HHS Public Access

Author manuscript

*Am J Med Genet A*. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

*Am J Med Genet A*. 2019 July ; 179(7): 1376–1382. doi:10.1002/ajmg.a.61182.

# Review of the phenotypic spectrum associated with haploinsufficiency of *MYRF*

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## Abstract

The myelin regulatory factor gene (*MYRF*) encodes a transcription factor that is widely expressed. There is increasing evidence that heterozygous loss-of-function variants in *MYRF* can lead to abnormal development of the heart, genitourinary tract, diaphragm, and lungs. Here, we searched a clinical database containing the results of 12,000 exome sequencing studies. We identified three previously unreported males with putatively deleterious variants in *MYRF*: one with a point mutation predicted to affect splicing and two with frameshift variants. In all cases where parental DNA was available, these variants were found to have arisen de novo. The phenotypes identified in these subjects included a variety of congenital heart defects (hypoplastic left heart syndrome, scimitar syndrome, septal defects, and valvular anomalies), genitourinary anomalies (ambiguous genitalia, hypospadias, and cryptorchidism), congenital diaphragmatic hernia, and pulmonary hypoplasia. The phenotypes seen in our subjects overlap those described in individuals diagnosed with PAGOD syndrome [MIM# 202660], a clinically defined syndrome characterized by pulmonary artery and lung hypoplasia, agonadism, omphalocele, and diaphragmatic defects that can also be associated with hypoplastic left heart and scimitar syndrome. These cases provide additional evidence that haploinsufficiency of *MYRF* causes a genetic syndrome whose cardinal features include congenital heart defects, urogenital anomalies, congenital diaphragmatic hernia, and pulmonary hypoplasia. We also conclude that consideration should be given to screening individuals with PAGOD for pathogenic variants in *MYRF*, and that individuals with MYRF

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DATA AVAILABILITY STATEMENT

**C**linical and molecular data included in this manuscript have been submitted to CliniVar (https://www.ncbi.nlm.nih.gov/clinvar/).

CONFLICT OF INTEREST STATEMENT

The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from clinical laboratory testing conducted at Baylor Genetics. Otherwise, authors have no conflicts of interest to declare.

deficiency who survive the neonatal period should be monitored closely for developmental delay and intellectual disability.

**Keywords**

Myelin regulatory factor; MYRF; congenital heart defects; pulmonary hypoplasia; congenital diaphragmatic hernia; urogenital anomalies; PAGOD syndrome

## INTRODUCTION

The myelin regulatory factor gene (*MYRF*; MIM# 608329) encodes an endoplasmic reticulum membrane protein that undergoes auto-processing to release its N-terminal fragment which enters the nucleus, forms a homo-trimer, and functions as a transcription factor [Bujalka et al., 2013; Kim et al., 2017; Li et al., 2013]. MYRF was first noted to be a key transcription factor for oligodendrocyte differentiation and central nervous system myelination [Emery et al., 2009; Hornig et al., 2013; Kim et al., 2017; Koenning et al., 2012]. In keeping with that function, Kurahashi et al. have described nine individuals from two unrelated Japanese families with mild encephalitis/encephalopathy and reversible myelin vacuolization (MIM# 618113) who carried the same heterozygous c.1208A>G, p.(Gln403Arg) variant in *MYRF* [Kurahashi et al., 2018]. This variant leads to a single amino acid substitution in the highly conserved DNA-binding domain of MYRF and causes decreased transcriptional activity in luciferase assays.

MYRF is also expressed outside of the central nervous system, and there is increasing evidence that it plays a critical role in the development of various organs including the heart, lungs, diaphragm, and genitourinary tract [Chitayat et al., 2018; Homsy et al., 2015; Jin et al., 2017; Nagase et al., 1999; Pinz et al., 2018; Qi et al., 2018; Stohr et al., 2000]. Pinz et al. described 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 who were found to have likely pathogenic variants in *MYRF* [Pinz et al., 2018]. Chitayat et al. then described a male fetus with hypoplastic left heart sequence, atretic/dysplastic aortic, mitral, and tricuspid valves, hypoplasia of the ascending aorta, total anomalous pulmonary venous connection to the right atrium, mild pulmonary hypoplasia, intestinal malrotation, and ambiguous genitalia who also had a likely pathogenic variant in *MYRF* [Chitayat et al., 2018]. Based on these reports, a new genetic syndrome, cardiac-urogenital syndrome [MIM# 618280], was defined.

