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Gain-of-Function Mutations in *SMAD4* Cause a Distinctive Repertoire of Cardiovascular Phenotypes in Patients with Myhre Syndrome

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Myhre syndrome is a rare, distinctive syndrome due to specific gain-of-function mutations in *SMAD4*. The characteristic phenotype includes short stature, dysmorphic facial features, hearing loss, laryngotracheal anomalies, arthropathy, radiographic defects, intellectual disability, and a more recently appreciated spectrum of cardiovascular defects with a striking fibroproliferative response to surgical intervention. We report four newly described patients with typical features of Myhre syndrome who had (i) a mildly narrow descending aorta and restrictive cardiomyopathy; (ii) recurrent pericardial and pleural effusions; (iii) a large persistent ductus arteriosus with juxtaductal aortic coarctation; and (iv) restrictive pericardial disease requiring pericardiectomy. Additional information is provided about a fifth previously reported patient with fatal pericardial disease. A literature review of the cardiovascular features of Myhre syndrome was performed on 54 total patients, all with a *SMAD4* mutation. Seventy percent had a cardiovascular abnormality including congenital heart defects (63%), pericardial disease (17%), restrictive cardiomyopathy (9%), and systemic hypertension (15%). Pericarditis and restrictive cardiomyopathy are associated with high mortality (three patients each among 10 deaths); one patient with restrictive cardiomyopathy also had epicarditis. Cardiomyopathy and pericardial abnormalities distinguish Myhre syndrome from other disorders caused by

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mutations in the TGF-β signaling cascade (Marfan, Loeys–Dietz, or Shprintzen–Goldberg syndromes). We hypothesize that the expanded spectrum of cardiovascular abnormalities relates to the ability of the *SMAD4* protein to integrate diverse signaling pathways, including canonical TGF-β, BMP, and Activin signaling. The co-occurrence of congenital and acquired phenotypes demonstrates that the gene product of *SMAD4* is required for both developmental and postnatal cardiovascular homeostasis.

Key words: cardiovascular malformation; coarctation; congenital heart defect; pericarditis; pericardial effusion; restrictive cardiomyopathy; *SMAD4* mutations; TGF-β signaling

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INTRODUCTION

Since the first description in two unrelated male patients [Myhre et al., 1981], Myhre syndrome (MIM #139210) has been steadily described in clinical reports and small series as a distinctive short stature syndrome with characteristic facial features, hearing loss, laryngeal anomalies, arthropathy, radiographic defects, and intellectual disability [Soljak et al., 1983; Garcia-Cruz et al., 1993; Titomanlio et al., 2001; Davalos et al., 2003; Lopez-Cardona et al., 2004; Rulli et al., 2005; van Steensel et al., 2005; Becerra-Solano et al., 2008; McGowan et al., 2011; Whiteford et al., 2012]. Two series from Europe [Le Goff et al., 2011; Caputo et al., 2012] reported that Myhre syndrome was caused by missense heterozygous changes affecting the Ile500 residue of *SMAD4* tumor-suppressor gene (chromosome 18q12.2). A restricted spectrum of Ile500 mutations (amino acid changes at threonine, valine, and methionine) was found in almost all patients, with the exception of a c.1486C>T(p.Arg496Cys) change, currently limited to three patients [Caputo et al., 2014; Michot et al., 2014]. Patients with laryngotracheal stenosis who were described as the Laryngeal, Arthropathy, Prognathism, and Short Stature (LAPS) syndrome [Hopkin et al., 1998] were found to have the same *SMAD4* mutations, leading to the conclusion that this was allelic to Myhre syndrome [Lindor et al., 2002, 2012; Oldenburg et al., 2015].

A variety of cardiovascular abnormalities were noted in 54% of the patients in the largest series of 32 patients [Michot et al., 2014], which was followed by detailed reporting of pericardial disease [Picco et al., 2013], and possible treatment with losartan [Piccolo et al., 2014]. Recently, abnormal wound healing with an exuberant fibroproliferative response during operative procedures, including orthotopic heart transplantation for restrictive cardiomyopathy (RCM) [Starr et al., 2015], has been highlighted as an important management issue.

This paper expands on the type, frequency, pattern, and clinical course of the cardiovascular abnormalities in Myhre syndrome (which includes LAPS). We report on four newly described patients, expand the description of one briefly cited patient [Michot et al., 2014], and update the literature review. We discuss relevant diagnostic and management guidelines, and hypothesize about the mechanisms of *SMAD4* mutations in the cardiovascular tissues of patients with Myhre syndrome.

MATERIALS AND METHODS

The clinical and molecular findings of four newly described patients are briefly summarized in Table I. Molecular genetic testing using targeted gene analysis for the *SMAD4* mutation (patients 3 and 4) or exome sequencing (patients 1 and 2) was performed at each center using a commercial lab. We also reviewed reported patients and series, avoiding duplicative reports (see Appendix for individual cases and references).

Clinical Reports

Patient 1. This 17-year-old girl was evaluated for protein-losing enteropathy (PLE) and mid-aortic syndrome with a known diagnosis of Myhre syndrome. She was the fourth pregnancy of a 36-year-old mother and unrelated 39-year-old father who had two healthy children (one first trimester miscarriage). Her mother had normal prenatal ultrasonography and spontaneous vaginal vertex delivery at 40 weeks gestational age. Birth weight (2.75 kg) and length (47.6 cm) were both at the 5–10th centile; OFC was not available. Her appearance was thought to be normal, but review of family photos show characteristic small deep-set eyes and small mouth. Physical features and clinical issues as noted (Table I). The brain MRI and MRA were normal shortly after birth. As part of extensive diagnostic testing for hypotonia, she had a muscle biopsy under general anesthesia, and the intubation was uncomplicated. Intubation for subsequent endoscopy was followed by gasping and loud coughing.

Cardiovascular evaluations began at age 3 years when a two-dimensional echocardiogram showed mild valvar pulmonic stenosis, and clinical follow-up suggested resolution. By age 6 years, she had had normal kidney-uterus-bladder radiographs and pelvic ultrasound. Because of increasing hepatomegaly at age 11 years, a CT of the abdomen was obtained which noted diffuse narrowing of the descending thoracoabdominal aorta (mid-aortic syndrome). At age 14 years, abdominal ultrasonography showed similar tapering of the descending thoracoabdominal aorta. Additional testing which was negative or non-diagnostic included alpha-1-antitrypsin level, quadriceps muscle biopsy, and endoscopy. At age 15 years, echocardiography did not detect pulmonic stenosis or pericardial effusion, but noted mild pleural effusions and ascites. Liver biopsy showed nonspecific sinusoidal dilation. At age 17, she

TABLE I. Clinical Features of 54 Patients (4 New, 50 Literature) With Myhre Syndrome

