A preclinical study on a murine model associated with LMS

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Lateral meningocele syndrome (LMS), also known as Lehman syndrome, is a rare hereditary musculoskeletal disorder with vertebral anomalies and familial osteosclerosis. Recently, whole exome sequencing of unrelated LMS patients revealed truncating mutations in the last exon of NOTCH3. Notch signaling is an evolutionarily conserved signaling pathway involved in cell fate decisions and stem cell renewal in the musculoskeletal system. To understand the gap in our knowledge of LMS and NOTCH3, we are characterizing a mouse model (also known as Humpback (hpbk)) associated with kyphosis and developmental disorders. The hpbk mutant mice harbor a pathogenic variant in the Notch3 gene that contains a G to A point mutation in the splice donor site at the exon 31-intron 31 boundary, resulting in a mutant protein product, which mimics the human NOTCH3 truncating protein found to occur in LMS patients. In order to study the hpbk mouse line at the younger age, we first designed and applied a multiplex tetra-primer amplification-refractory mutation system (ARMS-PCR) to precisely and swiftly genotype the mice. We then performed systematic studies on their phenotypes and pathological mechanisms. The homozygous hpbk mice typically develop kyphosis by 5 weeks of age, and we were able to observe a change in their weights at postnatal day 14. MicroCT analysis of homozygous mice indicates that female mutant mice exhibit osteosclerosis by 2 months, and both male and female mutant mice have a severe muscle phenotype evident by a decrease in TA muscle weight and lower gripping strength in our behavior analysis. We have also performed RNA-seq on calvaria and skeletal muscle samples of WT and homozygous mice. We are investigating the use of an HDACi epi-drug to treat this model, where we show promising data revealing rescue of kyphosis and trabecular bone volume. Ongoing studies include bone histomorphometry of wildtype, mutant, and SAHA treated mutant mice, and a single cell sequencing analysis of wildtype versus mutant mice. Because there is currently no treatment available, a thorough biological understanding of this novel mouse model may point to new treatments to reduce the severity of the disease in LMS patients.