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De Novo ACTA2 Mutation Causes a Novel Syndrome of Multisystemic Smooth Muscle Dysfunction

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Abstract

Smooth muscle cells (SMCs) contract to perform many physiological functions, including regulation of blood flow and pressure in arteries, contraction of the pupils, peristalsis of the gut and voiding of the bladder. SMC lineage in these organs is characterized by cellular expression of the SMC isoform of α -actin, encoded by the *ACTA2* gene. We report here on a unique and *de novo* mutation in *ACTA2*, R179H, that causes a syndrome characterized by dysfunction of SMCs throughout the body, leading to aortic and cerebrovascular disease, fixed dilated pupils, hypotonic bladder, malrotation and hypoperistalsis of the gut and pulmonary hypertension.

Keywords

ACTA2; alpha-actin; smooth muscle cell; thoracic aortic aneurysm; moyamoya disease; congenital mydriasis

INTRODUCTION

The walls of arteries and hollow visceral organs, including the stomach, colon, bladder, airways and uterus, are lined with smooth muscle cells (SMCs) that contract to perform these organs' various functions. These contractile cells are also found in the irises of the eye, the hair follicles and lacrimal ducts. Although the SMCs found in these various organs differ in terms of their embryonic origin and physiologic properties, the differentiation into functional SMCs is defined by expression of contractile proteins, including the SMC-specific isoform of the contractile protein α -actin (*ACTA2*) [Martin et al., 2007]. While this isoform is the primary actin in vascular SMCs, the SMC-specific muscular δ -actin (*ACTG2*) is the predominant isoform expressed in intestinal, urogenital, and respiratory SMCs [Gabbiani et al., 1981].

Heterozygous missense mutations in *ACTA2* cause a predisposition to a variety of vascular diseases, including thoracic aortic aneurysms and dissections, early onset coronary artery disease (CAD) and strokes [Guo et al., 2007; Guo et al., 2009; Morisaki et al., 2009]. In families with inherited *ACTA2* mutations, carriers can present with aortic disease, CAD or strokes, but none of the mutations identified are fully penetrant for any of these vascular diseases. Interestingly, correlations have emerged between specific *ACTA2* mutations and particular vascular diseases. For example, R118Q and R149C predispose primarily to aortic disease and early onset CAD. In contrast, *ACTA2* mutations altering R258 are associated with aortic disease, patent ductus arteriosus (PDA) and very early onset cerebrovascular disease, including Moyamoya disease, which is an early onset stroke syndrome characterized by bilateral occlusion of the distal internal carotid arteries and collateral vessel formation [Scott and Smith, 2009].

In mice, deficiency of *ACTA2* leads to normal development but decreased contractility of arterial segments and hypotension [Schildmeyer et al., 2000]. It has been proposed that *ACTA2* missense mutations in humans disrupt α -actin polymerization and lead to decreased contractility of aortic SMCs, and this dysfunction leads to thoracic aortic disease [Milewicz et al., 2008]. Accumulating evidence suggests a different etiology for the occlusive disease of smaller vessels associated with *ACTA2* mutations. Examination of coronary and intracardiac arteries, and arteries in the vasa vasorum of the aorta from *ACTA2* mutation patients shows increased number of SMCs located in the intimal or medial layers leading to a reduction in lumen diameter [Guo et al., 2009]. In addition, SMCs harboring *ACTA2* mutations proliferate more rapidly than control SMCs *in vitro*. Taken together, these data suggest that mutations in SMC α -actin have a gain of function effect leading to increased vascular SMC proliferation and, consequently, the stenosis or occlusion of arteries.

We report here patients with a *de novo* mutation in *ACTA2* not previously identified in our cohort of families with aortic disease. These patients have a unique clinical syndrome characterized by SMC dysfunction in organs throughout the body, including decreased contractile function in the iris, bladder and gastrointestinal tract, and SMC proliferative lesions in the vasculature. In addition, this *ACTA2* mutation appears to represent the most severe end of the spectrum of *ACTA2* mutations based on the very early onset and highly penetrant vascular diseases in affected patients.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston. Three families (proband and parents) had genetic testing at a clinical laboratory. Two families had testing in our research laboratory. DNA sequencing was performed according to protocol described previously [Guo et al., 2007].