Qi et al. subsequently reported ten individuals with de novo missense or frameshift variants in *MYRF* who were identified in large-scale screens of individuals with congenital heart defects and/or congenital diaphragmatic hernia [Homsy et al., 2015; Jin et al., 2017; Qi et al., 2018]. All these individuals had congenital heart defects, six had congenital diaphragmatic hernia, and one had a right hemidiaphragm eventration. This report provided strong evidence that MYRF not only plays a role in cardiac and urogenital development, but also in diaphragm development.

Here we describe three previously unreported males with deleterious *MYRF* variants that are predicted to lead to a loss of MYRF activity.

## MATERIALS AND METHODS

### Editorial Policies and Ethical Considerations

The parents of Subjects 1 and 2 provided informed consent and they were enrolled in an institutional review board-approved research study. The clinical and molecular description of Subject 3 is based solely on anonymized clinical data. This study was conducted in accordance with the ethical standards of the institution's committee on human research and were in keeping with international standards.

### Exome Sequencing and In Silico Prediction of the Effects of Sequence Variants

Exome sequencing studies were performed on a clinical basis at Baylor Genetics. MutationTaster (http://www.mutationtaster.org/) was used to predict the effects of sequence variants on protein function. This program takes into account both the potential effects of amino acid substitution and effects on splice junctions. Human Splicing Finder version 3.1 (http://www.umd.be/HSF3/) was also used to predict the effects of sequence variants on splicing.

## RESULTS

We searched a clinical database containing the results of 12,000 exome sequencing studies. We identified three previously unreported males (Subjects 1–3) with putatively deleterious variants in *MYRF*. Their clinical phenotypes and molecular findings are summarized in Table 1 and are described in detail below. All *MYRF* variants reported are based on *MYRF* transcript variant 2 (NM_001127392.2).

### Subject 1

Subject 1 is a male born at 38 weeks gestation. He was known to have a cardiac malformation based on ultrasound, but the pregnancy had otherwise been uncomplicated. Birth weight was 2889g ($16^{th}$ centile), length was 48cm ($16^{th}$ centile), and head circumference was 33cm ($11^{th}$ centile). A postnatal echocardiogram showed hypoplastic left heart syndrome with mitral valve atresia, hypoplasia of the aortic valve annulus, a muscular ventricular septal defect, an atrial septal defect, patent ductus arteriosus, and partial anomalous pulmonary venous return to the inferior vena cava. He was also been noted to have right-sided pulmonary hypoplasia with concomitant right-sided diaphragmatic elevation consistent with a diagnosis of scimitar syndrome. Additional imaging studies, including a renal ultrasound and brain MRI, were normal. No other anomalies were noted on physical examination. He underwent the first steps of surgical repair at 4 weeks of life and was eventually discharged home at 8 months of age.

Array-based copy number variant (CNV) analysis revealed two changes. The first was a 1.5 kb loss on chromosome 3q13.2, including the last 2 exons of *CD96* [MIM# 606037]. *CD96* has been proposed as a gene responsible for an autosomal dominant form of C syndrome

(Opitz trigonocephaly) (MIM# 211750), but has a predicted loss-of-function intolerance (pLI) score in the gnomAD database of 0.0 [http://gnomad.broadinstitute.org/ accessed 11/9/2018]. The second change was a 25 kb loss on chromosome 8q21.3, which included exon 3 of *CNGB3* (MIM# 605080). This gene has been associated with two autosomal recessive conditions, achromatopsia 3 (MIM# 262300) and juvenile macular degeneration (MIM# 248200).