	Patient 1[a]	Patient 2[b]	Patient 3[c]	Patient 4[d]	Total (% rounded), literature and new (# pts reporting if <54)
SMAD4 affected residue	Ile500Val	Ile500Val	Ile500Thr	Ile500Val	Ile500Val 32 (59) Ile500Thr 19 (33) Ile500Met 1 (2) Arg496C 3 (6)
Targeted gene/exome sequencing	Exome sequencing de novo	Exome sequencing de novo	Targeted gene	Targeted gene	Targeted gene 52 (94) Exome sequencing 3 (6)
Paternal age birth (yrs)	39	35	33	24	Mean 35.2; range 22–50; (46 pts)
Oldest age when examined (yrs)	18	10	6	25	Mean 19.8; (48 pts) <5 yrs: 3 5–18 yrs: 24 >18 yrs: 21
Deaths: causes	NA	NA	NA	NA	Total 10 (20) Pericardial 3 (6) RCM 3 (6) Choking 1 (2) Unknown 3 (6)
Sex	F	F	F	M	F 31 (57)
Intellectual disability, special needs, DD, LDs	+, FSIQ 70	+, SPED	+, SPED	+, SPED	46 (85)
Autism spectrum	+	—	—	—	7 (13)
Growth and appearance					
IUGR	+	—	—	—	41/49 (84)
Typical facial features[e]	+	+	+	+	50 (92)
Puberty, altered	+ (10 yrs)	+	NA	+	14 (26)
Brachydactyly	+	+	+	—	35/40 (88)
Radiographs					
Skeletal survey	NA	+	NA	+	25 (46)
At least one feature[f]	NA	+	NA	+	34 (63)
Thick calvarium	NA	+	NA	—	26/30 (87)
Other skeletal[g]	NA	11 ribs	Mod thoraco-lumbar scoliosis	—	11 ribs 3 (6) Cervical vertebral fusion 3 (6)
Limited joint mobility	+	+	+	+	23/27 (85)
Thickened skin	—	—	+	+	43/51 (84)
ENT, airway					
Hearing loss	+	+	+	—	45 (83)
Choanal stenosis	—	—	Possible	+	6 (11)
CN 7 palsy	—	—	—	—	2 (4)
Subglottic stenosis, other laryngotracheal obstruction	+	—	—	+, multiple levels	9 (17)
CL, CP, CL/P, VPI surgery	—	—	Cleft lip/palate	—	7 (13)
Ocular					
At least one feature[h]	+	—	+	—	26/49 (53)
Refractory abnormality	—	—	Hyperopia	—	17/53 (31)
Strabismus	—	—	+	—	13/53 (24)
Pulmonary	—	—	—	+	12 (22)
Renal	—	—	—	—	0
Brain, spinal cord	—	Chiari 1; malformation; szs	—	—	1 (2)
Gastrointestinal					
PLE	+	—	—	—	1 (2)
Duodenal atresia	—	—	+	—	3 (6)
Neoplasia	—	—	—	—	3 (6)

+, present; —, absent; CL, cleft lip; CN, cranial nerve; CP, cleft palate; DD, developmental delay; ENT, ear-nose-throat; F, Female; LDs, learning disabilities; M, male; Mod, moderate; NA, not available or not applicable; PLE, protein losing enteropathy; pts, patients; RCM, restrictive cardiomyopathy; SPED, special education; szs, seizures; VC, vocal cord; VPI, velo-pharyngeal insufficiency; yrs, years.

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[e]Short palpebral fissures, variable ptosis, mid-facial retrusion, prognathism, narrow mouth, thin upper lip vermilion, small ears.

[f]Thick femoral neck, platyspondyly, large vertebral pedicles, narrow pelvis, broad ribs.

[g]Skeletal anomalies: single occurrences of hemivertebrae, Madelung deformity, spina bifida occulta.

[h]Ocular abnormalities: refractory, strabismus, cataract, pseudopapilledema, astigmatism, optic nerve Drusen.

had an MRI with and without contrast of the descending thoracoabdominal aorta which showed unchanged, smooth tapering aorta, and narrowing at the origin of celiac axis. Repeat abdominal ultrasonography showed that the abdominal aorta decreased in caliber from 9 to 5 mm at the level of the iliac bifurcation. Genetic testing which had been normal or negative included chromosome analysis, FISH analysis, chromosome microarray, and urine glucosaminoglycans. Exome sequencing performed by GeneDx laboratories detected a characteristic de novo *SMAD4* mutation (c.1498A>G, which predicts p.Ile500Val). Physical examination showed a pleasant, cooperative teen appearing several years younger than age with short stature, macrocephaly, and typical facial features of Myhre syndrome (Fig. 1; Table I). Measurements included weight 50.0 kg (24th centile), height 145.5 cm (<5th centile), OFC 57 cm (97th centile), right hand length 15.5 cm (<5th centile), and right middle finger 6.5 cm (much <3rd centile). The heart rate was 113, blood pressure 133/50. There was no murmur or click, but a gallop rhythm (S3) was appreciated. The liver edge was 3 cm below the right costal margin.

To determine if the protein losing enteropathy (PLE) and hepatomegaly reflected high systemic venous pressure we performed echocardiography which was normal, and showed no pericardial effusion, ventricular hypertrophy, or any quantitative Doppler abnormality. The ECG-gated CTA showed no pericardial thickening. All major arteries of the head, neck, and thorax were normal in appearance (Fig. 2), and smooth tapering of the small caliber descending thoracoabdominal aorta, worst at the level of the celiac axis was again confirmed, noting mild narrowings of the celiac artery and right renal artery, without other aneurysms or stenoses. Mild elevation of right ventricular pressures was suggested by mild right ventricular enlargement with diastolic interventricular septal flattening. Cardiac catheterization showed biventricular diastolic dysfunction with concordant respiratory variation suggestive of RCM (right atrial mean pressure 19 mm Hg; pulmonary capillary wedge mean 25 mm Hg; right ventricular end-diastolic pressure 22 mm Hg; left ventricular end-diastolic pressure 24 mm Hg). Angiography confirmed mild proximal stenosis of celiac artery and right renal artery, but no other vasculopathy. The patient remains clinically stable, and has been listed for cardiac transplantation.

FIG. 1. Frontal view of Patient 1 (age 18 years) shows a healthy appearing young woman with mild facial features including relatively small palpebral fissures, small mouth, and prominent chin.

Patient 2. This now 10-year-old girl presented to clinical genetics shortly after birth because of dysmorphic features. She was a 36-week product of a naturally conceived pregnancy to first cousin Bangladeshi parents. She was the second pregnancy of a 30-year-old mother and 35-year-old father, and had a healthy older sister. The pregnancy was uncomplicated and her mother had normal prenatal ultrasonography. She was delivered breech via cesarean. She had hypoglycemia and breathing requiring NICU monitoring for several days, where her dysmorphic features prompted a genetics evaluation, and she had a normal karyotype. Three additional clinical genetics evaluations failed to identify a unifying diagnosis. When she again presented at age 6 years with developmental delay and dysmorphic features, she had had a normal genome wide SNP microarray remarkable only for large regions of homozygosity, consistent with reported consanguinity. At that visit she had normal plasma amino acid analysis, urine organic acid analysis, acylcarnitine profile, lactate/pyruvate analysis, and lysosomal enzyme biochemical testing. The skeletal survey showed partially fused skull sutures, prominent mandible, 11-rib bearing thoracic-type vertebral bodies with anterior beaking of the lower thoracic vertebral bodies, short and broad clavicles, and broad under-tubularized metacarpals and phalanges. Sequencing for Kabuki and Rubinstein–Taybi syndromes, (*MLL2*, *CREBBP*, and *EP300*) was normal. The patient was re-examined at 10 years of age during hospitalization for pericardial effusion with cardiac tamponade. Interval history and physical exam showed physical features, and clinical issues as noted (Table I). Measurements included weight 42.8 kg (88th centile), height 138.0 cm (50th centile), and OFC 55 cm (98th centile). The blood pressure was 84/36 and heart rate was 114. Because of the clinical concern for an atypical presentation of a rare autosomal recessive genetic condition, clinical exome sequencing was performed. Testing for congenital disorder of glycosylation was normal.