Pathology slides of the aorta were obtained for two patients (C and D, Table I) and of the lungs (patient D) and stained with Movat and Trichome, respectively. Medical records including imaging studies, surgical reports, pathology reports and physicians' notes were obtained and reviewed.

RESULTS

We describe here five patients of Northern European descent with a novel heterozygous *ACTA2* missense mutation, R179H. Sequencing of the parents' DNA confirmed that these mutations were independent *de novo* events in all five patients. These patients, ranging from 11 to 26 years of age, had similar clinical findings including vascular diseases consistent with the defined spectrum of arterial complications of previously reported *ACTA2* mutations, but R179H appears to be a more severe mutation based on the very early onset and high penetrance of the vascular diseases (Table I). In addition, these patients have unique clinical complications characterized by SMC dysfunction in organs throughout the body, including decreased contractile function in the iris, bladder and gastrointestinal tract. Clinical reports were published on two of these patients (Table I, B and D) but with partial reporting of the clinical features and no identification of the causative mutation [Khan et al., 2004; Lemire et al., 2004].

The *de novo* *ACTA2* R179H mutation caused ascending aortic aneurysms and PDA in all five patients. Large PDAs were diagnosed in infancy and required surgical closure. Patient D also had surgical repair of an aortic coarctation at the age of 4 months. All patients subsequently developed fusiform ascending aortic aneurysms extending to the arch during childhood, with the diameter of the ascending aorta greater than the sinuses of Valsalva, and three patients underwent aortic surgical repair between 10 and 12 years of age. Aneurysms of the arteries originating from the arch and the suprarenal abdominal aortic aneurysm were also identified. Pathologic analysis of the ascending aorta was similar in two of the patients (Figure 1). Fibrocellular intimal SMC proliferation was evident, along with significant SMC proliferation in the subintimal medial layer associated with fragmentation of the elastic fibers. Changes that are more typical of medial degeneration, such as proteoglycan accumulation and loss of elastic fibers, were evident in the middle of the medial layer. Stenosis of the vasa vasorum due to SMC proliferation was also noted (Fig 1, Panel E).

In addition to the aortic disease, all patients had cerebrovascular abnormalities. MRA and angiograms in all of these patients showed fusiform dilatation of the internal carotid arteries (ICA) from the cavernous to the clinoidal segments (Fig 2, Panel A). In addition, the terminal region of the ICA showed mild to moderate tapering indicative of stenosis of the artery and consistent with changes observed in Moyamoya disease. These stenoses often extended into the M1 and A1 segments of the middle cerebral and the anterior cerebral arteries. Patients A and B [Khan et al., 2004] underwent neurosurgical bypass for cerebral revascularization for Moyamoya disease.

All patients also had white matter abnormalities characterized by a similar pattern of increased T2 signal intensity on MRI in the periventricular white matter (Fig 2, Panel B). The Virchow-Robin spaces were mildly prominent. In addition to the periventricular white matter changes, the oldest patient (E) also had white matter changes in the distribution of the middle and anterior cerebral arteries consistent with a prior ischemic stroke. Patient C had colpocephaly with a thin corpus callosum and somewhat small cerebellar vermis, and was diagnosed with developmental delay.

All patients also had disrupted function of SMC-dependent organs that have not been previously associated with *ACTA2* mutation, including the irises, bladder and

gastrointestinal tract. All had congenital mydriasis, which is a rare condition characterized by aplasia of the iris sphincter muscles and hypofunction or hypoplasia of the dilator muscles, resulting in fixed dilated pupils from birth. They were all diagnosed with a hypotonic bladder, which was variably associated with dilated ureters, calyces or renal pelves, hydronephrosis, vesicoureteral reflux, and recurrent urinary tract infections. Two of the patients had congenital intestinal malrotation, one of whom required resection of a mesenteric band for an obstructed jejunum five days after birth. These same patients were diagnosed with hypoperistalsis of the gut. Patient D had gallstones that spontaneously resolved and subsequently presented with hydrops of the gallbladder without evidence of residual gallstones. Biopsies of the esophagus, stomach and small intestine revealed normal ganglionic cells and no specific neural or smooth muscle pathology.