Exome sequencing demonstrated a heterozygous *de novo* c.3118A>G, p.(Arg1040Gly) missense variant in *MYRF* (NM_001127392.2). This variant is not reported in gnomAD and is highly conserved down to mice (Figure 1). The 3118 position is also located just two base pairs away from the exon 23/intron 23 junction (Figure 1) and may result in breakage of the wild-type donor splice site based on an *in silico* analysis using Human Splicing Finder version 3.1. This variant is also predicted to be “disease causing” by MutationTaster which takes into consideration both conservation and possible effects on splicing.

### Subject 2

Subject 2 was a male born at 37 weeks gestation. He was prenatally diagnosed with hypoplastic left heart syndrome, and a left-sided congenital diaphragmatic hernia. The pregnancy had been complicated by maternal kidney infection requiring treatment with a course of antibiotics. Birth weight was 2685g ($7^{th}$ centile), length was 54.5cm ($86^{th}$ centile), and head circumference was 33.5cm ($2^{nd}$ centile). His physical exam was also notable for genitourinary anomalies, including bilateral undescended testes, hypospadias, and chordee.

He underwent repair of his diaphragmatic hernia at 4 days of life, however, he continued to require high ventilatory support likely due to the degree of his pulmonary hypoplasia. He continued to decline clinically, and eventually the decision was made to withdraw care. The patient passed away at 1 month of life in the cardiovascular intensive care unit.

Prenatal array-based CNV analysis was nondiagnostic. Exome sequencing revealed a heterozygous *de novo* c.3239dupA, p.(Glu1081Glyfs*5) frameshift variant in *MYRF* (NM_001127392.2) which was confirmed by Sanger sequencing. This variant was not reported in gnomAD, and is predicted to be disease causing by MutationTaster.

### Subject 3

Subject 3 is a male referred for clinical exome sequencing due to congenital diaphragmatic hernia, ambiguous genitalia, surgically repaired hypospadias, and a possible history of horseshoe kidney and hydronephrosis. Testing revealed that he carried a c.350_366delinsT, p.(Gly117Valfs*31) variant in *MYRF* (NM_001127392.2). This variant was not detected in a maternal sample, but no paternal sample was available for analysis. This variant was not reported in gnomAD, and is predicted to be disease causing by MutationTaster.

## DISCUSSION

Including the patients we report here, there are now sixteen reported cases of individuals carrying variants in *MYRF* with structural birth defects affecting the heart, lungs, diaphragm, and genitourinary system (Table 1; Figure 2). Loss-of-function variants—stop-

gain, frameshift, and variants that may affect splicing, including the c.3118A>G, p.(Arg1040Gly) variant carried by Subject 1—are distributed throughout the *MYRF* gene. Based on their locations, these variants are likely to trigger nonsense-mediated mRNA decay. In contrast, single amino acid changes are clustered in the DNA binding domain and the peptidase S74 domain. This suggests that loss of MYRF function is the likely mode of action of these variants. It follows that haploinsufficiency of *MYRF* causes a genetic syndrome whose cardinal features include congenital heart defects, pulmonary hypoplasia, congenital diaphragmatic hernia, and genitourinary anomalies.

Congenital heart defects (CHD) are the most common type of anomaly seen in MYRF deficiency with 15/16 (94%) of individuals being affected. The most common forms of congenital heart defects seen in these individuals were hypoplastic left heart syndrome (7/16, 44%) and scimitar syndrome (5/16, 31%). Other forms of CHD included tetralogy of Fallot, septal defects, valvular defects, hypoplastic aortic arch, and patent ductus arteriosus.

Urogenital anomalies are the second most common structural birth defect seen among individuals with MYRF deficiency with at least 12/16 (75%) of individuals being affected. Males can present with ambiguous to completely feminized genitalia, hypospadias, cryptorchidism, micropenis, chordee, and persistent urachus. One female has been described with genital anomalies. She had no internal sex organs and a blind-ending vagina [Qi et al., 2018].