The pericardial effusion required pericardiocentesis and pericardial drain placement with aspiration of sanguineous fluid (Fig. 3). The pericardial fluid had a hemoglobin of 6 g/dl, increased uric acid, and elevated LDH. Repeat echocardiogram following drain placement showed worsening right ven-

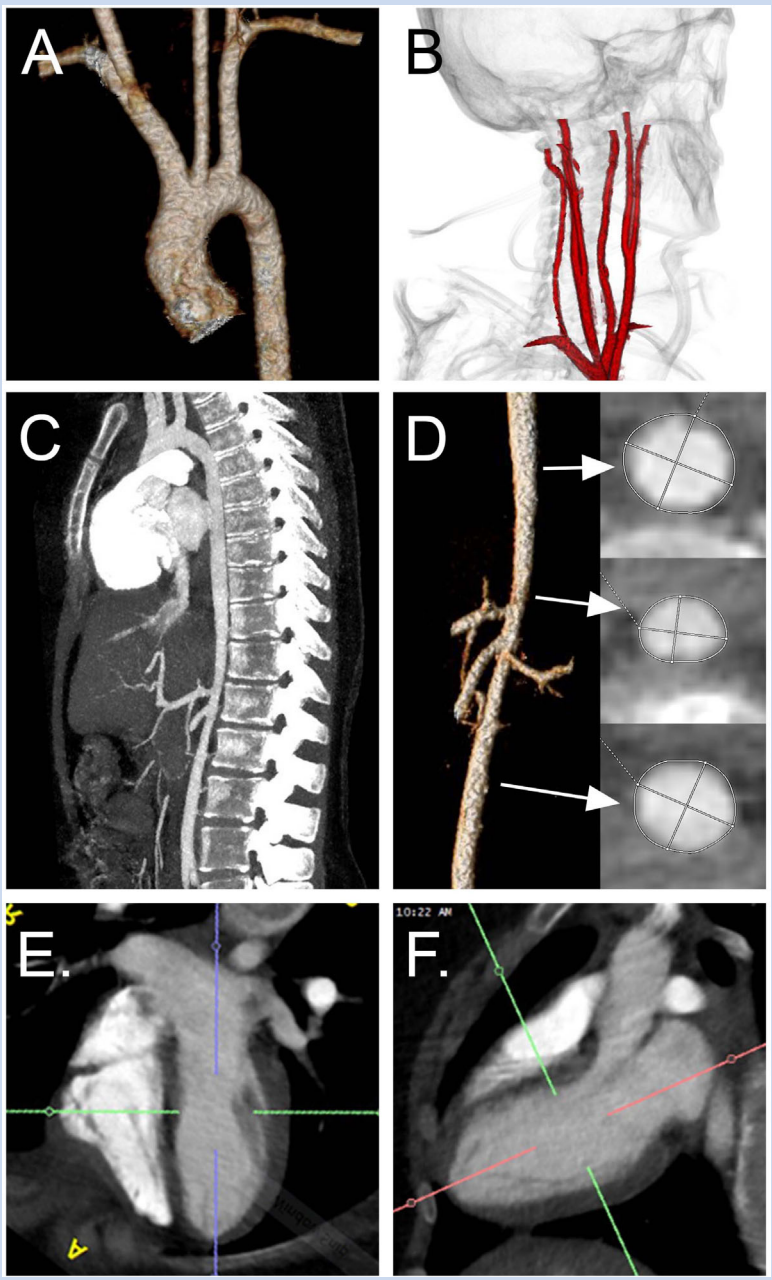

FIG. 2. Patient 1 (age 17 years). An ECG-gated CTA demonstrates absence of (A) proximal aortic enlargement or (B) cervical artery tortuosity. C: A CTA demonstrates an overall very small descending thoracoabdominal aorta, with mild long segment mid aortic narrowing. D: Three-dimensional reconstruction of the abdominal aorta with representative cross-sectional transverse sections at the level of the diaphragmatic hiatus (Area 0.85 cm^2), 1 cm above the celiac axis (Area 0.49 cm^2), and 1 cm above the aortic bifurcation (Area 0.73 cm^2), respectively. E: Four- and (F) three-chamber CTA views of left ventricle in diastole demonstrate lack of pericardial thickening or atrial enlargement.

tricular (RV) dilatation and wall thinning with worsening biventricular function. She was started on milrinone, with subsequent improvement in cardiac function, but developed pleural effusions treated with furosemide. She also received a course of IVIG for presumed myopericarditis and started on a treatment course of colchicine.

Cardiac MRI performed after resolution of the hemopericardium showed preserved left ventricular (LV) systolic function but regional thickening of the ventricular septum at the level of the mid ventricle. Qualitatively, there appeared to be global LV hypertrophy (LV end-diastolic volume measured 30 cc/m^2 and LV ejection fraction 64%, asymmetric regional thickening of the ventricular

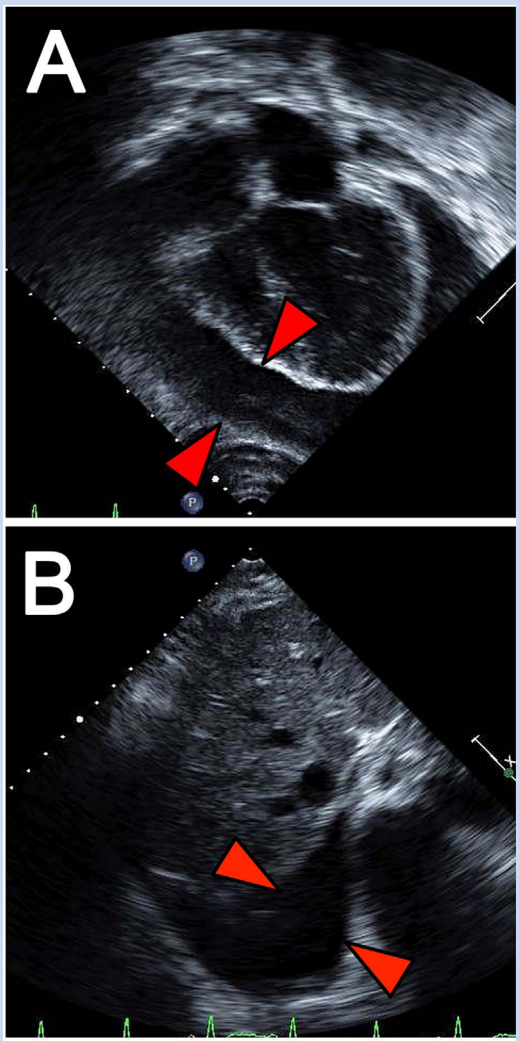

FIG. 3. Patient 2 (age 10 years). Transthoracic echocardiogram showing spontaneous pericardial (A) and pleural (B) effusions (Red arrows depict fluid accumulation).

septum measured 1.3–1.4 vs. 0.7–0.8 cm in the remaining basal walls). The abnormal appearance of the LV wall thickening/hypertrophy was presumed to be related to myocardial edema. She also had preserved RV ejection without dilation with mild RVH (RV end-diastolic volume measured 32 cc/m^2 and RV ejection fraction 65%). There was also evidence of global myocardial hyperemia most pronounced at the region of the ventricular septal thickening with regional myocardial edema at the region of ventricular septal thickening and delayed enhancement at the inferior RV/LV hinge point and possibly along the ventricular septum, which could be consistent with myocarditis.

