Thoracic Aortic Aneurysm in Patients with Loss of Function *Filamin A* Mutations: Clinical Characterization, Genetics, and Recommendations

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Abstract

The frequency and gender distribution of thoracic aortic aneurysm as a cardiovascular manifestation of loss-of-function (LOF) X-linked FilaminA (*FLNA)* mutations are not known. Furthermore, there is very limited cardiovascular morbidity or mortality data in children and adults. We analyzed cardiac data on the largest series of 114 patients with LOF *FLNA* mutations, both children and adults, with periventricular nodular heterotopia (PVNH), including 48 study patients and 66 literature patients, median age of 22.0 years (88F, 26M, range: 0 – 71 years), with 75 *FLNA* mutations observed in 80 families. Most (64.9%) subjects had a cardiac anomaly or vascular abnormality (80.8% of males and 60.2% of females). Thoracic aortic aneurysms (TAA) were found in 18.4% (n= 21), and were associated with other structural cardiac malformations in 57.1% of patients, most commonly patent ductus arteriosus (PDA) and valvular abnormalities. TAA most frequently involved the aortic root and ascending aorta, and were associated with sinus of Valsalva aneurysms in almost half. Six TAA patients (28.5%) required surgery (median age 37 yrs, range 14 – 41). TAA was also the only recorded cause of premature, non-accidental mortality in adults (2M, 2F), with unanticipated aortic rupture found on autopsy in 2 adult patients (1F, 1M, median 38.5 yrs), at aortic dimensions smaller than current recommendations for surgery for other aortopathies. Data from this largest series of LOF *FLNA* mutation patients underscore the importance of serial follow-up to identify and manage these potentially devastating cardiovascular complications.

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INTRODUCTION

Although *FLNA* mutations have long been associated with an X-linked brain malformation and epilepsy (Fox et al., 1998), cardiovascular manifestations of *FLNA* mutations, including aortic aneurysm, have increasingly been recognized as being common and often life-threatening in this population (Oegema et al., 2013; Reinstein et al., 2013; Robertson et al., 2003; Sheen et al., 2005). *FLNA* loss-of-function mutations are usually male-lethal, and are associated most commonly in females with periventricular nodular heterotopia (PVNH), a disorder in which some neurons fail to migrate normally from where they are generated, in the ventricular region (Figure 1), to the cerebral cortex (Eksioglu et al., 1996; Fox et al., 1998; Kakita et al., 2002; Sheen et al., 2001). Frequently PVNH patients have normal intelligence and demonstrate few outward dysmorphic signs, despite the distinctive brain MRI pattern. Patients typically come to clinical attention when they develop seizures during late childhood or young adulthood, which usually triggers the brain MRI that results in the clinical diagnosis of PVNH (Chen & Walsh, 2015).

FLNA encodes a homodimeric protein that crosslinks F-actin to transduce ligand-receptor binding into actin reorganization (Feng et al., 2006; Feng & Walsh, 2004; Sheen et al., 2002; Wang, Ash, & Singer, 1975). The FilaminA protein binds over 90 cellular proteins of varying function and is widely expressed throughout the body, a likely explanation for the many different organ systems including the brain, skin, heart and vasculature that can be adversely affected when the *FLNA* gene is mutated (Ehrlicher, Nakamura, Hartwig, Weitz, & Stossel, 2011; Nakamura, Stossel, & Hartwig, 2011; Razinia, Makela, Ylanne, & Calderwood, 2012; Retailleau et al., 2016; Zhou, Boren, & Akyurek, 2007). *FLNA* missense mutations also can produce a diverse spectrum of other rare developmental diseases such as frontometaphyseal dysplasia, intestinal pseudo-obstruction, Melnick-Needles syndrome, and otopalatodigital syndromes with involvement of the bones, muscles, lung, and gastrointestinal tract (Clark, Sawyer, Robertson, & Sutherland-Smith, 2009; Foley et al., 2010; Kapur et al., 2010; Masurel-Paulet et al., 2011; Reinstein et al., 2013; Robertson et al., 2006; Robertson et al., 2003; Sun et al., 2010).

Mouse studies suggest that *FLNA* is important during the embryonic development of the heart and vasculature. Male *FLNA*-null mice often died in utero from lethal hemorrhage and have abnormally developed blood vessels. *FLNA* knockout mice also showed anomalies of the left-ventricular outflow tract, atria, septum, and the great vessels of the heart (Feng et al., 2006; Hart et al., 2006). Blood vessels were found to be abnormally dilated, coarse, and with abnormal branching patterns. Importantly, vascular endothelial cells showed abnormal adherens junctions suggesting abnormalities in cell-to-cell contact (Feng et al., 2006). FLNA also regulates the structure and function of large arteries in adult mice (Retailleau et al., 2016).

Cardiovascular anomalies in *FLNA* deficient patients with PVNH have been described in case reports or small series (de Wit et al., 2011; de Wit et al., 2009; Hehr et al., 2006; Kapur et al., 2010; Sole et al., 2009; Srour et al., 2011), but these limited numbers have prevented systematic assessment of cardiovascular outcomes from aortic aneurysms and cardiac malformations. Recently, the cardiovascular consequences of *FLNA* mutations were studied in 11 patients (Reinstein et al., 2013) and X-linked PVNH found to be associated with a range of vascular and connective tissue anomalies. Serial long-term data on change in aortic aneurysm size in PVNH patients have only rarely been reported. Therefore, in this paper, we systematically analyze the aortic disease and associated structural cardiac malformations of *FLNA* patients, present serial data when available on aortic aneurysm growth, and present death data on a total of 114 mutation-positive patients—48 original study patients and 66 literature patients. Our study may help guide cardiac screening, frequency, and also follow-up in this population.

METHODS

Patients

Inclusion criteria for patients were 1) radiographically confirmed periventricular nodular heterotopia (PVNH), 2) a documented mutation of the *FLNA* gene, and 3) sufficient medical history, cardiac imaging, or records available to determine the presence or absence of a cardiac anomaly or cardiovascular issues in individuals.

Boston Research Patient Cohort—Patients were consecutively enrolled from an existing registry of patients with *FLNA* mutation with PVNH. Of >300 case reviews of PVNH patients, only patients with confirmed *FLNA* mutation were included. Informed consent was obtained according to Human Studies Protocols and the study was approved by the institutional review boards of the Beth Israel Deaconess Medical Center, Boston Children's Hospital, and other participating institutions. In cases where cardiac information or outcomes was missing, the team re-contacted the subject's physicians to review the patient's cardiac history and available imaging; additionally, the study cardiologist (MHC) reviewed each subject's existing medical information in the database for diagnoses of cardiovascular disease. Cardiac images in our database, when available (i.e. echocardiography, CT, cardiac MRI), were also reviewed. Outcomes on aortic deaths were directly derived from autopsy findings listed on death certificates. Our data collection produced a total of 48 individuals; of which only a minority had limited cardiac history included in previous publications.

Literature Review Cohort—We also performed a literature review of all *FLNA* mutation-positive PVNH patients published in the literature until 2016 with sufficient clinical information to ascertain cardiac history (see Supplement). A PubMed search was conducted using a combination of the following MESH terms: FilaminA, Filamin1, *FLNA*, periventricular nodular heterotopia, cardiovascular, congenital heart defects, thoracic aortic aneurysm, patent ductus arteriosus, mitral valve prolapse, and septal defects. Additionally, references relevant to this cohort cited in the literature but not found on the first series of PubMed searches, were also reviewed.

