smooth muscle cells also are present in veins, suggesting the potential predisposition for thrombosis secondary to vascular disease, especially in the context of environmental stressors, such as surgery.

Risk factors for aortopathy and/or dissection are poorly defined, but increasing evidence shows that genetic assessment may serve diagnostic and prognostic purposes. Reduced penetrance and variable expressivity, including the predisposition to dissection, are characteristics of genetic heterogeneity and complex inheritance and apply to nonsyndromic (familial and isolated) TAA. Moving forward it will be important to identify predisposing or protective genetic modifiers. The natural history of TAA is incompletely understood. Although many advances have been made in the context of Marfan syndrome, our understanding of disease progression remains limited. It is increasingly appreciated that the genetic basis of syndromic, familial, and isolated TAA will inform timely diagnosis and improved risk stratification. Multi-institutional consortia, such as the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions and International Registry of Aortic Dissection, are taking a coordinated approach to accelerating clinical breakthroughs and facilitating the use of genetic information. Importantly, the impact these diseases have in the pediatric age range is being addressed. For example, Januzzi et al demonstrated that although pediatric and young adult patients with TAA have different risk factors than older adults, the risk for dissection and death is equal, dispelling an engrained misperception about risk.

These findings also demonstrate the importance of correlating histopathology with genetic causes of TAA. Interestingly, these findings suggest that TAA may have an inflammatory component that contributes to disease progression and potentially dissection, contrary to the prevailing view of TAAD mechanism. However, it is unclear from these data whether inflammation was the cause of dissection or secondary to the dissection. Further studies focusing on cross-talk between the adventitia and medial layers are needed, as our data would suggest that the infiltrate is originating from the adventitial vasa vasorum, as evidenced by CD45 expression localized to the adventitia layer despite ostensibly normal gross pathology, rather than endothelial injury within the intima, which we did not identify. Delineating the gene-specific similarities and differences of disease pathogenesis is a necessary prerequisite to patient-specific medical therapy. Reconciling clinical and developmental theories (eg, etiology of TAA versus AAA) with pathogenesis paradigms (eg, excessive TGFβ versus smooth muscle cell dysregulation) will facilitate the identification of new approaches to early diagnosis and intervention.

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