INTRODUCTION: Hereditary Multiple Exostoses (HME) is a genetic disorder that affects 1 in 50,000 children. It is characterized by the development of many exostoses (also called osteochondromas) that usually grow outward from the most epiphyseal portion of long bone growth plates near the groove of Ranvier. The exostoses can compress surrounding tissues and cause pain and growth retardation. In about 5% of cases, the exostoses can become malignant chondrosarcomas or osteosarcomas.

The majority of HME patients bear heterozygous loss-of-function mutations in EXT1 or EXT2 that encode the components of a Golgi-associated glycosyltransferase complex responsible for heparan sulfate (HS) synthesis. These heterozygous mutations should theoretically lead to a 50% reduction in HS synthesis, but human exostoses often exhibit a far lower HS content.

Thus, to understand HME pathogenesis and exostosis formation in particular, transgenic mice were previously created. Mice bearing a hypomorphic Ext1 mutation were found to produce low HS levels and survived to E14.5, but did not exhibit exostoses. Standard heterozygous Ext2+/- mice exhibited about 50% reduction in their HS production, but exostosis-like outgrowths were seen in about 20% of these mice and were confined to ribs only. Thus, this project was initiated to create a more faithful mouse model of human HME and to clarify the origin of exostosis-forming cells.

METHODS: Heterozygous Ext1+/- and Ext2+/- mice were mated to create compound heterozygous Ext1-/-Ext2+/- mice. In addition, we created conditional Ext1-null mice by mating Gdf5Cre with Ext1+/- mice. The Gdf5Cre mice were used because they express Cre recombinase in cells anatomically located near or within the groove of Ranvier. Pregnant mice and postnatal mice were sacrificed using IACUC approved methods. Fixed long bones were embedded in paraffin. Sections (5 µm) were stained with safranin O/fast green, or treated with heparin lyases that generates a neo-epitope recognized by mAb 3G10. Staining was performed with DAB or a fluorescent antibody. Staining for β-galactosidase on tissue sections was accomplished on fixed tissue using standard techniques.

RESULTS: Because human exostoses exhibit greater than 50% loss of HS, we reasoned that a more faithful mouse model of HME would have to replicate such steep loss. Thus, we created compound heterozygous Ext1-/-Ext2+/- mice. Since each heterozygous allele reduces HS synthesis by 50%, the double mice were expected to produce 25% HS levels compared to WT. Indeed, whereas wild type growth plates stained heavily for HS (Fig. 1A), staining was far less intense in the growth plates of compound mutants (Fig. 1C). When we examined the incidence of rib exostoses, we found that it had nearly doubled (about 45%) compared to that seen in single heterozygous Ext1+/- or Ext2+/- mice (about 20%). Most importantly, the compound heterozygous mice exhibited stereotypic exostoses along the epiphyseal region of their long bones. These exostoses closely resembled those in patients, had a growth plate-like organization with characteristic gene expression patterns, and eventually ossified.

DISCUSSION: Our data show that incidence of exostosis formation is inversely related to Ext gene expression and that a steep, but not necessarily complete, loss of Ext expression and HS production are sufficient to trigger formation of stereotypic exostoses along the long bones. They also show that incidence can reach 100% when Ext function is completely lost, an outcome seen also in two recent mouse studies. Our data provide an explanation for the variable phenotypes seen in HME patients that can vary from mild to severe and could arise from varying degrees of EXT loss-of-function due to different EXT mutations, loss of heterozygosity or other epigenetic mechanisms. As importantly, our data reveal that the stem cell-rich groove of Ranvier may represent a major source of exostosis-forming cells. In sum, our findings provide major new insights into, and a better understanding of, the pathogenesis of HME.

ACKNOWLEDGEMENTS
This work was supported by NIH grants 1RC1AR058382 (to M.P. and E.K.) and R37GM33063 (to J.D.E.).