Literature review subjects met the same three criteria as original cohort patients. Excluded were those who tested negative for a *FLNA* mutation or did not have documented genetic testing or lacked cardiac data. Duplicate reports of the same patient in different articles were also eliminated. Also excluded were patients from studies that aggregated medical and cardiac data, wherein there was insufficient information to correlate an individual's clinical phenotype to their genotype. All members of a family who were *FLNA* mutation carriers were included, not only the index case. This method generated over 36 articles, from which 66 patients met inclusion criteria (Supplemental Table 1). Thus 48 original patients with the 66 literature review subjects yielded the study cohort of 114 subjects.

Diagnoses and Definitions

Each subject was classified as having either 1) one or more cardiovascular abnormality or 2) no known cardiovascular issues. Cardiovascular abnormalities included both intracardiac lesions and cardiovascular lesions. An intracardiac lesion was defined as an anatomic defect involving the structures within the heart, i.e. atrial septal defect, ventricular septal defect, mitral valve prolapse, or other dysplastic valve. A cardiovascular lesion was defined as one that affects the vascular structures that are associated with the heart (i.e. aortic dilatation/aneurysm or pulmonary artery dilatation). With the literature cohort, we had to rely on definitions used by the authors. For our cohort, we reported the subjects' clinical diagnoses (i.e. aortic aneurysm or aortic dilatation). Since the study cohort included both children and adults, aortic size and z-scores were used to allow comparison of aortic dilatation among individuals of differing sizes. Of note, aortic dilatation, by convention, is defined as a Z-score of ≥ 2 (i.e., ≥ 2 standard deviations above the predicted mean normal diameter (Chubb & Simpson, 2012; Lopez et al., 2010). In adults, aortic dimensions at different anatomic locations were presented as absolute values per convention. Nomenclature of anatomic segments of the aorta in our own cohort (i.e. aortic root, ascending aorta, aortic arch, descending aorta) were defined as per the American Society of Echocardiography (Goldstein et al., 2015).

Analysis

Microsoft Access was used as the database and analysis software. Median age of the cohort was calculated. Age data for the original cohort was based on the age of the subject at completion of the collection of cardiovascular data. Age data from the literature cohort was taken directly from the articles. Z scores were used in the reporting of serial aortic dimensions in growing children so that significance could be determined (Chubb & Simpson, 2012; Lopez et al., 2010).

RESULTS

Study Schema and Clinical Features (Figure 2)

A total of 114 subjects (76.4% female, 84F, 26M), with a median age of 22 years (range: 0 – 71 years), with 75 unique *FLNA* mutations from 80 families, comprised the cohort. All patients except four had cardiac imaging or autopsy performed. Given that *FLNA* is X-linked, the greater proportion of females than males was not unexpected. Seventy-four subjects (64.9%, 21 M, 53F) had at least one vascular and/or structural cardiac

malformation, with the types of abnormalities categorized in Figure 2b. The remaining 40 individuals (35.6%, 35F, 5M) had no known cardiovascular issues by medical history. Prevalence of cardiovascular malformations and vascular abnormalities by gender is detailed in Figure 2c. Overall, 80.8% of males and 60.2% of females had at least one cardiac anomaly or vascular abnormality, but this difference was not statistically significant.

Thoracic Aorta Aneurysms (Table 1)

Twenty-one individuals (18.4%, 15F, 6M) had an aortic aneurysm or pathological aortic dilatation, making it the most common vascular abnormality in this cohort. Since definitions and usage of the terms TAA and aortic dilatation vary somewhat between institutions, and between pediatric and adult patients, we use the term TAA to refer to both. The *FLNA* mutations of these 21 patients with TAA are summarized in Figure 3. The median age of all subjects with TAA was 23 years (range 0 months - 57 years). Unexpectedly, a similar proportion of males and females had an aortic aneurysm (Figure 2c). Median age of males vs. females with a TAA was 26 yrs. vs. 23 yrs. respectively. Many of our patients with PVNH and TAA have symptoms and signs that are frequently also seen in Ehlers-Danlos syndrome, including joint hypermobility, skin hyperelasticity, and cutaneous abnormalities (Reinstein et al., 2013)(Sheen et al, 2005).

Clinical Presentation

A brief history of five patients with PVNH with TAA is presented below, demonstrating the region of aortic involvement, progressive dilatation, and diverse outcomes (Table 1).

#114 was a 21 year-old college student, born at term, s/p PDA ligation at age 10 months, and with a history of mitral valve prolapse since infancy. She also has asthma responsive to inhalers. She developed seizures at age 16 years and was diagnosed with PVNH with a *FLNA* mutation. Echocardiogram at age 16 showed a new sinus of Valsalva aneurysm involving her right coronary cusp (RCC) and noncoronary cusp (NCC) and a perimembranous VSD (See Figure 4 A–D). Her aortic root measured 4.2 cm, and has been without significant progression in the last five years.

#85 was a 11 year-old female with PVNH with additional cardiac follow-up and serial echocardiography data analysis obtained for this study. Initially described as a 6 year-old, (Reinstein et al., 2013), she had a PDA s/p surgically ligation in infancy, a small sinus of Valvalva aneurysm, history of pulmonary hypertension and lung disease initially dependent on sildenafil, and has now improved with time. She was noted to have mild ascending aortic dilatation at birth, with a normal aortic root size. Serial analysis for this study showed progressive increase in her aortic root size, with continued eccentric enlargement of a sinus of Valsalva aneurysm involving her non-coronary cusp (See Figure 4 E–F). A perimembranous VSD is also visualized. Over the last decade, her aortic root z scores have transitioned from normal at birth to abnormal [z = 0.6 (birth) → 5.0 (11 years old)] (Figure 5A).

#37 was a 17 year-old female diagnosed with PDA at 3 months, s/p surgical closure, and with a dilated aorta since birth (Figure 5B, 6). She underwent a double lung transplant in infancy for bronchoalveolar dysplasia. At age 5 years, in the setting of seizure-like activity,

we diagnosed her with PVNH and a *FLNA* mutation. She was empirically treated with Losartan at age 10 years by her cardiologist, but her ascending aorta continued to dilate. At age 13 years, she underwent ascending aortic aneurysm repair surgery at an aortic root size of 4.2 cm (Z score = 11.9) (Figure 5B).

#39 was a 36 year-old male that died from a sudden aortic rupture. He was diagnosed with partial complex epilepsy which brought him to medical care at age 32 years. He was noted to have increasing voltages on his EKG for borderline left ventricular hypertrophy and also left atrial enlargement over the ensuing next 5 years. Last physical exam by neurology noted a pulsatile lesion in his neck area. He was lost to subsequent follow-up. His death certificate documented fatal aortic rupture with cardiac tamponade. No further records or details are available.

#36 was a 41 year-old female first found to have mild aortic root and ascending aortic dilatation at 29 years, after presenting with a seizure post-partum and being diagnosed with PVNH. She was otherwise well. Initial echocardiogram demonstrated eccentric sinus of Valsalva aneurysms involving the NCC and RCC, and an aortic root size of 2.7 cm. She remained asymptomatic, and serial echocardiograms over the next decade showed progressive aortic root dilatation from 2.7 cm (age 29 years) to 3.2 cm (36 years) to 4.2 cm (41 years) (Figure 5). At 41 years, several months after her last echocardiogram, she died suddenly. Autopsy identified the cause of death as sudden aortic rupture. Importantly, although an aortic root size of 4.2 cm is above normal limits, her aortic dimensions were smaller than current recommendations for aortic surgery in aortopathies.