The patient was readmitted twice due to worsening pleural effusions, requiring repeated thoracocenteses. During the second readmission, exome sequencing identified the characteristic de novo c.1498A>G (which predicts p.Ile500Val) *SMAD4* mutation consistent with Myhre syndrome. Given recurrent pleural effusions, her colchicine dose was increased. Her most recent echo showed normal left ventricular systolic function (ejection fraction of 62%) and normal right ventricular systolic function.

Patient 3. This now 6-year-old girl presented to the genetic service with multiple congenital anomalies at 6 days of life. She was the second pregnancy of a 25-year-old mother and the first of an unrelated 33-year-old father. Pregnancy was complicated by maternal history of asthma, scoliosis, Wolff–Parkinson–White syndrome treated with ablation, and complicated by pulmonary embolism at 6 weeks gestational age. Maternal AFP screening suggested a chromosome abnormality, but amniocentesis was normal (46,XX). She was delivered by Cesarean for failure to progress at 37 weeks' gestation. Birth weight (2,370 g) and length (44 cm) were at the 10–25th and 5–10th centile, respectively. OFC (32 cm) was 10–25th centile. She had dysmorphic features including blepharophimosis, cleft lip and palate, and a small thumb (Table I).

At age 6 days, echocardiography showed a small mid-muscular ventricular septal defect (VSD), dilated coronary sinus, and a large patent ductus arteriosus (PDA). After discharge she developed severe coarctation with maintenance of ductal patency (Fig. 4A and B). At age 2 months, she had end-to-end anastomosis of the coarctation and PDA ligation. During surgery, the PDA was larger than the aortic arch and the descending aorta; the ductal stenosis extended proximally, to the undersurface of the arch. At age 4 months, she had successful balloon angioplasty for recurrent severe coarctation with a systolic gradient under general anesthesia of 75 mm Hg. At age 21 months, repeat cardiac catheterization showed no gradient. High-resolution karyotype was normal and 44k chromosomal microarray showed two benign copy number variants. She had normal chromosome breakage studies, *SALL4* sequence analysis, 7-dehydrocholesterol, carbohydrate-deficient transferrin, and *KAT6B* sequencing.

In the first years of life, she was noted to have developmental delay and growth failure. At age 6, she had severe abdominal pain and an EGD showed significant duodenal stenosis for which she underwent surgical repair. She was reevaluated in genetics clinic and physical exam showed typical clinical features (Table I). Weight was 16.2 kg (1st centile), height was 101.3 cm (<5th centile and −3 SD below the mean), and OFC was 49 cm (15th centile). Heart rate was 106 and blood pressure was 116/56 mm Hg. Myhre syndrome was suspected and sequencing of *SMAD4* showed a c.1499T>C(p.Ile500Val) mutation consistent with this diagnosis. She continued to be followed by cardiology.

Patient 4. This now 25-year-old man presented at age 11 to a geneticist with a history of short stature, joint contractures, and cognitive impairment. He was the 39-week product of a pregnancy to unrelated parents, the fifth pregnancy for his 29-year-old mother. The pregnancy included exposure to cocaine and possibly alcohol and tobacco, but was otherwise uncomplicated and had normal ultrasonography. He was born by uncomplicated spontaneous vaginal delivery with a birth weight of 3.5 kg (62nd centile). Global developmental delays included toe walking around 2 years of age, delayed speech, and cognitive development during pre-school years. Because he also developed pain and reduced range of motion in almost all major joints and the spine, he was

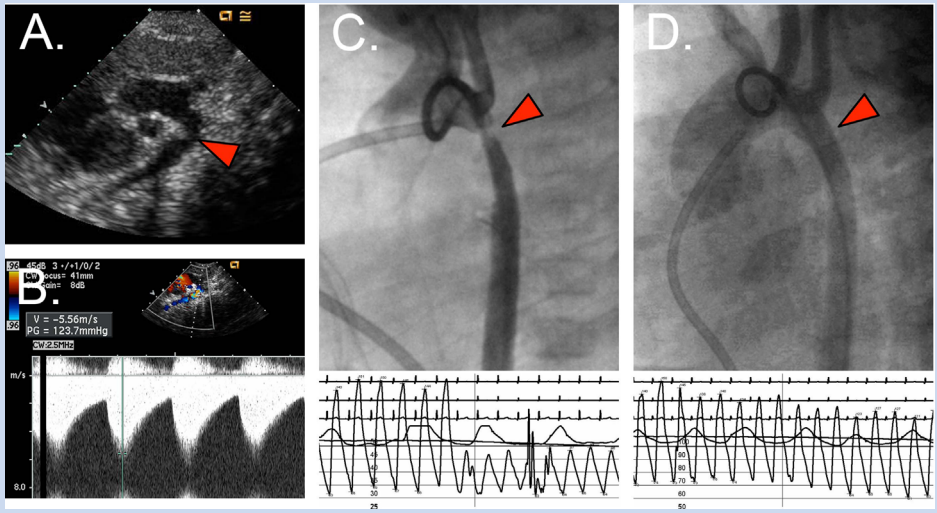

FIG. 4. Patient 3 (age 6 years) with juxtaductal aortic coarctation. A: Two-dimensional echocardiography of aortic arch at age 2 months shows postnatally acquired obstruction (Red arrow). B: Continuous wave Doppler through coarctation documents ~120 mm Hg obstruction. C: Aortogram and pullback aortic pressure tracing of post-surgical recoarctation at 4 months of age (red arrow). D: Aortogram and pullback aortic pressure tracing of region after balloon angioplasty (Red arrow).

evaluated by neurology and rheumatology. The muscle and skin biopsies provided no specific diagnosis for his joint contractures and pain. He presented to endocrine clinic at age 11 for short stature and precocious puberty (onset at age 10), and had elevated FSH, and an enlarged left testis. The neuroendocrine evaluation was otherwise normal and did not identify a unifying diagnosis. Genetic testing was negative, including a karyotype, FISH for 22q11.2, testing for Fragile X syndrome, testing for Emery–Dreifuss Muscular Dystrophy (analysis of exons 1–11 of the *LMNA* gene), and biochemical testing for congenital disorders of glycosylation and lysosomal storage disorders.

He was admitted for pericardial effusion at age 11 years. Over the next 6 months, he had multiple hospitalizations for chronic re-accumulation of apparently idiopathic pericardial effusion. Due to failure of medical management the effusions were treated with surgical creation of a pericardial window. Two days after surgery, echocardiography showed dramatic re-accumulation of pericardial fluid. A second pericardial window was created. He was treated with nonsteroidal anti-inflammatory agents, steroids, indomethacin, and colchicine, but after 6 months of continued effusion and pericardiocenteses, he underwent complete pericardiectomy through a median sternotomy. He remained asymptomatic and was discharged from routine cardiology follow-up at age 17 years. Additional imaging testing at age 11 years included abdominal ultrasonography showed hepatomegaly and splenomegaly, which resolved after treatment of the pericardial effusions.