Four patients were noted to have tachypnea at birth. Patient A required supplemental oxygen and positive pressure after birth and had an unexplained pulmonary infiltrate and severely dilated pulmonary arteries. The pulmonary complications resolved over the next two years. The tachypnea resolved in Patient B with ligation of the PDA at 20 days of age but he was noted at age 14 years to have hyperinflation of the upper lung segment, a hypoplastic lower segment and a dilated pulmonary trunk. Patient D was noted to have evidence of cystic lung disease as an infant. She had a negative sweat test for cystic fibrosis and normal alpha-1-antitrypsin, karyotype and chromosome 22q11 microdeletion analyses. Lung biopsy at 4 months of age confirmed alveolar dysgenesis, with a pattern of alveolar formation suggestive of a developmental defect (Fig 3, Panel A). The biopsy of the pulmonary vasculature showed marked changes consistent with pulmonary hypertension (grade II – III out of IV) but it was not clear whether these changes were primary or secondary to the dysgenesis (Fig 3, Panel B and C). Patient C was diagnosed with primary pulmonary hypertension and underwent bilateral lung transplant at the age of 18 months. Lung pathology at the time of transplant showed pulmonary arterial hypertensive changes (grade III-IV) with SMC hyperplasia and neointimal fibrocellular proliferative lesions.

DISCUSSION

In summary, the patients described here with a unique *ACTA2* mutation have a syndrome characterized by ascending aortic aneurysms, PDA, cerebrovascular disease, periventricular white matter lesions, congenital mydriasis, hypotonic bladder, malrotation and hypoperistalsis of the gastrointestinal tract and pulmonary hypertension-associated vascular changes. Other less common complications require further study to confirm an association with this mutation and include a cystic lung disease, undescended testes, gallstones, arterial venous malformation and aortic coarctation. There are previously published clinical reports of children with similar findings [Adès et al., 1999; Graf and Jungherr, 2002; Kato et al., 1999; Khan et al., 2004; Lindberg and Brunvand, 2005]. Dysmorphic facies and joint, skeletal and skin abnormalities were reported to be absent in these patients, which is surprising given that dermal myofibroblasts express *ACTA2*. In addition, the livedo reticularis present in a subset of *ACTA2* mutation patients was not reported to be present in these patients. Although the clinical findings are pleiotropic, the complications involve organs dependent on SMCs for their function. In fact, the congenital mydriasis, urinary retention and intestinal hypoperistalsis most likely result from a loss of SMC contraction. In addition, it has been proposed that ascending aortic aneurysm formation results, at least in part, from loss of aortic SMC contractile function [Milewicz et al., 2008].

The occlusive vascular diseases found in these patients, including stenosis of the terminal internal carotids in a Moyamoya pattern and pulmonary vascular changes, are not well explained by SMC contractile dysfunction. The occlusive vascular pathology previously described in other patients with *ACTA2* mutations is characterized by increased SMCs in

both the intimal and medial layers of arteries, suggesting that excessive SMC proliferation leads to the greater-than-expected incidence of ischemic stroke and Moyamoya disease in these patients. A similar vascular pathology of SMC proliferation in the neointimal layer is described in the occluded carotid arteries of Moyamoya disease patients [Masuda et al., 1993]. The pulmonary vascular lesions in the patients reported here show a similar neointimal and medial SMC proliferation. It is interesting to note that the aortic pathology at the time of aneurysm repair for Patients C and D also showed significant intimal and medial SMC proliferation, raising the possibility that SMC proliferation may also contribute to aneurysm formation.

The periventricular white matter lesions identified in these patients would suggest a leukodystrophy with gliosis or demyelination originating from abnormalities in nervous tissue. A connection between neural development and SMC function is already suggested by mutations in filamin A, a cytoskeletal protein that links actin filaments to membrane receptors. Filamin A mutations cause a syndrome characterized by periventricular nodular heterotopia due to defective neuronal migration and thoracic aortic disease [Sheen and Walsh, 2005]. However, since arterial occlusive lesions occur with *ACTA2* mutations, it is possible that these white matter changes result from the occlusion of small vessels. It is important to note that similar periventricular findings are observed in adult patients with occlusive small vessel disease as a result of CADASIL syndrome, diabetes or chronic hypertension [van, 1998]. Another possible explanation is that these MRI findings are watershed lesions resulting from chronic occlusion of the internal carotid artery, and similar lesions have been described in Moyamoya disease [Hasuo et al., 1998].

