Skeletal progenitors and Fibrous Dysplasia of bone

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Fibrous dysplasia and related disorders

Mc-Cune Albright syndrome

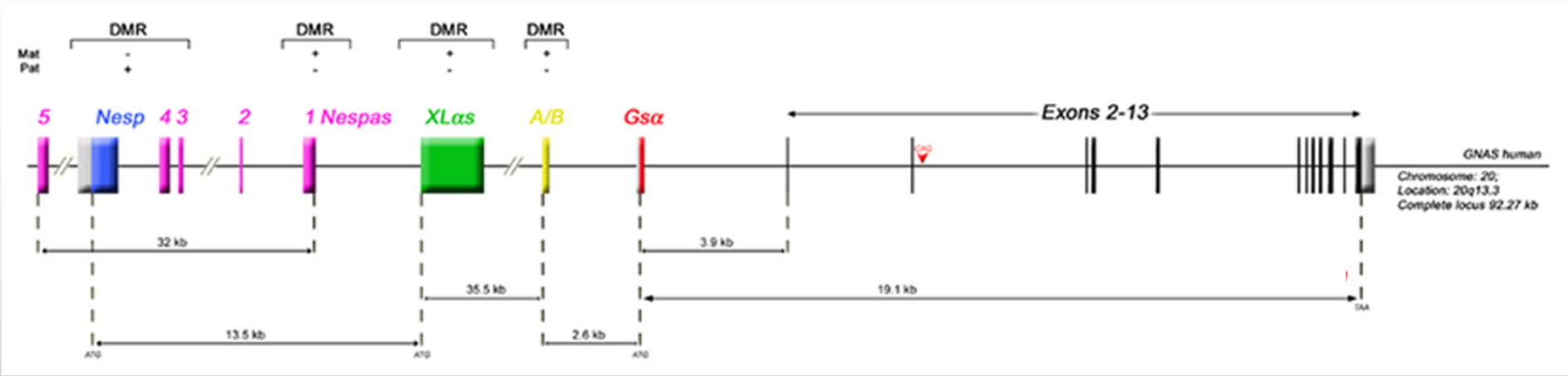
Isolated FD
(monostotic, polyostotic)

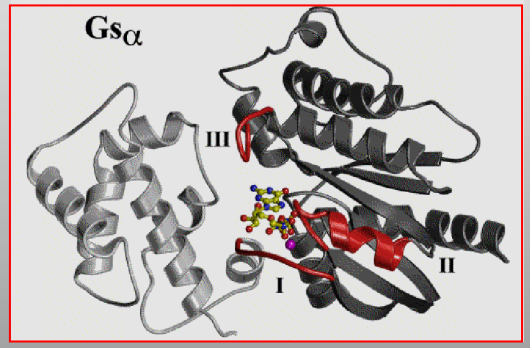
Mc-Cune Albright syndrome
(FD, endocrine lesions, skin hyperpigmentation)

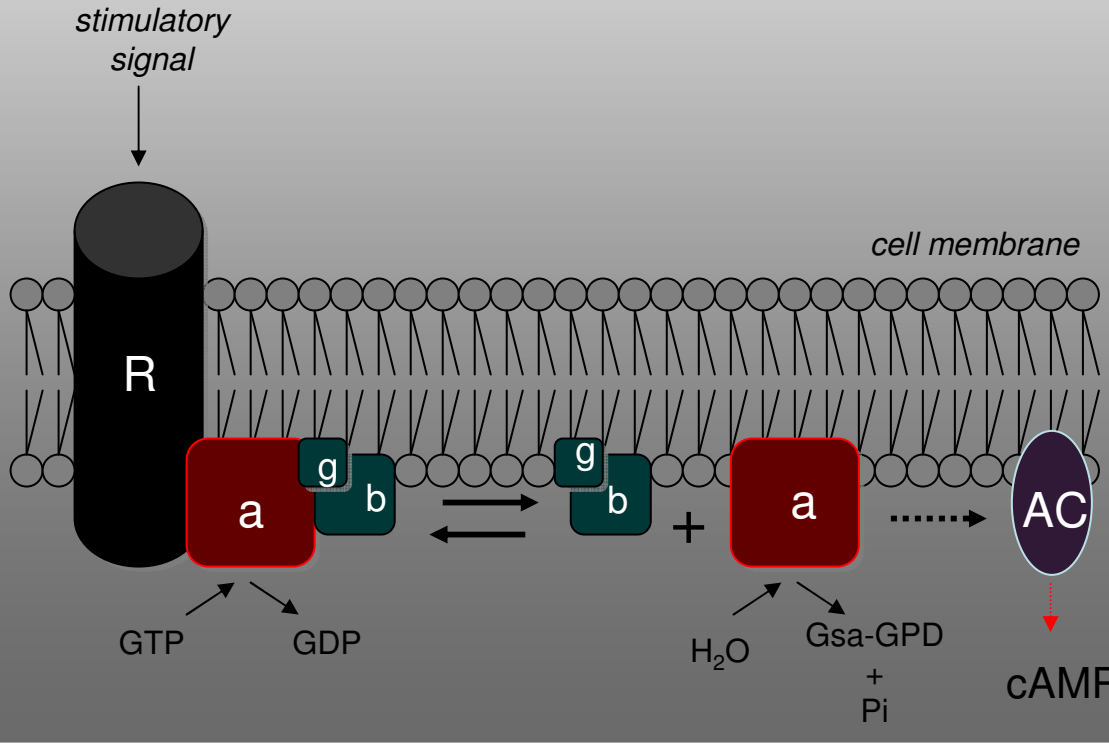
Mazabraud's syndrome
(FD, intramuscular myxomas)

.....

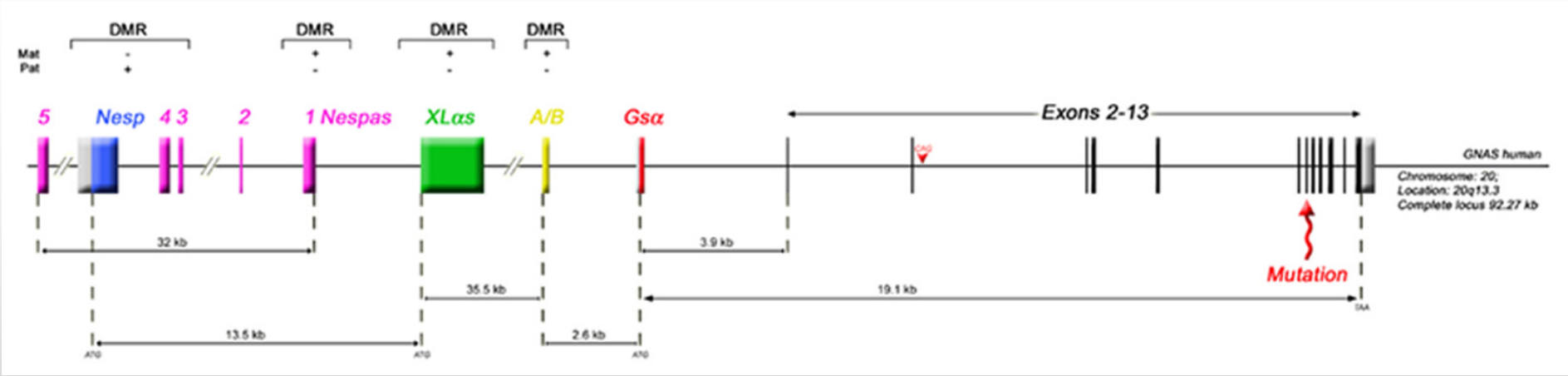
GNAS

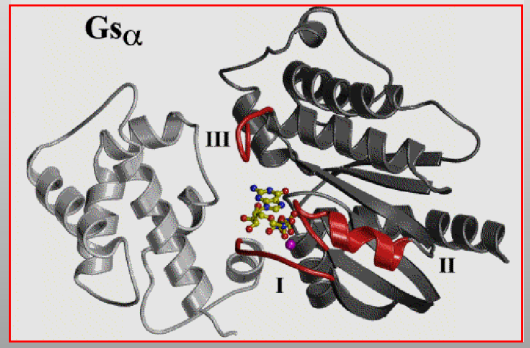

DMR
DMR
DMR
DMR
Mat
Pat
5
Nesp
4 3
2
1 Nespas
XLαs
A/B
Gsα
Exons 2-13
GNAS human
Chromosome: 20;
Location: 20q13.3
Complete locus 92.27 kb
32 kb
3.9 kb
35.5 kb
19.1 kb
13.5 kb
2.6 kb


Gsα
III
II
I

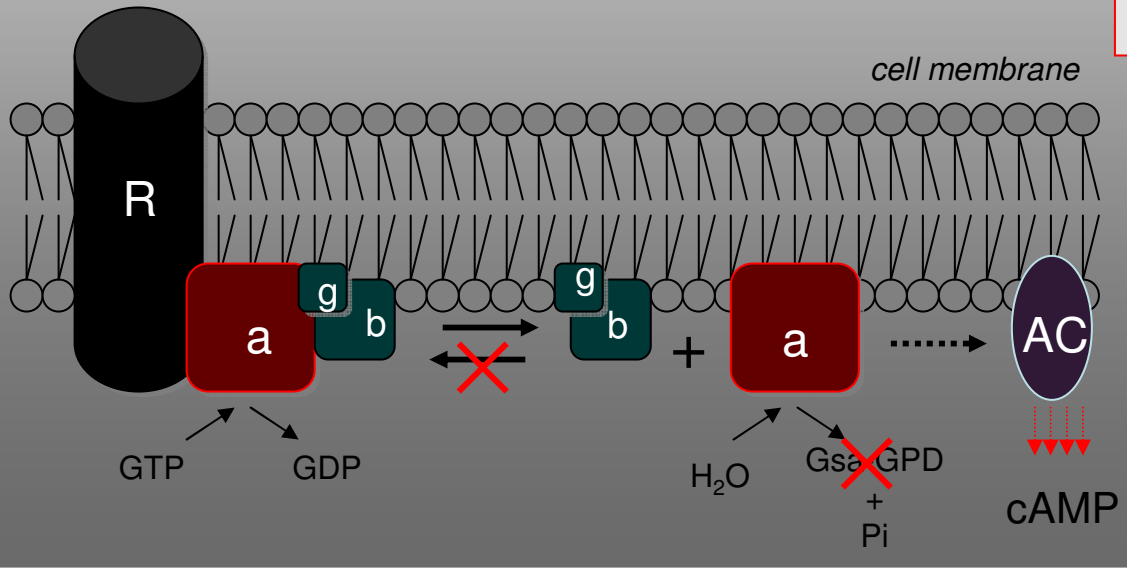

stimulatory signal
cell membrane
R
a
g
b
g
b
+
a
AC
GTP
GDP
H2O
Gsa-GPD
+
Pi
cAMP