Diaphragm abnormalities, including CDH and diaphragmatic eventration, constitute the third most common structural defect with 10/16 (63%) of individuals being affected. Lung hypoplasia was seen in 7/16 (44%) of individuals. Although lung hypoplasia is common in both scimitar syndrome and in individuals with CDH, the fetus described by Chitayat et al. had neither of these defects [Chitayat et al., 2018]. This suggests that MYRF plays a role in lung development that is independent of venous drainage to the heart or diaphragm development. The high rate of diaphragmatic anomalies and lung hypoplasia underscores the importance of these phenotypes in individuals with MYRF deficiency. With that in mind, we would suggest that the current name of this disorder, cardiac-urogenital syndrome [MIM# 618280], be revised to include these features (cardiac-urogenital-diaphragm-lung syndrome; CUDL syndrome), or be changed to place greater emphasis on the gene rather than the phenotype (*MYRF*-related congenital anomalies syndrome). The use of a more inclusive name for this disorder may make it more likely that laboratory and clinical geneticists will consider this genetic syndrome even in cases where cardiac or urogenital features are not present.

The principle features seen in MYRF deficiency have significant overlap with PAGOD syndrome [MIM# 202660], a clinically defined syndrome characterized by pulmonary artery and lung hypoplasia, agonadism, omphalocele, and diaphragmatic defects [Delgado-Luengo et al., 2016; Gavrilova et al., 2009; Gil et al., 2014; Kennerknecht et al., 1993; Kim et al., 2007; Takahashi et al., 2014]. Other congenital heart defects seen in individuals who have been diagnosed with PAGOD syndrome include hypoplastic left heart and scimitar syndrome which are commonly seen in MYRF deficiency [Kim et al., 2007; Takahashi et al., 2014]. Although PAGOD syndrome has been hypothesized to be an autosomal or x-

linked disorder, it seems likely that some individuals who have been given this diagnosis, particularly those without a family history, may actually have MYRF deficiency.

Congenital anomalies were not reported in the nine individuals described by Kurahashi et al. who had mild encephalitis/encephalopathy with reversible myelin vacuolization and carried heterozygous c.1208A>G, p.(Gln403Arg) variants in *MYRF* [Kurahashi et al., 2018]. In contrast, Qi et al. patient 9 had abnormalities of the heart, diaphragm, lungs, and genitourinary system and carried a p.(Gln403His) variant that affected the same amino acid [Qi et al., 2018]. One possible explanation for this difference is that the substitution of a histidine residue at this position generates a protein whose function is more severely compromised than that generated by an arginine substitution at the same location. If this is the case, it is possible that individuals who survive into adulthood with strong loss-of-function variants in *MYRF* may experience encephalitis/encephalopathy and myelin vacuolization. With this in mind, individuals with loss-of-function variants in *MYRF* who survive the neonatal period should be monitored closely for signs of developmental delay and intellectual disability.

## ACKNOWLEDGEMENTS

The authors thank family members for participating in this research. This work was funded in part by the Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD; R01 HD093660 to DAS).

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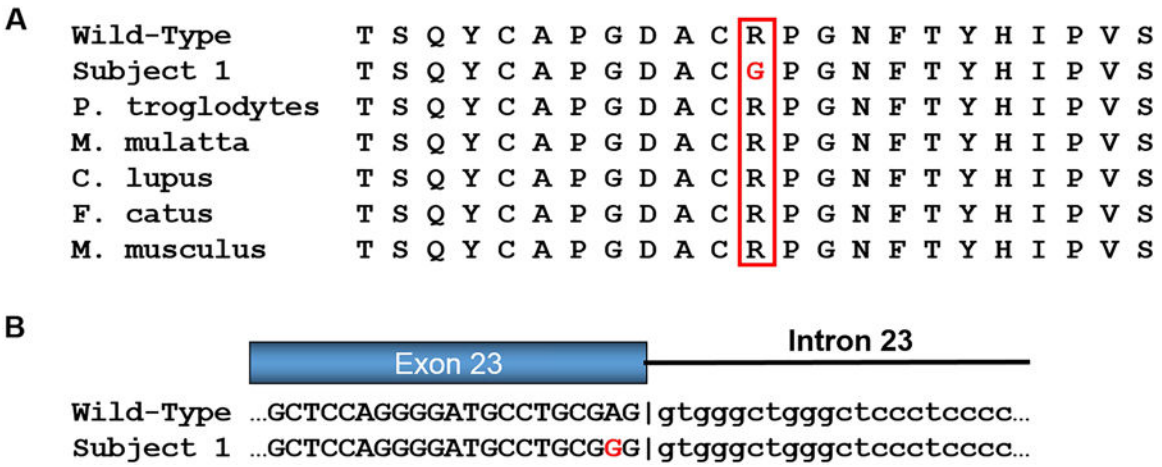


