Osteochondromagenesis: somatic loss of heterozygosity modeled via *Cre*-mediated inversion of the second exon of *Ext1* in chondrocytes

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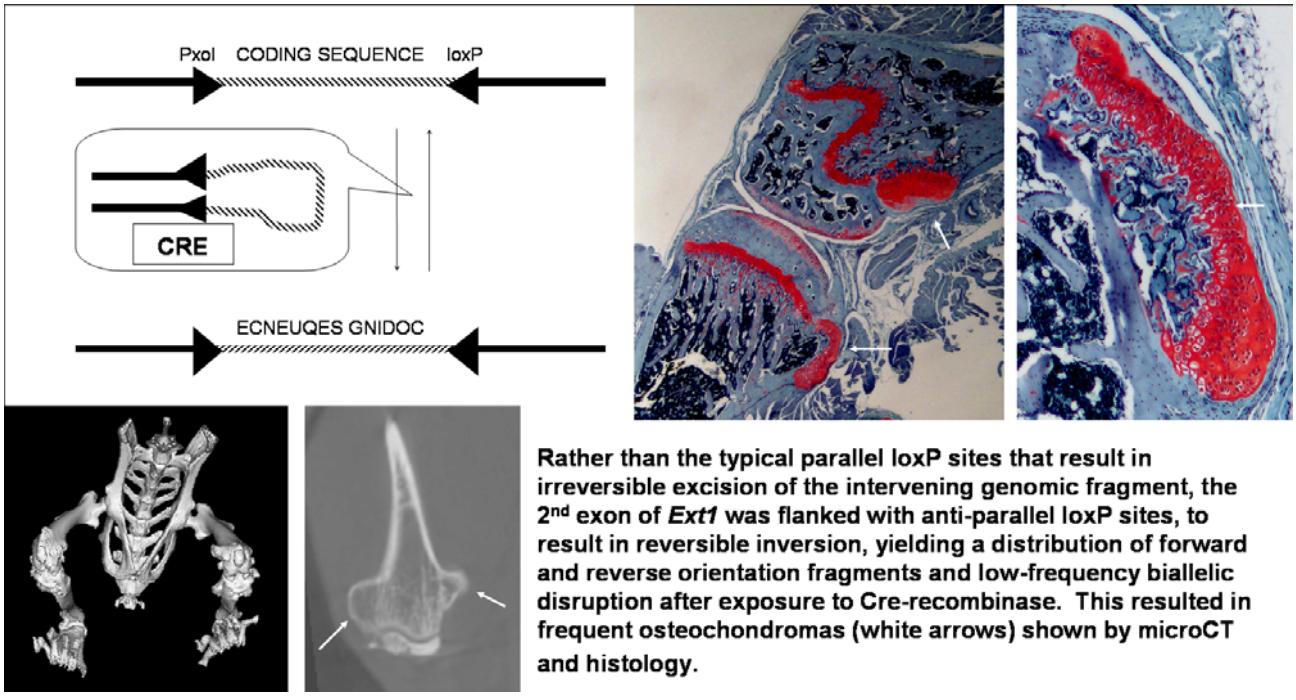
Background:
Multiple hereditary exostoses (MHE) is caused by heterozygosity for mutations in *EXT1* or *EXT2*. Osteochondroma pathogenesis in this disorder remains elusive. Mice homozygous null for homologous *Ext1* or *Ext2* die prenatally; only rarely will a heterozygote form a rib osteochondroma-like excrescence. Origin of osteochondromas in the physeal chondrocytes or adjacent perichondrium is also unclear.

Methods:
Gene-targeting generated a unique conditional allele of *Ext1* with *trans*-orientation *loxP* sites flanking exon 2. Mice bearing this $Ext1^{e2neofl}$ were crossed with mice bearing transgenic *Cre*-recombinase driven by a doxycycline-inducible collagen IIa1 promoter (*Col2-rtTA-Cre*). Doxycyline was administered during week 2 of life. Mice were sacrificed for phenotypic analysis at 4, 6, and 10 weeks. Phenotypic analysis included histology of ribs and knees and microCT. Crossing to mice bearing *Cre*-recombinase driven by *Osterix* expression and activated by tamoxifen (*Osx-CreERT*) at P8 tested competing cells of origin.

Results:
PCR from cartilage-containing tissues demonstrated both forward- and reverse-orientation exon 2 in $Ext1^{e2neofl/e2neofl}$;*Col2-rtTA-Cre* mice after receiving doxycycline. Homozygotes lacking *Cre* and heterozygotes with *Cre* had no demonstrable phenotype. Homozygotes with induced *Cre* consistently developed numerous osteochondromas. $Ext1^{e2neofl/e2neofl}$;*Osx-CreERT* mice, in contrast, formed no osteochondromas.

Discussion:
Reversible *Cre*-mediated inversion of a *trans*-floxed genomic fragment results in a 50:50 distribution of forward and reverse orientation alleles and a small fraction of cells with homozygous disruption. This genetic recapitulation of somatic loss of heterozygosity of *Ext1* in physeal chondrocytes generated numerous osteochondromas, when heterozygosity for a null-allele failed to. This argues that loss of heterozygosity is critical to the phenotypic expression of MHE. That osteochondromas do not form when *Ext1* disruption is induced in pre-osteoblasts argues for a proliferating chondrocyte as the cell of origin



Rather than the typical parallel loxP sites that result in irreversible excision of the intervening genomic fragment, the 2nd exon of *Ext1* was flanked with anti-parallel loxP sites, to result in reversible inversion, yielding a distribution of forward and reverse orientation fragments and low-frequency biallelic disruption after exposure to Cre-recombinase. This resulted in frequent osteochondromas (white arrows) shown by microCT and histology.