FD is caused by gain-of-function mutations of GNAS

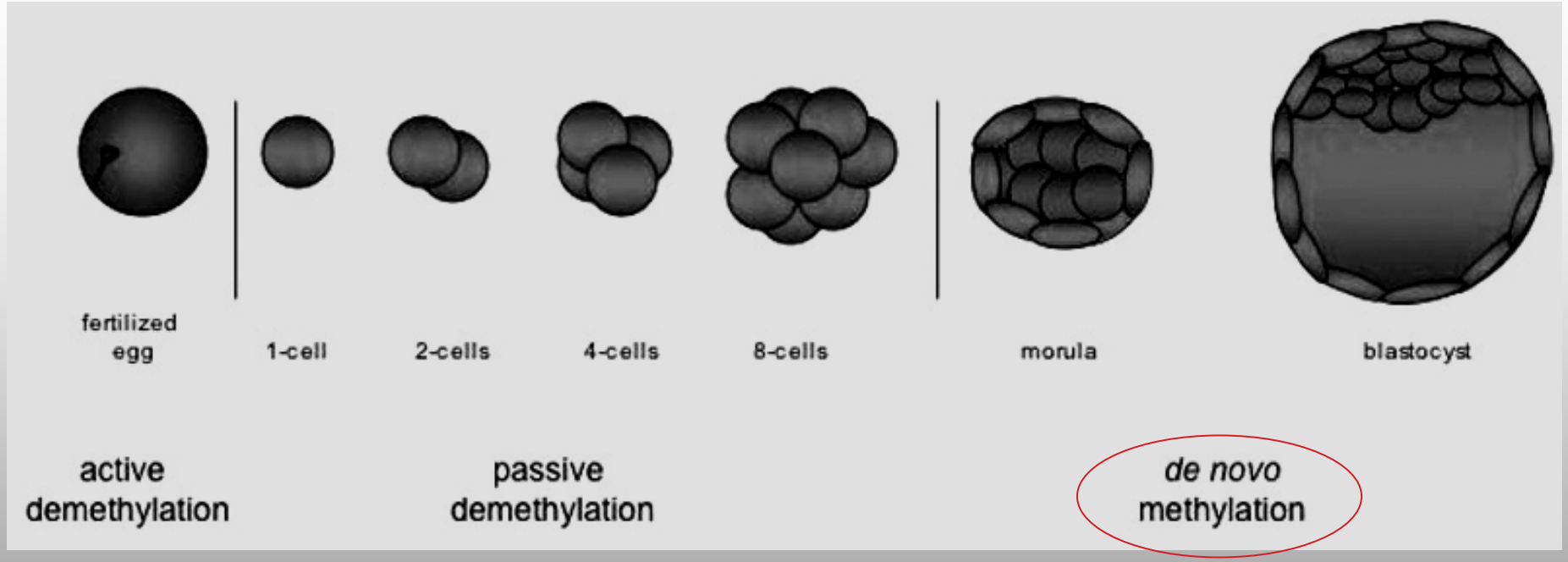


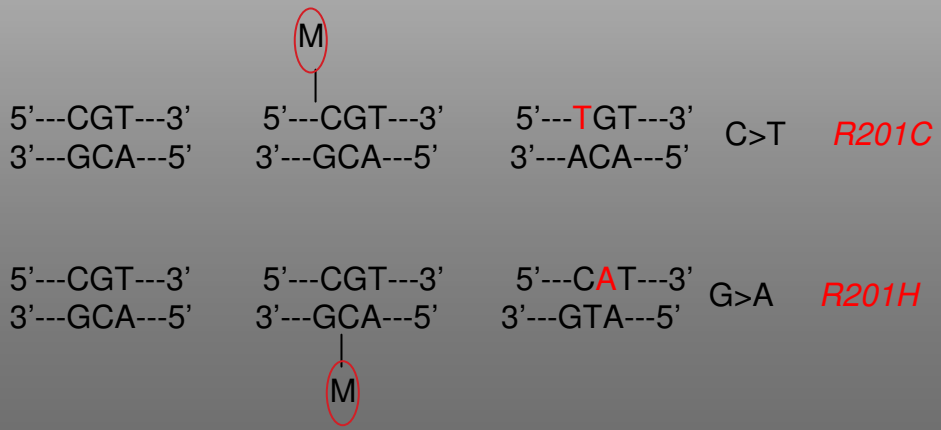


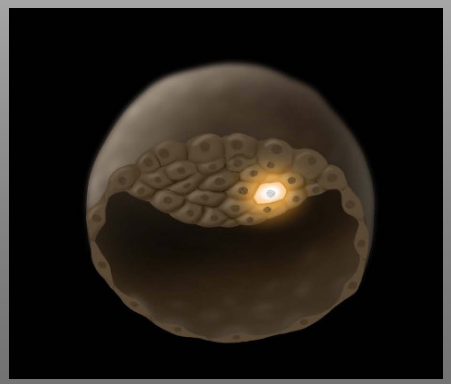
R201H
R201C



FD is established in pluripotent stem cells







pluripotent stem cells

FD develops in the post-natal life

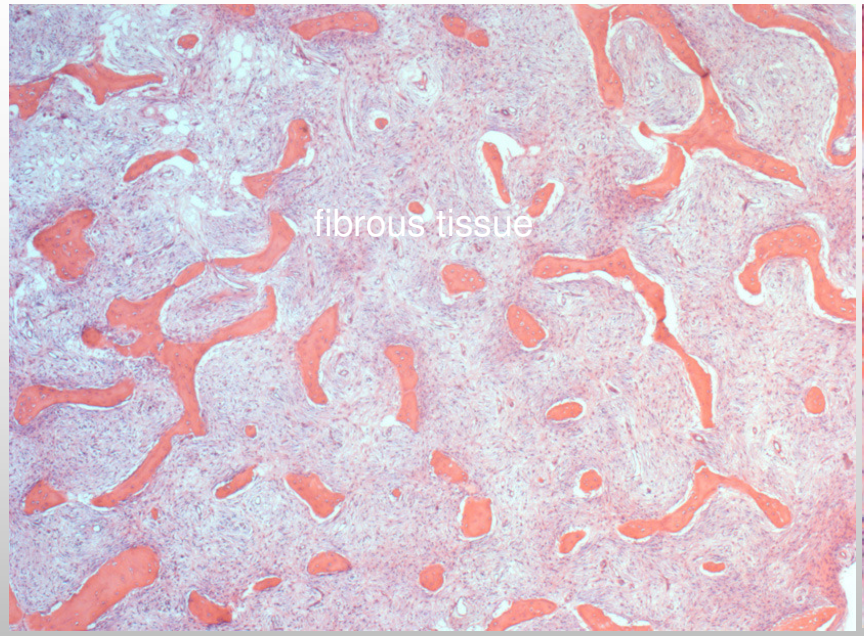
craniofacial FD

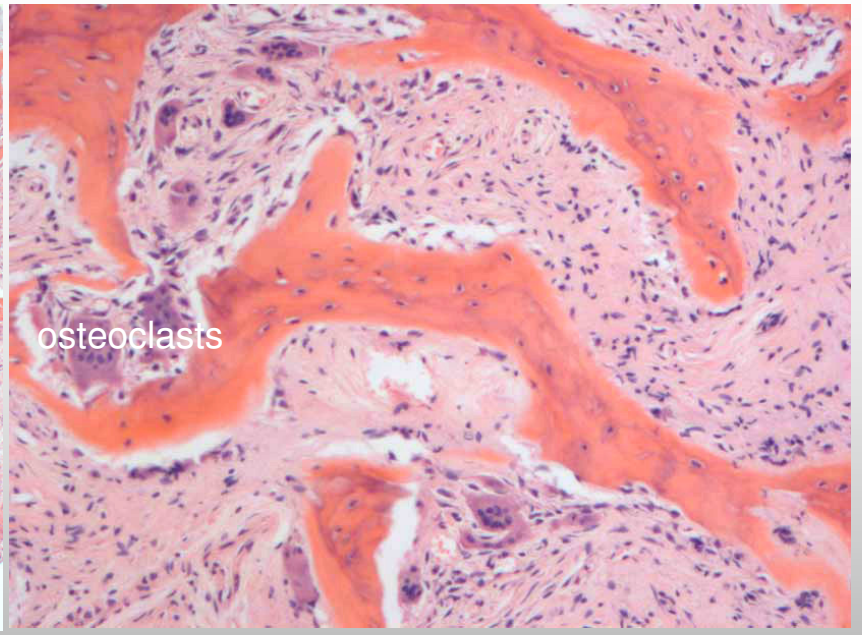
bleeding and compression

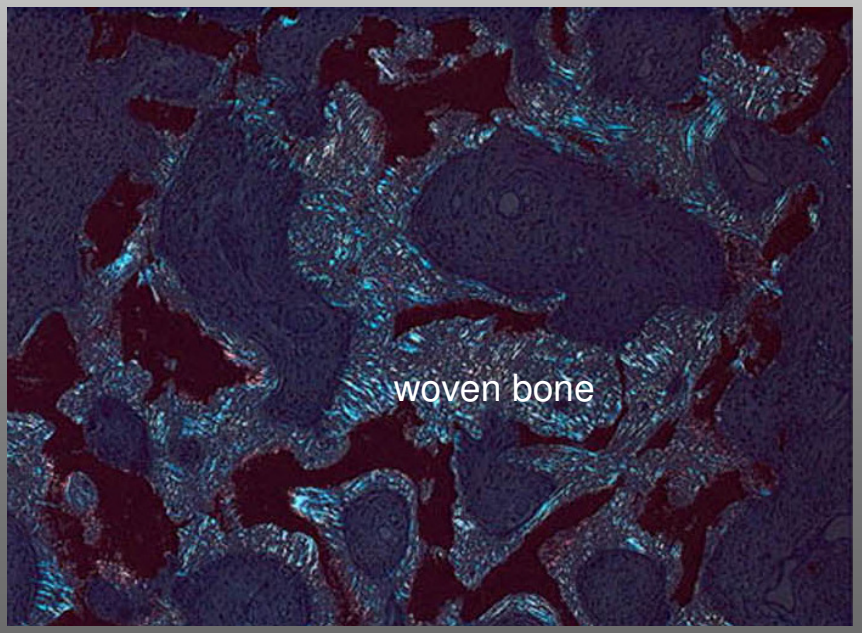
appendicular FD

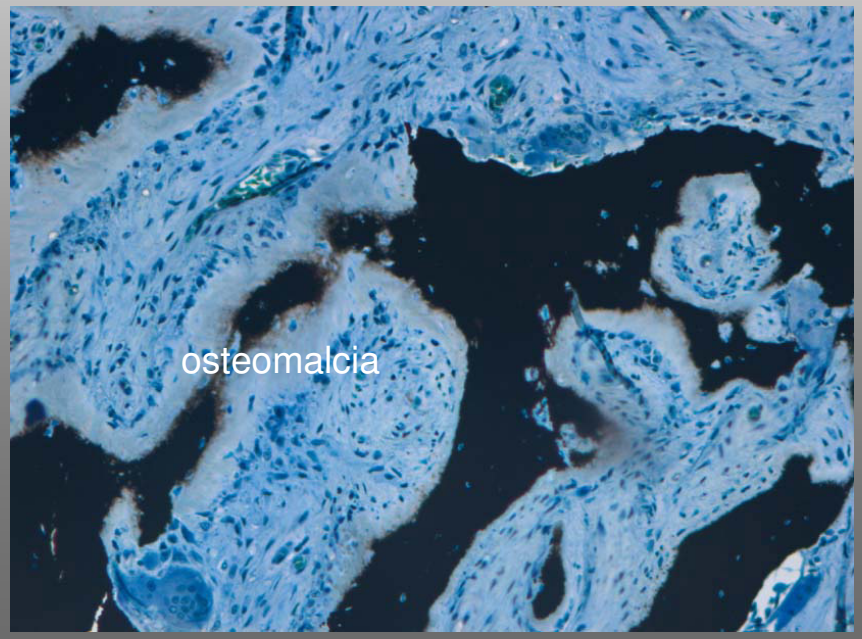
fractures and deformities

Abnormal bone/bone marrow organ in FD

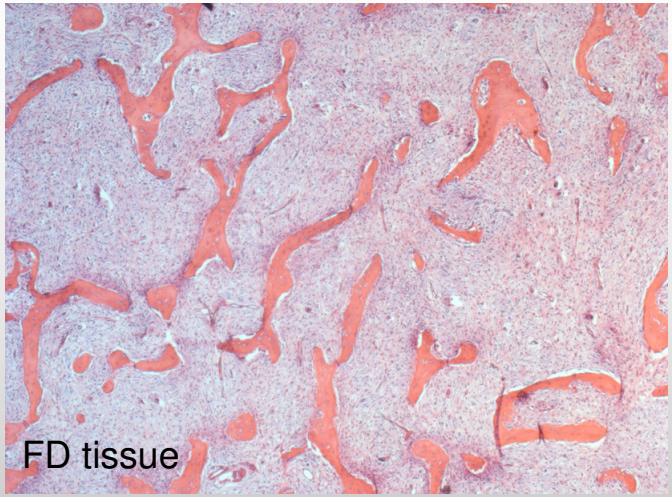


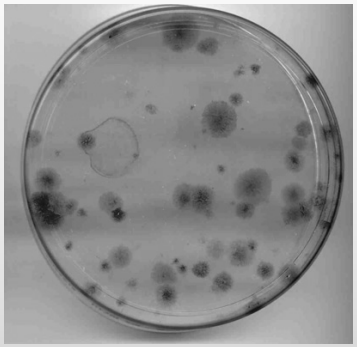


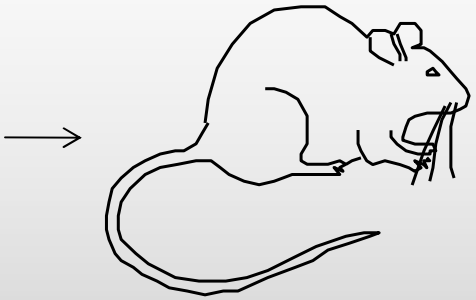


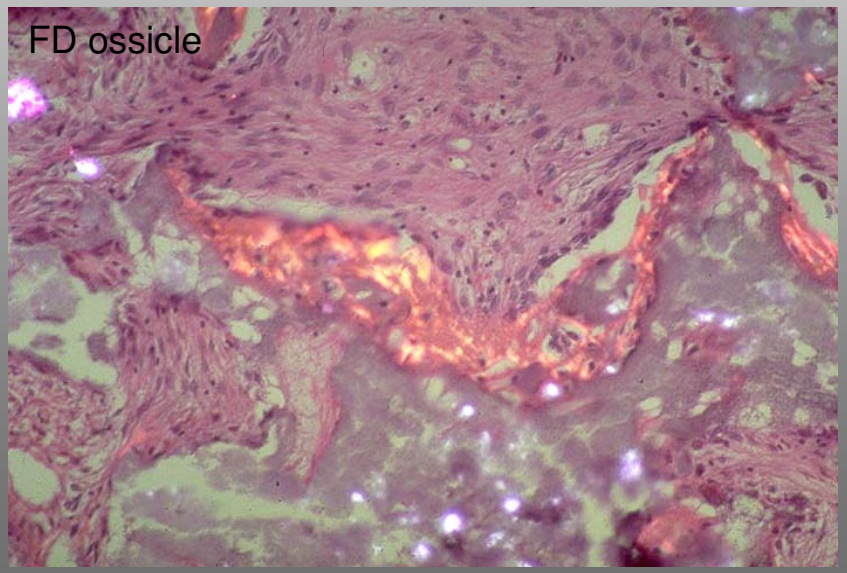


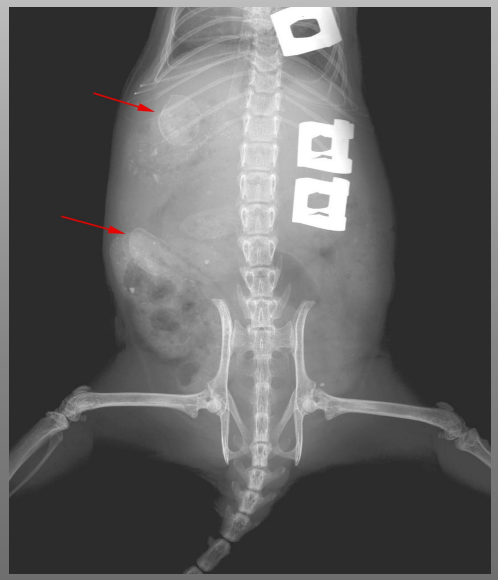
FD is a disease of post-natal skeletal stem cells