The patient developed progressive nasal obstruction following adenotonsillectomy and myringotomy tubes at 8 years old for recurrent otitis media. A CT scan at age 10 years demonstrated complete closure of the posterior nasal cavity and surgery was performed at age 14 years for complete bilateral choanal and nasopharyngeal stenosis. He had a second surgery a few months later for recurrent, progressive tracheal stenosis, and bilateral choanal atresia, which failed to improve the respiratory problems. He eventually required a tracheostomy at age 24 for respiratory distress.

When reevaluated in the adult genetics clinic at age 25 years, physical examination showed blood pressure 147/63, pulse 89 bpm, weight 69.2 kg, height 1.45 m (BMI 34.2), OFC 57 cm (relative macrocephaly for height) with facial appearance and habitus (Table I) suggesting Myhre syndrome. Sequencing of exon 12 of *SMAD4* identified the c.1498A>G(p.Ile500Val) mutation consistent with this diagnosis. Subsequent CTA imaging of the chest, abdomen, and pelvis demonstrated a normal appearance of the aorta and main branching arteries with a high origin of the coronary arteries. Pulmonary abnormalities were also noted including diffuse circumferential wall thickening of the proximal airways, right lower lobe mucus plugging, and ground glass opacities. He continued to be followed by pulmonology and rheumatology, and was reestablishing care with cardiology as a result of his recent diagnosis.

Literature

We review 54 reported patients with molecular confirmation of Myhre syndrome (*SMAD4*) (Tables II and III), and also expand on the striking cardiac findings of a patient described briefly by Michot et al. [2014], Table I, patient 24. She is not counted as an additional patient, and remains part of the literature series.

The diagnosis of Myhre syndrome in this 29-year-old woman was made by review of the autopsy report 15 years after her death,

TABLE II. Cardiovascular Abnormalities in 54 Patients (4 Newly Described, 50 Literature) With Myhre Syndrome and a *SMAD4* Mutation[a]

Cardiovascular abnormality	Patient 1	Patient 2	Patient 3	Patient 4	Total 54 (100%), literature + new
Total					38 (70)
CHDs, total					34 (63)
Simple shunts					16 (29)
ASD	—	—	—	—	2 (4)
VSD	—	—	+, small muscular	—	3 (6)
PDA	—	—	+, large	—	11 (20)
Right-sided defects					4 (7)
Branch PA stenosis	—	—	—	—	2 (4)
Pulmonic valve stenosis	—	—	—	—	1 (2)
Tricuspid valve	—	—	—	—	1 (2)
Left-sided defects, total					21 (39)
Aortic valve stenosis	—	—	—	—	8 (15) (includes 1 subaortic stenosis)
Mitral valve stenosis	—	—	—	—	1 (2)
Coarctation, juxtaductal	—	—	+	—	8 (15)
Long segment narrowing	+	—	—	—	3 (6)
Polyvalvar dysplasia, narrow aorta					1 (2)
CHD treatment	None	None	COA surgery PDA ligation COA balloon dilation	None	Four balloon angioplasty procedures: Two aortic valve two COA
Peripheral vascular stenoses	+, celiac, right renal	—	—	—	4 (7) One superior mesenteric One celiac Two celiac and renal
Pericardial disease[b]	—	+	—	+	9 (17)
Pericarditis non-progressive	NA	—	NA	—	1 (2)
Pericarditis, constrictive		—		—	4 (7)
Pericarditis, recurrent effusions		+		+	4 (7)
Treatment					
Pericardiectomy	NA	+	NA	+	5 (9)
Pericardiocentesis					
Pericardial window					
Hypertension, systemic	—	+	—	+	8 (15)
Restrictive cardiomyopathy[c]	+	—	—	—	5 (9)

ASD, atrial septal defects; CHDs, congenital heart defects; COA, coarctation; NA, not available or not applicable; PA, pulmonary artery; PDA, patent ductus arteriosus; VSD, ventricular septal defect.
[a]Excluded from this analysis are (i) two patients reported by Michot et al. [2014], who were negative for *SMAD4* mutations and (ii) 13 patients who were described clinically, but did not have molecular testing [Myhre et al., 1981; Soljak et al., 1983; Garcia-Cruz et al., 1993; Hopkin et al., 1998; Davalos et al., 2003; Lopez-Cardona et al., 2004; van Steensel et al., 2005; Becerra-Solano et al., 2008; Oldenburg et al., 2015, patient 4].
[b]One patient with epicarditis.
[c]Hypertrophic and dilated cardiomyopathy was not observed.

characterized originally as the LAPS phenotype. She had low birth weight, short stature, precocious puberty, bilateral hearing loss, learning disabilities, recurrent nasolacrimal duct stenosis, flexion contractures, and typical facial features. During a difficult intubation, there was a small glottis and larynx. Approximately, 10 weeks prior to her death, she had a febrile flu-like illness, and the chest radiograph obtained during the emergency department visit showed opacification suggesting either severe pleural effusions or lung consolidation. During her evaluation in the emergency department, cardiovascular collapse occurred from which she could not be resuscitated. Post-mortem examination showed constrictive pericarditis in which the heart was encased in a layer of organizing fibrous tissue up to 3 mm thick, suggesting the process had been present 3–5 weeks. There were pulmonary emboli of both lower lobes, and iliac vein thrombus. A membranous VSD was closed. There was no myocarditis. The clinical diagnosis of Myhre syndrome was confirmed by mutation analysis using DNA extracted from a paraffin embedded surgical cell block (*SMAD4* c.1498A>G, p.Ile500Val), courtesy of Valerie Cormier-Daire, M.D., reported in [Michot et al., 2014].

TABLE III. Myhre Syndrome and Other Disorders Associated With Pericardial Disease and/or Restrictive Cardiomyopathy (Excluding Inborn Errors of Metabolism[a])

Syndrome [Author, year]	Gene	Disorder or condition
Myhre syndrome	*SMAD4* missense mutations	Pericarditis, pericardial effusion Restrictive cardiomyopathy
MULIBREY dwarfism [Karlberg et al., 2004; Kivistö et al., 2004; Eerola et al., 2007; Kumpf et al., 2013]	*TRIM37*	Pericarditis, pericardial constriction Myocardial dysfunction, restrictive cardiomyopathy
Cantu syndrome [Grange et al., 2006; Von Bon et al., 2012]	*ABCC9*	Pericardial effusions Dilated, hypertrophic cardiomyopathy Pulmonary hypertension
CACP syndrome [Faivre et al., 2000; Taşar et al., 2014]	*PRG4*	Pericarditis (noninflammatory), pericardial effusions, pericardial constriction
Allelic forms of genes causing DCM and HCM [Kaski et al., 2008; Caleshu et al., 2011; Webber et al., 2012; Peled et al., 2014; reviewed by Brodehl et al., 2015; Tariq and Ware, 2015]	*DES*, *ACTC1*, *FLNC*, *MYH7*, *MYL2*, *MYL3*, *MYPN*, *TNNC1*, *TNNI3*, *TNNT2*, *TPM1*, *TTN*	Restrictive cardiomyopathy

CACP, camptodactyly-arthropathy-coxa vara-pericarditis; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; MULIBREY, Muscle, Liver, Brain, eye.
[a]Inborn errors of metabolism associated with restrictive cardiomyopathy include pseudoxanthoma elasticum, Gaucher disease, Hurler disease, glycogen storage disease, Fabry disease and hemochromatosis (reviewed by [Sen-Chowdhry et al., 2010]).

