Cardiology in the Young

cambridge.org/cty

The frequency and efficacy of genetic testing in individuals with scimitar syndrome

Tyler A. Fick[1], Daryl A. Scott[2,3], Philip J. Lupo[4], Justin Weigand[1] and Shaine A. Morris[1]

[1]Department of Pediatrics, Section of Pediatric Cardiology, Texas Children's Hospital and Baylor College of Medicine, Houston, Texas, USA; [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [3]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA and [4]Department of Pediatrics, Section of Hematology-Oncology, Baylor College of Medicine, Houston, TX, USA

Original Article

Cite this article: Fick TA, Scott DA, Lupo PJ, Weigand J, and Morris SA (2022) The frequency and efficacy of genetic testing in individuals with scimitar syndrome. *Cardiology in the Young* **32**: 550–557. doi: 10.1017/S1047951121002535

Received: 14 March 2021
Revised: 4 May 2021
Accepted: 6 June 2021
First published online: 2 July 2021

Keywords:
Scimitar syndrome; pulmonary vein; cardiogenetics; CHD

Author for correspondence: Shaine A. Morris, MD, MPH, Department of Pediatrics, Section of Pediatric Cardiology, Texas Children's Hospital and Baylor College of Medicine, 6651 Main St, Legacy Tower, E1920, Houston, Texas, USA. Tel: 832–824–8278.
E-mail: shainem@bcm.edu

Abstract

Background: Scimitar syndrome is a rare CHD composed of partial anomalous pulmonary venous connection from the right lung, via a scimitar vein, to the inferior vena cava rather than the left atrium. Genetic conditions associated with scimitar syndrome have not been well investigated at present. *Methods:* Our study included patients with scimitar syndrome diagnosed at Texas Children's Hospital from January 1987 to July 2020. Medical records were evaluated to determine if genetic testing was performed, including chromosomal microarray analysis or whole-exome sequencing. Copy number variants identified as pathogenic/likely pathogenic and variants of unknown significance were collected. Analyses of cardiac and extracardiac findings were performed via chart review. *Results:* Ninety-eight patients were identified with scimitar syndrome, 89 of which met inclusion criteria. A chromosome analysis or chromosomal microarray analysis was performed in 18 patients (20%). Whole-exome sequencing was performed in six patients following negative chromosomal microarray analysis testing. A molecular genetic diagnosis was made in 7 of 18 cases (39% of those tested). Ninety-six per cent of the cohort had some type of extracardiac finding, with 43% having asthma and 20% having a gastrointestinal pathology. Of the seven patients with positive genetic testing, all had extracardiac anomalies with all but one having gastrointestinal findings and 30% having congenital diaphragmatic hernia. *Conclusions:* Genetic testing revealed an underlying diagnosis in roughly 40% of those tested. Given the relatively high prevalence of pathogenic variants, we recommend chromosomal microarray analysis and whole-exome sequencing for patients with scimitar syndrome and extracardiac defects.

Scimitar syndrome is a rare CHD composed of a partial anomalous pulmonary venous connection from the right lung to the inferior vena cava via a scimitar vein. The birth prevalence of scimitar syndrome is approximately 1 in 50,000 live births.[1] Scimitar syndrome is often associated with varying degrees of right lung hypoplasia and patients with scimitar syndrome often suffer from chronic lung parenchymal disease, asthma, and recurrent respiratory infections.[2] There is also an increased prevalence of right pulmonary artery hypoplasia, atrial and ventricular septal defects, coarctation of the aorta, tetralogy of Fallot, and other congenital heart lesions including single-ventricle lesions.[1,2] Patients with scimitar syndrome and multiple congenital anomalies can be challenging to manage due to their multiple organ system pathologies.

Although some forms of CHD have been linked to various genetic syndromes, the mechanisms that contribute to scimitar syndrome remains elusive.[3–9] Studies have demonstrated an association between scimitar syndrome and extracardiac anomalies including congenital diaphragmatic hernia, imperforate anus, and variants of VACTERL association.[10–14]

Although pathogenic variants in specific genes can clearly cause CHDs, the genetic factors contributing to most cases of scimitar syndrome remain unidentified.[3–9] In this study, we sought to determine the frequency and efficacy of genetic testing in patients with scimitar syndrome from a single institution. We also use the results of clinically based genetic testing to identify genes and pathways whose alteration may lead to scimitar syndrome development. We hypothesiszed that genetic testing would be performed in a minority of patients with scimitar syndrome but would have a considerable rate of positive findings.

Methods

This retrospective cohort study included all patients evaluated at Texas Children's Hospital (TCH) from January 1987 to July 2019 diagnosed with Scimitar syndrome. Patients were first identified from institutional echocardiographic and clinical databases. All patient charts were reviewed to confirm an anatomic diagnosis of scimitar syndrome. A portion of these patients

have been used in a prior publication from our institution, focusing on different aspects than our study and not consisting of our entire cohort. Patients were only included if they had been seen by a provider and had sufficient data for chart review. Coexisting diagnoses were also identified and extracardiac defects were classified as: asthma (any diagnosis of asthma, irrespective of medical management), right lung hypoplasia (if documented on imaging), tracheal stenosis, frequent respiratory infections (if indicated as such by the provider), pulmonary sequestration (if indicated on cross-sectional imaging), any gastrointestinal symptoms (poor feeding and failure to thrive), or status post-nasogastric feeding requirement or gastrointestinal tube placement, liver dysfunction, omphalocele, pyloric stenosis or imperforate anus. Developmental delay, intellectual disability, and psychiatric diagnosis (attention-deficit hyperactivity disorder, autism spectrum disorder, anxiety disorder, and obsessive–compulsive disorder) were also identified. Cardiac abnormalities were identified by review of echocardiograms, MRI, and CT and reported, with the exclusion of patent foramen ovale and small patent ductus arteriosus.