Thoracic Location (Table 1)

The location of the aortic aneurysm was known in 10 patients, and not specified in 2 literature subjects. In all 19 patients, the aortic aneurysm involved the aortic root and/or thoracic ascending aorta, as typically seen in other genetic causes of TAA in the young (Isselbacher, Lino Cardenas, & Lindsay, 2016). The descending aorta was very infrequently involved, with only one patient having diffuse dilatation of his vasculature system including the entire thoracic aorta, pulmonary arteries, and also head and neck vessels. Another single patient had involvement of the abdominal aorta in addition to the thoracic aorta, and none had an abdominal aneurysm alone. For patients with aortic root aneurysms, there was frequent eccentric involvement of the sinuses of Valsalva (n= 9, 2M, 7F), with preferential involvement of the right and non-coronary cusps rather than the left coronary cusp. Of these 9 patients with sinus aneurysms, 4 also had dilatation of the thoracic ascending aorta.

Change in Size of Aortic Aneurysm

There was progressive dilatation of the TAA in individuals followed serially, leading to aortic aneurysm surgery in some patients, and deaths noted in some unoperated patients (Table 1). Serial aortic measurements were available in 5 individuals with TAA. Two young female subjects, with normal aortic root size at birth, developed sinus of Valsalva aneurysms during childhood (#85, #114 Figure 4). Longitudinal data of aortic root size over 10 years is presented in 3 patients (Figure 5).

Surgical Repair (Table 1)

Seven of 21 patients (33.3%, 2M, 5F) with TAA underwent aortic aneurysm repair, with one dying of a post-surgical bleed. The age of repair, known in 5 patients, was a median of 37 years (range 14 – 41 years). Two patients (1M, 1F) had progressive aortic dilatation after TAA surgery, with one needing a reoperation 16 years later. The size of the aorta at the time of surgical repair was not available in most subjects, except for three. One child underwent ascending aorta repair at 4.8 cm (z score=11.9); an adult female repaired with an ascending aorta of 5.1 cm, and a third patient, originally reported by Reinstein, was operated on when TAA increased to 5.7 cm (Reinstein et al., 2013)(Table 1).

Structural Cardiac Malformations (SCM)

The types and frequency of *FLNA*-associated SCM for the entire cohort are displayed in Figure 2b. Specifically, over half the patients with TAA (57.1%, 12/21) also had an associated SCM. Patent ductus arteriosus (PDA, n=7) and ventricular septal defects (VSD, n=6) were most common in patients with TAA. In 3 patients where the type of VSD was known, it was perimembranous. Not surprisingly, aortic valve abnormalities (bicuspid/dysplastic AV) were common, occurring in 28.6% (6/21) of TAA patients. Mitral valve abnormalities were noted in 5/21 TAA patients, with elongated leaflets resulting in prolapse or thickened, dysplastic valves.

Pulmonary Artery and other Involved Blood Vessels

Pulmonary artery dilatation was also common, occurring in 5 patients. Most patients (4/5) with pulmonary artery dilatation also had a TAA. Aneurysms of other vessels present in our study included the subclavian artery (1), the abdominal aorta (1), the middle cerebral artery (1), and the internal carotid artery (1).

Vascular Events and Death

In addition to the 21 patients with thoracic aortic aneurysms described above, four additional subjects (all female and without TAA) had a vascular event (myocardial ischemia, stroke/TIA, subarachnoid hemorrhage) at relatively young ages (median 40 yrs, range 19–71 yrs). One had a myocardial infarction at age 39. Another had a stroke at age 19 due to a thrombus without known source of embolus, but did have protein C deficiency. Another had a first stroke at age 41 and recurrent TIA/stroke thereafter at age 50 without source or clotting disorder found. Finally, one had a fatal subarachnoid hemorrhage at age 71 secondary to a rupture of an internal carotid artery aneurysm. One additional patient had dilatation of the pulmonary artery root without a TAA (Reinstein et al., 2013). Therefore, a total of twenty-six subjects (22.8%, 6M, 19F) of the entire cohort had a vascular aneurysm or dilatation, or suffered a vascular event. Vascular aneurysms were found in both children and adults, as well as in males and females.

Pediatric and Adult Deaths (Table 2)

Eight patients (7.3%, 3F, 5M) suffered nontraumatic death at the time of their respective study, with a median age of death of 18 years old (range: 0–71 years). A ninth patient died at age 48 years in a motorcycle accident and was not included in the analysis below. Half of all

non-traumatic deaths (50.0%, 3M, 1F) occurred in infants < 1 year of age, with the remainder occurring during adulthood (2M, 2F, median age 41 years, range 36–57 years). The cause of death in infancy was predominantly secondary to pulmonary disease with or without heart failure, or the sequelae of multiple congenital defects. In contrast, the most common cause of death in adults (3/4) was autopsy-confirmed aortic rupture, or post-operative bleeding s/p aortic root repair for aortic aneurysm.

DISCUSSION

Here we provide the largest systematic analysis to-date of the frequency of aortic aneurysms and cardiovascular outcomes in both pediatric and adult patients with PVNH and *FLNA* loss-of-function mutations. Although ascertainment of cardiovascular abnormalities in the majority was performed by reviewing existing genetic records and medical records in this relatively young population, we find a surprisingly high 18.4% of PVNH patients had aneurysms of the aortic root and/or the thoracic ascending aorta, and almost 42.1% (48/114) had SCM. Furthermore, we found that a) the location of the aortic aneurysms was almost entirely thoracic, commonly involving the aortic root and ascending aorta, and frequently associated with sinus of Valsalva aneurysms, b) there was progressive dilatation of the TAA in both children and adults, frequently resulting in the need for surgery during early adulthood, and c) TAA rupture was a cause of premature death in middle-age adults with PVNH. Rupture of the aorta and the frequency of deaths from TAA have not been reported previously in *FLNA* patients. Our data underscores the importance of long-term cardiovascular screening of all *FLNA* mutation positive PVNH patients for this silent, life-threatening disease.

Eighty percent of male *FLNA* mutation carriers in our cohort show a SCM, vascular abnormality, or lung disease, and are at risk for death during childhood (Table 1). Of course, most males with *FLNA* mutations die before birth, and those that survive childhood often have mutations that preserve some residual *FLNA* function. Nevertheless, occasional males in our cohort survived into adulthood, although 2 of them subsequently died from TAA (Table 2). We presume that the postnatal survival of these males reflects mutations that leave some residual FLNA function intact, and the nature of the mutations in males associated with postnatal survival (missense, splicing, truncating mutations late in the protein sequence in the case of older males) are generally consistent with this notion (Guerrini et al., 2004; Hehr et al., 2006; Oegema et al., 2013; Parrini, Mei, Wright, Dorn, & Guerrini, 2004; Sheen et al., 2005; Zhang et al., 2016).