Diagnosis and Definitions

All patients in this review had Myhre syndrome confirmed by a *SMAD4* mutation that was either reported in the literature or described here. A congenital heart defect (CHD) was defined as a structural defect of the heart and major aortic branches. Cardiac findings such as those associated with prematurity, or neonatal patent foramen ovale or physiologic peripheral pulmonary stenosis were excluded. Restrictive cardiomyopathy (RCM) was defined as normal or decreased volume of both ventricles associated with biatrial enlargement, normal left ventricle wall thickness and atrioventricular valves, impaired ventricular filling with restrictive physiology, and normal (or near normal) systolic function (reviewed by [Colan, 2007]). Pericardial disease included effusions and pericarditis [Dudzinski et al., 2012]. Some patients in the literature were reclassified using current definitions, for example, exclusion of patent foramen ovale as a CHD; superior mesenteric artery stenosis as a small artery. Calculations for the occurrence of an anomaly were usually based on the possible total cohort as denominator (which would bias to lower frequency), except when the frequency of reporting was known.

RESULTS

Of 54 (4 new, 50 Literature) patients with Myhre syndrome confirmed by a *SMAD4* mutation, the Ile500 at Ile500Val transition (32, 59%) was more common than the Ile500Thr (19, 33%), p.Ile500Met (1, 2%), and p.Arg496Cys (3, 6%) transition. Three (6%) patients ([Need et al., 2012]; patients 1 and 2) were diagnosed using clinical exome sequencing rather than targeted gene analysis.

Demographics and Outcome

There were slightly more females (31, 57%) than males (Table I). Among 48 patients reporting ages (mean 19.8 years), 21 (43%) patients were older than 18 years. There were 10 (20%) deaths, three each due to pericardial disease or RCM (one of whom also had epicarditis). Additional causes of death included choking in a 22-year-old male [McGowan et al., 2011, patient 3; Michot et al., 2014] and possible arrhythmia without prior cardiovascular disease in a 43-year-old female [Oldenburg et al., 2015, patient 3]; the cause of death was unspecified in two patients.

General Clinical Features

All patients had typical Myhre syndrome features, with a generally moderate level of medical need and neuropsychologic disability. The typical facial appearance (i.e., short palpebral fissures, ptosis, mid-facial hypoplasia, short philtrum, prognathism, narrow mouth, thin upper lip, small ears) was noted in almost all patients (50, 92%). Some form of intellectual disability, learning disability, or developmental delay was present in 46 (85%), 7 (13%) of whom had an autism spectrum disorder. Hearing loss was present in 45 (83%). At least one ocular abnormality was noted in 26 (53%) of patients, typically a refractory abnormality (17, 31%) or strabismus (13, 24%); three patients had pseudopapilledema. A complete skeletal survey was performed in fewer than half of the patients (25, 46%), and thus, we did not analyze each possible radiographic feature. We did note that at least one of the typical radiographic features (i.e., brachydactyly, thick femoral neck, platyspondyly, large vertebral pedicles, narrow pelvis, hypoplastic iliac wing, broad ribs, thick calvarium) was reported in 34 (63%). Most commonly noted was brachydactyly (35 of 40, 88%) and thick calvarium (26 of

30, 87%). Also contributing to the distinctive habitus were limited joint mobility (23 of 27, 85%), and thickened tight skin (43 of 51, 84%).

Less common anomalies included those of the skeleton (three patients each with 11 ribs and cervical vertebral fusion, and one each with hemivertebrae, Madelung deformity, and spina bifida occulta); craniofacial region (seven patients with either cleft lip, cleft palate, cleft lip/palate, or velo-pharyngeal insufficiency requiring treatment, two with facial nerve weakness, three with pseudopapilledema), gastrointestinal system (three patients with duodenal atresia, one with Hirshsprung disease), cancer (one patient each endometrial carcinoma, mesencephalic glioma, optic nerve sheath meningioma), and nervous system (one patient each with Chiari 1 malformation and tethered cord). Anomalies of the airway included choanal stenosis (four patients) and laryngotracheal defects (nine patients, two of whom also had choanal stenosis) The LAPS phenotype could be applied in these nine patients.

Cardiovascular Abnormalities

A cardiovascular abnormality of any type was reported in over two-thirds of the patients (38, 70%), most frequently a CHD (34, 63%) (Table II). Atrial and ventricular septal defects (5, 10%) closed spontaneously. Of 11 (20%) patients with a PDA, new patient 3 had a large PDA requiring surgical closure. The most common group of CHDs were various levels of left heart obstruction (22, 41%) including typical juxtaductal coarctation (8, 15%), diffuse descending aorta hypoplasia (3, 6%), aortic valve stenosis, often dysplastic (8, 15%), mitral valve stenosis (2, 4%), and polyvalvar dysplasia with aorta hypoplasia (1, 2%). There were no patients with a major CHD such as visceral heterotaxy, single ventricle, conotruncal, or atrioventricular septal defect. Abdominal aorta stenosis occurred in four (7%) patients which involved the celiac artery (three total, with additional renal artery involvement in two), and superior mesenteric artery in one patient. Less common than left-sided obstruction were 4 (7%) patients with various levels of right-sided obstruction including pulmonic valve stenosis (1, 2%), pulmonary artery stenosis (2, 4%), and tricuspid valve cleft and stenosis (1, 2%).

Pericardial disease (9, 17%) was usually a recurrent, chronic and occasionally lethal disease, with only one patient [Michot et al., 2014] reported as an "isolated episode." Five (56%) of those with pericardial disease could be viewed as having LAPS. We classified the remaining nine patients as either as constrictive (4, 7%) or having chronic effusions (4, 7%), and for the sake of this discussion, include in this discussion the patient with epicarditis reported by Starr et al. [2015]. Seven (78%) of those affected were 18 years and younger. Restrictive cardiomyopathy was diagnosed in at least 5 (9%) patients, including new patient 1. This patient had protein losing enteropathy (PLE), and hepatomegaly was reported in at least one other patient [Rosser et al., 1995; McGaughran and Donnai, 1996; Michot et al., 2014, patient 10]. Systemic hypertension was reported in 8 (15%) patients. Pulmonary hypertension cannot be reliably reported since there were patients with primary lung disease, "restriction," pulmonary artery stenosis, and RCM.

In addition to the 54 patients confirmed by mutation analysis who formed the core of this study, there were 13 clinically diagnosed patients, who are briefly noted for completeness sake and historical importance [Myhre et al., 1981; Soljak et al., 1983; Garcia-Cruz et al., 1993; Hopkin et al., 1998; Davalos et al., 2003; Lopez-Cardona et al., 2004; van Steensel et al., 2005; Becerra-Solano et al., 2008; Oldenburg et al., 2015, patient 4]. Cardiovascular abnormalities included CHD (2, 15.3%), and pericarditis (2, 15.3%) which was constrictive in one patient and self-limited in the other; there were no patients with RCM.