All clinically obtained genetic testing was reviewed, including chromosome analysis, chromosomal microarray analyses, and whole-exome sequencing. Chromosome analysis and/or single gene or panel sequence testing in isolation were not performed on any patients in this cohort. Positive findings on genetic testing were labelled by the third-party laboratory as "abnormal" or indicative of a pathologic change. All copy number variants reported on chromosomal microarray analysis were recorded and results were described. Variants identified by whole-exome sequencing were interpreted according to the American College of Medical Genetics and Genomics guidelines.[15] Studies that revealed "pathogenic" or "likely pathogenic" variants in genes associated with the patient's phenotype were considered positive if their inheritance pattern was also consistent the proposed diagnosis. Variants of unknown significance found in genes that associated with the patient's phenotype were also recorded and results described.

Descriptive analyses included the assessment of categorical variables expressed as counts and percentages. This study was approved by the Baylor College of Medicine Institutional Review Board.

Results

Ninety-eight patients with scimitar syndrome were identified. Eighty-nine of these patients met study criteria (Table 1). The majority of patients were female (60/89, 67.0%). Of these 89 patients, 18 patients (20.2%) had chromosome analysis or chromosomal microarray analysis testing performed and six (7%) had whole-exome sequencing performed in addition to chromosomal microarray analysis (Table 2). No genetic tests were performed prior to 2000, and the majority of genetic testing was performed since 2010, with 16 of 18 chromosomal microarray analyses performed after 2010 and all whole-exome sequencing performed after 2010 (supplemental Figure 1). Of the individuals for whom chromosomal microarray analysis was obtained, 4 of 18 (22.2%) revealed clearly pathogenic copy number variants (Patients 1, 2, 6, and 7). Three patients had positive genetic findings delineated by whole-exome sequencing. Hence, a genetic diagnosis was made in 7 of 18 (38.9%) of patients in whom genetic testing was obtained (Fig 1).

Extracardiac defects and coexisting diagnoses are described in Table 1, and at least one of these was identified in a total of 86 of 89

Table 1. Patient characteristics

Patient characteristics	N (%)
Demographics N = 89	
Sex	
Female	60 (67.0)
Male	29 (33.0)
Race/ethnicity	
Non-Hispanic White	46 (51.7)
Non-Hispanic Black	7 (7.9)
Hispanic	28 (31.4)
Asian	4 (4.5)
Other	4 (4.5)
Coexisting diagnoses/extracardiac defects	
Any coexisting diagnosis/extracardiac defect	86 (96.6)
Respiratory/pulmonary pathology	75 (84.3)
Asthma	38 (42.7)
Right lung hypoplasia	69 (77.5)
Frequent respiratory infections	11 (12.4)
Tracheal stenosis	5 (5.6)
Tracheoesophageal fistula	1 (1.1)
Pulmonary sequestration	4 (4.5)
Congenital diaphragmatic hernia	12 (13.2)
Gastrointestinal pathology	21 (23.6)
Poor feeding	19 (21.3)
NG tube	3 (3.4)
Gastrostomy tube	12 (13.2)
Liver dysfunction	2 (2.2)
Omphalocele	1 (1.1)
Pyloric stenosis	1 (1.1)
Imperforate anus	2 (2.2)
Any musculoskeletal pathology	18 (20.2)
Scoliosis	14 (15.7)
Vertebral anomalies	6 (6.7)
Pectus deformity	2 (2.2)
Developmental delay	7 (7.9)
Psychiatric diagnosis	11 (12.4)
Additional cardiovascular abnormalities	
Aortopulmonary collateral	50 (56.2)
Status post-occlusion	34 (38.2)
Atrial septal defect	38 (42.7)
Sinus venosus defect	4 (4.5)
Secundum defect	33 (37.1)
Left superior vena cava	19 (21.3)
Ventricular septal defect	7 (7.9)
Arrhythmia	3 (3.4)

(*Continued*)

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press

Table 1. (*Continued*)

Patient characteristics	N (%)
Coarctation of the aorta	3 (3.4)
Single ventricle	3 (3.4)
Hypoplastic left heart syndrome	2 (2.2)
DORV, mitral atresia	1 (1.1)
Pulmonary valve stenosis	3 (3.4)
Aberrant right subclavian artery	2 (2.2)
Tetralogy of Fallot	2 (2.2)
Subaortic stenosis	2 (2.2)
Hypertrophic cardiomyopathy	1 (1.1)
Unroofed coronary Sinus	1 (1.1)
Interventions/outcomes	
Status post-tracheostomy	3 (3.4)
Status post-Scimitar vein surgery	28 (31.4)
Residual pulmonary vein stenosis	6 (6.7)
Deceased	4 (4.5)

DORV = double-outlet right ventricle; NG = nasogastric.