In mice, *FLNA* has essential functions both in the vascular endothelium, neural crest, and arterial muscle layers, all of which might contribute in principal to the pathophysiology of the TAA in humans. Germline *FLNA* mutant mice show widespread abnormalities of vascular patterning and growth, as well as abnormal endothelial adherens junctions between vascular endothelial cells, which results in decreased integrity of the vascular walls and widespread hemorrhage prenatally in males (Feng et al., 2006). Moreover, *FLNA* has essential roles in cardiac and valvular development that are at least in part reproduced by conditional removal from neural crest cells (Feng et al., 2006; Sauls et al., 2012) so that essential roles in neural crest might also lead to TAA (Sherif, 2014; Weinreich, Yu, & Trost,

2015). The preferential involvement of the RCC and the NCC, compared to the LCC, may be explained by their embryologic derivation from neural crest cells (Sherif, 2014). Sinus of Valsalva aneurysms are thought to result from a lack of continuity between the aortic media and the aortic annulus (Feldman & Roman, 2006). Interestingly, this region of the aorta is subject to rupture and not dissection. The association of perimembranous VSD with sinus of Valsalva aneurysms is consistent with the observation that this portion of the septum serves as the base which forms the right and noncoronary sinuses (Taguchi, Sasaki, Matsuura, & Uemura, 1969). Our *FLNA* mouse model also developed a VSD, also likely reflecting a known embryologic derivative of the distal left ventricular outflow tract from neural crest cells (Feng et al., 2006).

Although FLNA binds a host of other intracellular molecules, the distribution of missense mutations associated with TAA might give some tentative insight into cellular/molecular mechanisms. FLNA is thought to anchor the actin cytoskeleton to transmembrane integrin molecules (Karimi & Milewicz, 2016). Interestingly in our current analysis we find that 5 unique missense mutations in 7 patients with TAA reside in the first five exons of the coding region (Figure 3). Since this segment of the gene encodes the actin-binding domain, these mutations suggest that TAA might involve at some level a dysregulation of the polymerization of actin filaments, but this suggestion would need further substantiation in animal models.

It is not known in patients when the aortic aneurysms first present, since cardiac imaging is not always performed in this population and serial follow-up is even rarer, and TAA is thus often not diagnosed until symptomatic. Of note, our data demonstrates that aortic dilatation can be present at birth, as evident in several of our patients. In other patients with available serial data, we observe progressive dilatation of the aorta over 5–10 years. Our preliminary findings suggest that *FLNA* mutations may behave somewhat differently than genetic forms of Ehlers-Danlos Syndrome, which tend to show greater stability of aortic size over time (Reinstein et al., 2013), and underscores that serial follow-up and imaging of the thoracic aorta are critical in *FLNA* patients.

Our findings are consistent with data that TAA are often genetic in etiology and occur generally in a younger age group than abdominal aortic aneurysms, which tend to be atherosclerotic in nature (Isselbacher et al., 2016; Lindsay & Dietz, 2014). Currently, almost 20 genes have been previously identified as causing TAA: *ACTA2, COL3A1, FBN1, TGFB 2–3, TGFBR1–2, SMAD3, MFAP5, MYH11, MYLK, PRKG1, AAT1, AAT2,* and *MAT2A* (Milewicz et al., 2008; Milewicz & Regalado, 2016). They have been generally categorized into genes affecting extracellular matrix proteins (i.e. *COL3A1, FBN1),* TGF-Beta signaling (*TGFB 2–3, TGFBR1–2, SMAD2–4*), or cytoskeletal/smooth muscle contraction apparatus proteins (e.g., *MYH11, MYLK, PRKG1*) and others. There are several similarities between our PVNH *FLNA* cohort and patients with other cytoskeletal/smooth muscle contraction apparatus gene mutations. *MYH11* encodes a regulator of affects smooth muscle myosin and the actin-binding site (Isselbacher et al., 2016) and patients with *MYH11* mutations also develop TAA and PDA (Zhu et al., 2006). *ACTA2* mutations are also associated with both TAA and PDA (Regalado et al., 2015). Our data further support the hypothesis that *FLNA, MYH11,* and *ACTA2* share defects in mechanical strength of the vascular walls or the ability

of the arterial walls to contract, potentially in part through a common pathway (Karimi & Milewicz, 2016; Retailleau et al., 2016).

For *FLNA*, *ACTA2*, *TGFBR2*, and *FBN1* individuals with thoracic aneurysms, there were a high percentage of patients who suffered a vascular event. However, unlike Marfan, Loeys-Dietz syndrome, and *ACTA2* patients, our cohort often suffered sudden aortic rupture rather than acute or chronic aortic dissection. None of the death certificates reviewed on our patients indicated that they had an aortic dissection, although further work needs to be done in this area, and there is a great need to obtain pathological material to characterize the histopathology of the *FLNA* TAA. Sinus of Valsalva aneurysms typically rupture and tend not to dissect. Given the frequency of sinus of Valsalva aneurysms in this study and the abnormal adherens junctions in *FLNA* mutant mice, it may be not be unexpected that sudden rupture occurs, and often at aortic root diameters much smaller than in standard thoracic aortic dissections. Like Loeys-Dietz and Marfan disease, *FLNA* patients mainly have dilatation of the aortic root and the thoracic ascending aorta, and but also show dilatation of the pulmonary arteries, and also mitral valve dysplasia or prolapse (Lindsay & Dietz, 2014). Like Marfan and *TGFBR2* patients, a high percentage of *FLNA* patients require aortic surgical repair. In contrast, *ACTA2* patients have frequent involvement of their entire arch, whereas most of our cohort only had thoracic ascending aorta dilatation, with very rare involvement of the descending aorta or abdominal aorta, by history or by type of surgical repair required (Regalado et al., 2015).

Our results suggest that a comprehensive baseline cardiac evaluation should be undertaken at the time of PVNH diagnosis to identify any existing SCM, as well as to document baseline dimensions of the thoracic aorta. Imaging should include at least an echocardiogram for identification of any structural anomaly including septal defects, valvular abnormalities, and PDA (Chen & Walsh, 2015). At the same time, measurements of the aortic root, and the visualized portion of the ascending and descending thoracic aorta should also be performed (Lai, Mertens, Geva, & Cohen 2009). For any patient with difficult acoustic windows, a CT or a MR should be performed to examine the vasculature of the transverse aorta and the great vessels (Lai et al., 2009). Data on vascular tortuosity also can also be obtained via MRI or CT, although its usefulness to the *FLNA* population is unknown (Reinstein, Morris, Rimoin, Robertson, & Lacro, 2014). We recommend that serial cardiac imaging and cardiac follow-up be performed for all *FLNA* positive patients given the documented progressive nature of TAA associated with this disease, even in patients who have normal sized aortas. Childhood and times of rapid growth may be when even closer follow-up is warranted. Also women who are contemplating pregnancy or those undergo other surgical procedures should be followed more closely.

As with other aortopathies, the frequency of imaging in patients with an aortic aneurysm or dilated aortic dimensions is based on size and rate of progression. Furthermore, there are currently no specific recommendations of aortic size to consider aortic root or ascending aorta surgery in *FLNA* patients, and much more data is needed. Our cohort may more resemble patients with Loeys-Dietz syndrome, where surgical repair is considered when the patient's aortic size approaches 4.0–4.5 cm (the range at which our *FLNA* patient ruptured), and obviously more urgently if the aneurysm is growing rapidly. There is very little known

about specific medical therapy that might modulate the course of aortic enlargement due to *FLNA* mutation, and specifically no published data about potential efficacy of atenolol or losartan, which have shown some efficacy in Marfan syndrome (Franken et al., 2015; Lacro et al., 2014; Sandor et al., 2015). Increased awareness of the natural history of *FLNA* mutations may allow identification of multi-center cohorts needed to perform such clinical studies. Given the catastrophic consequences of aortic rupture and the importance of early diagnosis of silent aortic aneurysms, DNA testing or MRI analysis of any first degree of affected individuals should be performed, and cardiac imaging is recommended.