**Figure 1. The heterozygous *de novo* c.3118A>G, p.(Arg1040Gly) missense variant in *MYRF* seen in Subject 1 affects a conserved amino acid and may also affect splicing.**

A) The arginine at position 1040 is conserved down to mice. B) The c.3118A>G change occurs close to the exon 23/intron 23 junction and is predicted to disrupt the associated donor splice site.

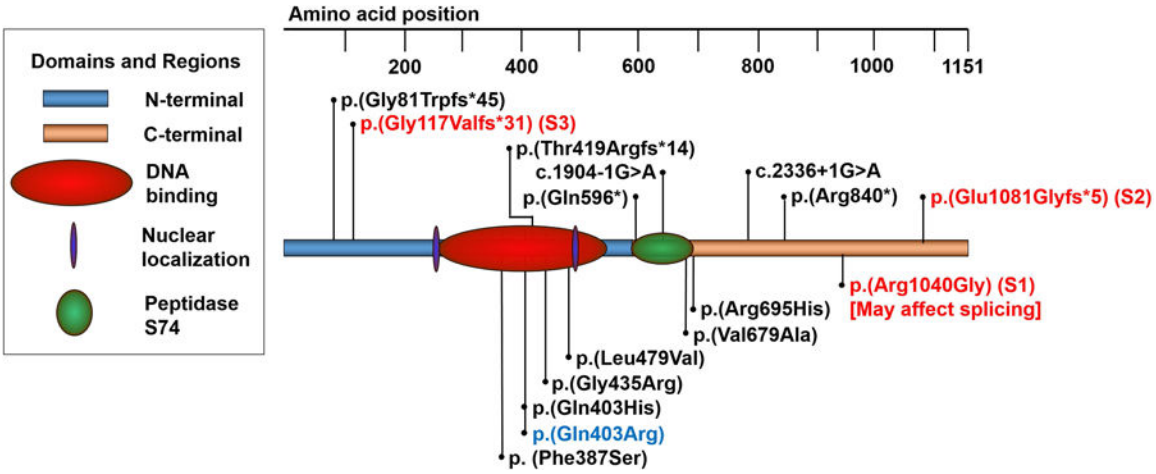


**Figure 2. The locations of the variants in MYRF are shown in relation to its protein domains.**

MYRF variants identified in Subjects 1–3 (S1–3) are shown in red. Variants previously reported in individuals with congenital anomalies affecting the heart, lungs, diaphragm and genitourinary system are shown in black. The variant that has been shown to cause mild encephalitis/encephalopathy with reversible myelin vacuolization (MIM# 618113) is shown in blue. Stop-gain, frameshift (shown above the protein) and variants that could affect splicing are located throughout *MYRF*. Based on their locations, these variants are likely to trigger nonsense-mediated mRNA decay. In contrast, single amino acid changes (shown below the protein) are clustered in the DNA binding domain and the peptidase S74 domain.