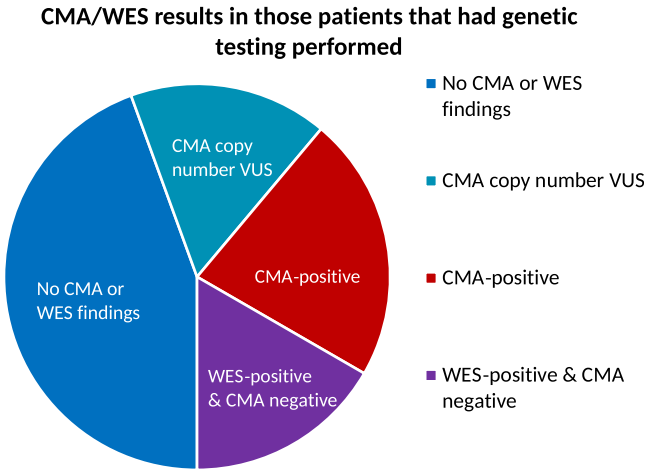

Figure 1. Pie graph indicating the distribution of chromosomal microarray (CMA) and/or whole-exome sequencing (WES) results on patients with SS. No CMA or WES findings were noted in 8 of 18 patients (44%). CMA revealed copy number variants of unknown significance (VUS) in 3 of 18 (17%). CMA was positive in 4 of 18 (22%) and WES was positive, with negative CMA, in 3 of 18 (17%).

patients (96.6%). Among the entire cohort, 43% of patients were diagnosed with asthma compared to 14% of the genetic-positive subgroup. Four patients were found to have pulmonary sequestration. Congenital diaphragmatic hernia was identified in 13% of the entire cohort and 29% in the genetic-positive subgroup. Any gastrointestinal pathology was identified in only 24% of the entire cohort versus 86% of the genetic-positive subgroup. All patients that had positive genetic findings had some type of extracardiac finding (Table 3) and their genetic findings are summarised in Table 4.

Four patients were deceased, none of which had genetic testing performed. One patient had VACTERL syndrome with a transitional atrioventricular septal defect, subaortic stenosis, and coarctation of the aorta. No further information surrounding the death was available. A second patient had hypoplastic left heart syndrome (mitral and aortic atresia) and underwent stage 1 palliation with Norwood/Sano repair and then stage 2 palliation with Glenn repair. The patient had poor PO feeding, intestinal malrotation, and pulmonary sequestration but was eventually discharged from the hospital following Glenn palliation. No further information surrounding the death was available. A third patient had a secundum atrial septal defect, right-sided congenital diaphragmatic hernia, hemivertebrae, and a sacral anomaly. She had severe lung hypoplasia and oxygen requirement and was managed with home hospice care. A fourth patient had a secundum atrial septal defect as well as horseshoe lung, tracheal stenosis requiring surgical repair and subsequent tracheal stent, chiari malformation, and pulmonary hypertension and died due to pulmonary hypertensive crisis.

Table 2. Genetic testing results in patients with SS

Test	N (%)
Chromosomal microarray (CMA)	18 (20.2)
CMA with no structural variation	11 (61.1)
CMA with structural variation of unknown significance	3 (16.7)
CMA with pathogenic structural variation	4 (22.2)
Whole-exome sequencing (WES)	6 (6.7)
WES with no concerning variants	3 (50.0)
WES with variants of unknown significance	0 (0.0)
WES with pathogenic/likely pathogenic variants	3 (50.0)

Clinical and molecular summaries

Seven patients (Patient 1–7) had a definitive molecular diagnosis made through genetic testing. Three patients (Patients 8–10) had copy number variants identified on chromosomal microarray analysis and that were of variants of unknown significance. None of these patients had whole-exome sequencing performed. Patient 11 did not have genetic testing but was diagnosed on a clinical basis with Marfan syndrome.

Patient 1

Patient 1 had scimitar syndrome with a congenital diaphragmatic hernia, right lung hypoplasia, right pulmonary artery narrowing, dextroposition of the heart, secundum atrial septal defect, and aortic arch elongation. Chromosomal microarray analysis demonstrated ˜1.6 Mb gain on 22q11.21 (minimum chr22:17,998,078–19,606,540; maximum chr22:17,982,527–19,627,555; hg19). The proximal portion of the DiGeorge critical region was involved in the duplication, but the *TBX1* gene is located outside of the duplication. Duplications involving the DiGeorge critical region have a variable phenotype with significant clinical overlap to 22q11.2 deletion syndrome that has been described in association with total anomalous pulmonary venous return.[16,17]

This patient was also found to have a ˜0.6 Mb gain on 10q21.1 (minimum chr10:53,155,144–53,788,294, maximum chr10:53,108,516–53,813,645; hg19) involving two genes; a portion of *PRKG1* and all of *CSTF2T*. Missense variants in *PRKG1* are associated with autosomal-dominant aortic aneurysm, familial

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press

Table 3. Cardiac and extracardiac findings in patients with pathogenic/likely pathogenic or VUS on genetic testing