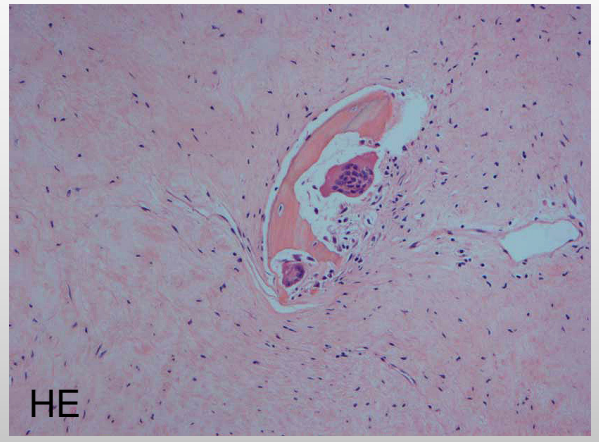


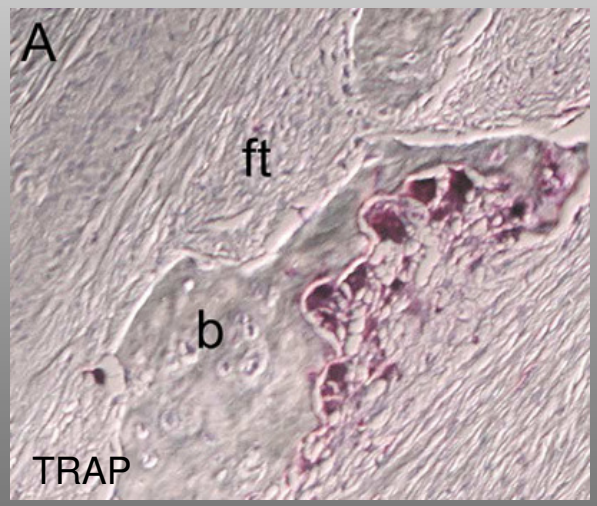




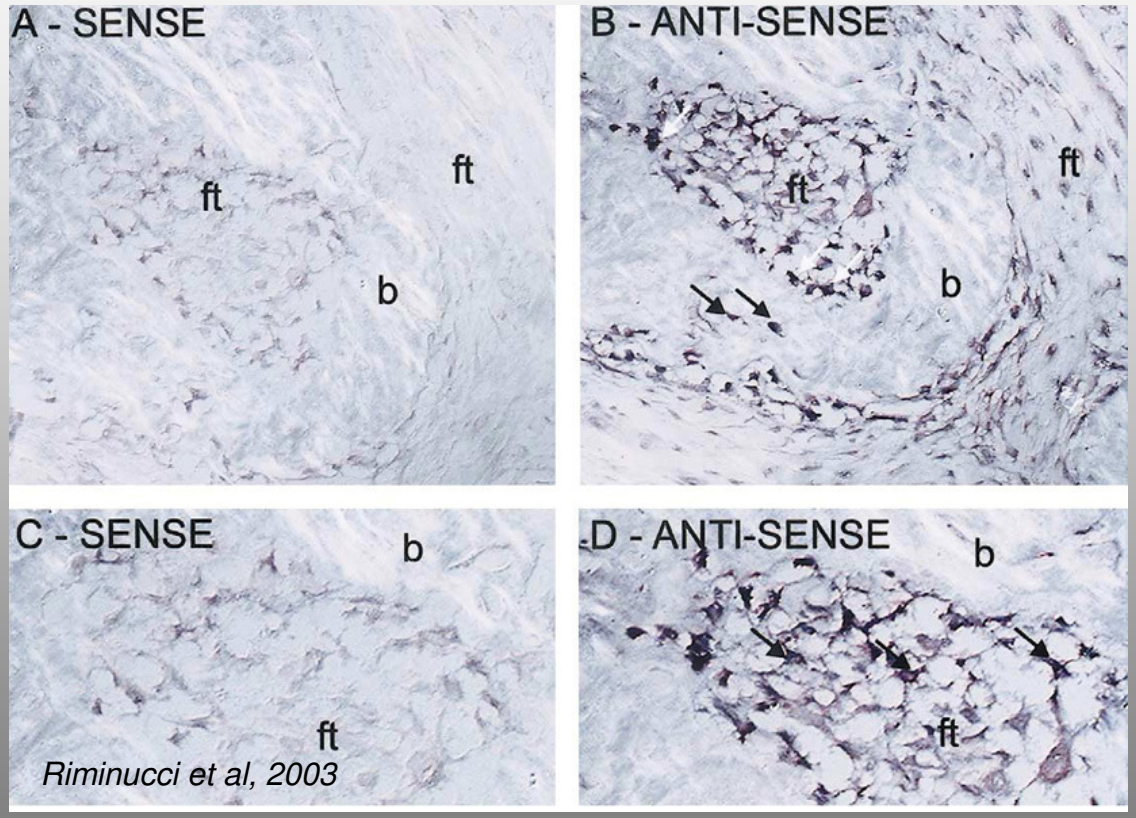


Bone resorption in FD



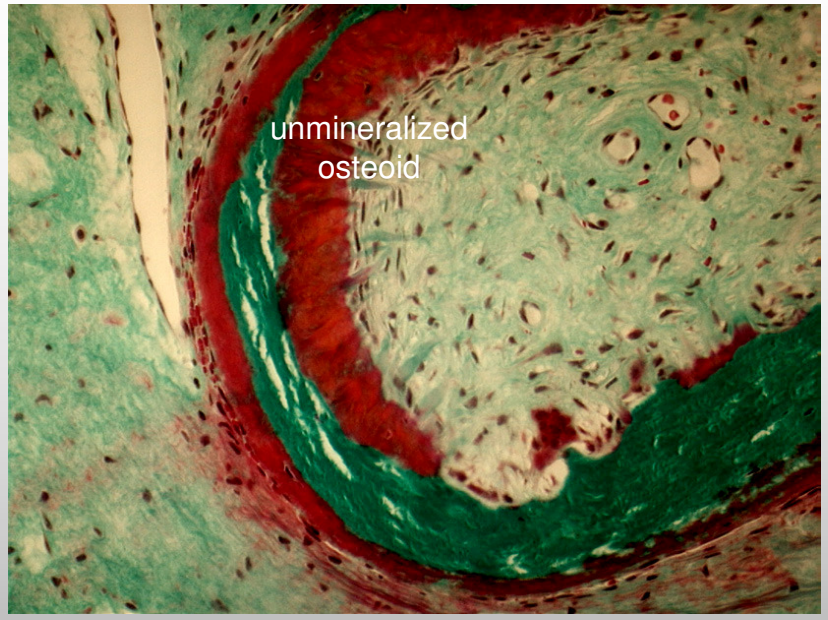


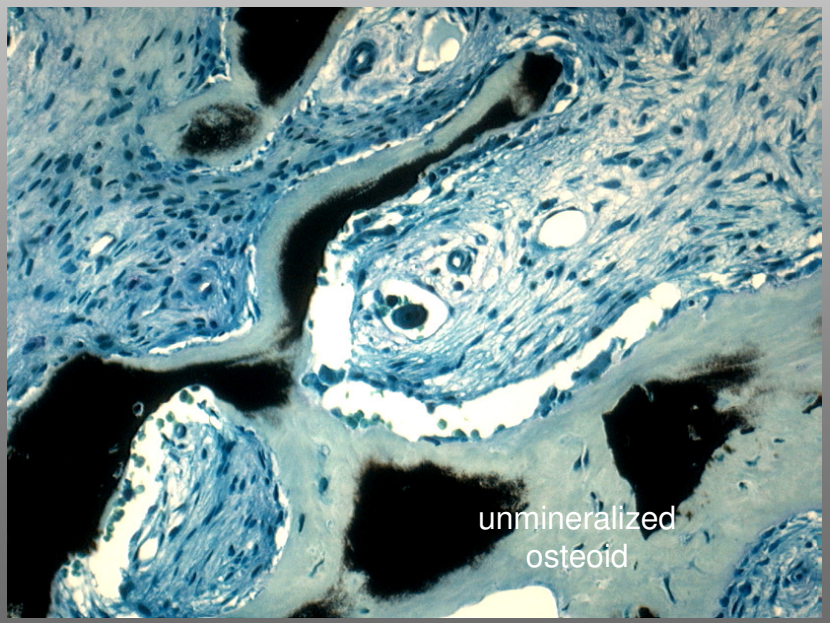
IL6 ISH

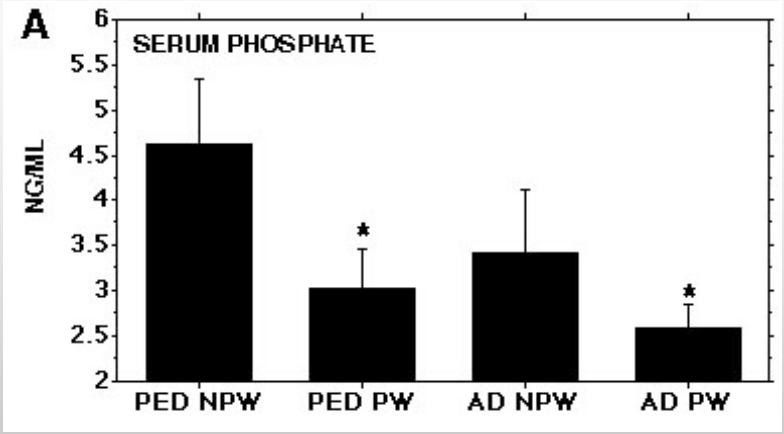


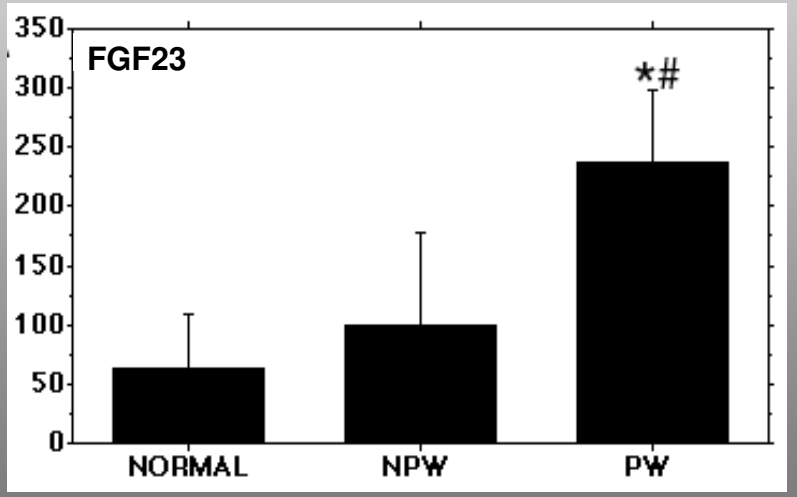
Riminucci et al, 2003

Osteomalacia and FGF23 in FD





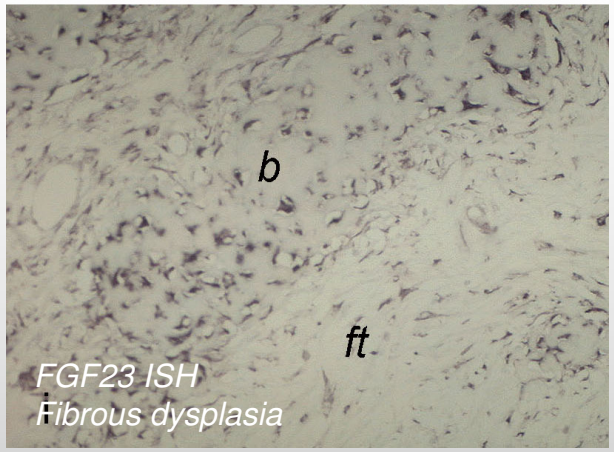


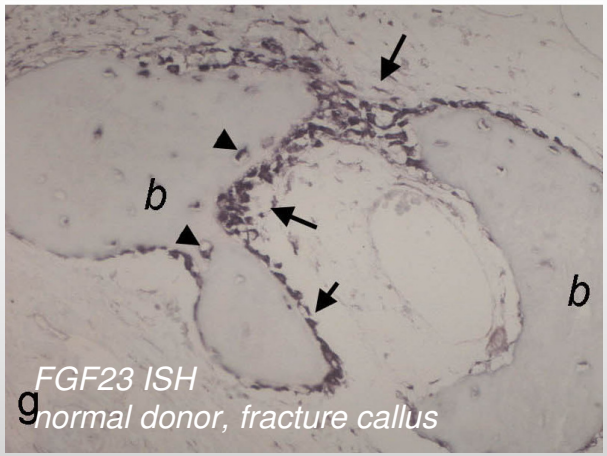


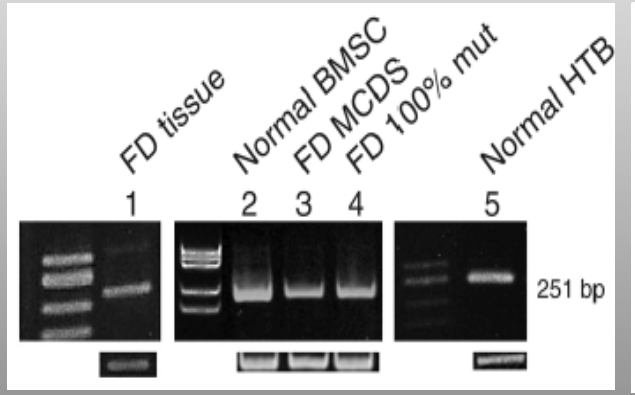
PED pediatric patients; AD adult patients; PW phosphate wasters
NPW non-phosphate wasters

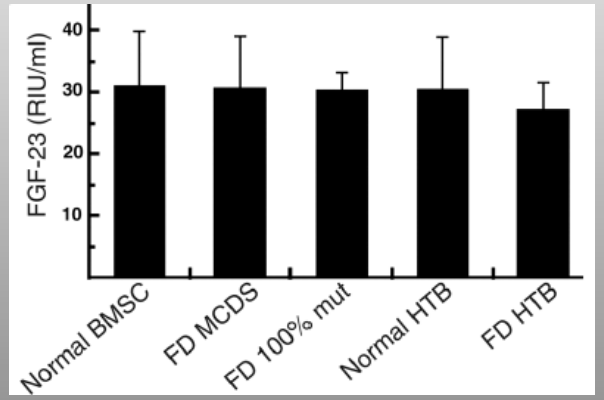
Riminucci et al, 2007

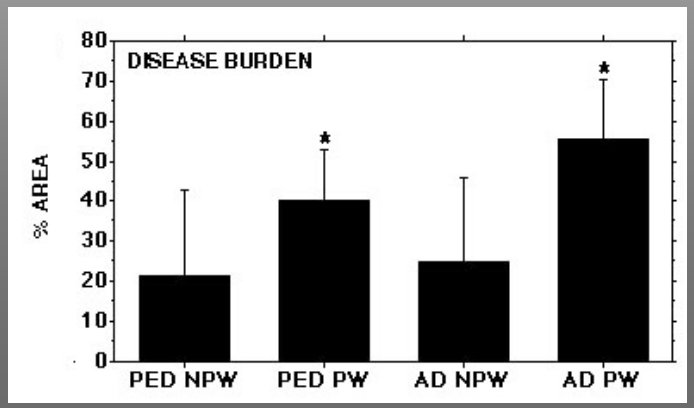
Osteomalacia and FGF23 in FD











FD and the endocrine function of bone

J. Clin. Invest. **112**:683–692 (2003). doi:10.1172/JCI200318399.

FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting

See the related Commentary beginning on page 642.

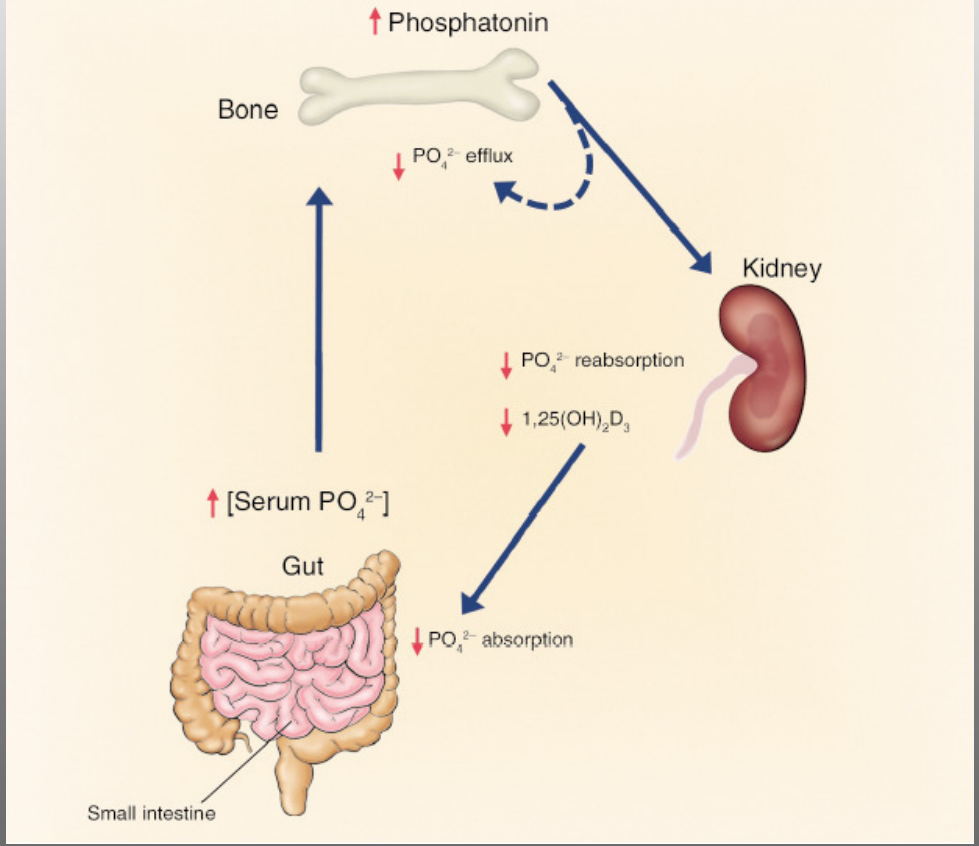
Mara Riminucci,[1,2] Michael T. Collins,[3] Neal S. Fedarko,[4] Natasha Cherman,[3] Alessandro Corsi,[1,5] Kenneth E. White,[6] Steven Waguespack,[7] Anurag Gupta,[3] Tamara Hannon,[8] Michael J. Econs,[6,9] Paolo Bianco,[2,3,5] and Pamela Gehron Robey[3]

COMMENTARIES

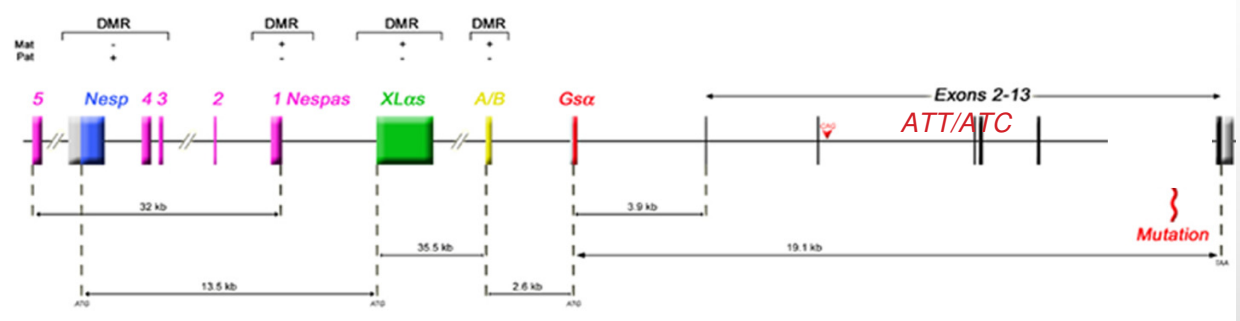
Evidence for a bone-kidney axis regulating phosphate homeostasis

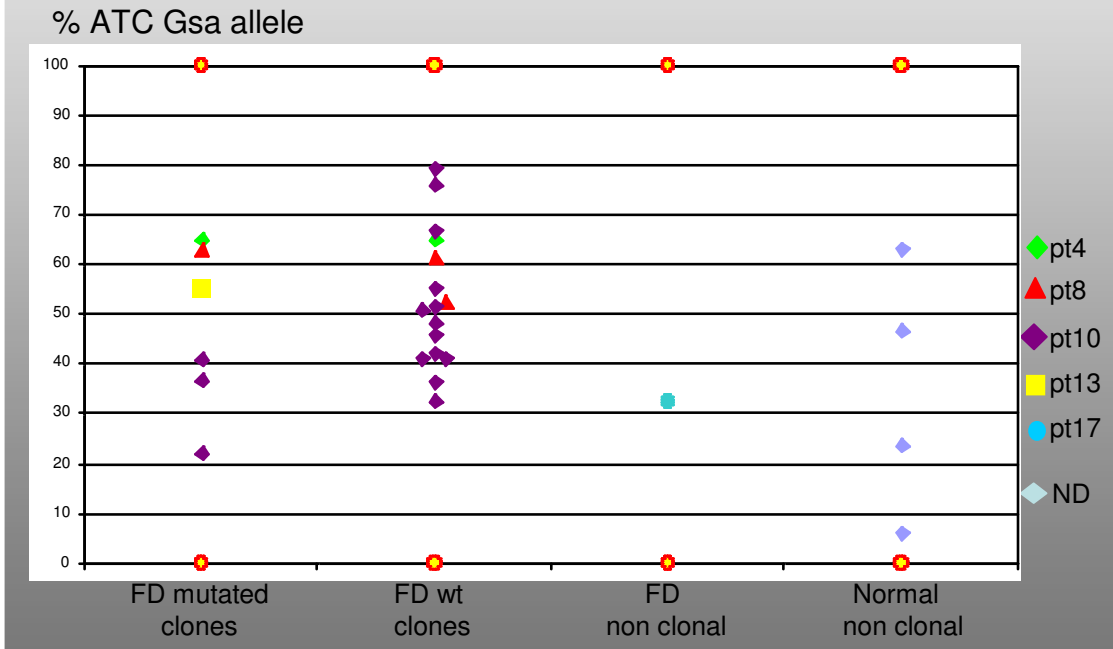
L. Darryl Quarles

Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA



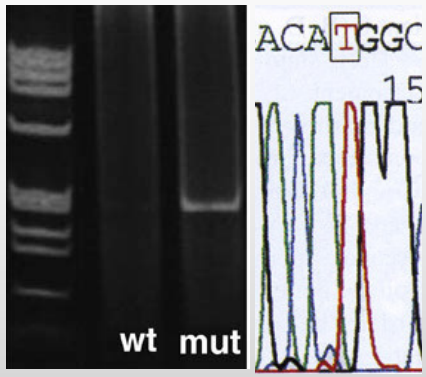
Asymmetric expression of Gsα in human skeletal progenitors