Since early studies were mainly conducted to look for neurologic disease, the association of PVNH with aortic aneurysms and cardiac anomalies was not always systematically gathered. Since detailed cardiac history and serial cardiac screening was not always performed, even our reported frequency of TAA may well be an underestimation of the true frequency of SCM and TAA in PVNH patients. The majority of findings was derived from medical history, post-mortem examination, and not by comprehensive prospectively conducted serial cardiovascular screening, which would be the gold standard. Even in our cohort where we attempted to contact each patient, and recommended cardiac screening, some did not receive it. Despite these issues, the high frequency of TAA and the unexpected premature death of patients make greater awareness and management of *FLNA* and aortic aneurysms imperative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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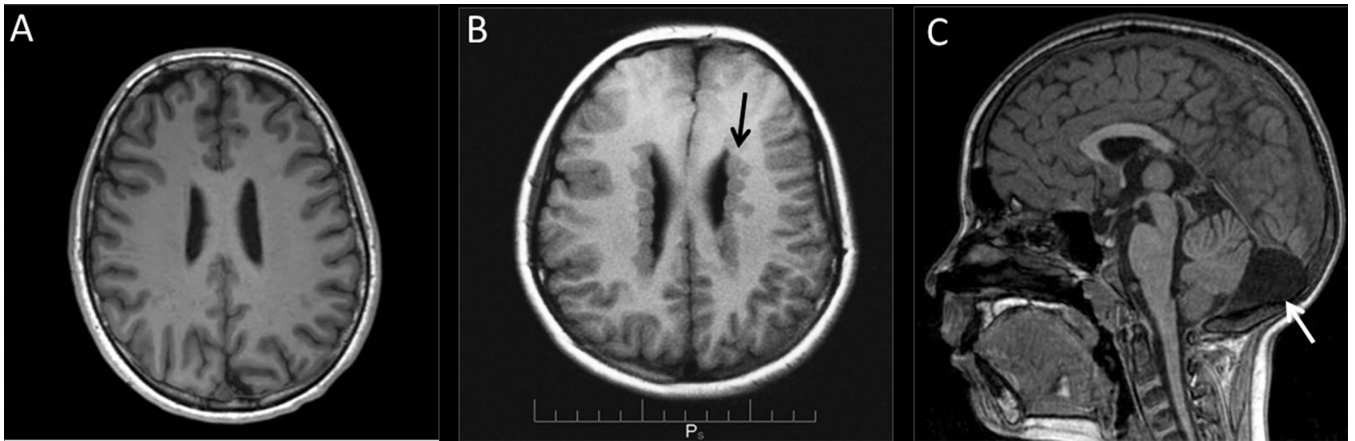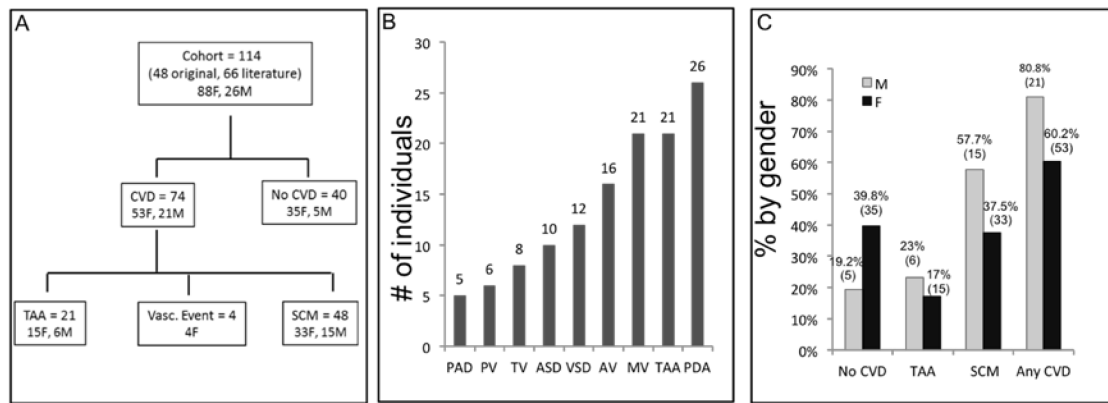
Figure 1: Representative axial T1-weighted brain Magnetic Resonance Imaging (MR) from normal and PVNH patients.

(A) Patient without PVNH. (B) PVNH patient with nodular heterotopias (arrow) along the lateral ventricles of the brain. (C) Mega cisterna magna (arrow) is seen on sagittal images in some PVNH patients.

Figure 2:

(A) *Study schema (n=114 individuals).* 64.9% (n=74) of the cohort had either a vascular aneurysm or event (n=26) or a structural cardiac anomaly (n=48). Twenty-one had a thoracic aortic aneurysm (TAA), with 4/21 patients having both a TAA and a dilated pulmonary artery. One patient with a dilated pulmonary artery alone was not specifically depicted in schema. (B) *Frequency of the vascular and structural cardiac abnormalities in individuals.* TAA and PDAs were very common in subjects and frequently occurred in conjunction. (C) *Gender distribution of cardiovascular malformations and aneurysms:* There was no significant difference between males and females, by Fisher's exact test. Absolute numbers are in parentheses. M = male, F = female. No CVD = No cardiovascular anomalies or abnormalities, TAA = Thoracic aortic aneurysm, SCM= Structural cardiovascular malformation, Vasc = Vascular event (i.e. stroke, myocardial infarction).