	Pathogenic/likely pathogenic variants N = 7 (%)	Structural variation or sequence VUS N = 3 (%)
Coexisting diagnoses/extracardiac defects		
Any coexisting diagnosis/extracardiac defect	7 (100)	3 (100)
Respiratory/pulmonary pathology	6 (85.7)	3 (100)
Asthma	1 (14.3)	1 (33.3)
Right lung hypoplasia	6 (85.7)	2 (66.6)
Frequent respiratory infections	2 (28.6)	0
Tracheoesophageal fistula	0	1 (33.3)
Tracheal stenosis	1 (14.3)	0
Congenital diaphragmatic hernia	2 (28.6)	0
Gastrointestinal pathology	6 (85.7)	1 (33.3)
Poor feeding	4 (57.1)	1 (33.3)
NG tube	1 (14.3)	0
Gastrostomy tube	4 (57.1)	1 (33.3)
Any musculoskeletal pathology	2 (28.6)	2 (66.6)
Scoliosis	1 (14.3)	1 (33.3)
Vertebral anomalies	1 (14.3)	1 (33.3)
Developmental delay	2 (28.6)	1 (33.3)
Psychiatric diagnosis	0	1 (33.3)
Additional cardiovascular abnormalities		
Aortopulmonary collateraln	5 (71.4)	1 (33.3)
Status post occlusio	3 (42.9)	1 (33.3)
Atrial septal defect	4 (57.1)	3 (100)
Sinus venosus defect	0	0
Secundum defect	4 (57.1)	3 (100)
Left superior vena cava	2 (28.6)	0
Ventricular septal defect	1 (14.3)	1 (33.3)
Arrhythmia	1 (14.3)	0
Coarctation of the aorta	1 (14.3)	0
Single ventricle	1 (14.3)	0
Hypoplastic left heart syndrome (MA/AS)	1 (14.3)	0
DORV, mitral atresia	0	0
Aberrant right subclavian artery	1 (14.3)	1 (33.3)
Tetralogy of Fallot	1 (14.3)	1 (33.3)
Pulmonary valve stenosis	1 (14.3)	0
Hypertrophic cardiomyopathy	1 (14.3)	0

AS = aortic stenosis; DORV = double-outlet right ventricle; MA = mitral atresia; NG = nasogastric.
All values as n (%).

thoracic 8 through a gain-of-function mechanism.[18] It is possible that *PRKG1* may be disrupted by this gain, but data from normal individuals catalogued in the gnomAD database (https://gnomad.broadinstitute.org/) suggest that haploinsufficiency of *PRKG1* is unlikely to be associated with a significant phenotype. Similarly, a gain of *CSTF2T* is not currently associated with a known phenotype. Hence, is it unlikely that this change contributed to the development of scimitar syndrome in this patient.

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press

Table 4. Summary of genetic testing results

Patient	Testing	Genetic finding indicated
1	CMA	~1.6 Mb gain on 22q11.21 (minimum chr22:17,998,078–19,606,540; maximum chr22:17,982,527–19,627,555; hg19. This patient was also found to have a ~0.6 Mb gain on 10q21.1 (minimum chr10:53,155,144–53,788,294, maximum chr10:53,108,516–53,813,645; hg19) involving two genes; a portion of *PRKG1* and all of *CSTF2T*.
2	CMA	Copy number gain of approximately 16.7 Mb on 10q21.3q23.1 (minimum chr10:69,734,057–86,426,833; maximum chr10:69,678,160–86,472,655; hg19).
3	WES	Maternally inherited, heterozygous c.239_240delAT, p.(H80Rfs*17) frameshift variant in *NAA15*.
4	WES	Likely pathologic c.3118A > G, p.(R1040G) variant in *MYRF* consistent with cardiac urogenital syndrome.
5	WES	Likely pathologic c.2660C > T, p.(T8871) missense variant in *EP300* consistent with a diagnosis of Rubinstein–Taybi syndrome 2.
6	CMA	Copy number gain on chromosome 16p13.11p12.3 (chr16:15,333,155–18,242,713; hg19) consistent with 16p13.11 microduplication syndrome.
7	Chromosomal analysis	De novo unbalanced translocation between chromosomes X and 2 with a chromosomal designation of 46,X,der(X)t(X;2)(q26;q31.1).
8	CMA	~0.16 Mb loss at 16q22.1 (minimum chr16:68,579,360–68,695,101; maximum chr16:68,550,178 – 68,709,888; hg19) which involved two genes, *ZFP90* and *CDH3*.
9	CMA	~ 7 kb loss at 8p11.23 (minimum chr8:38,091,564–38,098,218; maximum chr8:38,090,832–38,099,625; hg19) leading to loss of exons 3–6 of *DDHD2*. This patient also had an ~0.1 Mb gain on 12q24.31 (minimum chr12:124,090,709–124,111,801; maximum chr12:124,008,592–124,114,750; hg19) and an ~ 0.21 Mb loss on 12q24.31 (minimum chr12:124,155,205–124,156,920; maximum chr12:124,137,166–124,158,087; hg19).
10	CMA	~ 0.026 Mb loss at 8p11.21 (minimum chr8:42,694,713–42706855; maximum chr8:42,685,068–42,710,811; hg19) leading to loss of exons 4–5 of *CHRNB*

CMA = chromosomal microarray; WES = whole-exome sequencing.