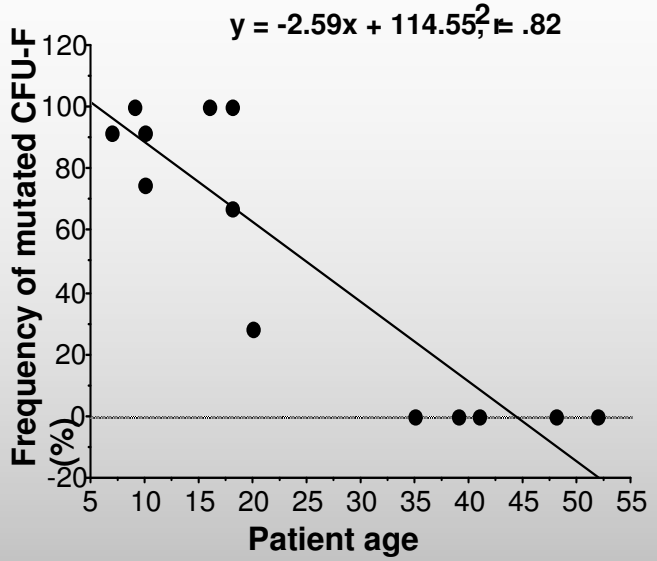


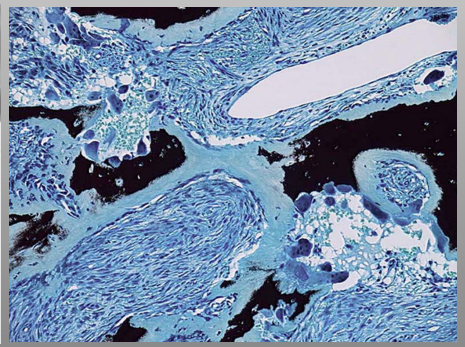
Michienzi et al, 2007

Intralesional mosaicism and the "normalization" of FD lesions

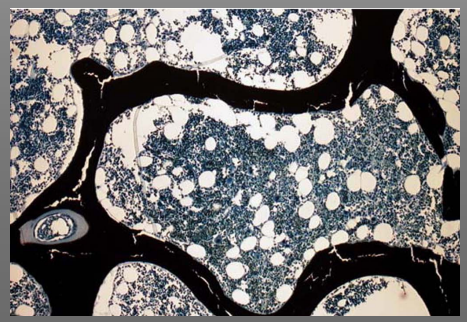


PNA clamping/PCR



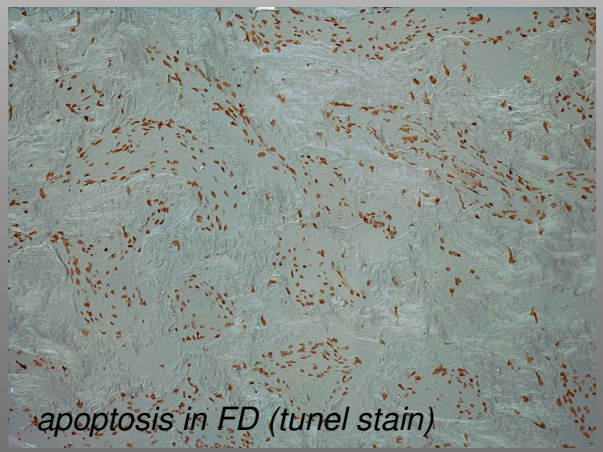


Pt A, 16 yr
84% mutated CFU-F



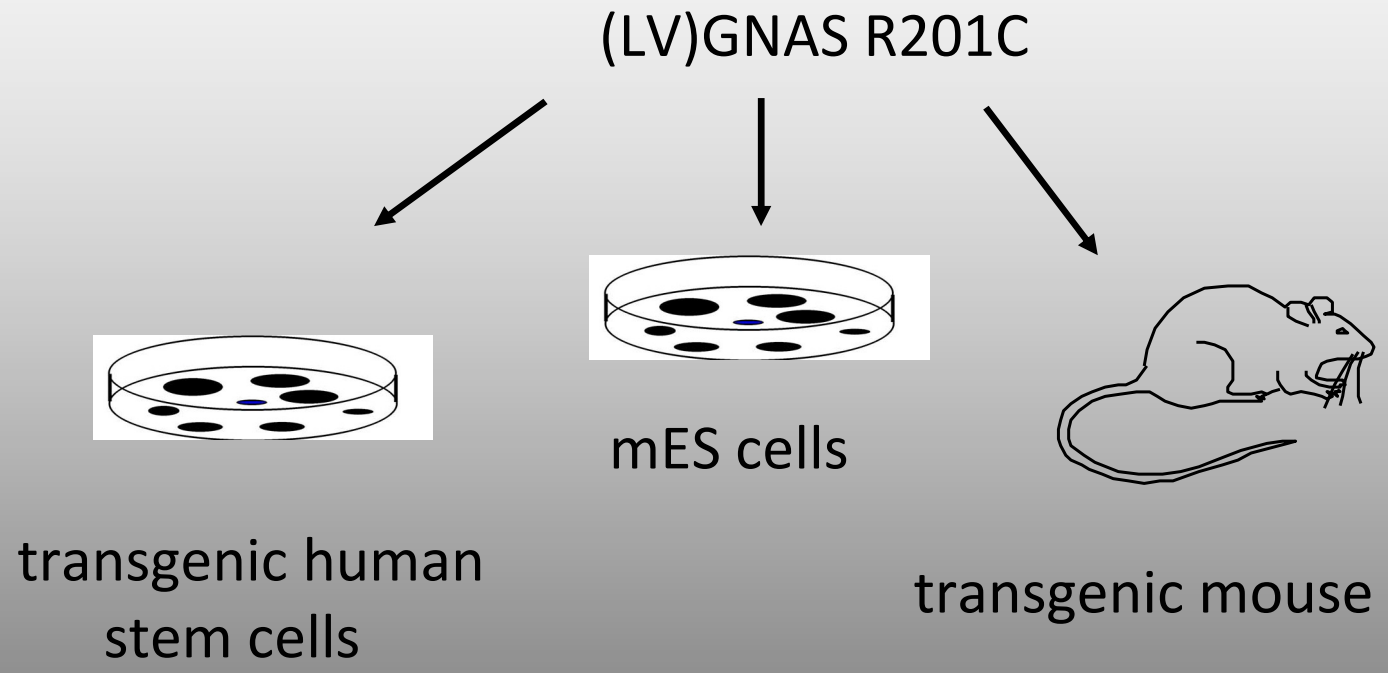
Pt B, 48 yr
0 mutated CFU-F

Kuznetsov et al, 2008



apoptosis in FD (tunel stain)

Modeling Fibrous dysplasia



Cellular models of FD: what can we learn, what can we do

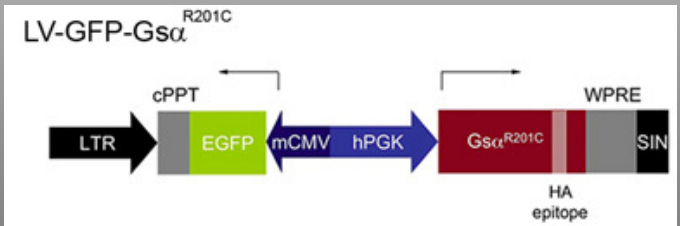
1. Molecular pathology of FD

   Adaptive mechanism countering the mutation

   Pathogenetic mechanism downstream the mutation

2. Proof-of-principle for gene therapy

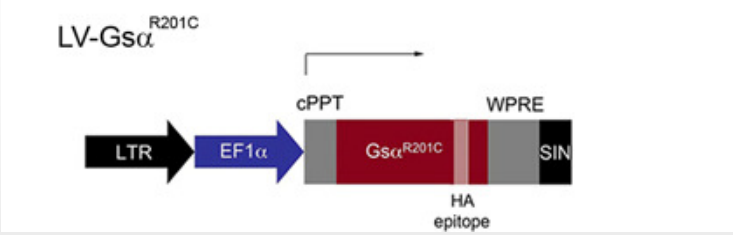
Lentiviral vector+*Gsα*^{R201C}

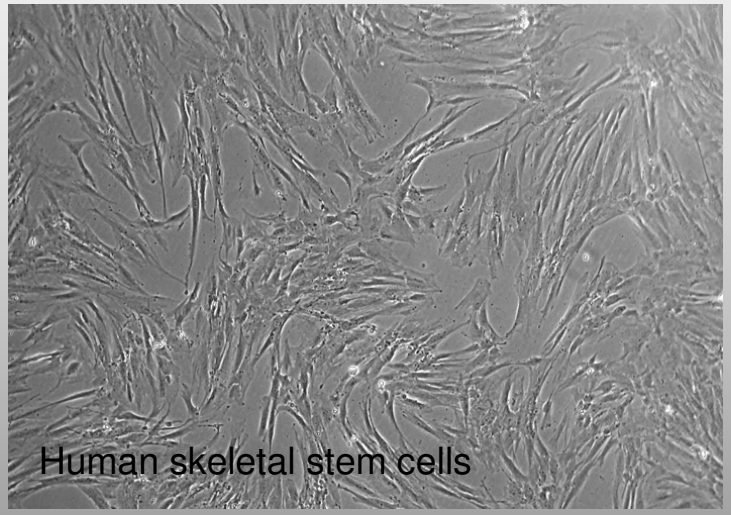


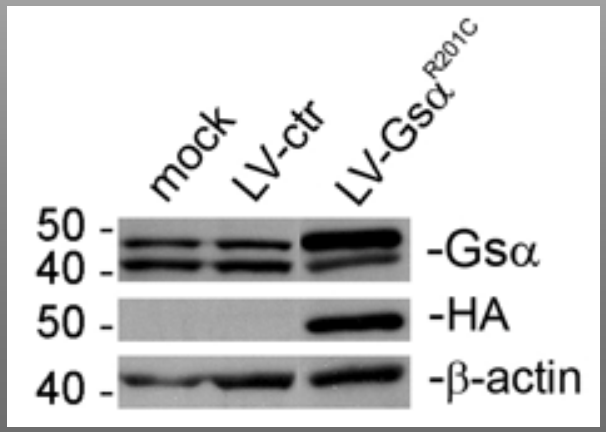
human skeletal stem cells (normal donors)

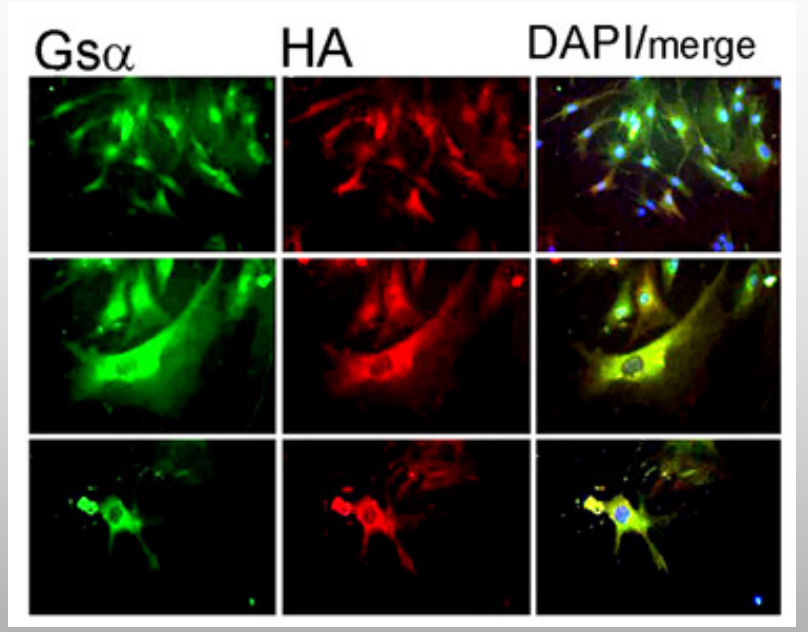
mouse pluripotent stem cells

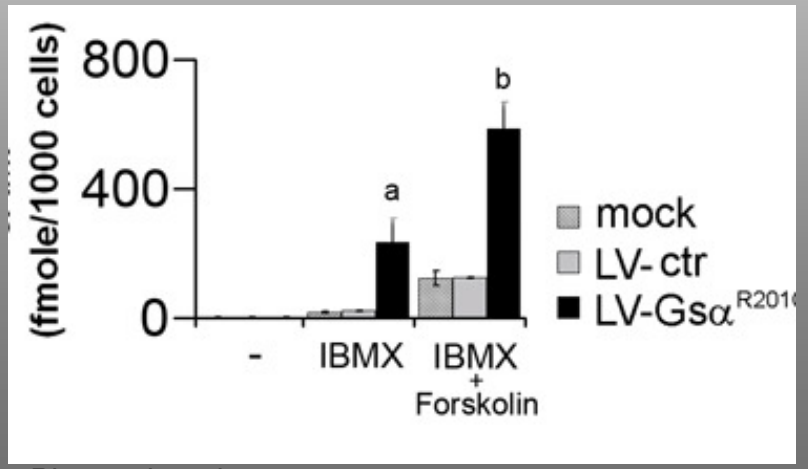
Transfer of the FD cellular phenotype in hSSC





