Mutations in LAMA5 disrupts a skeletal noncanonical focal adhesion pathway and produces a distinct bent bone dysplasia

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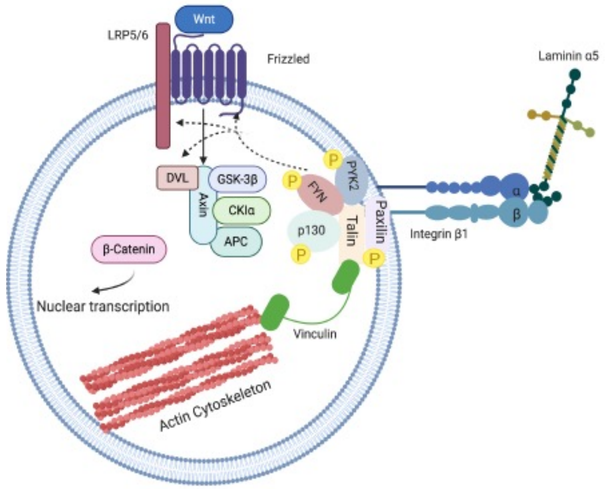
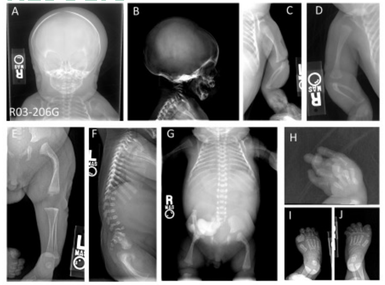
INTRODUCTION

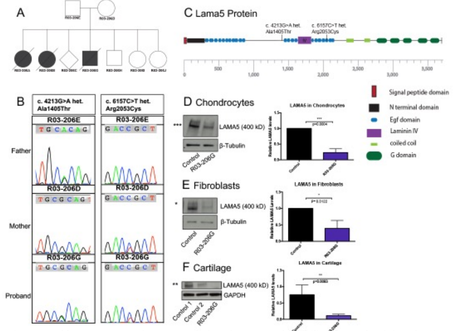
In addition to its structural role in skeletogenesis, the extracellular matrix (ECM), particularly basement membrane proteins, facilitates communication with intracellular signaling pathways and cell to cell interactions to control differentiation, proliferation, migration and survival. Alterations in extracellular proteins cause a number of skeletal disorders and one attributed mechanism results from the deleterious effects of mutated proteins on ECM structure. Yet, the consequences of abnormal ECM on cellular communication remains less well understood. Herein, we describe an unclassified form of bent bone dysplasia caused by recessively inherited mutations in LAMA5, the gene encoding the alpha-5 laminin basement membrane protein. This finding uncovered a mechanism of disease driven by ECM-cell interactions between alpha-5-containing laminins, and integrin-mediated focal adhesion signaling, particularly in cartilage. Loss of LAMA5 altered β1 integrin signaling through the non-canonical kinase PYK2 and the skeletal enriched SRC kinase FYN. Loss of LAMA5 negatively impacted the actin cytoskeleton, vinculin localization, and WNT signaling, tying focal adhesion molecules to key skeletal signaling molecules. This newly described mechanism reveals that a LAMA5-β1 Integrin-PYK2-FYN focal adhesion complex regulates skeletogenesis and, when dysregulated, produces a distinct skeletal disorder. .



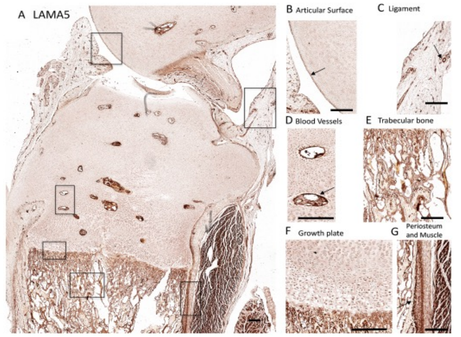
RESULTS



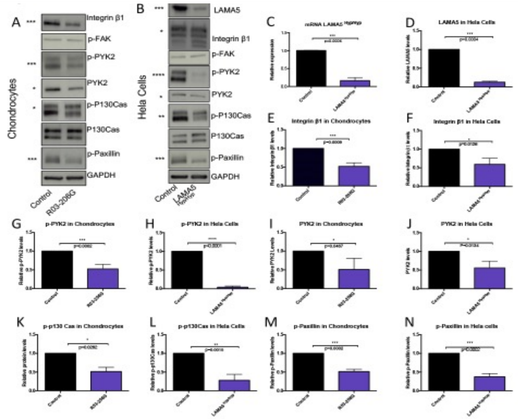
Radiographic findings in R03-206G. Full term fetus with enlarged skull **(A)**, micrognathia **(B)**, presence of scapula and elbow dislocations **(C)**, slight bowing of the humerus, radius and ulna **(C, D)**, severely bent femurs with cortical abnormalities **(E)**, platyspondyly with coronal clefts **(F)**, aplastic acetabular roofs **(G)** ulnar deviation and contractures in the hands **(H)** and bilateral equinovarus **(I, J)**.