The pulmonary problems in these patients were already present in infancy. The etiology of the cystic lung disease and chronic interstitial pneumonitis is not known. Patient C was diagnosed with primary pulmonary hypertension, but whether the similar pulmonary vascular changes in two other patients were primary or secondary is not clear. It is intriguing to speculate that the pulmonary vascular pathology results from disruption of the maturational changes of the pulmonary vasculature that occur at birth. *In utero*, the pulmonary vasculature maintains high vascular resistance, in part due to proliferative and poorly differentiated SMCs in the arteries, causing thickening of the walls [Haworth, 1995]. The resistance falls rapidly after inhalation at birth and is associated with flattening and spreading of the individual SMCs and differentiation and decreased numbers of SMCs overall, resulting in decreased thickness in the arterial walls. The abnormal thickening of the medial layer of the pulmonary arteries observed in these patients suggests that postnatal regression of pulmonary vascular SMCs had not occurred and that these SMCs may have continued to proliferate in the intimal lesions.

The urinary and gastrointestinal problems found in these patients are similar to complications of megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS; OMIM 249210), an autosomal recessive condition typically lethal shortly after birth. This disorder is characterized by megacystis and hydronephrosis, microcolon/short bowel with malrotation and hypoperistalsis. The underlying genetic defect has not been identified but the overlap of MMIHS with the *ACTA2*R179H phenotype suggests a defect of SMC contractile function, possibly from recessive or *de novo* *ACTA2* mutations. Interestingly, congenital mydriasis has been reported in a patient with MMIHS [Narayanan et al., 2007].

SMCs express a number of different isoforms of actin, with SMC specific α -actin and δ -actin being the predominant forms expressed, in addition to lesser amounts of cytoplasmic β - and δ -actin [Rubenstein, 1990; Vandekerckhove and Weber, 1978]. The amount of each isoform found in SMCs varies according to the organ. In vascular SMCs, α -actin is the major isoform expressed, accounting for approximately 60% of the cellular actin [Fatigati

and Murphy, 1984]. In contrast, the major actin isoform in intestinal and bladder SMCs is muscular δ -actin [Kim et al., 1994]. Therefore, the disruption of intestine and bladder function in these patients due to mutant α -actin is surprising. It is possible that the mutant α -actin disrupts the polymerization or function of the muscular δ -actin, or that α -actin plays a more important role in the contraction of these organs than previously concluded from isoform expression levels. The observation that mice lacking smooth muscle α -actin have decreased contractility of the bladder supports the second hypothesis [Zimmerman et al., 2004]. Since R179 is located close to a key protein-protein interaction site on the macromolecular surface of α -actin, the mutation may also disrupt critical interaction and disrupt downstream signaling events necessary for SMC function [Gerthoffer, 2005].

The R179H mutation is the most common recurrent mutation identified in *ACTA2* in our cohort of patients with thoracic aortic disease. Analysis of the nucleotide sequence around the mutation failed to identify a mutable motif to explain the increased frequency of this mutation [Rogozin and Pavlov, 2003]. Therefore, the recurrent identification of this mutation may be due to a recruitment bias resulting from the unique phenotype in these patients, specifically the fixed dilated pupils, early onset aortic disease and Moyamoya-like cerebrovascular disease.

In summary, the recurrent *de novo* *ACTA2* missense mutation R179H leads to more severe and penetrant vascular disease than with previously reported inherited *ACTA2* mutations. In addition, this mutation is associated with novel clinical features affecting SMC-dependent organs not described for other *ACTA2* mutations. Since the vascular diseases in the patients described here overlap with Marfan syndrome and vascular Ehlers Danlos syndrome, many of these patients were classified as having an underlying connective tissue disease. The identification of the causative gene as *ACTA2*, along with the constellation of findings involving organs dependent on SMCs for function, suggests that these patients should instead be classified as having a unique syndrome globally disrupting SMC function, what may essentially be a smooth muscular dystrophy. As such, the phenotype of these patients aids in predicting the spectrum of clinical complications resulting from gene mutations disrupting smooth muscle function.