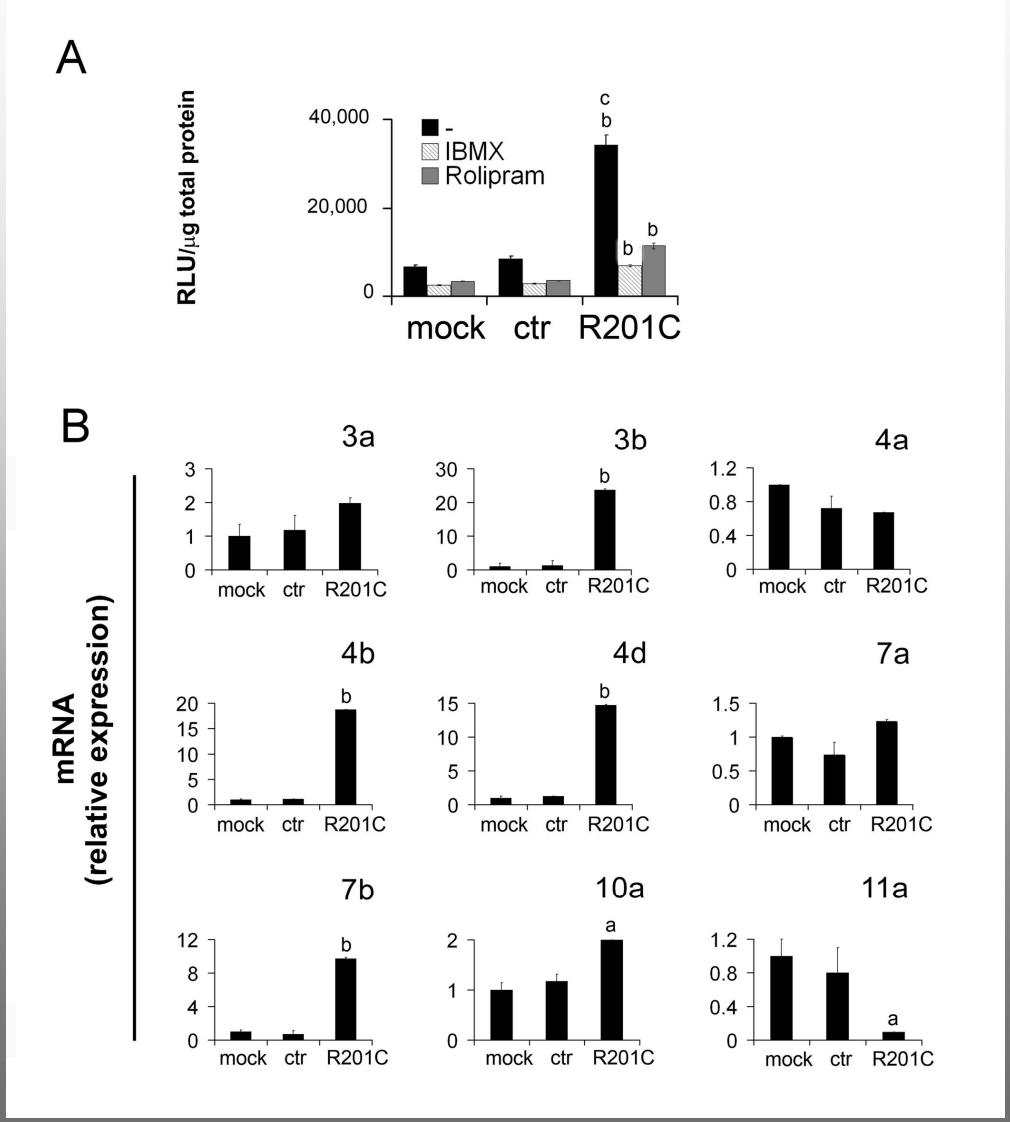




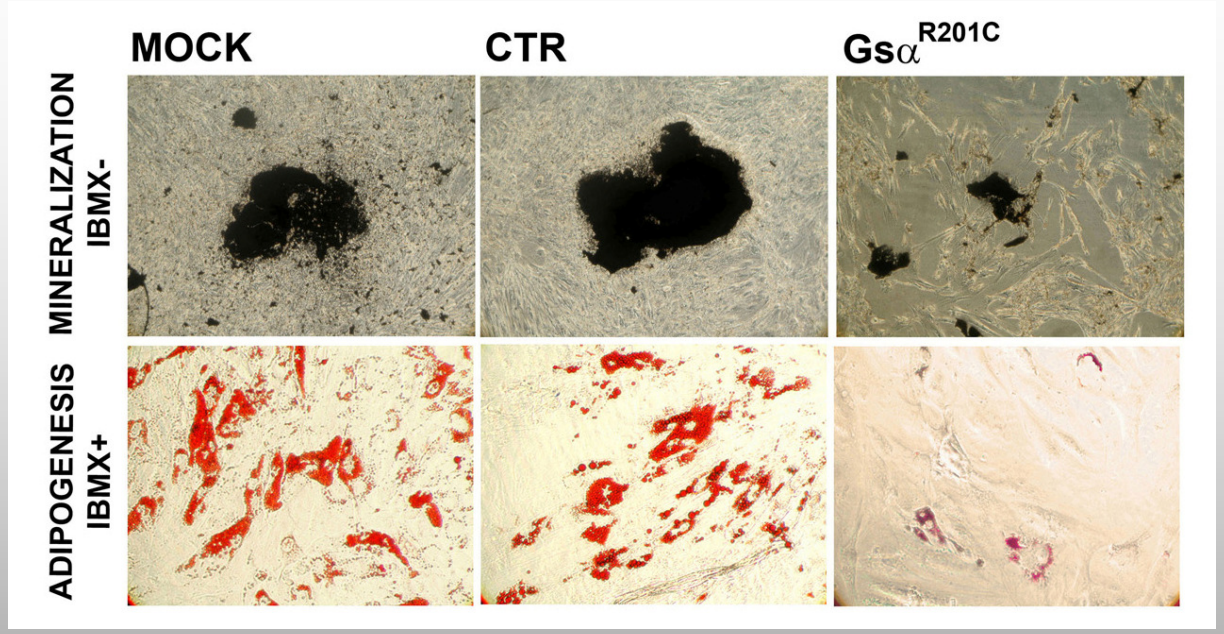


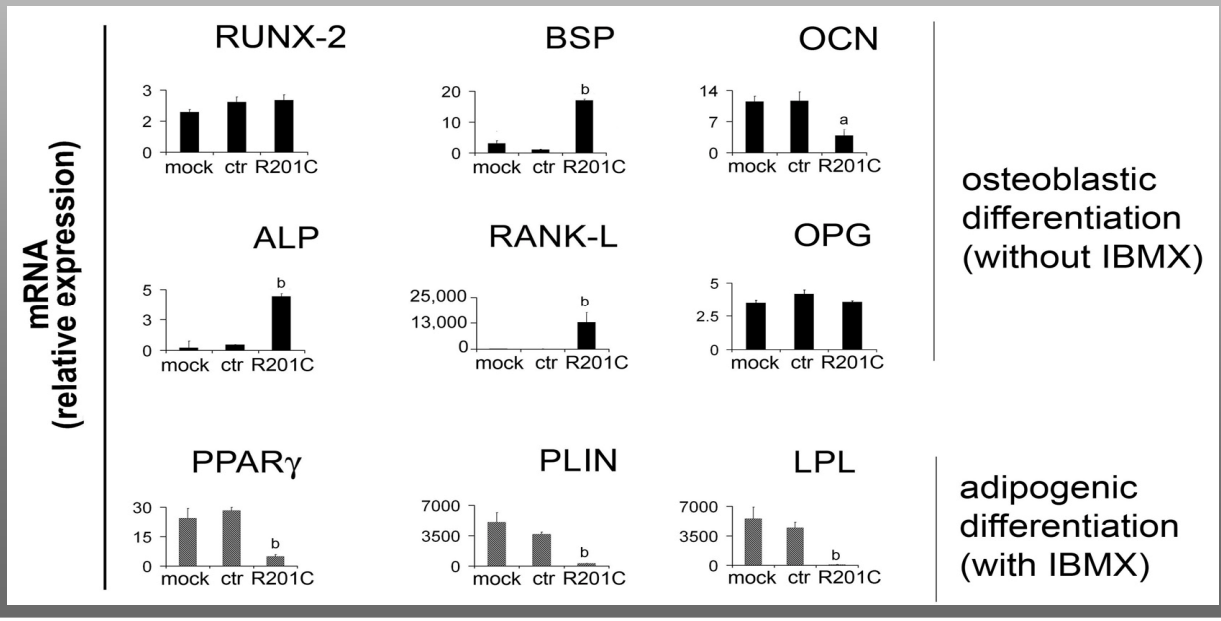
Piersanti et al, 2010

PDE-based adaptive response in LV- Gsα^{R201C} hSSC

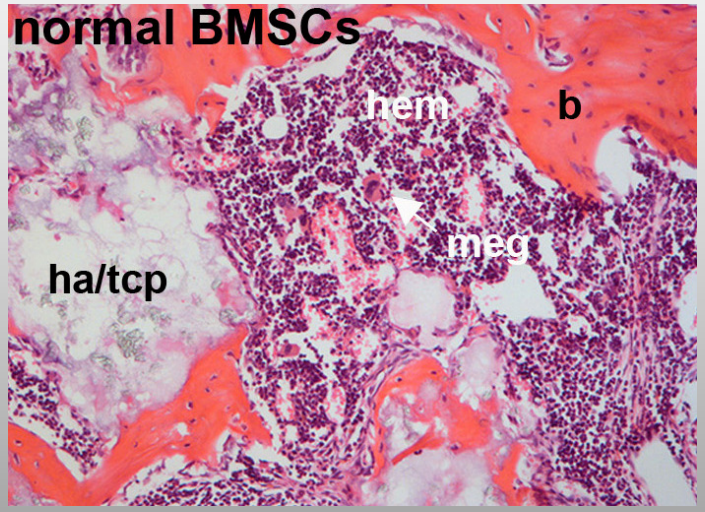


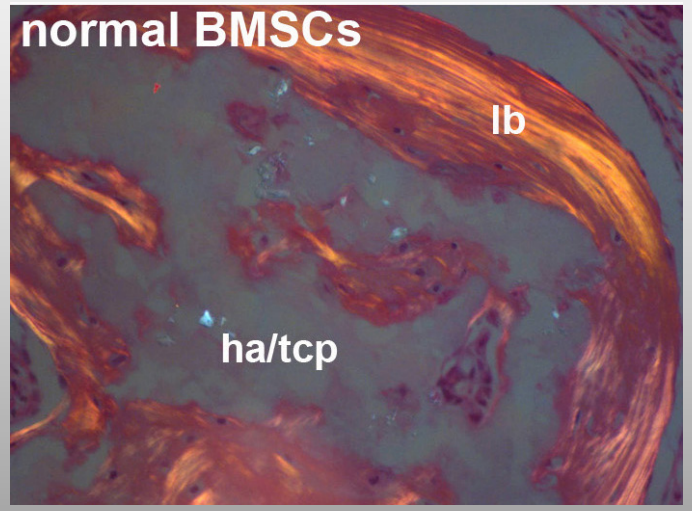
Abnormal skeletal differentiation of LV- Gsα^{R201C} hSSC