Pulmonary Complications

Pulmonary disease (12, 22.0%) has been mainly characterized based on pulmonary function tests and described as restrictive (3) or obstructive (4) [McGowan et al., 2011; Oldenburg et al., 2015; Starr et al., 2015]. There has been one report of bronchiolitis obliterans, now referred to as cryptogenic organizing pneumonia, suggesting an inflammatory pathology [McGowan et al., 2011]. Starr et al. [2015] documented diffuse interstitial fibrosis with copious collagen and smooth muscle hyperplasia of the airways from open lung biopsy, suggesting an interstitial pathology. Pulmonary hypertension has also been reported in three patients ([McGowan et al., 2011; Starr et al., 2015], patients 2 and 3). Although the pulmonary disease in Myhre syndrome seems to be mostly interstitial, further imaging, pulmonary function testing, and lung biopsy would be helpful in determining the true pathogenesis.

Treatment

Interventional balloon angioplasty was performed in two patients each with aortic valve stenosis and coarctation, and when refractory, required more than one procedure or larger balloon size ([Starr et al., 2015]; patient 3 reported here).

Surgery was needed to ligate a PDA and stent left pulmonary artery stenosis [Michot et al., 2014], and to repair coarctation in two patients (one who had also had ligation of a large PDA) [Michot et al., 2014; patient 2, reported here]. Orthotopic heart transplant was used by Starr et al. [2015] to treat two patients with Myhre syndrome. Patient 1 in this report had polyvalvar dysplasia necessitating aortic valve replacement, tricuspid valvuloplasty, mitral valve replacement, and ASD closure. The development of RCM and progressive heart failure led to heart transplantation. In patient 2, of the Starr report, aortic coarctation refractory to balloon angioplasty, and the development of RCM, interstitial lung disease, and pulmonary hypertension led to bilateral heart and lung transplantation. In both patients, dramatic fibrosis and adhesions were reported, and both patients died.

Pericardial disease in five patients required pericardiocenteses and pericardiectomy. Traditional pharmacologic measures to treat pericarditis included non-steroidal anti-inflammatory drugs and high dose prednisone (two patients), colchicine (two patients) as well as mycophenolate mofetil, and interleukin antagonist ([Picco et al., 2013], also reported as patient 2 in [Michot et al., 2014]), and losartan [Piccolo et al., 2014]. The PLE in patient 1 reported here was treated with diuretics including hydrochlorothiazide and spironolactone.

DISCUSSION

Myhre syndrome (OMIM #139210) is an increasingly recognized syndrome that is usually diagnosed clinically by the distinctive pattern of short stature, mild intellectual disability, hearing loss, unusual facial features, frequent airway compromise, arthropathy (joint contractures and pain, without inflammation) with radiographic skeletal anomalies, and various birth defects. The type and frequency of craniofacial, airway, skeletal, and visceral involvement in this series was similar to previous reviews [Michot et al., 2014] and will not be discussed in detail. We noted a slight predominance of females, and did not detect advanced paternal age (mean 35.2 years). With this larger series, we confirm autism in 13% and the high frequency of neuropsychologic disability and learning challenges in Myhre syndrome. We call attention to pseudopapilledema, cleft lip and palate, rib and vertebral anomalies, and duodenal stenosis. The low frequency of apparently unrelated cancer (i.e., endometrial cancer, mesencephalic glioma, optic nerve sheath meningioma) should be monitored as new patients are identified. Although Myhre syndrome is usually confirmed with targeted gene analysis, increasingly patients are identified using exome sequencing ([Need et al., 2012]; patients 1 and 2 reported here). This may indicate both the challenge of recognizing this phenotype, and a general trend towards early utilization of exome sequencing as a clinical application for patients with rare disorders [Biesecker and Green, 2014; Nguyen and Charlebois, 2015; Xue et al., 2015].

We propose that cardiovascular abnormalities are sufficiently common and distinctive to be added to the core diagnostic features, and that pericarditis and/or RCM could establish the diagnosis in a patient with short stature and unusual facial features. Congenital heart defects were reported in the majority of patients in the earliest series [Le Goff et al., 2011; Caputo et al., 2012], and we emphasize the predominance of left-sided obstruction, and apparent absence of early developmental defects. A recent review of five patients with Myhre syndrome called attention to RCM and pericardial disease, the role of heart and lung transplantation, and importantly, a fibroproliferative response [Starr et al., 2015]. We discourage further use of the acronym LAPS since pericarditis is not specific to it, and since the airway disease extends above the larynx and trachea to include choanal stenosis. As noted above, further research should be directed to the general connective tissue disease which leads to fibrosis, stenosis, scarring, and proliferation in many locations.

Comparison to Other Syndromes

Because Myhre syndrome includes CHDs and vasculopathy, the pattern of cardiovascular anomalies resembles other syndromes although the facial appearance and habitus are distinct. Myhre syndrome, William–Beurens syndrome, Alagille syndrome and the Ras–MAPK pathway syndromes are all associated with coarctation of the aorta, and/or reduced aorta caliber, and valve dysplasia [Pober, 2010; Lin et al., 2011; Spinner et al., 2000]. In contrast to the syndromes caused by TGF-β receptor signaling [Lindsay and Dietz, 2014], Myhre syndrome is not currently associated with aorta or pulmonary artery dilation. Interestingly, Myhre syndrome shows a tendency toward PDA, including at least one very large lesion (patient 3).

None of the three other syndromes associated with pericardial disease (summarized in Table III) should be mistaken for the overall appearance of Myhre syndrome. Patients with Cantu syndrome have a coarse facial appearance with hypertrichosis [Grange et al., 2006; Von Bon et al., 2012]. The Camptodactyly-Arthropathy-Coxa vara-Pericarditis syndrome lacks the distinctive facial and functional disorders, despite the digital anomalies [Faivre et al., 2000; Taşar et al., 2014]. Children with MULIBREY dwarfism present with the features of the acronym, and more significant growth restriction [Karlberg et al., 2004; Eerola et al., 2007]. Cardiovascular disease in patients with MULIBREY dwarfism presents as a mixed pericardial constrictive process with additional aspects of myocardial restrictive disease in some patients, best delineated on MRI [Karlberg et al., 2004; Kivistö et al., 2004]. An instructive patient is that of Kumpf et al., [2013] who report a 12-year-old boy who developed severe right heart failure with protein losing enteropathy and died despite pericardiectomy for pericardial disease. Thus, Myhre syndrome and MULIBREY dwarfism are the only multiple anomaly syndromes associated with RCM, in addition to several inborn errors of metabolism (e.g., Gaucher disease, Hurler syndrome, glycogen storage disease, Fabry disease, hemochromatosis, familial amyloidosis), and a connective tissue disorder (pseudoxanthoma elasticum) [Sen-Chowdhry et al., 2010]. Several familiar and new genes that are mutated in RCM can also produce dilated and hypertrophic cardiomyopathy (reviewed in Table III) [Kaski et al., 2008; Caleshu et al., 2011; Webber et al., 2012; Peled et al., 2014; reviewed by Brodehl et al., 2015; Tariq and Ware, 2015].

