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De novo variants in Myelin regulatory factor (MYRF) as candidates of a new syndrome of cardiac and urogenital anomalies

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Myelin Regulatory Factor (MYRF) is a transcription factor that has previously been associated with the control of the expression of myelin-related genes. However, it is highly expressed in human tissues and mouse embryonic tissues outside the nervous system such as the stomach, lung, and small intestine. It has not previously been reported as a cause of any Mendelian disease. We report here two males with Scimitar syndrome [MIM 106700], and other features including penoscrotal hypospadias, cryptorchidism, pulmonary hypoplasia, tracheal anomalies, congenital diaphragmatic hernia, cleft spleen, thymic involution, and thyroid fibrosis. Gross neurologic functioning appears to be within normal limits. In both individuals a de novo variant in *MYRF* was identified using exome sequencing. Neither variant is found in gnomAD. Heterozygous variants in *MYRF* should be considered in patients with variants of Scimitar syndrome and urogenital anomalies.

KEYWORDS
congenital heart disease, Myelin regulatory factor, MYRF, Scimitar syndrome

1 | INTRODUCTION

Myelin Regulatory Factor (MYRF) has been previously shown to play a vital role in the normal maintenance of myelin by oligodendrocytes, but has not been implicated in any Mendelian disease (Duncan et al., 2017). The protein is highly expressed outside of the nervous system, and its expression is higher in the stomach than the brain (Fagerberg et al., 2014). Although it is a transcription factor, little is known about its role outside the nervous system (Duncan et al., 2017). We report here two individuals with de novo variants in *MYRF* who have an overlapping phenotype of mainly cardiac and urogenital congenital abnormalities. Both individuals have Scimitar syndrome [MIM 106700], a form of partial anomalous venous return. The urogenital abnormalities included cryptorchidism, penoscrotal hypospadias, and micropenis in one individual, and bilateral testes in the inguinal canal of the other individual. Additional findings in single individuals included pulmonary hypoplasia, tracheal anomalies, congenital diaphragmatic hernia, cleft spleen, thymic involution, and thyroid fibrosis (Table 1).

Hailey Pinz and Louise C. Pyle contributed equally to this work.

2 | RESULTS

2.1 | Individual 1

Individual 1 was a male born at 41 weeks gestation to a 27-year-old G2P1 mother; his mother had previously experienced a spontaneous abortion at 14 weeks gestation. There were no reported exposures. At 20 weeks gestation, mesocardia without other signs of heterotaxy

TABLE 1 Molecular and phenotype data on two patients with de novo MYRF variants. Note the shared cardiac and urogenital abnormalities

Individual	Variant (NM_001127392.2)	Incidence in gnomAD	Prediction (SIFT/LRT/mutation taster/CADD)	Sex	Age	Craniofacial	Cardiac	Intestinal	Uro-genital	Development	Other
1	c.2336+1G>A	0	0/0/1/26.8	M	3 yr	None	Scimitar syndrome, cortriatriatum	None	Penoscrotal hypospadias, micropenis, unilateral cryptorchidism	Normal motor, resolved mild speech delay	Pulmonary hypoplasia, tracheal anomalies.
2	c.2518C>T:p.R840X	0	0/0.02/1/44	M	Died DOL10	Wide nasal bridge	Scimitar syndrome	Malrotation	Persistent urachus, bilateral descending testes	Unable to assess	Congenital diagphramatic hernia, cleft spleen, thymic involution, thyroid fibrosis