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Addendum added during revision of the manuscript

We have identified two additional patients with the *ACTA2*R179H mutation but do not have complete medical records for these individuals. The first patient is a male who was previously reported in the literature [Adès et al, 1999] and had the following features: PDA, surgically ligated at 3 months of age, bilateral iris hypoplasia diagnosed at 3 months of age, bilateral inguinal hernia repair at 6 months of age, absence of one testis, asthma during childhood, hypoplasia of the terminal phalanges of both thumbs with mild shortening of the distal phalanges of the fingers, descending aortic dissection at age 14 years, and sudden death at age 17 years. The second patient is a woman who is currently 27 years old, in whom the following clinical features are reported by the patient and physician: gastrointestinal malrotation, PDA, congenital mydriasis, asthma as a child, gallstones diagnosed at 16 years of age, ascending aortic aneurysm extending to the arch and the brachiocephalic artery (repaired at the age of 25 years), and suprarenal aortic aneurysm. Cerebrovascular imaging at 22 years of age showed focal stenosis of the right supraclinoid carotid artery and an aplastic right A1 segment of the middle cerebral artery, stretched appearance of all the intracranial arteries, and colpocephaly.

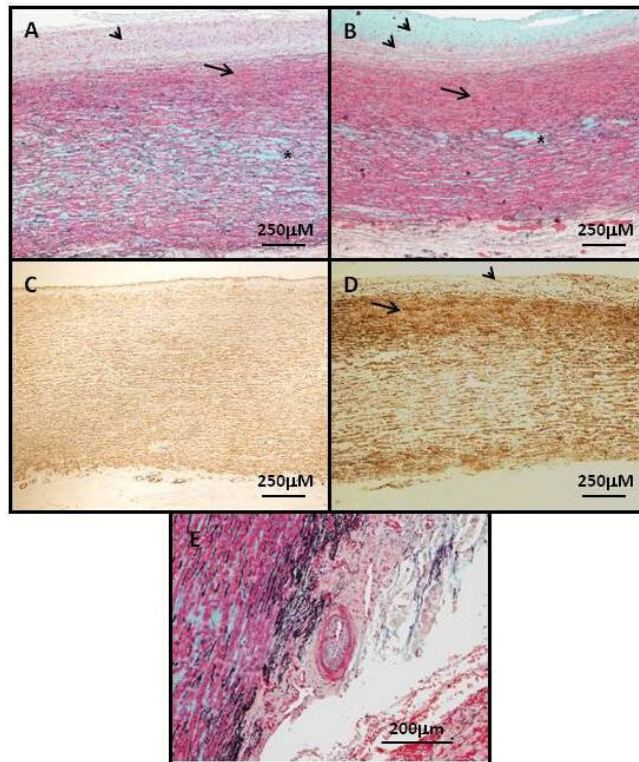


Figure 1.

Panel A and B show the aortic pathology of the ascending aneurysms associated with *ACTA2* R179H mutations (patients D and C, respectively). Movat staining of the aortas shows significant fibroproliferative lesions in the intima (arrowheads) characterized by SMCs (red) and extracellular matrix accumulation, including collagens (yellow) and proteoglycans (blue). In the medial layer of the aorta, there is subintimal proliferation of SMCs (arrows) and medial degeneration with proteoglycan accumulation and loss of elastic fibers (black), which is most apparent in the middle of medial layer (asterisks). Panel C is an age-matched control aorta and panel D is an ascending aneurysm (patient C) immunostained with α -actin to identify SMCs. These panels confirm intimal SMC hyperplasia (arrowheads) and increased SMCs in the subintimal medial layer (arrows) in the patient compared with the control. Panel E shows an artery in the aortic vasa vasorum with intimal fibrocellular proliferation (the thin black line in the artery is the internal elastic lamina, which demarcates the intimal from the medial layer). Magnification is indicated on the images.

