MOUSE MODELS RECAPITULATE BIRTH DEFECTS ASSOCIATED WITH ZNF777 MUTATIONS AND IDENTIFY ZNF777 AS A REPRESSOR IN HUMAN DEVELOPMENT

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Background: Krüppel-associated box (KRAB) zinc finger proteins are the largest family of DNA binding factors in humans and despite being indispensable for human reproduction and embryonic survival, most remain uncharacterized (1). Human genetics databases list ZNF777, a 320-million-year-old KRAB zinc finger protein, as intolerant to mutations in healthy humans. In line with this intolerance a point mutation in ZNF777 associates with a structural birth defect, Tetralogy of Fallot, indicating ZNF777 may be relevant for human development and reproduction.

Objective: The objective of this study was to use mouse models to test ZNF777's role in human development and reproduction and to characterize molecular mechanisms underlying ZNF777 function.

Materials and Methods*:* Viability was analyzed in newborns from intercrosses of heterozygous mice harboring the *Zfp777*^{tm1a} null allele established by the EUCOMM consortium. Structural abnormalities upon Zfp777 loss were identified by cesarean-section followed by microCT scans. The embryonic expression pattern of Zfp777 was assessed using the *Zfp777*^{tm1b} lacZ reporter allele. Molecular mechanisms of Zfp777 function were investigated by identifying direct genomic target genes using Chromatin Immunoprecipitation Sequencing (ChIP-seq) in mouse embryonic stem cells and embryonic fibroblast cells and RNA sequencing (RNA-seq) in embryos. Mass spectrometry was used to identify proteins interacting with ZFP777.

Results: Analysis of 100 newborn mice (24 of which were *Zfp777*$^{tm1a/tm1a}$) revealed 0/24 *Zfp777*$^{tm1a/tm1a}$ survived past 24 hours of life establishing Zfp777 as an essential gene. MicroCT scans of Zfp777$^{tm1a/tm1a}$ embryos showed multiple abnormalities including a ventral septal defect (Figure 1), consistent with Tetralogy of Fallot associated with the ZNF777 patient mutation. β-galactosidase staining of *Zfp777*^{tm1b} embryos revealed ubiquitous Zfp777 expression (Figure 2). Using ChIP-seq, 2719 genes were identified as bound by ZFP777 in embryonic stem cells. Gene ontology analysis of 880 of these genes that were also bound by ZNF777 in human cell lines was consistent with a function in cardiovascular development. When the transcription of Zfp777-bound genes was assessed at mid-gestation by RNA-seq in control embryos and embryos lacking Zfp777 we identified 95 differentially expressed direct target genes. 93 of which were upregulated upon *Zfp777* loss suggesting ZFP777 acts as a repressor. In immunoprecipitation coupled mass spectrometry experiments ZFP777 interacted with all subunits of the Nuclease Remodeling and Deacetylase (NuRD) complex, a prominent epigenetic silencing complex, suggesting a mechanism for ZFP777-mediated gene repression.

Conclusion: We identified Zfp777 as essential for mouse development. MicroCT scans suggest lethality upon loss of Zfp777 results from structural heart defects consistent with the human patient phenotype. Our findings indicate ZNF777 functions as a repressor through a direct or indirect interaction with the NuRD complex. Current work tests this hypothesis in luciferase assays and by co-immunoprecipitation.

References:

Urrutia R. KRAB-containing zinc-finger repressor proteins. Genome Biol. 2003;4(10):231. doi: 10.1186/gb-2003-4-10-231. Epub 2003 Sep 23. PMID: 14519192; PMCID: PMC328446.

Figure 1: microCT scan of Zfp777 KO embryo obtained by C-section showing ventral septal defect

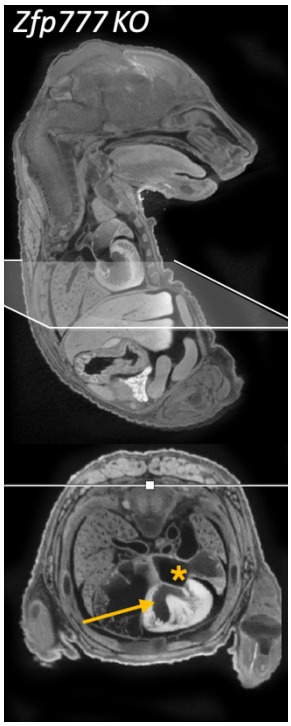

Figure 2: Whole embryo β-galactosidase staining to detect Zfp7777 expression by lacZ activity from the EUCOMM ^{Zfp777tm1b} reporter