ASD = Atrial septal defect, AV= Aortic valve abnormality, MV= Mitral valve abnormality, PAD = Pulmonary artery dilatation, PDA = Patent ductus arteriosus, PV = Pulmonary valve abnormality, TV= Tricuspid valve abnormality, VSD = Ventricular septal defect.
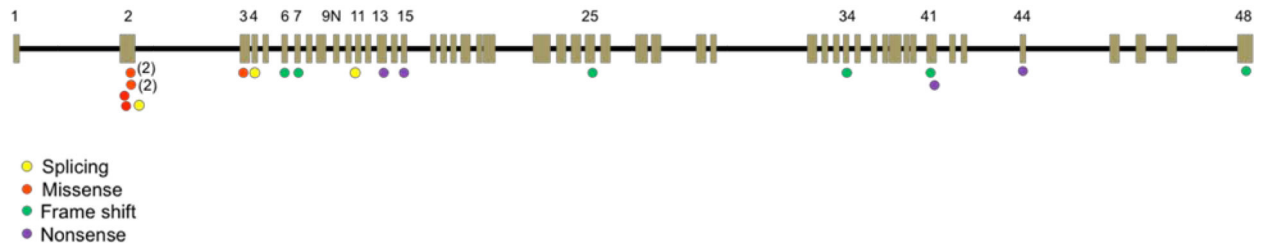
Figure 3: Genomic locations of exonic FLNA mutations associated with TAA.
A schematic representation of the *FLNA* gene. The *FLNA* gene is 25.9 kbp in size and encoded by 49 exons including a newly discovered "poison" exon 9N (Zhang et al, 2016), present in the contig: NT 025965. Exons are depicted by boxes, and introns are shown as black lines between the exons. Exons with relevant mutations are numbered. Types of *FLNA* mutations occurring in patients with TAA are localized on the *FLNA* map. The mutation in two families that each show two 2 individuals is indicated by one circle with total family members in parentheses. Five unique missense *FLNA* mutations (occurring in 7 individuals) associated with TAA localize most commonly to the actin-binding domains of *FLNA* gene (encoded by exons 2–5), whereas other loss-of-function mutations (nonsense, splicing, frameshift) are seen throughout the gene. One patient had a complete deletion of the *FLNA* gene.
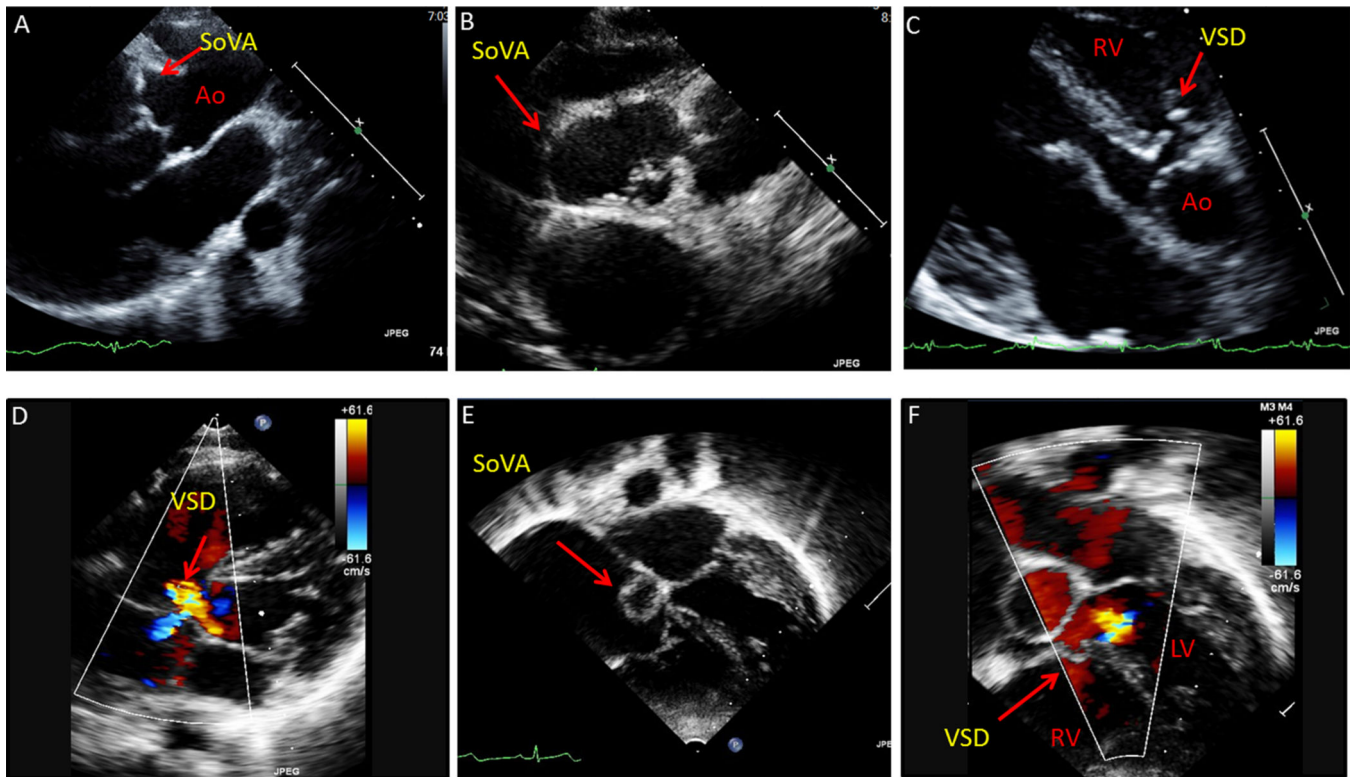
Figure 4: Sinus of Valsalva aneurysms (SoVA) with perimembranous ventricular septal defect in 2 young females.

(A-B) Echocardiography images from 21 year-old female with SoVA involving the right and non-coronary cusps of the aorta (parasternal long-axis and short-axis views). (C-D) Patient also has a perimembranous ventricular septal defect (VSD) with shunt flow, seen on parasternal and apical views. (E-F) Echocardiography images from an 11 year-old female depicting sinus of Valsalva aneurysm and a perimembranous VSD (apical 5-chamber view). Ao = aorta, LV = left ventricle, RV= right ventricle.
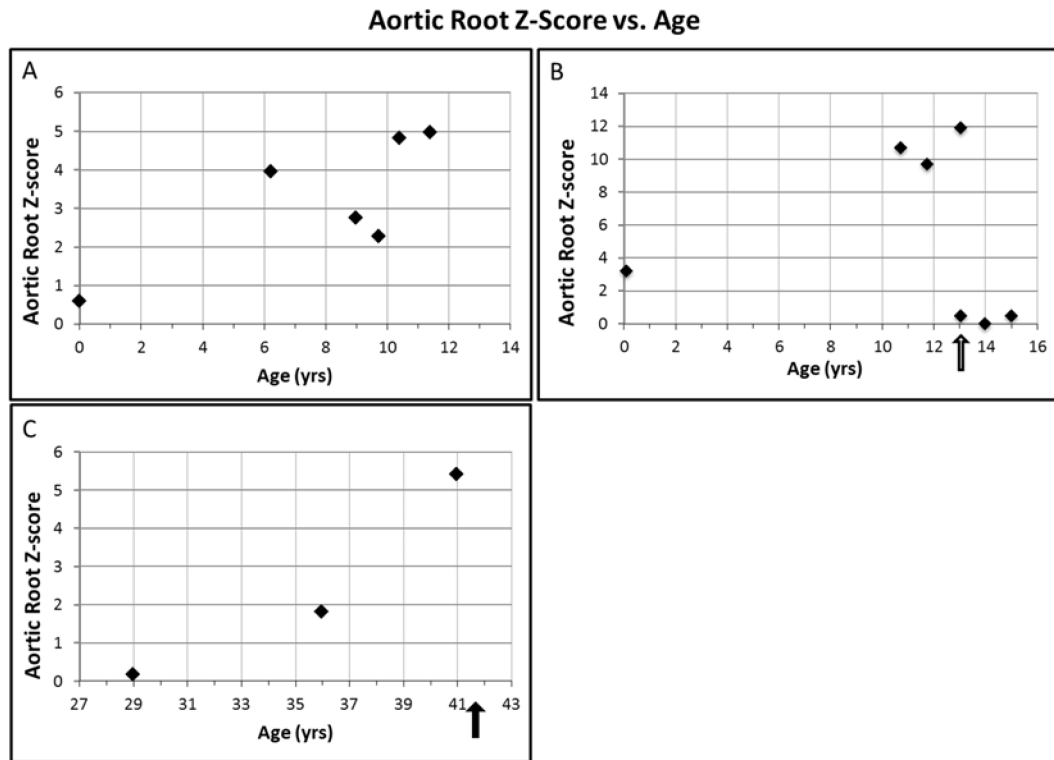
Aortic Root Z-Score vs. Age

Figure 5: Aortic root growth vs. age in 3 patients, pediatric and adult.