**Table 1.**

Summarized genotype and phenotype information for individuals currently reported in the literature carrying variants in MYRF with structural birth defects affecting the heart, lungs, diaphragm, and/or genitourinary system

| | Genotype^ | Sex | Congenital heart disease | Genitourinary anomalies | Diaphragm anomalies | Pulmonary hypoplasia | Other | Outcome |
|---|---|---|---|---|---|---|---|---|
| **Pinz et al. Subject 1** | c.2336+1G>A | M | Scimitar syndrome | Hypospadias, micropenis, cryptorchidism | - | + | Speech delay | Alive at 18 months old |
| **Pinz et al. Subject 2** | c.2518C>T p.(Arg840*) | M | Scimitar syndrome | Persistent urachus | CDH | + | - | Died at 10 days |
| **Chitayat et al. Subject 1** | c.1254_1255dupGA p.(Thr419Argfs*14) | M | Hypoplastic left heart syndrome | Ambiguous genitalia | - | + | Intestinal malrotation | Pregnancy terminated at 19 weeks |
| **Qi et al. Subject 1** | c.235dupG p.(Gly81Trpfs*45) | M | Tetralogy of Fallot, ASD, VSD | Cryptorchidism | Left CDH | - | - | Unknown |
| **Qi et al. Subject 2** | c.1303G>A p.(Gly435Arg) | F | VSD | No internal sex organs, blind-ending vagina | Left CDH | - | Accessory spleen | Unknown |
| **Qi et al. Subject 3** | c.2036T>C p.(Val679Ala) | M | ASD, VSD | Unknown | Left CDH | - | | Deceased |
| **Qi et al. Subject 4** | c. 2084G>A p.(Arg695His) | M | Hypoplastic left heart syndrome | Ambiguous genitalia, cryptorchidism | CDH | - | Intellectual disability and motor delay | Alive at 2 years old |
| **Qi et al. Subject 5** | c.1904–1G>A | F | Scimitar syndrome | Unknown | Right CDH | - | - | Deceased |
| **Qi et al. Subject 6** | c.1904–1G>A | F | Hypoplastic left heart syndrome | Unknown | - | - | - | Deceased |
| **Qi et al. Subject 7** | c.1786C>T p.(Gln596*) | M | Dextrocardia | Female-appearing external genitalia | - | + | - | Unknown |
| **Qi et al. Subject 8** | c.1160T>C p.(Phe387Ser) | M | Aortic arch hypoplasia, coarctation of aorta, hypoplastic left heart syndrome | Ambiguous genitalia, hypospadias, cryptorchidism | - | - | - | Unknown |
| **Qi et al. Subject 9** | c.1209G>C p.(Gln403His) | M | Scimitar syndrome, aortic arch hypoplasia, ASD, bicuspid aortic valve, hypoplastic left heart syndrome, mitral stenosis, VSD | Cryptorchidism | Right hemidiaphragm eventration | + | - | Unknown |
| **Qi et al. Subject 10** | c.1435C>G p.(Leu479Val) | M | Bicuspid aortic valve, coarctation of aorta | Female-appearing external genitalia | - | - | Short stature | Unknown |
| **This report Subject 1** | c.3118A>G p.(Arg1040Gly) | M | Scimitar syndrome, hypoplastic left heart syndrome, mitral valve atresia, hypoplastic aortic valve, VSD, ASD, PDA | - | Right-sided diaphragm elevation | + | - | Alive at 3 months |
| **This report Subject 2** | c.3239dupA p.(Glu1081Glyfs*5) | M | Hypoplastic left heart syndrome | Hypospadias, cryptorchidism, chordee | Left-sided CDH | + | - | Died at 4 weeks |

| | Genotype^ | Sex | Congenital heart disease | Genitourinary anomalies | Diaphragm anomalies | Pulmonary hypoplasia | Other | Outcome |
|---|---|---|---|---|---|---|---|---|
| **This report Subject 3** | c.350_366delinsT p.(Gly117Valfs*31) | M | - | Ambiguous genitalia, hypospadias, horseshoe kidney | CDH | - | - | Unknown |
| **Percent** | | | **15/16 (94%)** | **12/16 (75%)** | **10/16 (62.5%)** | **7/16 (44%)** | | |

Based on transcript NM_001127392.2. + = reported, - = not reported, ASD = atrial septal defect, CDH = congenital diaphragmatic hernia, F = female, M = male, PDA = patent ductus arteriosus, VSD = ventricular septal defect