were noted on ultrasound. He was delivered by Cesarean section for prolonged rupture of membranes and had a birth weight of 3,875 g (75%), length of 54.5 cm (95%), and head circumference of 34 cm (40%). A post-natal echocardiogram and cardiac MRI demonstrated severely obstructive left atrial cor triatriatum (additional membrane dividing the atrium), and Scimitar syndrome variant of partial anomalous venous return. His particular Scimitar syndrome involved connection of the right middle and upper pulmonary veins to the inferior vena cava just inferior to the diaphragm, via an additional Scimitar vein. Bronchoscopy and CT imaging revealed right pulmonary hypoplasia (25% of left lung volume), distal tracheal narrowing/malacia, atypical right bronchus including early take-offs to the right upper and right middle lobes, and severe right middle lobe bronchomalacia with narrowing and external compression. Shortly after birth he was also noted to have penoscrotal hypospadias with micropenis and unilateral cryptorchidism. His motor development was normal. His language development was mildly delayed at 18 months, but resolved with a year of weekly speech therapy. Further evaluations including a brain MRI, eye exam, and ciliary pathology were all normal. A physical exam at age 17 months demonstrated normal height 81 cm (43rd%), low weight at 8.51 kg (2nd%), and normal head circumference at 47 cm (40th%). One hyperpigmented macule, and mild 2/3 syndactyly on his left foot were the only differences noted. His weight has rectified after surgical cardiac repair. He had a normal SNP array, and a clinical exome revealed a de novo variant, c.2336 + 1G>A, in *MYRF* (Figure 1). In addition, the following variants were reported, but not thought to be causative by the clinical genetics team: maternally inherited *ATRX* p.I1738V (the gene associated with alpha thalassemia X-linked intellectual disability syndrome) and compound heterozygous variants in *KIAA0586* p.N441S/p.A1071D (the gene associated with Joubert syndrome 23).

2.2 | Individual 2

Individual 2 was a male born at 37.5 weeks gestation to a 30-year-old G2P1 who had previously experienced a spontaneous abortion at 6 weeks gestation. There were no reported exposures during the pregnancy. A complex congenital heart defect with pericardial effusion was identified at 20 weeks gestation by ultrasound. He was born by spontaneous vaginal delivery with a birth weight of 2,650 g (6th%), length of 46 cm (2nd%), and head circumference of 33.5 cm (22nd%). On a postnatal echocardiogram it was determined that his complex congenital heart disease was comprised of near interruption of the aortic arch with transverse arch hypoplasia with a mildly hypoplastic bicuspid valve, a large ventricular septal defect, large PDA, dextroposition with hypoplasia of the right pulmonary artery, and likely an interrupted inferior vena cava. In addition the right ventricle was mildly dilated and hypertrophied. It was also determined that he had a right congenital diaphragmatic hernia (CDH), with extension of the hepatic dome into the right hemithorax. He had tachycardia up to 315 beats per minutes, which was treated with adenosine. On exam shortly after birth he was found to have a

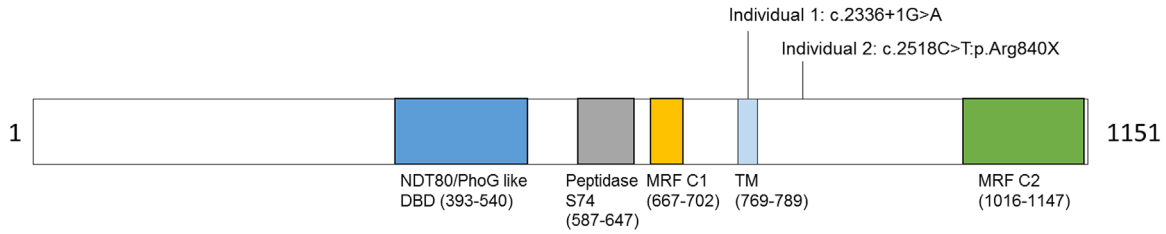

FIGURE 1 Schematic of MYRF protein showing conserved domains and variants identified in the affected individuals. Abbreviations are as follows: DBD, DNA-binding domain; Peptidase S74, chaperone of endosialidase domain; MRF C1, myelin gene regulatory factor C-terminal domain 1; TM, transmembrane domain; MRF C2, myelin gene regulatory factor C-terminal domain 2. [Color figure can be viewed at wileyonlinelibrary.com]