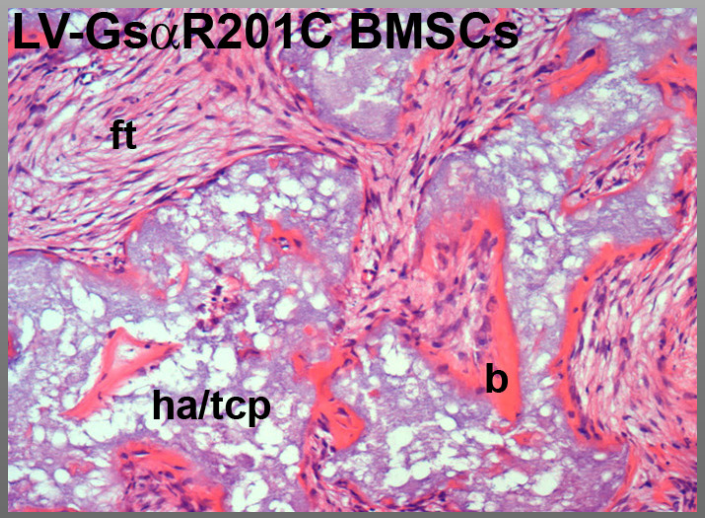


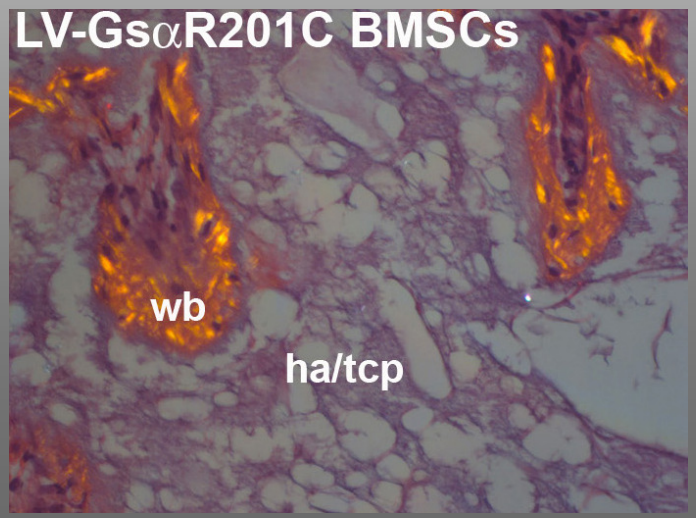


LV- Gsα^{R201C} hSSC reproduce FD ossicles

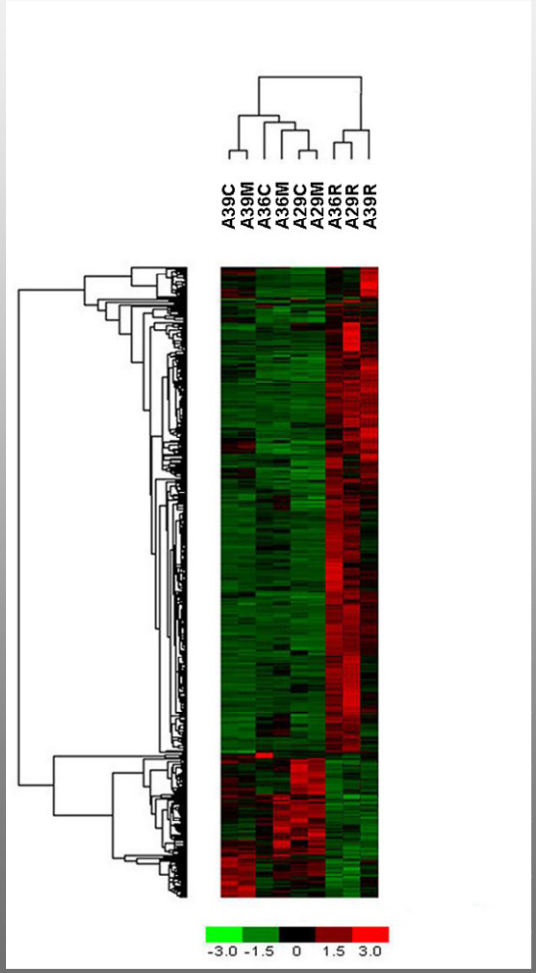


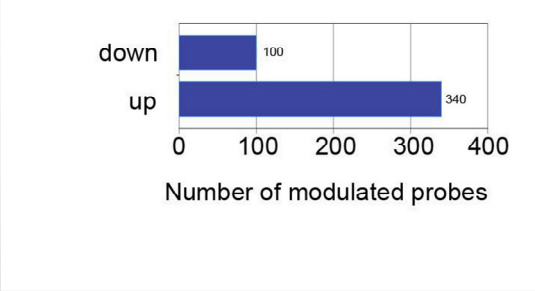


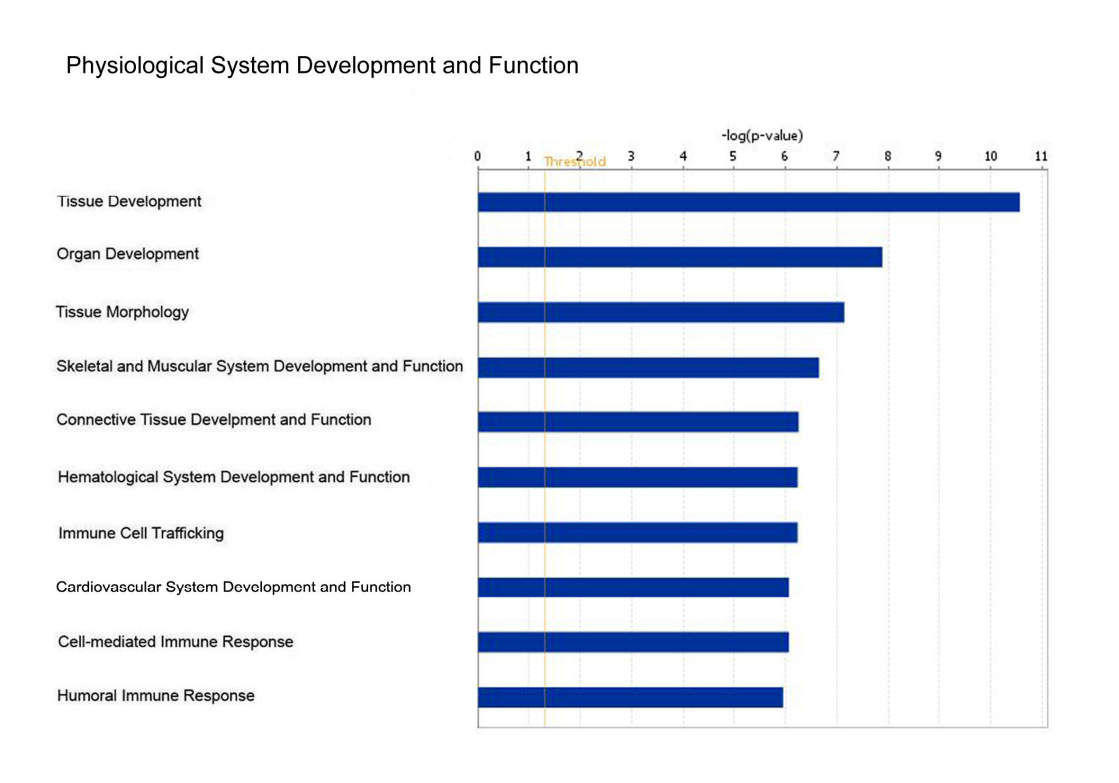




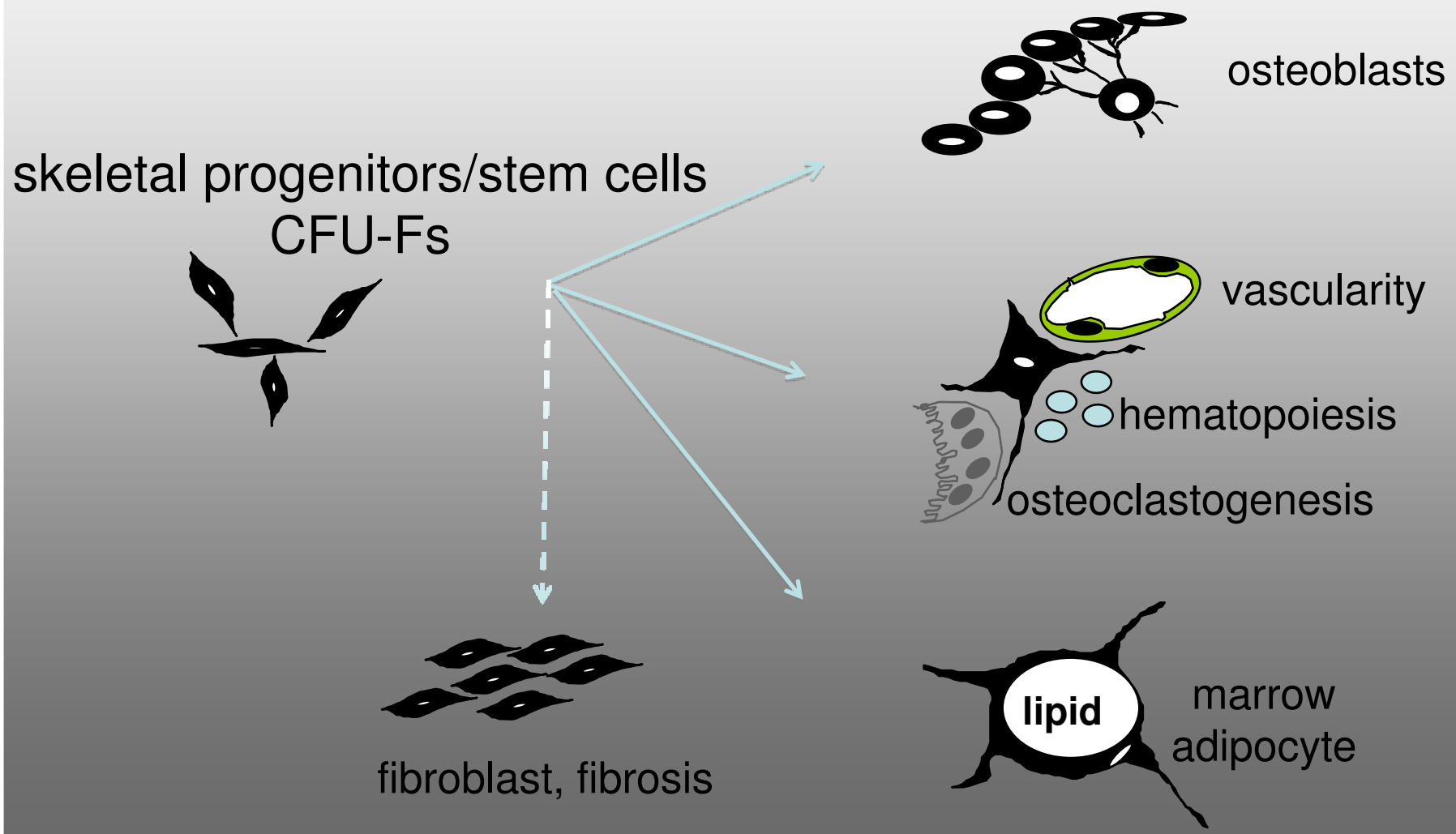
High throughput analysis of gene modulated in LV- Gsα^{R201C} hSSC







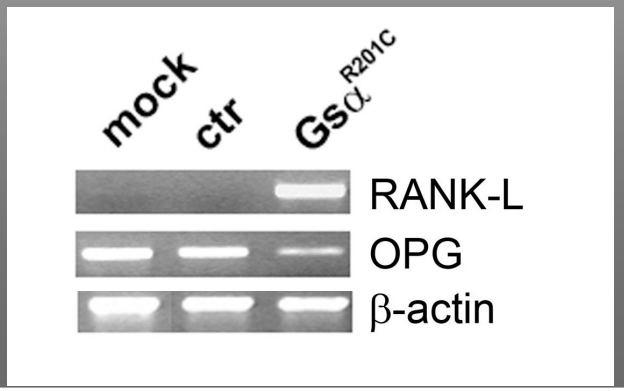
Skeletal cells and the abnormal bone/bone marrow organ in FD

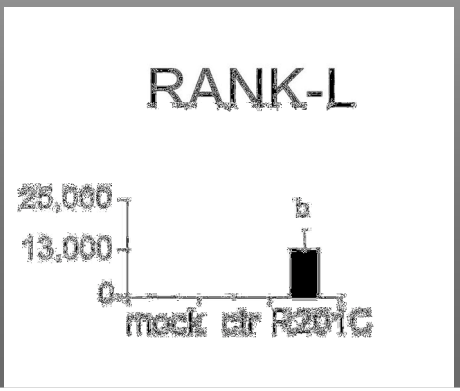


Skeletal cells and the abnormal bone/bone marrow organ in FD

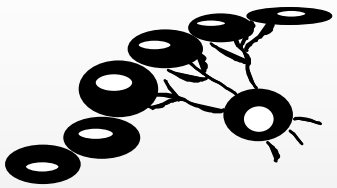
osteoclastogenesis

Chemotaxis of osteoclast precursors	CXCL1 CXCL2
Proliferation of osteoclast precursors	CXCL2
Differentiation of osteoclast precursors	**RANK-L** OPG
Regulation of RANK-L	CXCL13
TNFAIP6	
Stimulation of bone resorption	PLA2G4A





Skeletal cells and the abnormal bone/bone marrow organ in FD



osteogenesis

Osteoblast differentiation	(BMP/TGFb)	BMP2 TGFB3 TNFAIP6 ID2 BAMBI
BMP6		
Osteoblast differentiation	(Wnt)	WNT4 **WNT5a** **DKK1** SFRP1 SFRP2 SFRP4
FZD1		
		CLU
Transcriptional regulation		BHLHE40 SOX4 TBX3 HDAC4
Bone growth	(IGF-1)	IGF-1 IGFBP5
Bone formation	(prostaglandins)	PLA2G4A PTGS1
		PHC2 TGFBI **SLC1A3**
Matrix assembly/remodeling		MMP2 MMP13 ADAM12 POSTN
Matrix mineralization		MGP
Mineral metabolism		STC1

Matrix gla protein (MGP) and mineralization in FD

FGF23

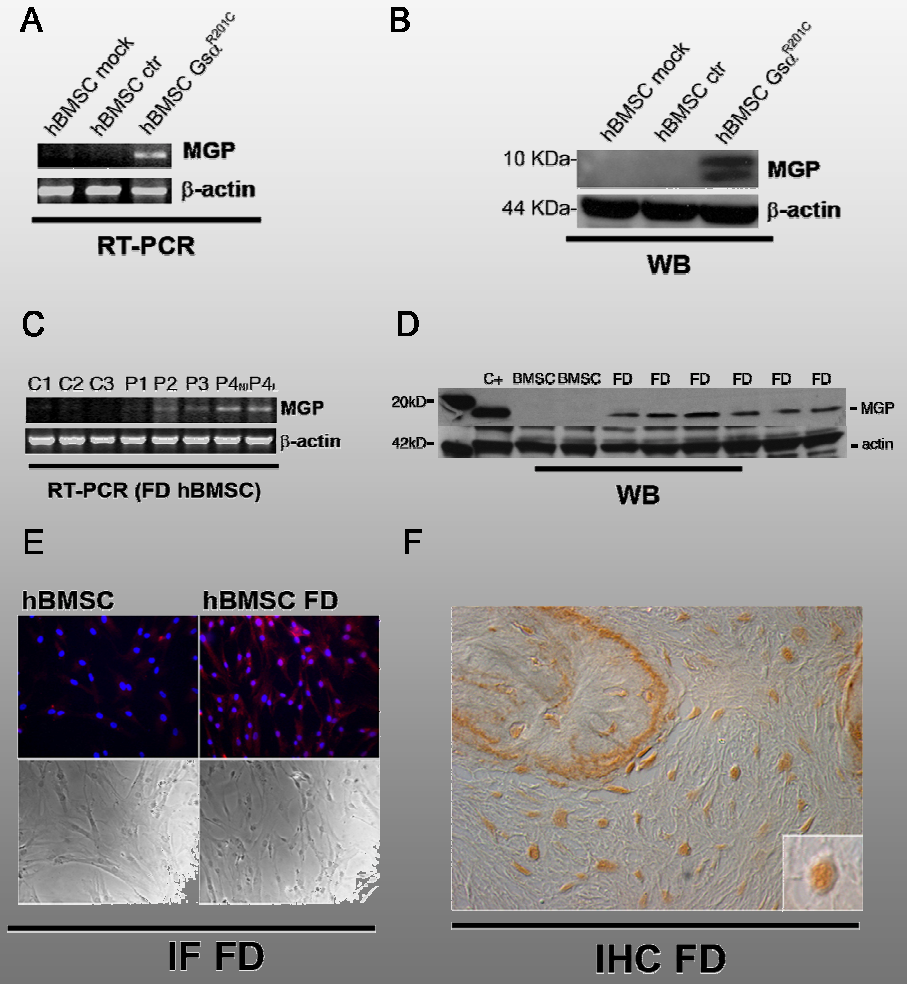
⊥ humoral, indirect

mineralization

MGP

⊥ local, direct

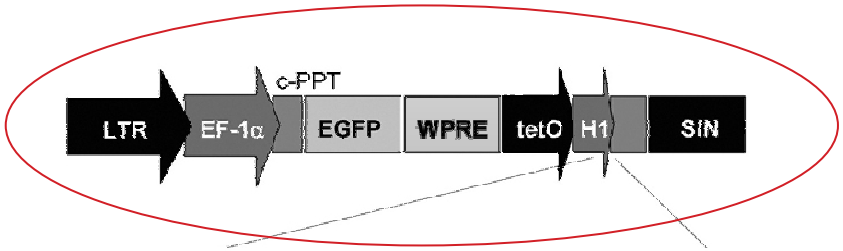
mineralization



Gene therapy in FD

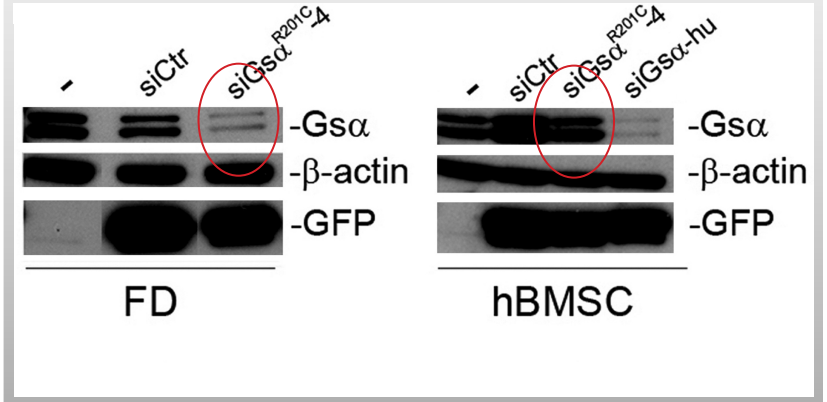
dominant, gain of function mutations
ubiquitously expressed gene → selective down-regulation of the mutated GNAS allele

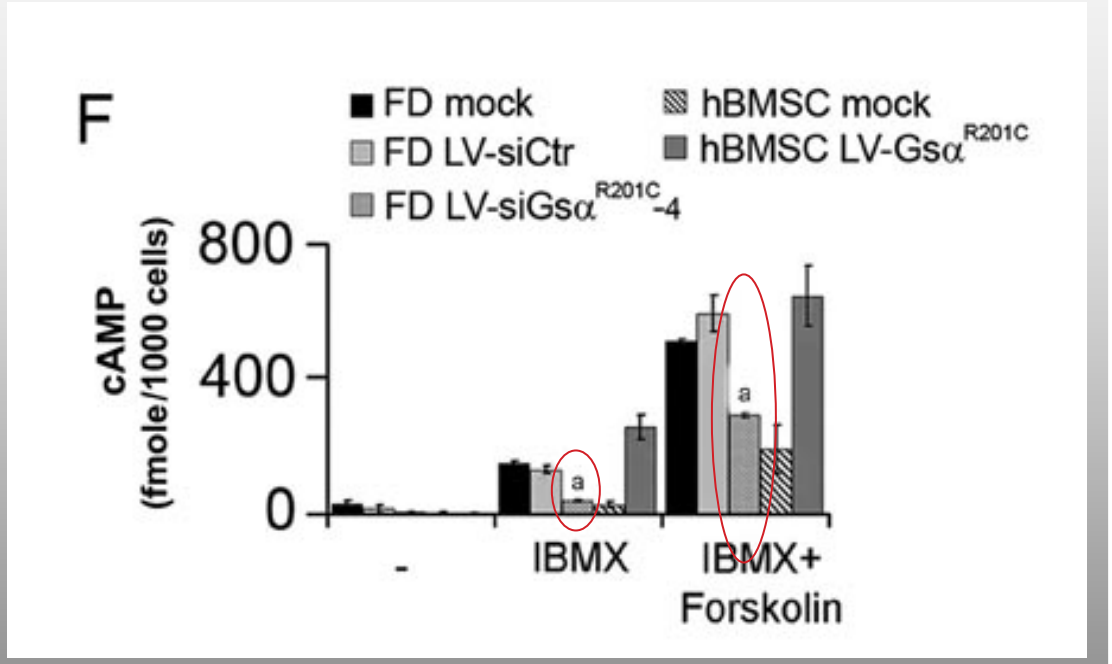
Gsα^{R201C} siRNA



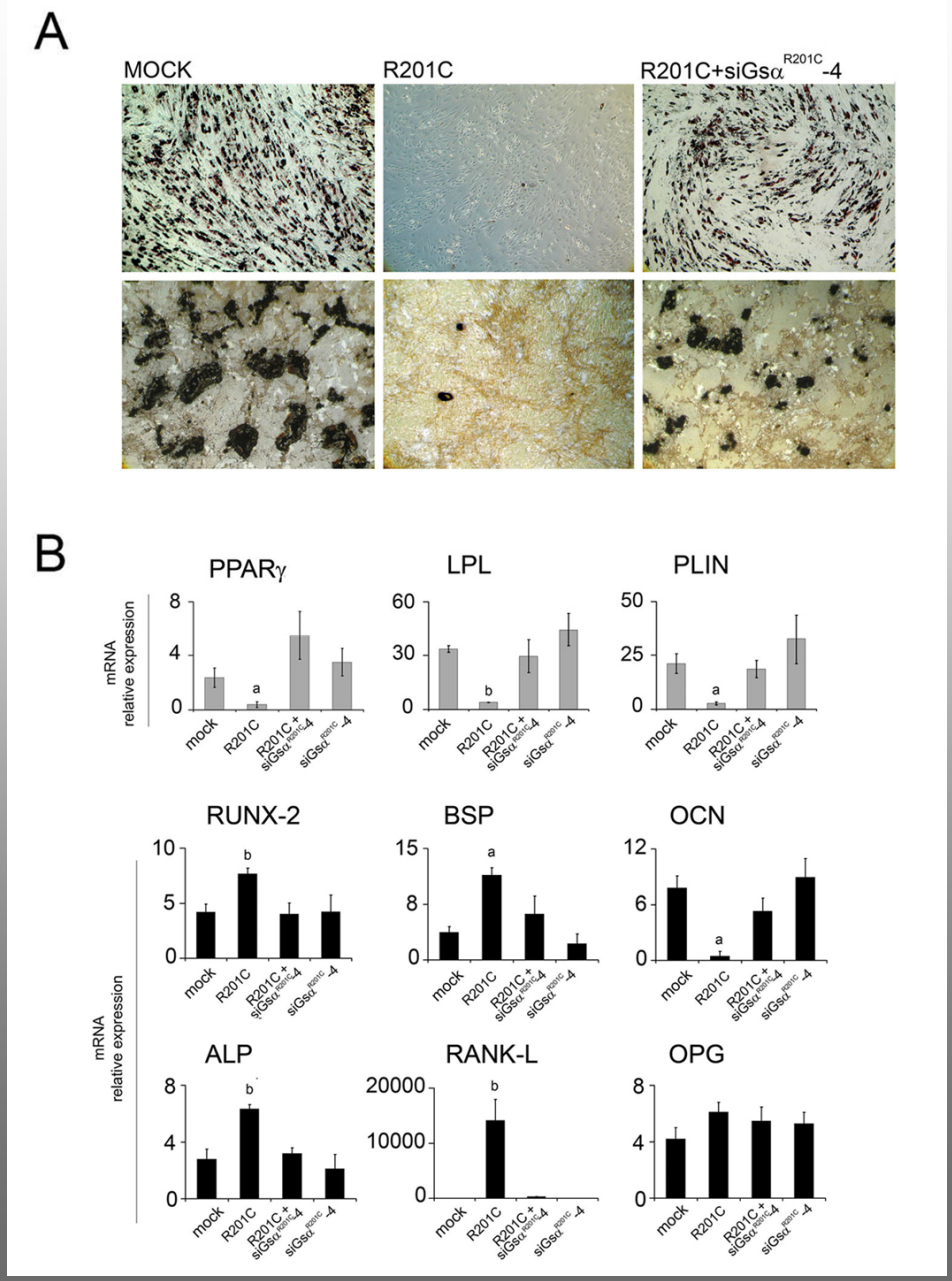
vector	nucleotides	sequence
LV-siGsα-human	407-425	ggcgcagcgtgaggccaac
LV-siGsα^{R201C}-1	839-857	ttcgctgctgtgtcctgac
LV-siGsα^{R201C}-2	838-856	cttcgctgctgtgtcctga
LV-siGsα^{R201C}-3	835-853	ctgcttcgctgctgtgtcc
LV-siGsα^{R201C}-4	845-863	gctgtgtcctgacctctgg
LV-siCtr	-	caccgacgtcgactgttgg