Patient 2

Patient 2 had scimitar syndrome with right lung hypoplasia as well as secundum atrial septal defect, coarctation of the aorta, and pulmonary valve stenosis. This patient also had extracardiac findings including congenital diaphragmatic hernia, osteopenia and rib fractures, failure to thrive with nasogastric feeding tube requirements, and nystagmus. Chromosomal microarray analysis demonstrated a copy number gain of approximately 16.7 Mb on 10q21.3q23.1 (minimum chr10:69,734,057–86,426,833; maximum chr10:69,678,160–86,472,655; hg19) that overlaps the region associated with 10q22.3-q23.2 deletion syndrome. Duplications overlapping that which was seen in this individual have been reported in patients with CHD, dysmorphisms, and delays in speech and motor development.[19,20]

Patient 3

Patient 3 had scimitar syndrome with right lung hypoplasia as well as tetralogy of Fallot, an aortopulmonary collateral to the right side requiring device occlusion, a secundum atrial septal defect, and hypertrophic cardiomyopathy. Of note, the patient developed progressive severe biventricular hypertrophy and underwent orthotopic heart transplant with reimplantation of the scimitar vein at 22 months of age. The reimplanted scimitar vein developed stenosis requiring transcatheter angioplasty and stenting of the vein. The patient had a normal chromosomal microarray analysis. Trio whole-exome sequencing revealed a maternally inherited, heterozygous c.239_240delAT, p.(H80Rfs*17) frameshift variant in *NAA15*. This gene encodes an acetyltransferase subunit and has a role in maintaining cell proliferation.[21] Changes in this gene have been identified in separate patients with complex CHDs, hypertrophic cardiomyopathy, and developmental delay.[22] Variants in this gene have also been described in patients with isolated congenital diaphragmatic hernia.[23] No report was available on whether the mother was symptomatic.

Patient 4

Patient 4 had scimitar syndrome and right lung hypoplasia, with hypoplastic left heart syndrome with mitral atresia/aortic stenosis, left ventricular sinusoids, and severe coarctation. The patient underwent single-ventricle palliation consisting of a Norwood procedure and, subsequently, a Glenn palliation. Extracardiac findings for this patient included feeding intolerance requiring gastrostomy tube placement, global developmental delay, and a para-oesophageal hernia. Chromosomal microarray analysis was normal. Whole-exome sequencing revealed a likely pathologic c.3118A > G, p.(R1040G) variant in *MYRF* consistent with cardiac urogenital syndrome.[24–26] This patient has previously been reported in the literature.[26]

Patient 5

Patient 5 had scimitar syndrome, an aberrant right subclavian artery, and a left superior vena cava. The patient also had extracardiac findings of failure to thrive, global developmental delay, right lung hypoplasia, congenital diaphragmatic hernia, and a laryngeal cleft. His chromosomal microarray analysis was normal. Whole-exome sequencing revealed a likely pathologic c.2660C > T, p.(T8871) missense variant in *EP300* consistent with a diagnosis of Rubinstein–Taybi syndrome 2, which has a known association with CHDs.[27]

Patient 6

Patient 6 had scimitar syndrome with an atrial septal defect and a history of supraventricular tachycardia along with pyloric stenosis. Chromosomal microarray analysis revealed a gain on chromosome 16p13.11p12.3 (chr16:15,333,155–18,242,713; hg19) consistent with 16p13.11 microduplication syndrome. Individuals with this syndrome can have CHDs[28,29] as well as aortopathy.[30,31] Developmental delay, seizure disorder, autism spectrum disorder, and speech and learning disorders have also been seen in patients with 16q13.11 microduplications.

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press

Patient 7

Patient 7 had scimitar syndrome with perimembranous and muscular ventricular septal defects, a history of poor feeding, developmental delay and intellectual disability. A chromosome analysis revealed a de novo unbalanced translocation between chromosomes X and 2 with a chromosomal designation of 46,X,der(X) t(X;2)(q26;q31.1). Additional studies demonstrated preferential inactivation of the abnormal X chromosome, with no inactivation of the translocated material from chromosome 2. This results in the presence of three active copies of the distal long arm of chromosome 2 in most cells.

Patient 8

Patient 8 had scimitar syndrome with tetralogy of Fallot as well as ectrodactyly, right lung hypoplasia, scoliosis, thoracic vertebrae anomalies and developmental delay. Chromosomal microarray analysis revealed a ˜0.16 Mb loss at 16q22.1 (minimum chr16:68,579,360–68,695,101; maximum chr16:68,550,178 – 68,709,888; hg19) which involved two genes, *ZFP90* and *CDH3*, neither of which have been associated with CHDs.

Patient 9

Patient 9 had scimitar syndrome with a large atrial septal defect, aberrant right subclavian artery, and pulmonary hypertension requiring extracorporeal membrane oxygenation. This patient also had a tracheoesophageal fistula requiring surgical repair, right lung hypoplasia, necrotising enterocolitis requiring medical management, intraventricular hemorrhage noted at birth, and adrenal insufficiency. Chromosomal microarray analysis revealed a ˜ 7 kb loss at 8p11.23 (minimum chr8:38,091,564–38,098,218; maximum chr8:38,090,832–38,099,625; hg19) leading to loss of exons 3–6 of *DDHD2*, which is not known to be associated with CHDs. This patient also had an ˜0.1 Mb gain on 12q24.31 (minimum chr12:124,090,709–124,111,801; maximum chr12:124,008,592–124,114,750; hg19) and an ˜ 0.21 Mb loss on 12q24.31 (minimum chr12:124,155,205–124,156,920; maximum chr12:124,137,166–124,158,087; hg19), both of which carry no association with CHDs.

