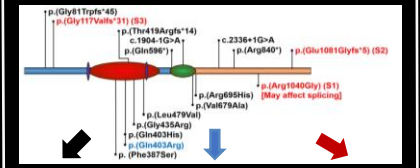
Myelin regulatory factor is required for mouse embryonic development

Lida Langroudi and Jennifer A Mitchell
Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada.

Introduction

MYRF is a transcription factor involved in regulation of myelination genes in Oligodendrocytes (1,2). Oligodendrocytes are the key cells that control myelination of the central nervous system (CNS) providing insulating layers for optimal electrical communication between neurons. Considering that myelination occurs after birth, embryonic expression of Myrf suggests a more comprehensive role than previously imagined. Several neurological disorders such as Huntington's disease (3) have been associated with Myrf, conforming with its well-known transcriptional role in oligodendrocyte differentiation and CNS myelination. However, several case reports have linked *Myrf* de novo mutations with a variety of birth defects in humans. The phenotypical spectrum includes cardiac-urogenital defects (4), disorders in sex development (5) and nanophthalmia/myopia (6). According to OMIM classification, *MYRF* associated defects (OMIM 618280) has been categorized under Congenital Diaphragmatic Hernia (CHD). The multi-organ involvement of *MYRF* in human cases and expression in several mouse embryonic tissues including heart, lung, liver and intestines by mid-gestation (FANTOM 5 project (10)) suggests a critical role for MYRF in embryonic development. In this study we have applied a 'loss of function' approach using a mouse model to examine the role of Myrf during mouse development.



cardiac-urogenital syndrome (CUGS) (OMIM#618280) : Congenital heart, genitourinary system

Mild encephalitis/ encephalopathy with reversible myelin vacuolization (OMIM# 618113)

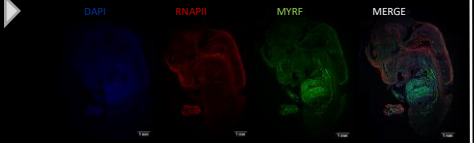
Scimitar syndrome (OMIM#): Congenital heart defects (CHD), genitourinary anomalies, pulmonary hypoplasia, agondia

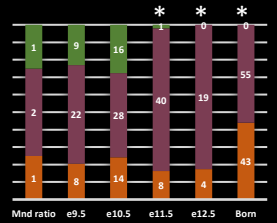
Subcategorization of Myrf de novo mutations in associated with birth defect (6,7,8).

Methodology and Results

Myrf is expressed during embryonic development

Transcriptome data provided by the Fantome5 project (10) increased *Myrf* expression in several mouse tissues at embryonic day 12.5 including heart, lung, liver, and forelimb. Using a verified antibody, immunostaining of wild type mouse embryos at e12.5 confirms MYRF expression in lung, liver, heart, intestines and forelimb. Figure to the right demonstrates dual immunostaining for MYRF/RNA polymerase 2, counter stained with DAPI to signify Myrf specific immunostaining in cryosections.



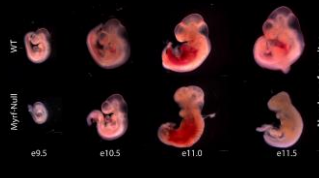


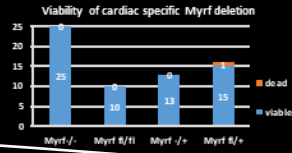
Myrf deletion is embryonically lethal

To generate Myrf null embryos, Myrf heterozygotes were established using Myrf Folxed transgenic mice (B6;129-Myrf tm1Barr/J) (1) crossed with constitutive Cre line (B6.C-Tg(CMV-cre)1Cgn/J). Following IMPC guidelines, viability of null mice (born) was assessed. Our results showed that Myrf depleted mice do not survive after birth and therefore timed mating studies were conducted at e12.5 to verify viability by mid gestation. Embryo collections at e12.5 revealed that Myrf null embryos were reabsorbed. Viability of corresponding genotypes were assessed at earlier time points (e9.5-e11.5). Mendelian ratios expected from heterozygous crossings are 1:2:1 for wild type (Myrf+/+), heterozygous (MyrfΔ/+) and homozygous (MyrfΔ/Δ) genotypes respectively. Statistical analysis of expected Mendelian ratios and obtained genotypes was assessed using a two-tailed Fisher's exact test. Statistically significant difference (*P-value at <0.0001) in viable genotypes after embryonic day 11 verifies embryonic lethality for Myrf deleted embryos compared to control litter mates.