$Gs\alpha^{R201C}$ siRNA reverts the cellular phenotype of FD





$Gs\alpha^{R201C}$ siRNA restores skeletal differentiation of LV- $Gs\alpha^{R201C}$ hSSC



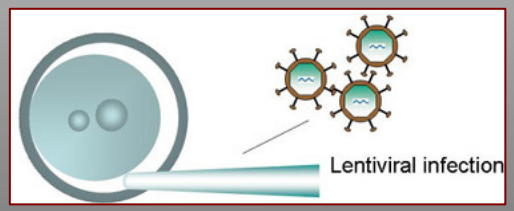
Transgenic models of FD: what can we do, what can we learn

1. Development of therapies

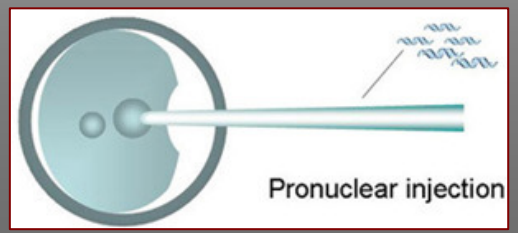
drug testing, cell therapy, gene therapy

2. Natural history and pathogenesis of FD

role of mosaicism
inheritance
post-natal development of lesions
evolution and heterogeneity of lesions



1. Mice with constitutive and ubiquitous expression of the transgene
LV-EF1α-Gsα^{R201C} mice, LV-CMVeGFP-hPGK-*Gsα^{R201C} mice*

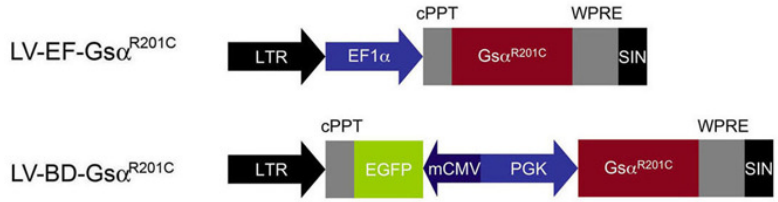


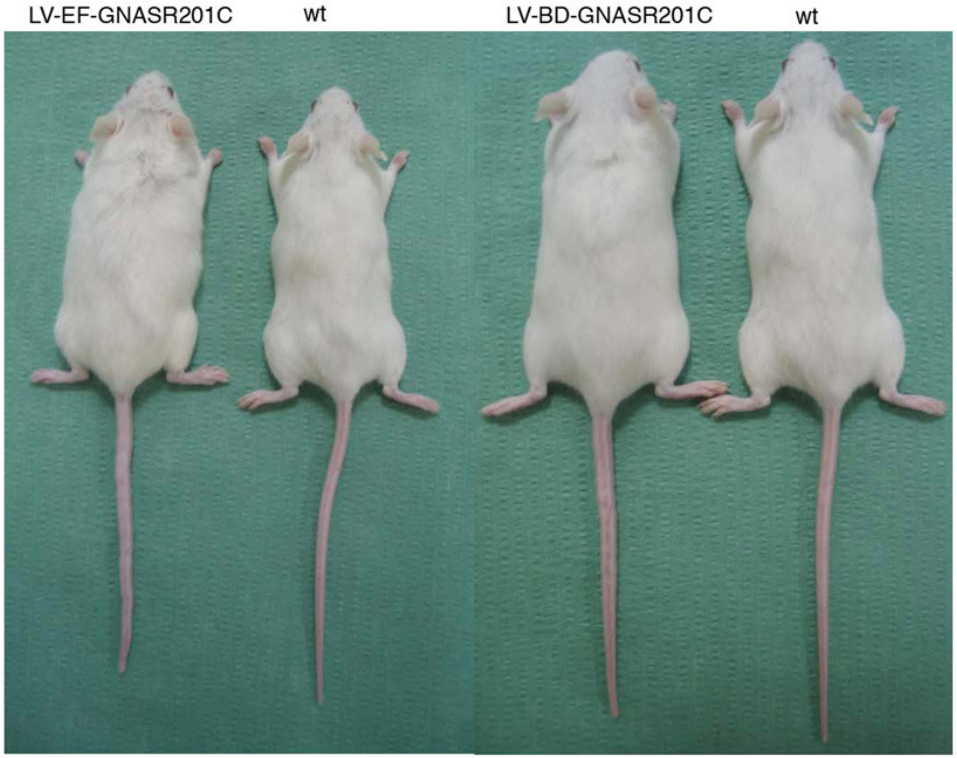
2. Expression of the transgene restricted to osteogenic cells
2.3 Col1A1Gsα^{R201C} mice

Transgenic mice with ubiquitous expression of Gsα^{R201C}

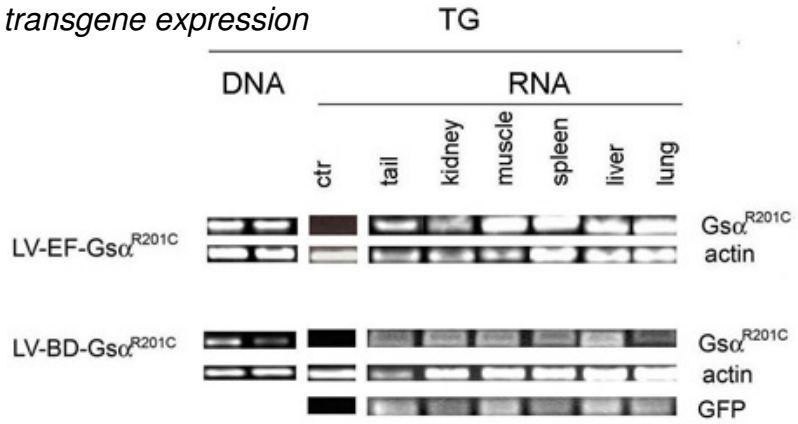
(expression of Gsα^{R201C} in all cell types, including skeletal stem cells and progenitors)