Patient 10

Patient 10 had scimitar syndrome, a perimembranous ventricular septal defect, a secundum atrial septal defect, scoliosis, asthma, and ADHD. This patient also had a tracheoesophageal fistula requiring surgical repair, necrotising enterocolitis requiring medical management, intraventricular hemorrhage noted at birth, and adrenal insufficiency. Chromosomal microarray analysis revealed an ˜ 0.026 Mb loss at 8p11.21 (minimum chr8:42,694,713–42706855; maximum chr8:42,685,068–42,710,811; hg19) leading to loss of exons 4–5 of *CHRNB3*, which has not been associated with a human disorder and carries no association with CHD.

Additionally, Patient 11 was clinically diagnosed with Marfan syndrome. The patient has a family history of clinical Marfan syndrome in multiple siblings, aunts/uncles, and cousins that all carry the clinical diagnosis of Marfan syndrome. The patient had the following findings on evaluation: aortic root dilation with most recent measurement of 4.0 cm (z-score for age + 5.0 years), ectopia lentis, positive thumb and wrist sign. Of note, no genetic testing has been performed on this patient and specifically *FBN1* sequencing has not yet been performed. The patient is being medically managed for Marfan syndrome with atenolol and losartan. She has an extracardiac history of spontaneous pneumothorax requiring apical bleb resection. She has not required surgical intervention on her aortic root.

Discussion

We present a cohort of 89 patients with scimitar syndrome and describe the frequency and findings of genetic testing, describing the genetic variants as well as the extracardiac findings seen in these patients. This is largest single-centre cohort of patients with scimitar syndrome described to date. Our findings correlate with similar literature, describing a 2:1 female-to-male preponderance.[1] The percentage of patients with scimitar syndrome who had genetic testing was relatively low at only 20% of patients in the cohort (18 of 89). However, genetic testing provided a molecular diagnosis in 39% of the individuals tested (7 of 18). Variants of unknown significance were identified in another three individuals that had genetic testing performed and one individual was clinically diagnosed with Marfan syndrome.

Among the genes implicated in this study, *EP300* and *NAA15* may lead to involved changes related to different phases of cell growth and proliferation, including transcription coactivators and post-translational acetylation processing.[32,33] *MYRF* has been shown to play a role in oligodendrocyte cell proliferation,[34] and we can assume that MYRF plays an important developmental role outside of the central nervous system as well.

Patient 1 had duplication of the 22q11.21 region, previously reported to be associated with total anomalous pulmonary venous return. However, duplications of this region have not been previously reported in individuals with scimitar syndrome.[35] Altered dosage of the *TBX1* gene has been implicated in the commonly noted outflow tract abnormalities in both 22q11.2 deletion syndrome and 22q11.2 duplication syndrome.[16,17] However, it is important to note that the *TBX1* gene was not involved in our patient's duplication. This suggests that increased copy number of genes other than TBX1 are responsible for the heart defects seen in this patient.

Patient 7 had an unbalanced chromosomal translocation between chromosome X and 2 that effectively resulted in trisomy of the terminal portion of the q arm of chromosome 2 extending to band 2q31.1. This large region is likely to harbour several genes that play a role in cardiac development. Although CHD can be seen in Turner syndrome, losses of material distal to Xq25 rarely give rise to phenotypes associated with Turner syndrome beyond secondary amenorrhoea or premature menopause.[36] In keeping with this observation, this patient had no stigmata or findings consistent with Turner syndrome. Of note, the patient population was predominantly female, calling into question if other sex chromosome variants could be implicated.

Patient 6 had a 16p13.11 microduplication, which has been associated with CHD as well as multiple neuropsychiatric anomalies and developmental delay, as well as hypermobility and various other musculoskeletal changes.[28–30,37] At present, there is no clear mechanistic link between the genetic findings described and cardiac embryogenesis or the development of scimitar syndrome.

The copy number variants classified as variants of unknown significance involved several different cellular pathways. For example, Patient 8 had a loss at 16q22.1 that involved *CDH3*. Autosomal recessive variants in *CDH3*, located within the 16q22.1 region, are associated with ectodermal dysplasia, ectrodactyly, and macular dystrophy (OMIM# 225,280) and hypotrichosis, congenital, with juvenile macular dystrophy (OMIM# 601,553). This gene has not been associated with CHD in the literature to date.

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press

Extracardiac findings were seen in a large majority of our cohort, and in all of the patients with positive genetic findings and copy number variants of unknown significance. The most common extracardiac findings throughout the cohort were asthma and right lung hypoplasia, gastrointestinal, and skeletal pathologies. Four patients were found to have pulmonary sequestration, a rare but known finding amongst scimitar syndrome patients.[38,39]

Our study must be considered in the light of certain limitations. First, this is a retrospective study, which limits the applicability of our results and introduces the risk of selection bias. This was a single-centre study, and the decision to perform genetic testing was likely altered by institutional practices as well as intrinsic sampling bias towards those patients with extracardiac findings. We relied on chart review for description of extracardiac findings, which places great emphasis on accurate documentation as the sole source for description of extracardiac findings and instances may have been missed due to this limitation. Right lung hypoplasia was seen in a large percentage of patients, but this and other extracardiac findings may be susceptible to reporting bias. Given the retrospective nature of the study, we also cannot describe what individual aspects drove each clinician to obtain genetic testing on some patients with scimitar syndrome and not others. It is possible that providers performed more genetic tests on patients with scimitar syndrome and other extracardiac abnormalities, as compared to those without additional findings. We also note that genetic testing was performed on a relatively small percentage of our cohort. Many of these limitations could be addressed in future studies performed on a prospective basis. Future studies may also consider analysis of the 2:1 female preponderance, which to date has been described but its mechanism remains unknown.

