

Patient-specific *Adamtsl2* D167N Knock-in Mice Recapitulate Key Skeletal Features of the Short Stature Syndrome Geleophysic Dysplasia

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INTRODUCTION: Geleophysic dysplasia (GD) is a rare syndromic connective tissue disorder caused by mutations in a disintegrin and metalloprotease with thrombospondin motifs-like 2, (*ADAMTSL2*, GD1, ~50% of cases), fibrillin-1 (*FBNI*, GD2, ~50% of cases), or latent transforming growth factor (TGF) β binding protein 3 (*LTBP3*, GD3, <1% of cases)¹⁻³. All three genes encode extracellular matrix (ECM) proteins that regulate TGF β , BMP, WNT, and possibly other signaling pathways^{4,5}. Signs of GD become apparent in the first year of life and include severe short stature, short fingers and toes (brachydactyly), bone shape abnormalities, joint contractures, thick skin, characteristic facial features ("happy face"), and a pseudomuscular build. Complications from progressive heart valve disease and narrowing of the large airways cause mortality in 33% of affected children before the age of 5^{6,7}. Despite significant childhood mortality and lifelong morbidity no disease-modifying treatments for GD are available. To address this unmet medical need, we generated a novel mouse model for severe GD1 by introducing the patient-specific *ADAMTSL2* c.499G>A (p.D167N) mutation in the mouse *Adamtsl2* locus⁸. The patient harboring this mutation presented with narrow airways at birth, followed by frequent chest infections that required hospitalization and oxygenation. At 2 years of age, the patient had short stature, brachydactyly, joint contractures, abnormal bone shapes, severe pulmonary stenosis due to a dysplastic pulmonary valve, and was walking on tiptoes.

METHODS: To generate a preclinical disease model for GD1, we introduced the *ADAMTSL2* c.499G>A (p.D167N) mutation into the mouse *Adamtsl2* locus by CRISPR/Cas9 gene editing. We quantified survival at the time of genotyping and measured bone length after X-ray imaging. Heart and lung architecture was analyzed by histology. For statistical analyses, we used a Student's t-test. A p-value of <0.05 was considered statistically significant. Mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Icahn School of Medicine at Mount Sinai.

RESULTS SECTION: While heterozygous D167N/+ mice are viable and fertile, homozygous D167N mice showed reduced postnatal survival (at postnatal day 7: 14% D167N mice observed, 25% expected, n=92 mice analyzed) and were substantially smaller. At 2 weeks of age, body length and body weight were significantly reduced (n=7 mice, p<0.0001). These data clearly show that D167N mice recapitulate key signs of severe GD1, including reduced postnatal survival and short stature. Since skeletal abnormalities, such as bone shortening, tubular bones, ovoid vertebral bodies, and delayed bone age/mineralization, are prominent features of GD1, we analyzed bone lengths and shapes in 2 week old WT and D167N mice after X-ray imaging (A-C). D167N metatarsals and phalanges appeared "moth-eaten", indicating delayed mineralization (Fig. 2B, right). Bone length measurements revealed significant shortening of long bones, metatarsals, and metacarpals (D-F, n=14 bones from n=7 mice, p<0.0001). To analyze bone shapes, we determined the aspect ratio of the femur. D167N femur length, but not its width was significantly reduced driving the significant increase in the femur aspect ratio (width/length) (G). To examine vertebral shapes, we measured height and width of coccygeal vertebrae based on X-ray images and calculated their aspect ratio (H-J). D167N vertebrae were less elongated and had a reduced width (J, p<0.001). This resulted in a significant increase in their aspect ratio suggesting an "ovoid" shape as described in GD1 patients³. 6-16 coccygeal vertebrae from n=7 WT and D167N mice were analyzed (total: 77 vertebrae per genotype). Collectively, the D167N skeletal features are consistent with the bone phenotypes reported in GD1 patients. Since progressive airway narrowing and heart valve anomalies contribute to early childhood mortality in GD1, we analyzed WT and D167N lung and heart histology at P17-P19. In D167N lungs, we observed bronchi with an irregular and dysplastic epithelium that were occluded with vesicular structures. This is consistent with our findings in *Adamtsl2* knock-out mice, where similarly occluded bronchi caused perinatal respiratory failure. Heart histology showed dysplastic aortic, pulmonic, and mitral valves.

DISCUSSION: The presence of skeletal, cardiac and pulmonary abnormalities in D167N mice recapitulate the syndromic nature of GD1 and underscore the value of D167N mice in modeling severe GD1. Similar to patients with severe GD, we observed reduced survival in D167N mice. This is likely caused by heart and/or respiratory failure. It also reflects an as yet to be explained phenotypic variability observed in patients. The skeletal phenotypes are consistent with clinical observation. The alterations in bone shapes may be caused by differential growth and the moth-eaten appearance reflects delayed mineralization, which again is described in GD patients as delayed bone age. Finally, the observation that several heart valves are altered in D167N mice is consistent with autopsy reports, showing fibrotic changes in all heart valves.

SIGNIFICANCE/CLINICAL RELEVANCE: This novel model for severe GD will be used for pre-clinical trials to test mechanism-based therapeutic approaches, such as chemical chaperones and inhibitors of TGF β signaling, using increased postnatal survival and normalization of skeletal phenotypes as readouts.

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