wide nasal bridge, but no other physical differences. There was a specific concern for 22q11.2 deletion syndrome given the interrupted aortic arch. He was also found to have narrowing of the mid and distal thoracic trachea. On Day of life (DOL) 7 he underwent complex repair of the CDH, and intestinal malrotation was also found and repaired. He became anuric with increased bleeding times despite aggressive treatment, and died on DOL 10. On autopsy he was also found to have a hypoplastic mitral valve, persistent left vena cava draining into a dilated coronary sinus, partial anomalous pulmonary venous return consistent with Scimitar syndrome, specifically his left pulmonary veins were normally placed and drained to the left atrium, his two right pulmonary veins drained into the inferior vena cava. On autopsy he was found to have absence of the right atrial appendage, it is unclear if this was congenital or iatrogenic from surgery. He had biventricular hypertrophy and focal right ventricular dystrophic calcification. His right diaphragmatic hernia resulted in a pulmonary-hepatic fusion with right pulmonary hypoplasia. He was found to have a persistent urachus and a central fork-like cleft of the spleen. He had bilateral renomegaly, which may have been iatrogenic. Both testes were found in the inguinal canal. His thyroid showed interstitial fibrosis and he had thymic involution. Family history was noncontributory and there was no reported consanguinity. He had a normal microarray, and clinical exome sequencing reported a de novo novel nonsense variant, c.2518C>T: p.R840X, in *MYRF*. In addition, the following variant was reported, but is not thought to be causative by the clinical genetics team: paternally inherited *DHCR7* c.964-1G>C (IVS8-1G>C) variant (associated with carrier status for Smith-Lemli-Opitz Syndrome).

3 | DISCUSSION

3.1 | MYRF

MYRF has been shown to be essential for myelination during development and plays an important role in the remyelination of multiple sclerosis lesions (Duncan et al., 2017). It has never been associated previously with a Mendelian disorder. Nevertheless, it is highly expressed in developing diaphragm in mouse (with mean expression values of 9.04, 8.74, and 8.01 at embryonic Day (E)11.5, E12.5, and E16.5, respectively), and demonstrates constraint in the context of both missense ($z = 2.39$) and loss of function (pLI = 1.00) variants per the Exome Aggregation Consortium (ExAC) (Russell et al., 2012). The two variants here are both predicted to cause loss of function in the protein, one is a stop gain before the last exon that likely triggers nonsense mediated decay. The other is a variant that ablates a canonical splice site and would lead to aberrant splicing. MYRF is a membrane-associated transcription factor that is activated through autocatalytic cleavage via the peptidase S74 domain to bind the enhancer region of target genes (Bujalka et al., 2013). It functions as a homotrimer in vivo in the nucleus to perform its role in transcription (Kim et al., 2017). Autoprocessing is apparently constitutive and is essential for transcriptional activity. A mouse model lacking MYRF in oligodendrocytes demonstrates severe neurologic dysfunction with seizure and premature death, but no phenotype in heterozygotes is described (Emery et al., 2009). It has also been shown to be essential for synaptic plasticity in *C. elegans* model systems (Meng et al., 2017). MYRF is known to be a target of SOX10, which causes some forms of Waardenburg syndrome (MIM 609136, 611584, and 613266) (Hornig et al., 2013). According to the Human Protein Atlas (HPA) database with tissue-specific RNA-Seq data, *MYRF* expression is highest in the stomach, brain, gall bladder, lung, and small intestine.[2] Similarly in the Mouse ENCODE Project, *MYRF* showed higher expression levels in embryonic developing tissues, such as stomach (FPKM = 9.27–14.62), intestine (FPKM = 5.94–14.18), kidney (FPKM = 1.84–3.33), liver (FPKM = 2.25–23.63), lung (FPKM = 2.83–7.39), and heart (FPKM = 2.14–17.14), compared to developing brain (FPKM = 0.09–1.61), which supports a role beyond the central nervous system (Mouse et al., 2012).

4 | CONCLUSIONS

In conclusion, here we report two individuals with Scimitar syndrome and other congenital anomalies who also have de novo predicted loss-of-function heterozygous variants in *MYRF*. Without functional studies we cannot confirm that these are the causative variants of their phenotypes, but their de novo predicted loss-of-function variant status, lack of instances of these variants in gnomAD, and overlapping phenotypic features are suggestive of a new syndrome. Causative variants in *MYRF* should be considered in patients with cardiac and

urogenital malformation, especially Scimitar syndrome, a rare cardiac malformation found in both of these individuals. Future work could explore the role of MYRF in embryonic development through animal models, or through transcriptional assays to identify a functional defect in these individuals.

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CONFLICTS OF INTEREST

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