Aortic size is displayed as Z-scores in all individuals to allow for comparison. (A) Female child (#85) has a normal Z score at birth, but over the next 10 years, her aortic root z-scores have increased to ~5. (B) #37 Female child (#37) was born with a dilated aorta that increased to a Z score of 11.9, before her aneurysm surgery. Root size was normalized by surgery. Black arrow indicates timing of aortic aneurysm surgery. (C) Middle-age adult female (#36) who had normal aortic root Z scores on initial imaging, with a rapid increase in aortic root size between age 35 and 41 years of age. Aortic root size was 4.2 cm (Z score = 5.4) at time of death. Black arrow denotes timing of patient demise.
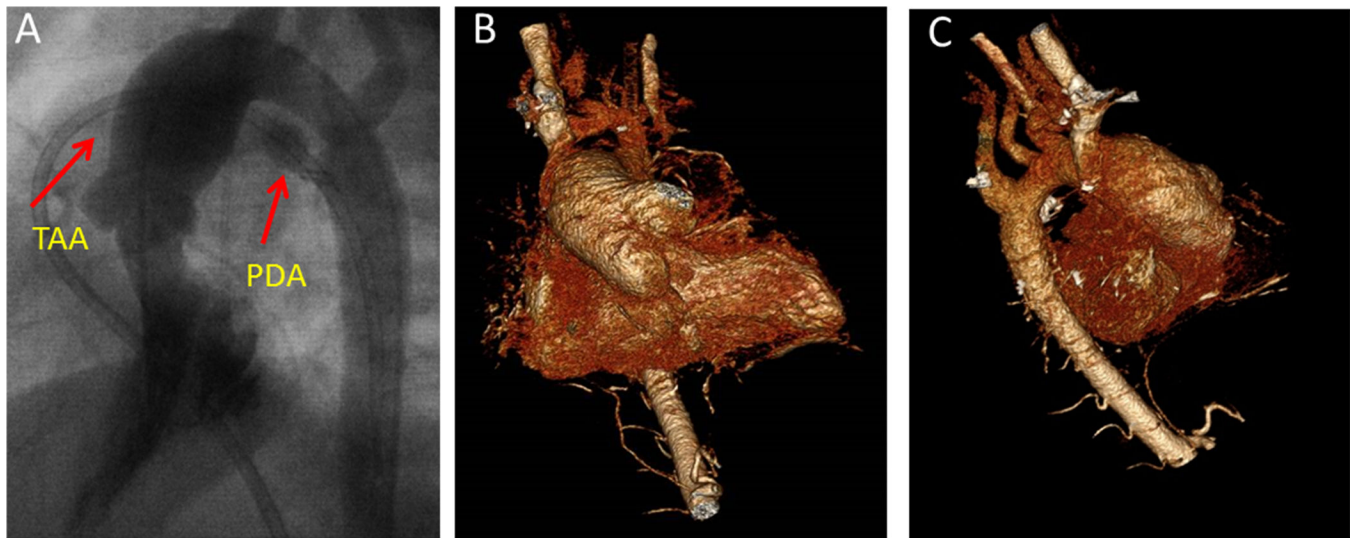
Figure 6: Female with progressive thoracic aortic dilatation requiring surgery.
(A) Dilated aorta and PDA seen on cardiac catheterization images during infancy. (B-C) 3D CT images of the patient's aorta, obtained a decade later, demonstrate persistent dilatation of the aortic root and ascending aorta without involvement of the distal arch or descending aorta.

Table I:

Patients with Dilated Thoracic Aorta or Thoracic Aortic Aneurysm (n=21/114, M=6, F=15):

Study ID	Sex (Age)	*FLNA* mutation	Aneurysm Location	Other significant cardiopulmonary or medical findings	Cardiovascular Outcome
Boston Cohort #9	F (43y)	GT to GC at exon 4+2	- Preop: Sinus of Valsalva aneurysm involving the non-coronary sinus (4.0×3.5 cm), eroding down toward the roof of the LA - Dilated thoracic aorta (5.1cm) from the sinuses to the mid-transverse aorta (between the left common carotid and the left subclavian artery)	- Congenital bicuspid aortic valve with moderate aortic stenosis with mild AR prior to Ross - Seizures, prolactin-secreting adenoma	1) S/p Dacron interposition graft in ascending aorta at age 36; S/p Ross procedure for aortic stenosis 2) Aortic arch began to dilate at age 40 years
Boston cohort #27	F (16y)	c.4147 del G single base pair deletion exon 25, p.(Ala1383Leufs*10)	-Thoracic ascending aorta (unknown)	- Hyperflexibility and joint dislocations - No seizures	n/a
Boston Cohort #29	F (15y)	c.116 C>G exon 2 p.(Ala39Gly)	- Sinus of Valsalva aneurysm	- Hyperelasticity of the large vessels, hyperextensibility of skin, joint laxity and subluxations - Seizures, mild developmental delay	n/a
Boston Cohort #36	F (41y)	G>A intron 2	- Sinuses of Valsalva aneurysm (non-coronary and right-coronary sinuses) - Mildly dilated thoracic ascending aorta	- Dysplastic AV, perimembranous VSD, moderate AR, mild MR. Normal abdominal aorta - Hyperflexible with joint dislocations - Seizures	1) Progressive increase in aortic size from age 29 to 41 years 2) Died age 41yrs of lethal aortic rupture, at an aortic root size of 4.2 cm
Boston Cohort #37	F (17y)	C>T exon 48, p.(Gln2385*)	- Dilated aortic root (4.8cm) - Dilated thoracic ascending aorta (2.7cm)	- PDA, PFO, perimembranous VSD, MVP. Pulmonary arterial dilation (3.7cm). Coronary fistula to proximal coronary artery - Developmental delay, no documented seizures	1) s/p PDA repair neonatal period 2) s/p Bilateral lung transplant during infancy 3) s/p Aortic root and valve replacement at age 13 years 4) s/p mitral valve annuloplasty ring and mitral valve repair
Boston Cohort #39	M (36y)	c.6915bp C>G exon 41, p.(Tyr2305*)	- Dilated thoracic ascending aorta	- AR - Seizures	1) Died age 36 yrs from aortic rupture and cardiac tamponade
Reinstein 2013, F3 ++ Boston Cohort #85	F(11y)	c.2193C>A exon15, p.(Tyr731*)	- Sinus of Valsalva aneurysm involving the non-coronary cusp. - Mildly dilated thoracic ascending aorta - Mildly dilated descending aorta.	- PDA, perimembranous VSD; dysplastic pulmonary, tricuspid and aortic valves. Moderate AR, PFO - Pulmonary hypertension with lung disease at birth that later stabilized. Dilatation of main pulmonary artery and its branches. Dilated head and neck branches. Hyperflexible. - No seizures	1) PDA ligated at 10 weeks 2) Development of sinus of Valsalva aneurysm by age 6 2) Aortic root Z score normal at birth and increased to 5 by age 11