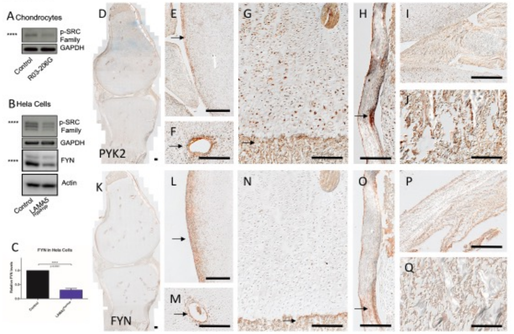
Compound heterozygous missense mutations in *LAMA5* destabilize the α5 laminin subunit. **(A)** Three affected siblings (R03-206A, B, G) showed the bent bone dysplasia phenotype. **(B)** Each parent (R03-206E, D) carried one of the mutations. **(C)** The mutations localized close to EGF domains of the LAMA5 protein. **(D, E)** Affected patient derived chondrocytes and fibroblasts showed reduced levels of LAMA5, *p<0.05, **p<0.01. **(F)** Similar reduction was observed in cartilage derived from the affected individual R03-206G, ***p<0.001.



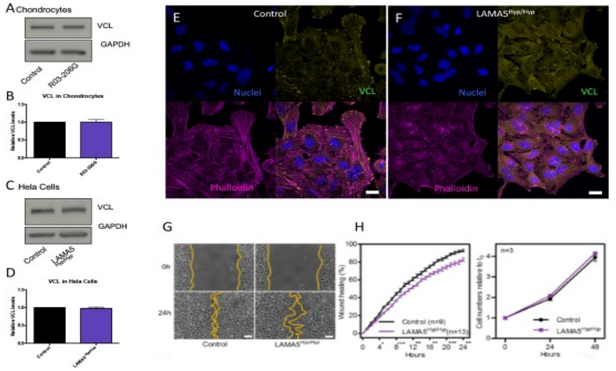
LAMA5 localization in the skeleton. **(A)** LAMA5 staining in a fetal 18 week musculoskeletal tissues. LAMA5 localized around blood vessels in cartilage, ligaments and bone **(C-E)** and independently of blood vessels in articular cartilage, periosteum and growth plate **(B, G, F).** Bars represent 50μm.



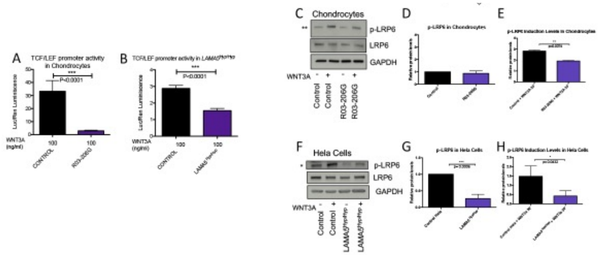
Deficient LAMA5 disrupts the focal adhesion pathway. **(A)** Chondrocytes from R03-206G showed decreased levels of integrin β1, total PYK2, pPYK2, p-P130CAS, and pPaxillin. **(B)** Similar changes were observed in HeLa LAMA5$^{Hyp/Hyp}$. **(C)** Quantitative PCR statistical analysis of the expression of gene-edited HeLa cells with the LAMA5$^{Hyp/Hyp}$ allele. **(D-N)** Quantification of protein levels in chondrocytes and Hela cells from **A** and **B,** *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



LAMA5 mutant chondrocytes and LAMA5$^{Hyp/Hyp}$ Hela cells dysregulate SRC kinase expression levels. **(A)** Chondrocytes from R03-206G showed decreased levels of p-SRC family. **(B)** A similar decrease in p-SRC family, as well as in FYN, was observed in HeLa LAMA5$^{Hyp/Hyp}$ cells. **(C)** Quantitation of the decreased FYN levels in LAMA5$^{Hyp/Hyp}$ HeLa cells shown in **B**. **(D-J)** PYK2 localizes similarly to LAMA5 around blood vessels in cartilage, ligaments and bone as well as articular cartilage growth plate and periosteum/perichondrium in human skeletal tissue. **(K-Q)** FYN shows a very similar pattern with the exception of proliferative and hypertrophic growth plate chondrocytes, where it was not seen. Bars represent 50um.



Effects on cell adhesion due to loss of LAMA5
(A) Chondrocytes from R03-206G and **(C)** LAMA5$^{Hyp/Hyp}$ HeLa cells showed no change in total VCL levels compared to controls. **(B, D)** Quantitation of the data shown in **A, B**. **(E-F)** Focal adhesions were visualized by immunostaining with anti-VCL (green) and actin marker phalloidin (red). Nuclei were stained with DAPI (blue). Bars represent 20μm. (**G**) LAMA5$^{Hyp/Hyp}$ HeLa cells have deficient migration. The LAMA5$^{Hyp/Hyp}$ cells healed more slowly than the control cells at 24 hours (92.8±1.6% vs. 82.8±2.2% (p=0.0013). The orange lines indicate borders of the cell-less regions. Scale bars, 100 μm. **(H)** Equal numbers of Hela cells were plated and the cell numbers were calculated on three consecutive days and plotted as values relative to the first day of measurement (t0). No differences in proliferation between control and LAMA5$^{Hyp/Hyp}$ cells were found.



Defective LAMA5 alters WNT signaling pathway. **(A)** TCF/LEF promoter showed a decreased activity in R03-206G patient chondrocytes when induced with 100 ng/ml WNT3A for 30 minutes. **(B)** Similar changes were observed in LAMA5$^{Hyp/Hyp}$ cells after application of 100 ng/ml WNT3A. **(C-H)** Defective LAMA5 cells showed reduced LRP6 phosphorylation after a 30-minute incubation with 50ng/ml WTN3A. *p<0.05, **p<0.01, ****p<0.0001.

CONCLUSIONS

LAMA5 has an important role in skeletogenesis and its mutations cause a distinctive skeletal dysplasia

These results define a new pathway for skeletogenesis involving LAMA5-Integrins-PYK2 and FYN

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