Myrf deleted embryos die at embryonic age of e10.5

Viability of embryos were assessed by presence of heartbeat and embryonic age was determined by somite counts and referencing to Theiler staging hallmarks. Further examination of embryos at e11.5 showed signs of demise and degeneration for Myrf nulls and developmental delay at earlier embryonic days (e9.5 and e10.5) compared to controlled litter mates. Statistical difference between somite counts observed for each genotype were analyzed using one-way ANOVA (***p-value<0.001). Statistically significant difference in developmental age between Myrf-Nulls and control littermates confirms morphological assessments. The maximum number of somites in viable Myrf-null embryo is ~ 37 which suggests developmental arrest occurs at gestational day (GD) 10.5.





Cardiac specific deletion of Myrf is not embryonic lethal

Based on reported case, we explored the possibility of cardiac involvement as the cause of lethality. To generate heart specific *Myrf* deleted mice, Myrf-Floxed transgenic mice – Myrf fl/fl; Cre-/- (B6;129-Myrf tm1Barr/J) were crossed with NKX2.5 Cre transgenic mice Myrf +/+; Cre+/+ (B6.129S1(SJL)-Nkx2-5tm2(cre)Rph/J; JAX®Stock No: 024637). The viability of all four possible genotypes resulting from Cre-carrying Hets and Myrf-Floxed homozygotes were assessed after birth: Myrf fl/+; Cre-/-, Myrf fl/-; Cre-/-, Myrf -/+; Cre+/-, Myrf -/-; Cre+/-). Screening of nine litters and a total of 64 pups revealed that heart specific deletion of Myrf does not result in embryonic lethality. Mice with heart specific Myrf Nulls, are viable and can reach full maturity (P60).

Conclusion

MYRF has been linked to several neurological disorders such as Huntington's disease, but several case reports have associated de novo mutation of Myrf with a variety of birth defects, including cardiac-urogenital defects (4), disorders in sex development (5) and nanophthalmia (6).

Our assessment confirms that constitutive deletion of MYRF is embryonic lethal and null embryos show developmental delay prior to embryonic death. The common causes of embryonic lethality during early organogenesis (e8.5-e11.5) include development of cardiovascular system, hematopoiesis and placenta. The expression of Myrf in heart tissue at e11 (The Fantome5 Project data (10)) and the link to cardio defects in de novo cases suggested a potential cardiovascular cause of lethality. However, our results indicate Myrf deletion in cardiac progenitor cells does not result in the same embryonic phenotype as constitutive deletion of Myrf. We are currently exploring potential placental phenotype and vascular development as secondary causes of lethality.

According to OMIM classification, MYRF associated defects (OMIM 618280) has been categorized under Congenital Diaphragmatic Hernia (CHD). Our developed model system can provide essential information on the role of MYRF during embryonic development and tissue specification which can contribute to prenatal diagnosis and understanding of genetic predispositions.

References

1. Emery, B. *et al.* Identification of Myelin-gene Regulatory Factor as a Critical Transcriptional Regulator Required for CNS Myelination. *Building* **138**, 172–185 (2010).
2. Koenning, M. *et al.* Myelin gene regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in the adult CNS. *J. Neurosci.* **32**, 12528–42 (2012).
3. Yin P, Liu Q, Pan Y, Yang W, Yang S, Wei W, et al. Phosphorylation of myelin regulatory factor by PRKG 2 mediates demyelination in Huntington's disease . EMBO Rep. 2020;
4. Pinz H, Pyle LC, Li D, Izumi K, Skraban C, Tarpinian J, 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. 2018;
5. Qi H, Yu L, Zhou X, Kitaygorodsky A, Wynn J, Zhu N, et al. Genetic analysis of de novo variants reveals sex differences in complex and isolated congenital diaphragmatic hernia and indicates MYRF as a candidate gene. bioRxiv [Internet]. 2017;206037.
6. Cross SH, Mckie L, Hurd TW, Riley S, Wills J, Barnard AR, et al. The nanophthalmos protein TMEM98 inhibits MYRF self-cleavage and is required for eye size specification. PLoS Genet. 2020;16(4):e1008583.
7. Rosseti, L. Z. *et al.* Review of the phenotypic spectrum associated with haploinsufficiency of MYRF. *Am. J. Med. Genet. Part A* **179**, 1376–1382 (2019).
8. Hamanaka, K. *et al.* MYRF haploinsufficiency causes 46,XY and 46,XX disorders of sex development: Bioinformatics consideration. *Hum. Mol. Genet.* **28**, 2319–2329 (2019).
9. Siggs, O. M. *et al.* Autosomal dominant nanophthalmos and high hyperopia associated with a C-terminal frameshift variant in MYRF. *Mol. Vis.* **25**, 527–534 (2019).
10. Lizio M, Harshbarger J, Shimoji H, Severin J, Kasukawa T, Sahin S, et al. Gateways to the FANTOM5 promoter level mammalian expression atlas. Genome Biol. 2015;