Molecular Insights

Among the *SMAD* proteins, *SMAD4* has a unique functional role at the intersection of TGF-β, BMP, and activin signaling. Heritable disease caused by alterations in TGF-β or BMP family member genes typically have restricted manifestations of cardiovascular disease based on the affected pathway. For instance, mutations in the TGF-β family members (including *TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3*, *SMAD2*, and *SMAD3*) cause aortic aneurysms, mitral valve disease, and a range of extra-cardiac phenotypes within the connective tissue disease spectrum [Micha et al., 2014; Bertoli-Avella et al., 2015]. In contrast, mutations in the type II bone morphogenetic protein receptor (BMPR2) and the *SMAD9* protein, encoded by *BMPR2* and *SMAD9*, respectively, cause primary pulmonary hypertension [Best et al., 2014]. In both classes of disease (TGF-β and BMP), the primary manifestation reflects deficits in vascular smooth muscle cellular function and consequently occurs primarily in the vasculature. In contrast, the spectrum of cardiovascular involvement in Myhre syndrome is diverse, involving vascular (PDA and mid aortic obstruction), cardiomyopathic (restrictive CM), and pericardial phenotypes (effusions and fibroproliferative pericarditis). A further consideration is the effect of *SMAD4* alteration on Activin signaling. Genetic variation in activin family members have not been described in human disease; however, dysregulation of activin A has been observed in heart injury and it remains unclear whether manifestations in Myhre syndrome

represent downstream manifestations of Activin dysregulation [Oshima et al., 2009]. We hypothesize that the involvement of multiple TGF-β superfamily signaling pathways (TGF-β, BMP, and Activin) may account for the expanded spectrum of cardiovascular phenotypic abnormalities seen in Myhre syndrome.

Cardiovascular disease is also a prominent manifestation of the other Mendelian disorder associated with *SMAD4* mutations, juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome (JPS-HHT) [Gallione et al., 2010]. Described mutations are unambiguously loss of function in nature and include missense, frame shift, and large genomic deletions [Bossler et al., 2006]. Like Myrhe syndrome, cardiovascular manifestations in JPS-HHT are diverse and encompass BMP-related phenotypes such as PPH as well as TGF-β-related manifestations such as proximal aortic enlargement [Heald et al., 2015]. In contrast to JPS-HHT, the mutational repertoire in Myhre syndrome is severely limited, encompassing only two recurrent missense variants (p.Ile500Val or p.Arg496Cys), suggest a gain of function mechanism. This concept is supported by data from clinical specimens that have demonstrated increased levels of the *SMAD4* protein, possibly related to decreased protein destruction [Le Goff et al., 2011]. The JPS-HHT and Myhre syndrome therefore represent opposite functional consequences of variation at a single gene locus. As such, it seems probable that basic investigation into the molecular consequences of these mutations will inform multiple aspects of cardiovascular biology.

Study Strengths and Weaknesses

This is the largest review of the cardiovascular features of patients with mutation-proven Myhre syndrome, with attention to removing duplicative reports of single patients, and careful classification. Most patients were identified from literature reports, and mild congenital heart defects, small pericardial effusions, mild myocardial dysfunction due to undiagnosed RCM may have been overlooked. Follow-up of the most recent patient reports varied.

Guidelines for Evaluation and Management

We recommend a baseline consultation by a cardiologist trained in congenital heart disease for patients with Myhre syndrome at the time of diagnosis. This should include upper and lower blood pressure measurements, and two-dimensional echocardiography with Doppler. Additional modalities may be needed to image the descending aorta or pericardial sac, and cardiac catheterization should be pursued for signs of elevated right atrial pressure. We think it is reasonable to obtain a B-type natriuretic peptide (BNP) level as an adjunct to assess myocardial function. Patients who have clinical and laboratory features of PLE should be presumed to have elevated right heart pressures, and an underlying cause should be sought such as pericardial disease or RCM. For asymptomatic patients who have a normal echocardiogram and examination, we suggest a minimum of 3 years follow-up. Those with abnormal findings would be followed as indicated with the knowledge of the progressive nature of the disorders. Patients with RCM have a lethal disease and should be considered early for cardiac transplantation.

We agree with Starr et al. [2015] who recommend functional pulmonary testing if symptoms suggest restrictive pulmonary insufficiency. Shortness of breath from RCM should be distinguished from primary lung disease. New concerns have been raised that these patients should be monitored post-operatively for abnormal fibrotic wound healing, but it has not been established whether pharmacologic therapy could modify the response. Elective surgical procedures should be performed with care, if at all, given the attendant risks.

Protein-losing enteropathy, present in patient 1, is a well described, but poorly understood complex disorder of enteric protein loss often seen in the setting of complex cardiac disease especially single ventricle physiology after palliative Fontan procedure [Rychik and Spray, 2002; Johnson et al., 2012]. Clinical manifestations in the setting of large gastrointestinal protein loss can include pleural effusions, ascites, dependent edema, steatorrhea with nutritional deficits including fat soluble vitamin deficiencies and relative immunodeficiency including hypogammaglobulinemia and lymphopenia. Acute therapeutic strategies can include fluid management, correction of protein status, and overall nutrition as well as reduction of effusions and ascites. Long-term strategies include the use of medical and dietary therapies which may include high protein diets, medium chain triglycerides, diuretics, heparin, and both systemic (methylprednisolone) and enteric topical (budesonide) steroids.

We conclude that the cardiovascular abnormalities in Myhre syndrome comprise a recognizable cardiac syndrome which frequently includes otherwise rare pericardial disease and RCM. Protein losing enteropathy must be distinguished from liver disease, and similarly, RCM and pericarditis may require intensive imaging to distinguish.

There is need for a prospective clinical study of Myhre syndrome, or at least, systematic data collection in a registry. Currently, an informal international network of concerned parents interacts through social media, although a formal Myhre syndrome advocacy group does not yet exist. This would facilitate the education of patients and families, stimulate research and facilitate the diagnosis of new patients.

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APPENDIX

A list of patient numbers and references is presented here (complete list available from authors upon request).

1, 4, 6, 13, 16, 20, 21, 23, 24, 25, 28, 29 [Michot et al., 2014]

2 [Le Goff et al., 2011; Picco et al., 2013; Michot et al., 2014]

3, 5, 8, 9 [Le Goff et al., 2011; Michot et al., 2014]

7 [Le Goff et al., 2011; McGowan et al., 2011, patient 3; Michot et al., 2014]

10 [Rosser et al., 1995, patient 3; McGaughran and Donnai, 1996; Michot et al., 2014]

11, 27 [McGowan et al., 2011, patients 11 and 1; Michot et al., 2014]

12, 26 [Le Goff et al., 2011; Al Ageeli et al., 2012; Michot et al., 2014]

17 [Burglen et al., 2003, patient 4; Michot et al., 2014]

30, 31 [Lindor et al., 2002, 2012; Oldenburg et al., 2015]

32–39 [Caputo et al., 2012]

40 [Asakura et al., 2012]

41–45 [Starr et al., 2015]

46 [Piccolo et al., 2014]

47 [Need et al., 2012]

48 [Hawkes and Kuri, 2015]
49 [Caputo et al., 2014]
50 [Oldenburg et al., 2015, patient 3]
51–54 [Newly described patients 1–4]

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