In conclusion, our study highlights the ability of genetic testing to identify a molecular diagnosis in a significant percentage of scimitar syndrome patients (7/18, 39%). Since all of these individuals had extracardiac defects, we conclude that genetic testing should be performed in all individuals with scimitar syndrome who have extracardiac defects. If a specific genetic syndrome is not suspected, chromosomal microarray analysis should be performed as the first-tier test. If a molecular diagnosis is not identified on chromosomal microarray analysis, whole-exome sequencing should then be performed. This testing will allow the clinician to better counsel families going forward and prepare the provider for extracardiac pathologies.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951121002535

Acknowledgements. The Frequency and Efficacy of Genetic Testing in Individuals with Scimitar Syndrome – none.

Financial support. Research reported in this publication was supported by the National Institutes of Health under award number R01HD093660.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the institutional committees at Baylor College of Medicine.

References

1. Gudjonsson U, Brown JW. Scimitar syndrome. Pediatr Card Surg Annu. Published online 2006. doi: 10.1053/j.pcsu.2006.02.011.
2. Wang H, Kalfa D, Rosenbaum MS, et al. Scimitar syndrome in children and adults: natural history, outcomes, and risk analysis. Ann Thorac Surg. Published online 2018. doi: 10.1016/j.athoracsur.2017.06.061.
3. van Nisselrooij AEL, Lugthart MA, Clur SA, et al. The prevalence of genetic diagnoses in fetuses with severe congenital heart defects. Genet Med. Published online 2020. doi: 10.1038/s41436-020-0791-8.
4. Pierpont ME, Brueckner M, Chung WK, et al. Genetic Basis for Congenital Heart Disease: Revisited: A Scientific Statement from the American Heart Association. Circulation. Published online 2018. doi: 10.1161/CIR.0000000000000606.
5. Acevedo JM, Lee S, Gotteiner N, Lay AS, Patel A. Total anomalous pulmonary venous connection (TAPVC): A familial cluster of 3 siblings. Echocardiography. Published online 2017. doi: 10.1111/echo.13665.
6. Li J, Yang S, Pu Z, et al. Whole-exome sequencing identifies SGCD and ACVRL1 mutations associated with total anomalous pulmonary venous return (TAPVR) in Chinese population. Oncotarget. Published online 2017. doi: 10.18632/oncotarget.15434.
7. Osio D, Jain N, Archer N, Turnpenny PD. Total anomalous pulmonary venous drainage in a patient with Koolen syndrome (del17q21.31). Clin Dysmorphol. Published online 2015. doi: 10.1097/MCD.0000000000000082.
8. Nash D, Arrington CB, Kennedy BJ, et al. Shared segment analysis and next-generation sequencing implicates the retinoic acid signaling pathway in Total Anomalous Pulmonary Venous Return (TAPVR). PLoS One. Published online 2015. doi: 10.1371/journal.pone.0131514.
9. Evans WN, Acherman RJ, Ciccolo ML, Castillo WJ, Restrepo H. An increased incidence of total anomalous pulmonary venous connection among Hispanics in Southern Nevada. Congenit Heart Dis. Published online 2015. doi: 10.1111/chd.12199.
10. Ashida K, Itoh A, Naruko T, et al. Familial scimitar syndrome: three-dimensional visualization of anomalous pulmonary vein in young sisters. Circulation. Published online 2001. doi: 10.1161/hc2501.092742.
11. Trinca M, Rey C, Breviere GM, Vaksmann G, Francart C, Dupuis C. Familial form of the scimitar syndrome | LE SYNDROME DU CIMETERRE A FORME FAMILIALE. Arch Mal Coeur Vaiss. 1993; 86: 635–638.
12. Ruggieri M, Abbate M, Parano E, Distefano A, Guarnera S, Pavone L. Scimitar vein anomaly with multiple cardiac malformations, craniofacial, and central nervous system abnormalities in a brother and sister: familial scimitar anomaly or new syndrome? Am J Med Genet. Published online 2003. doi: 10.1002/ajmg.a.10115.
13. Aklilu TM, Adhana MC, Aboye AG. Imperforate anus associated with anomalous pulmonary venous return in scimitar syndrome. Case report from a tertiary hospital in Ethiopia. BMC Pediatr. Published online 2019. doi: 10.1186/s12887-019-1643-z.
14. Lloyd IE, Rowe LR, Erickson LK, Paxton CN, South ST, Alashari M. Two cases of Scimitar syndrome associated with multiple congenital skeletal anomalies and lacking abnormalities by genomic microarray analysis. Pediatr Dev Pathol. Published online 2014. doi: 10.2350/14-04-1474-CR.1.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. Published online 2015. doi: 10.1038/gim.2015.30.
16. De La Rochebrochard C, Joly-Hélas G, Goldenberg A, et al. The intrafamilial variability of the 22q11.2 microduplication encompasses a spectrum from minor cognitive deficits to severe congenital anomalies [3]. Am J Med Genet Part A. Published online 2006. doi: 10.1002/ajmg.a.31227.
17. Hasten E, McDonald-McGinn DM, Crowley TB, et al. Dysregulation of TBX1 dosage in the anterior heart field results in congenital heart disease resembling the 22q11.2 duplication syndrome. Hum Mol Genet. Published online 2018. doi: 10.1093/hmg/ddy078.
18. Guo DC, Regalado E, Casteel DE, et al. Recurrent gain-of-function mutation in PRKG1 causes thoracic aortic aneurysms and acute aortic dissections. Am J Hum Genet. Published online 2013. doi: 10.1016/j.ajhg.2013.06.019.
19. Tang M, Yang YF, Xie L, et al. Duplication of 10q22.3-q23.3 encompassing BMPR1A and NGR3 associated with congenital heart disease,