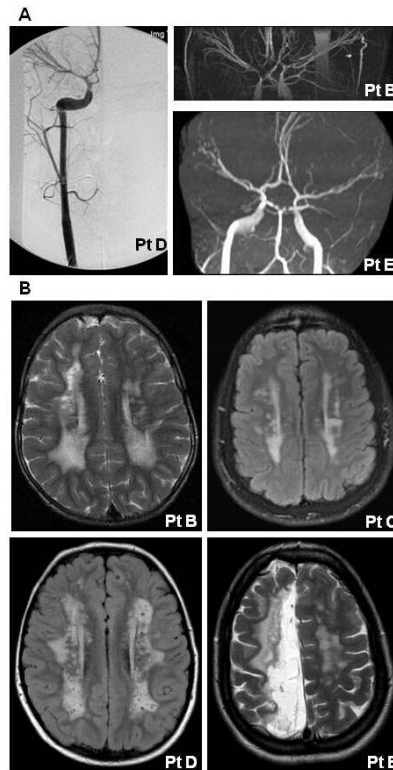


Figure 2.

Panel A shows angiogram and MRAs images from three patients with the *ACTA2*R179H mutation. These images illustrate the bilateral ectasia of internal carotid arteries from the cavernous to the clinoidal segments and stenosis of the carotid terminus in these patients. Panel B shows MRIs from four patients illustrating increased T2 signal intensity in the periventricular white matter bilaterally. In addition, MRI of patient E shows white matter changes consistent with an ischemic stroke in the distribution of the anterior cerebral artery.

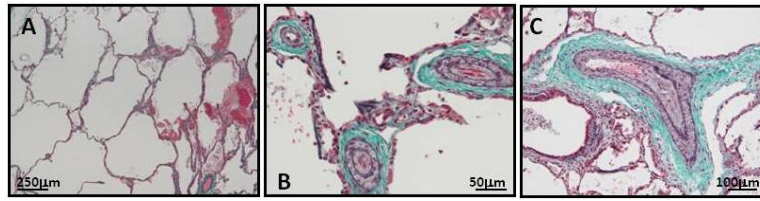


Figure 3. Panel A illustrates the emphysematous changes in the lungs in patient D (modified Trichrome staining elastin purple and collagen blue). Panel B and C show the increased thickness of the medial layer and subintimal proliferation of SMCs in the pulmonary vasculature of the same patient (dark purple lines surrounding the artery lumen are the internal and external elastic laminae, demarcating the intimal and medial layers and the medial and adventitial layers, respectively).

Table 1

Clinical characteristics of patients with the ACTA2R179H mutation.

Patient	A	B	C	D	E
Age	d. 11 y.o. ^a	14 y.o.	17 y.o.	11 y.o.	26 y.o.
Gender	female	male	male	female	female
CV	PDA, age at repair +, 5 months	+, 20 days	+, 18 months	+, 4 months	+, 4 months
	TAA, age at repair +	+	+, 12 yrs	+, 10 yrs	+, 11 yrs
	Dilated pulmonary arteries +	+		+	
	Other CV findings Dilatation of aortic arch, brachiocephalic, common carotid and left subclavian arteries, ASD		Left brachial artery AVM	Aortic coarctation	Dilatation of aortic arch, suprarenal abdominal aorta, brachiocephalic and common carotid arteries
	Bilateral stenoses of the terminal ICA +	+	+	+	+
	Bilateral dolichoectatic ICA +	+	+	+	+
	Bilateral periventricular WMH +	+	+	+	+
	Other CNS findings	Microaneurysms in the retina	Colpocephaly, basilar artery stenosis	Posterior communicating arteries and right ophthalmic artery occlusion	ACA and MCA distribution infarctions
Ocular	Congenital mydriasis +	+	+	+	+
GI	Malrotation -	-	+	+	-
	Hypopenialisis -	-	+	+	-
GU	Hypotonic bladder +	+	+	+	+
	Other -	Undescended testes			
Pulm	Tachypnea at birth +	+	+	+	+

Patient	A	B	C	D	E
Pulmonary hypertension					+
Lung disease		Hyperinflation of the lung	^b Chronic interstitial pneumonitis	Dysgenetic cystic lung disease	Undefined

Symbols are as follows: +, present; -, absent; blank, not known. Abbreviations are as follows: d, died; ASD, atrial septal defect; ICA, internal carotid arteries; WMH, white matter hyperintensities; AVM, arterial venous malformation; ACA, anterior cerebral artery; MCA, middle cerebral artery.

^a cause of death not determined at autopsy

^b bilateral lung transplant at 18 months of age for primary pulmonary hypertension.