Study ID	Sex (Age)	FLNA mutation	Aneurysm Location	Other significant cardiopulmonary or medical findings	Cardiovascular Outcome
Boston Cohort #107	F(1Y)	Complete deletion	- Nl size aortic root - Dilated thoracic ascending aorta (z score=4.6)	- Small PDA - Hyperflexible, no seizures	No cardiac symptoms
Boston Cohort #114	F(21)	c.62_64TCG exon 2,p.(Val21dup)	- Sinus of Valsalva aneurysm involving the right and non-coronary cusps - Mildly dilated thoracic ascending aorta	- PDA, perimembranous VSD, MVP with mild mitral regurgitation - Asthma, normal intellect, no seizures	1) PDA ligated at 10 months
Guerrini 2004, 3-II:1	M (49y)	A>G at intron 11	-Thoracic ascending aorta	- Dysplastic AV - Bifid epiglottis, seizures	1) s/p aortic valve replacement and ascending aorta replacement at age 26 yrs
Sole * 2009, F8	F(43y)	c.365 T>G exon 2 p.(Val122Gly) Mother of Sole F9.	- Aortic aneurysm (no further detail)	- Joint hypermobility - Seizures	1) s/p Aortic aneurysm surgery at age 38yrs
Sole * 2009, F9	F(15y)	c.365T>G exon 2 p.(Val122Gly)	- Aortic aneurysm (no further detail)	- EDS with ankle dislocation and joint hyperlaxity - No Sz	Treated with beta-blockers
Sole ** 2009,F15	F(35y)	c.356T>A exon 2 p.(Val122Gly)	- Aortic aneurysm (no further detail)	- EDS- with joint hyperlaxity, AR - Seizures	Treated with beta-blockers
Fergelot *** 2012, P1	M(57y)	c.356T>A exon 2, p.(Val122Gly) Father of Sole 2009, F15	- Severely dilated aortic root by age 41 years - Dilated thoracic ascending aorta (6cm)	- Severe AR, thrombocytopenia - Normal cognitive development, no seizures	1) s/p Aortic root repair and also AV replacement at age 41 yrs 2) s/p Bentall at age 57 yrs for progressive dilatation of the ascending aorta and also stenosed aortic valve prosthesis 3) Died post- Bentall of severe bleeding complications at age 57
de Wit 2011, P5	F(42y)	c.6635delTCAG exon 41, p.(Ser2213*)	-Dilated ascending aorta	- Severe AR from dysplastic AV, VSD - No seizures	1) s/p VSD repair and aortic valve replacement at age 24 yrs 2) s/p Bentall at age 36 yrs
Masurel-Paulet 2010, P1	M(6y)	c.994delG exon 7 p.(Lys331*)	- Sinus of Valsalva aneurysm	- PDA, AR, enlarged RV with wall hypertrophy. Pan-pulmonary emphysema and pulmonary arterial hypertension - Developmental delay; poor feeding, no seizures	s/p PDA ligation after 5 days of life
Reinstein 2013, F1	F(38y)	c883-880 del8 exon 6, p.(Asn296Glu fs*38)	- Sinus of Valsalva aneurysm	- Diffuse ectasia of aortic branch vessels - No seizures	s/p valve-sparing aortic root repair by age 38 years Root increased from 48mm to 57 cm (preop)
Reinstein 2013, F5	F(23y)	c.7813delC exon 48, p.(Leu2605Trpfs*2)	- Dilated aortic root (4.5cm) - Dilated thoracic ascending aorta	- AR, Pulmonary artery dilatation - Seizures	n/a
Reinstein 2013, M2	M(2mo)	c.5498-5504delCACCACCACinsAC exon 34, p.(Ala1833Aspfs*13)	- Dilated thoracic aorta - Dilated abdominal aorta	- PDA. Dysplastic AV, thickened mitral and tricuspid valves on autopsy. ASD, VSD	Died at age 2 months, secondary to sepsis following pulmonary hypertension and heart failure

Study ID	Sex (Age)	*FLNA* mutation	Aneurysm Location	Other significant cardiopulmonary or medical findings	Cardiovascular Outcome
			- Elongated/tortuous supra-aortic vessels	- Pulmonary hypertension, with pulmonary artery dilatation progressive heart failure. - No seizures	
Reinstein 2013, M3	M(15y)	c.381G>C exon3, p.(Lys127Asn)	- Dilated aortic root (3.1cm, nl < 2.8cm)	- MVP, history of pneumothorax, slightly hyperextensible skin, joint hypermobility, and umbilical hernia. - No seizures	n/a
LaPointe 2014	F(38y)	c.2002C>T exon13, p.(Gln668Ter)	- Dilated aortic root - Dilated thoracic ascending aorta	- Mild AR. PDA, bruising - Seizures	PDA repaired at 18 months

AR = aortic regurgitation. AVR = aortic valve replacement. AV= aortic valve. Bentall = Bentall cardiac surgical procedure to replace the aortic valve, aortic root, and ascending aorta. EDS = Ehlers Danlos Syndrome. LA = left atrium. MR = mitral regurgitation. PASP = pulmonary arterial pressures PDA = patent ductus arteriosus. PFO = patent foramen ovale. MVP = mitral valve prolapse. VSD = ventricular septal defect.

*
= relationship with another patient with *.

**
= relationship with another patient with **.

++ = Additional cardiac follow-up information ascertained since publication.

Table 2.

Cardiovascular and Pulmonary Deaths in Children and Adults (n=8):

Pediatric Deaths (n=4):				
Study ID	**Sex (Age at Death)**	***FLNA* Mutation**	**Cause of Death**	**Cardiovascular Findings**
Boston Cohort #105	F (7months)	39 Kb deletion encompassing all of *FLNA*	Respiratory failure secondary to emphysema	TV and MV dysplasia, PFO, pulmonary hypertension, RV hypertrophy, normal size aorta on post-mortem.
DeWit 2011, P1, Reinstein 2013, M2	M (2months)	c.5504delCACCACinsAC	Heart failure, pulmonary hypertension	TAA, dysplastic aortic valve, PDA, VSD, ASD, general enlargement of vessels
Gerard-Blanluet 2006, T2	M (8months)	c.7922C>T p.(Pro2642leu)	Respiratory complications from bronchopulmonary dysplasia	PDA
Kapur 2010, P5	M (1.5month)	c.7021C>T p.(Gln2341X)	Cerebral and spinal cord infarcts. Multiple congenital abnormalities with left diaphragmatic hernia. s/p surgical repair.	ASD

Adult Deaths (n=4)				
Study ID	**Sex (Age at Death)**	***FLNA* Mutation**	**Cause of Death**	**Cardiovascular Findings**
Boston Cohort #39	M (36yrs)	c.6915C>G p.(Glu2305Ter)	Aortic rupture (cardiac tamponade following rupture)	TAA
Boston Cohort #36	F (41yrs)	IVS2DS G-A +1 (splicing mutation)	Aortic rupture	TAA, VSD
Fergelot 2011, P1	M (57yrs)	c.356T>A p.(Ile119Asp)	Bleeding s/p 2nd aorta surgery	TAA requiring 2 surgeries (2nd lethal)
DeWit 2009, F1	F (71yrs)	c.3045-3049del5 exon 21	Subarachnoid hemorrhage secondary to internal carotid aneurysm.	s/pAVR @56y. Intracranial vascular disease. Heart failure requiring cardiac transplantation.

ASD = Atrial Septal Defect. AR = Aortic Insufficiency. AS = Aortic Stenosis. CAD = Coronary Artery Disease. PDA = Patent Ductus Arteriosus, HF= Heart Failure, LV = Left Ventricle, RV = Right Ventricle, TAA = Thoracic Aortic Aneurysm, VSD = Ventricular Septal Defect.