microcephaly, and mild intellectual disability. Am J Med Genet Part A. Published online 2015. doi: 10.1002/ajmg.a.37347.
20. Erdogan F, Belloso JM, Gabau E, et al. Fine mapping of a de novo interstitial 10q22-q23 duplication in a patient with congenital heart disease and microcephaly. Eur J Med Genet. Published online 2008. doi: 10.1016/j.ejmg.2007.09.007.
21. Sugiura N, Adams SM, Corriveau RA. An evolutionarily conserved N-terminal acetyltransferase complex associated with neuronal development. J Biol Chem. Published online 2003. doi: 10.1074/jbc.M301218200.
22. Ritter A, Berger JH, Deardorff M, et al. Variants in NAA15 cause pediatric hypertrophic cardiomyopathy. Am J Med Genet Part A. Published online 2020. doi: 10.1002/ajmg.a.61928.
23. Longoni M, Pober BR, High FA. Congenital Diaphragmatic Hernia overview 1. Clinical characteristics of congenital Diaphragmatic Hernia clinical description. Gene Reviews. University of Washington, Seattle, 2019.
24. Qi H, Yu L, Zhou X, et al. De novo variants in congenital diaphragmatic hernia identify MYRF as a new syndrome and reveal genetic overlaps with other developmental disorders. PLoS Genet. Published online 2018. doi: 10.1371/journal.pgen.1007822.
25. Pinz H, Pyle LC, Li D, et al. De novo variants in Myelin regulatory factor (MYRF) as candidates of a new syndrome of cardiac and urogenital anomalies. Am J Med Genet Part A. Published online 2018. doi: 10.1002/ajmg.a.38620.
26. Rossetti LZ, Glinton K, Yuan B, et al. Review of the phenotypic spectrum associated with haploinsufficiency of MYRF. Am J Med Genet Part A. Published online 2019. doi: 10.1002/ajmg.a.61182.
27. Stevens CA, Bhakta MG. Cardiac abnormalities in the Rubinstein-Taybi syndrome. Am J Med Genet. Published online 1995. doi: 10.1002/ajmg.1320590313.
28. Warburton D, Ronemus M, Kline J, et al. The contribution of de novo and rare inherited copy number changes to congenital heart disease in an unselected sample of children with conotruncal defects or hypoplastic left heart disease. Hum Genet. Published online 2014. doi: 10.1007/s00439-013-1353-9.
29. El Khattabi LA, Heide S, Caberg JH, et al. 16p13.11 microduplication in 45 new patients: Refined clinical significance and genotype-phenotype correlations. J Med Genet. Published online 2020. doi: 10.1136/jmedgenet-2018-105389.
30. Kuang SQ, Guo DC, Prakash SK, et al. Recurrent chromosome 16p13.1 duplications are a risk factor for aortic dissections. PLoS Genet. Published online 2011. doi: 10.1371/journal.pgen.1002118.
31. Erhart P, Brandt T, Straub BK, et al. Familial aortic disease and a large duplication in chromosome 16p13.1. Mol Genet Genomic Med. Published online 2018. doi: 10.1002/mgg3.371.
32. Gayther SA, Batley SJ, Linger L, et al. Mutations truncating the EP300 acetylase in human cancers. Nat Genet. Published online 2000. doi: 10.1038/73536.
33. Sugiura N, Patel RG, Corriveau RA. N-Methyl-D-aspartate Receptors regulate a group of transiently expressed genes in the developing brain. J Biol Chem. Published online 2001. doi: 10.1074/jbc.M100011200.
34. Koenning M, Jackson S, Hay CM, et al. Myelin gene regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in the adult CNS. J Neurosci. Published online 2012. doi: 10.1523/JNEUROSCI.1069-12.2012.
35. Cao R, Liu S, Liu C, et al. Duplication and deletion of 22q11 associated with anomalous pulmonary venous connection. Pediatr Cardiol. Published online 2018. doi: 10.1007/s00246-017-1794-3.
36. Therman E, Susman B. The similarity of phenotypic effects caused by Xp and Xq deletions in the human female: a hypothesis. Hum Genet. Published online 1990. doi: 10.1007/BF00193192.
37. Nagamani SCS, Erez A, Bader P, et al. Phenotypic manifestations of copy number variation in chromosome 16p13.11. Eur J Hum Genet. Published online 2011. doi: 10.1038/ejhg.2010.184.
38. Kayawake H, Motoyama H, Date H. Variant scimitar syndrome with intralobar pulmonary sequestration containing adenocarcinoma. Gen Thorac Cardiovasc Surg 2020; 68: 74–76. doi: 10.1007/s11748-019-01098-3.
39. Gonzalez M, Bize P, Ris HB, Krueger T. Scimitar syndrome in association with intrapulmonary sequestration. Eur J Cardio-thoracic Surg 2011; 40: 273. doi: 10.1016/j.ejcts.2010.11.033.

https://doi.org/10.1017/S1047951121002535 Published online by Cambridge University Press