

A Novel, Preclinical Model Recapitulates Neonatal Marfan Syndrome and Uncovers Potential Mechanisms Underlying Disease Severity

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Introduction: Neonatal Marfan syndrome (nMFS) is a severe, early-onset form of Marfan syndrome, a connective tissue disorder caused by mutations within exons 23-32 of fibrillin-1 (*FBN1*). Patients are born with cardiovascular, skeletal and ocular dysfunction, with 95% dying within 1-2 years. Cardiovascular complications include congestive heart failure, valve dysfunction and aortic aneurysms with risk of dissection. Currently, there is no cure for nMFS, and the molecular mechanisms driving its early-onset and severe progression remain poorly understood, in part due to a lack of suitable preclinical models.

Methods: Based on clinical findings of two nMFS patients with deletion of *FBN1* exon 30, we generated a conditional *Fbn1* exon 30 allele that was ubiquitously deleted via *Cmv-Cre*. Exon 30 deletion and nMFS phenotype were assessed by qPCR, survival analysis, and histology. RNAscope and immunofluorescence were performed on postnatal day 14 (PND14) heart sections to investigate underlying mechanisms, and bulk RNA-seq of PND0 hearts was used to identify potential molecular targets.

Results: *Fbn1* exon 30 deleted heterozygous mice (*ex30^{del/+}*) phenocopy nMFS characteristics as early as PND0, with 98% dying within 24 days. qPCR validated a 50% reduction in *Fbn1* exon 30 in *ex30^{del/+}* hearts. Hart's staining revealed increased elastic fiber breakage, enlarged wall thickness and dissections in *ex30^{del/+}* aortas. *Ex30^{del/+}* hearts have enlarged aortic and mitral valves with increased proteoglycan content and disorganized collagen. *Fbn1* RNAscope revealed significantly increased *Fbn1* RNA staining in the aortic and mitral valves, left ventricular compact myocardium and aorta. However, immunofluorescence staining revealed decreased fibrillin-1 protein. Bulk RNA-seq and Ingenuity Pathway Analysis uncovered genes and upstream pathways involved in the manifestation of nMFS, such as *Tnc*, *Mmp9*, *Mmp12* and TGF- β .

Conclusion: We successfully generated a preclinical model that phenocopies nMFS patient characteristics that has identified potential genes involved in its disease pathology. Future studies will test molecular targets to uncover therapeutic interventions.