lentiviral vectors

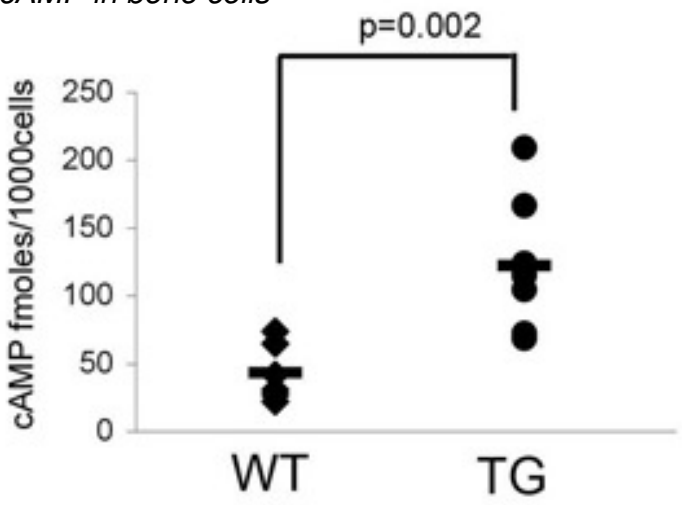




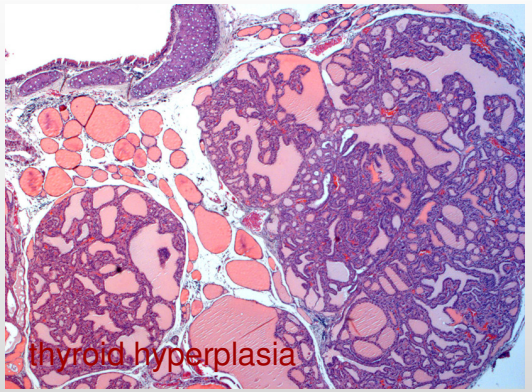
transgene expression

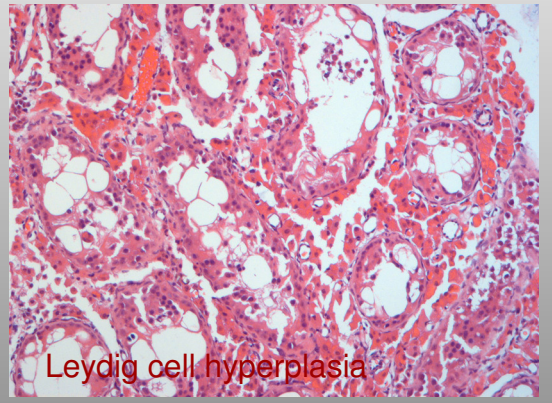


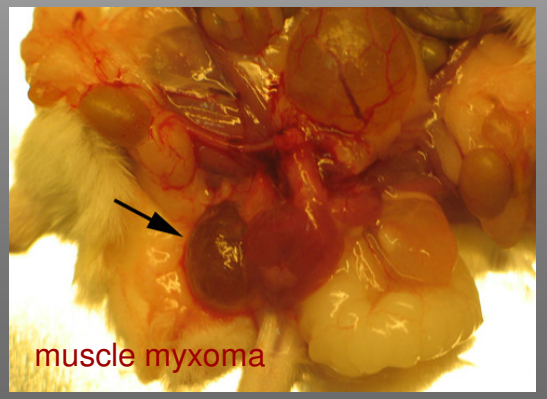
cAMP in bone cells



FD and related disorders in LV-FD mice

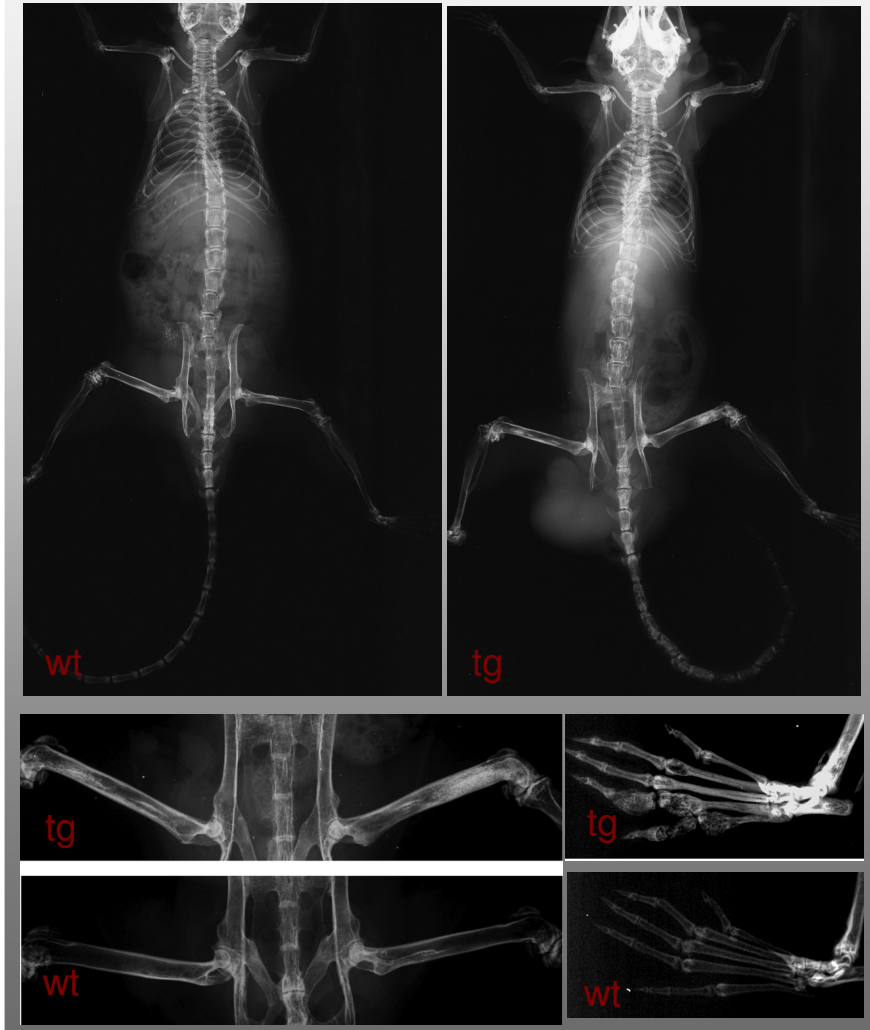






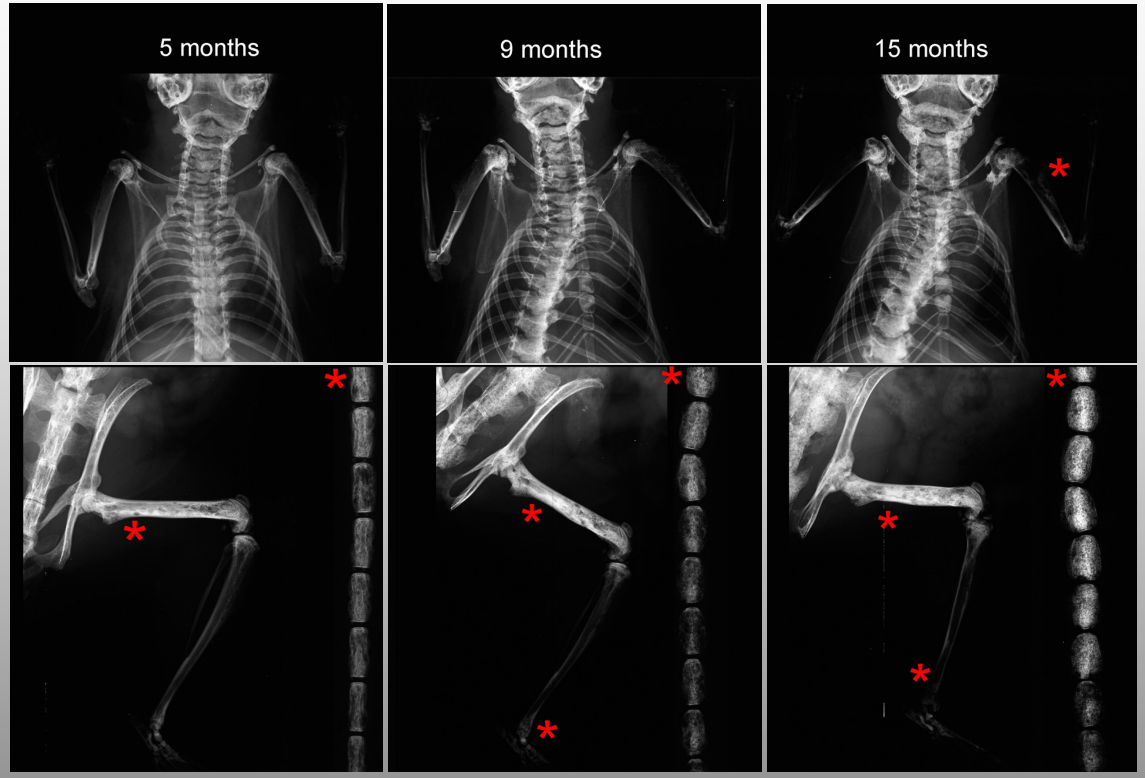
LV- FD mice reproduce the human disease

LV-CMVeGFP-hPGK-*Gsα*^{R201C} *mice*

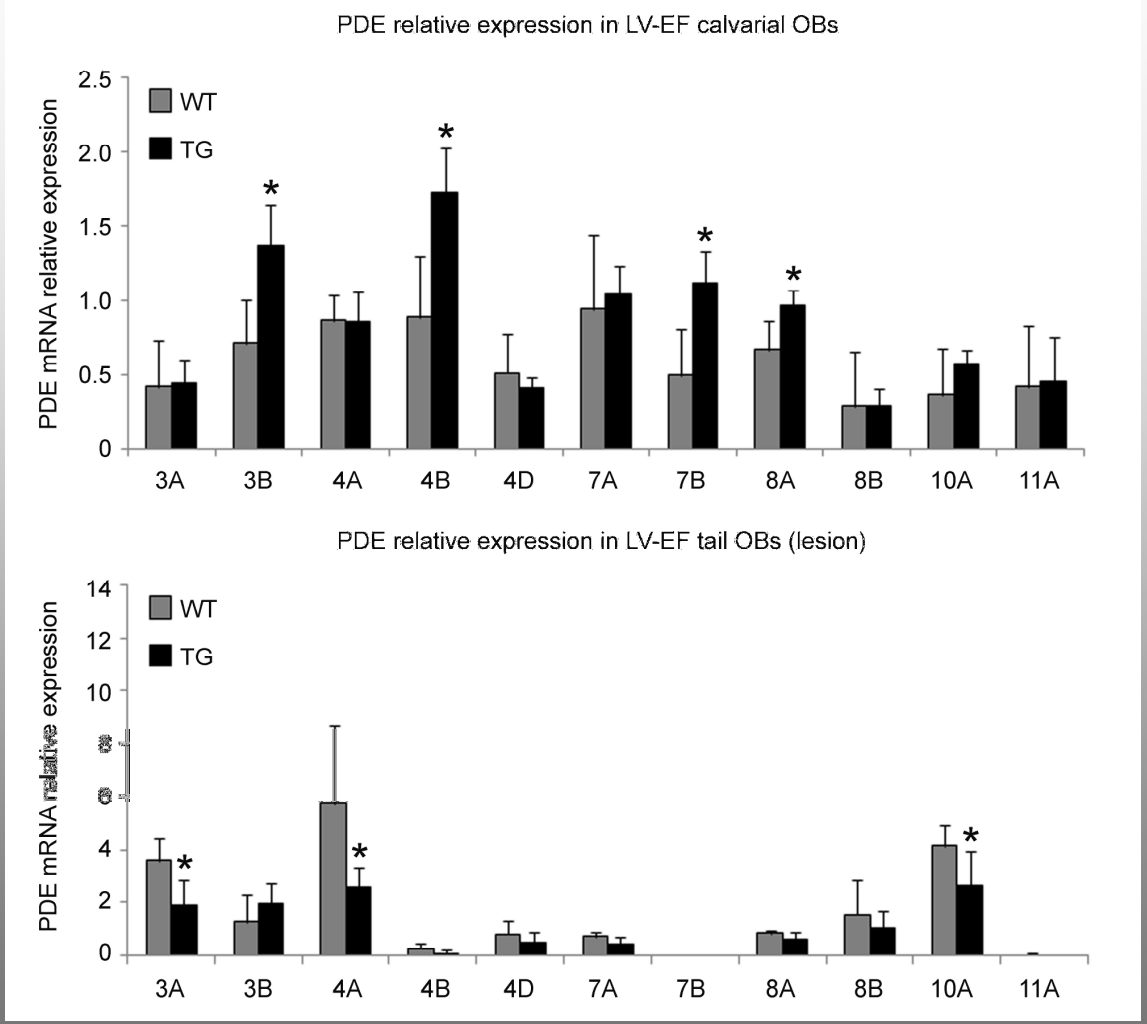


LV-EF1α-Gsα^{R201C} *mice*

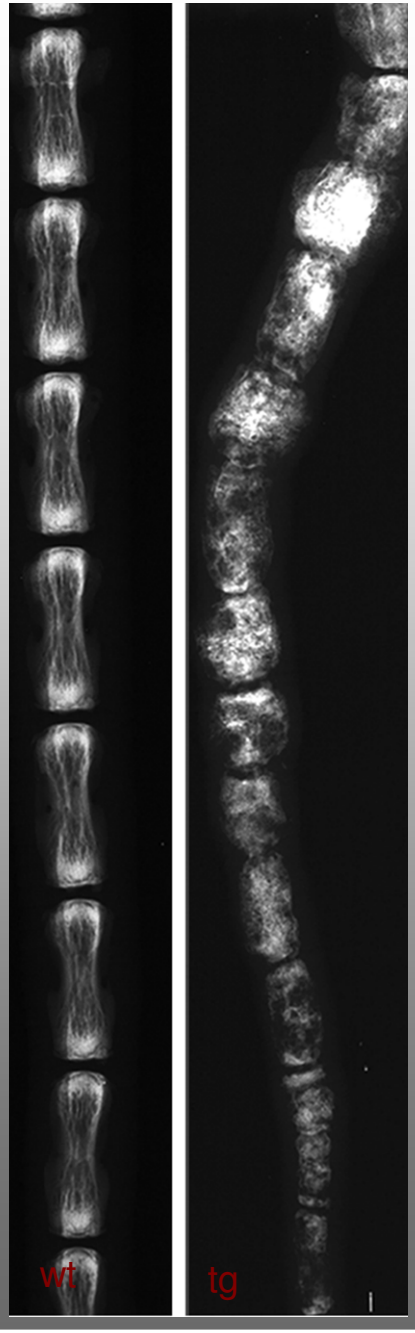
Spatial and temporal pattern of FD lesions in LV-FD mice

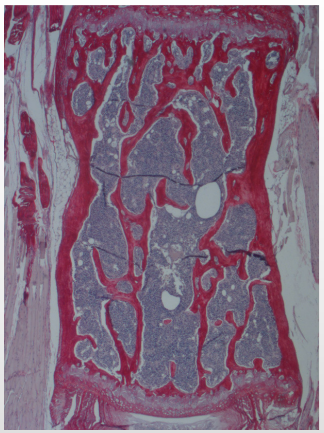


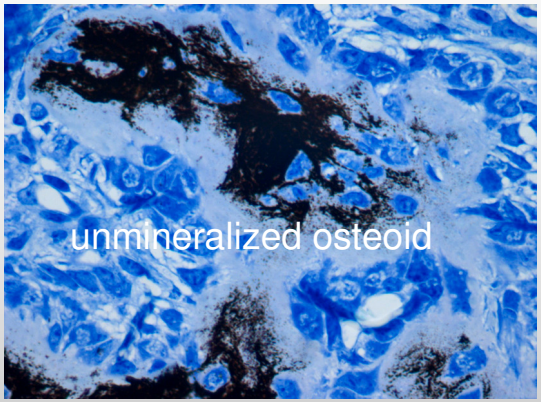
PDEs as potential molecular deteminants of FD

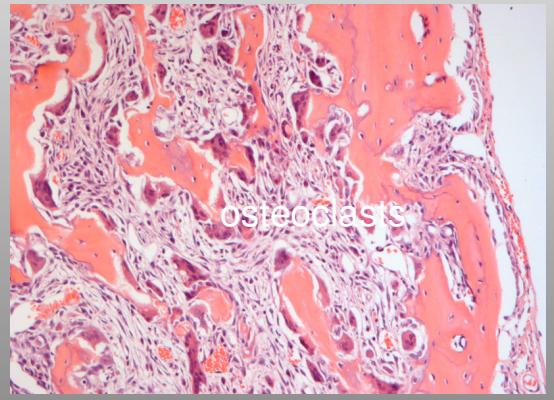


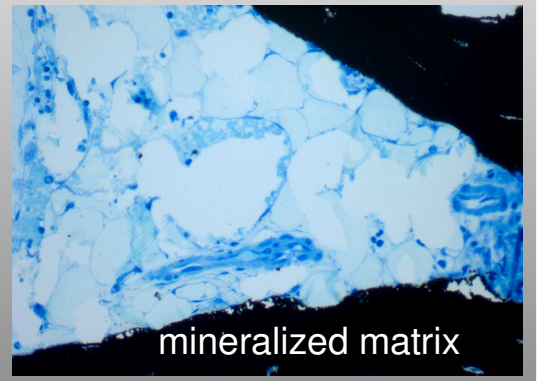
LV- FD mice reproduce the histopathology of FD





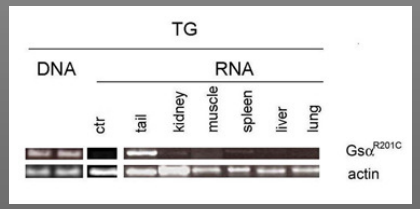


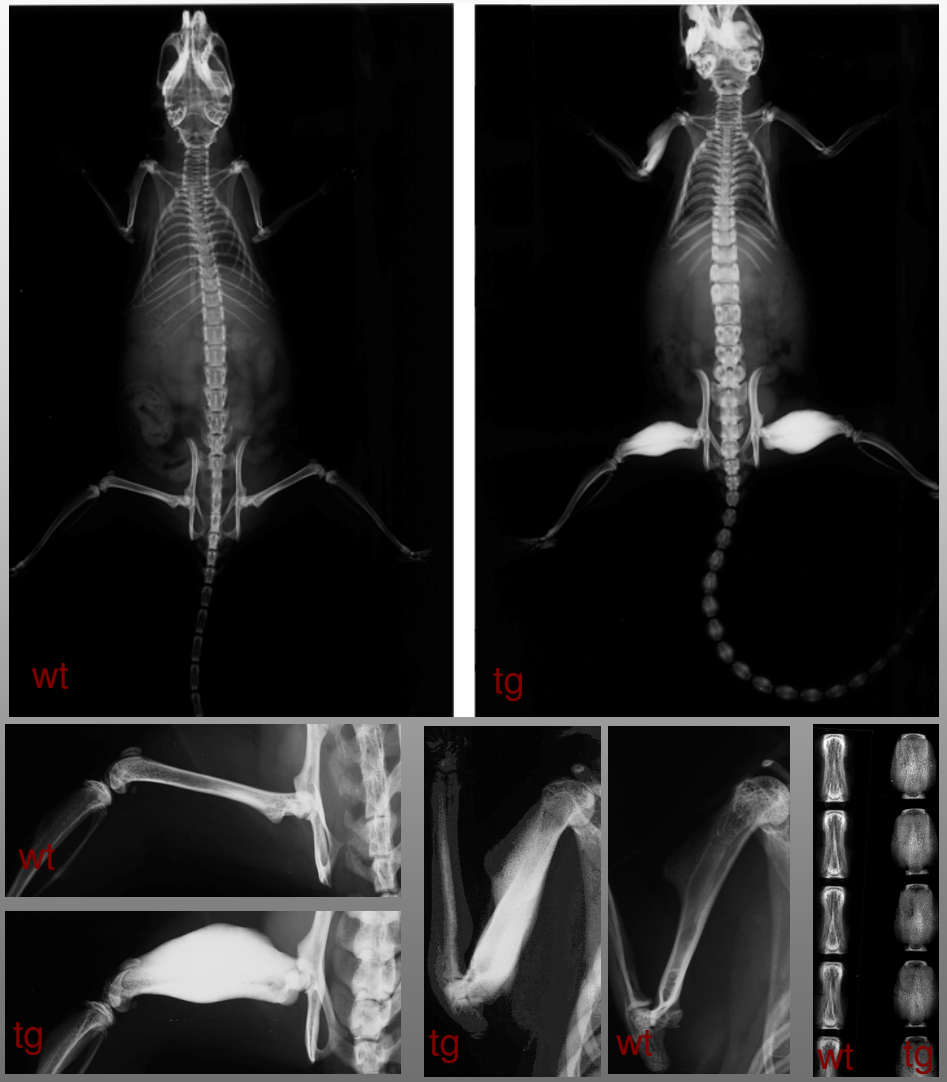




High bone mass phenotype in 2.3 Col1A1Gsα^{R201C} mice

(expression of Gsα^{R201C} restricted to mature osteoblasts)





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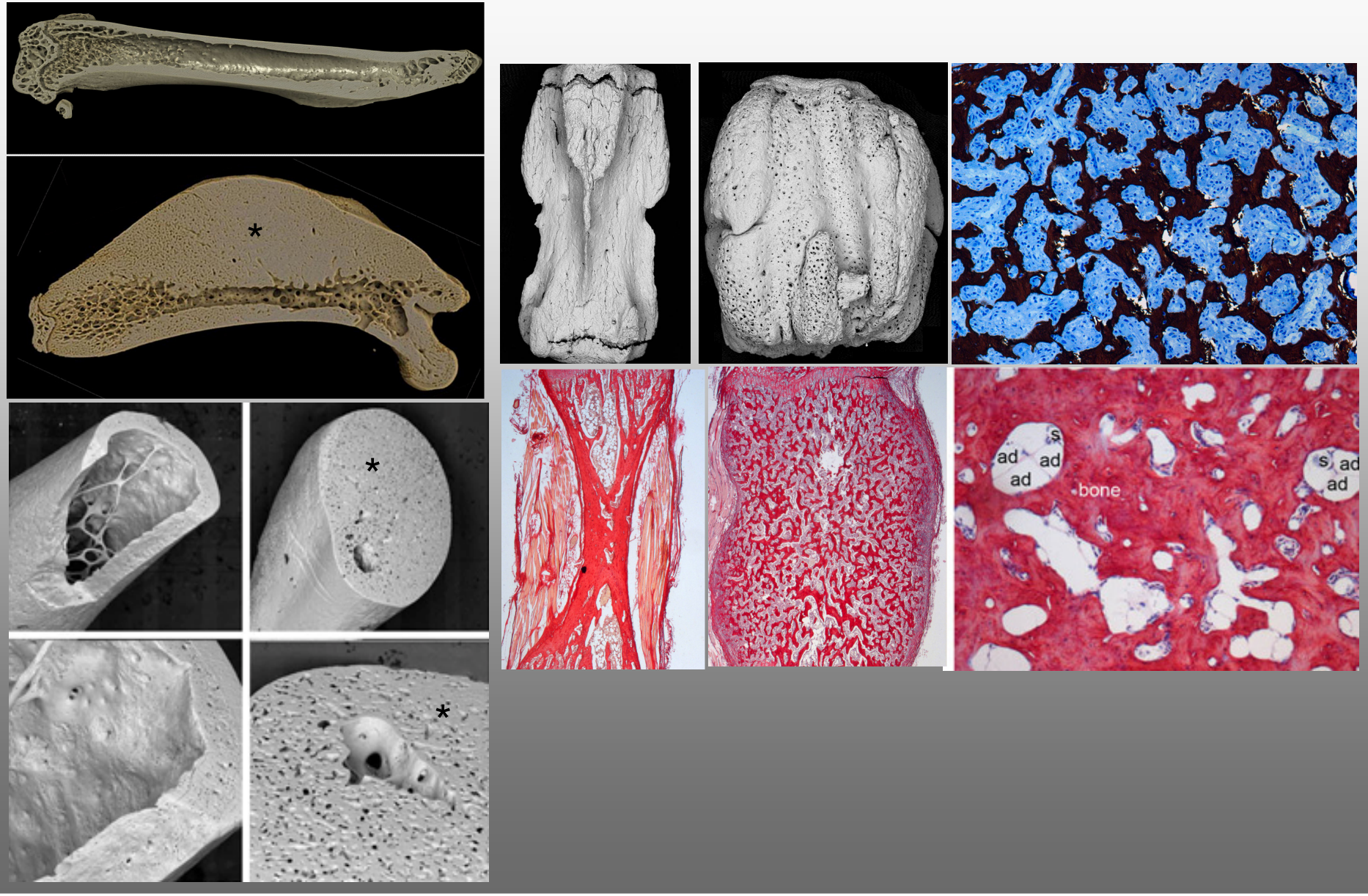
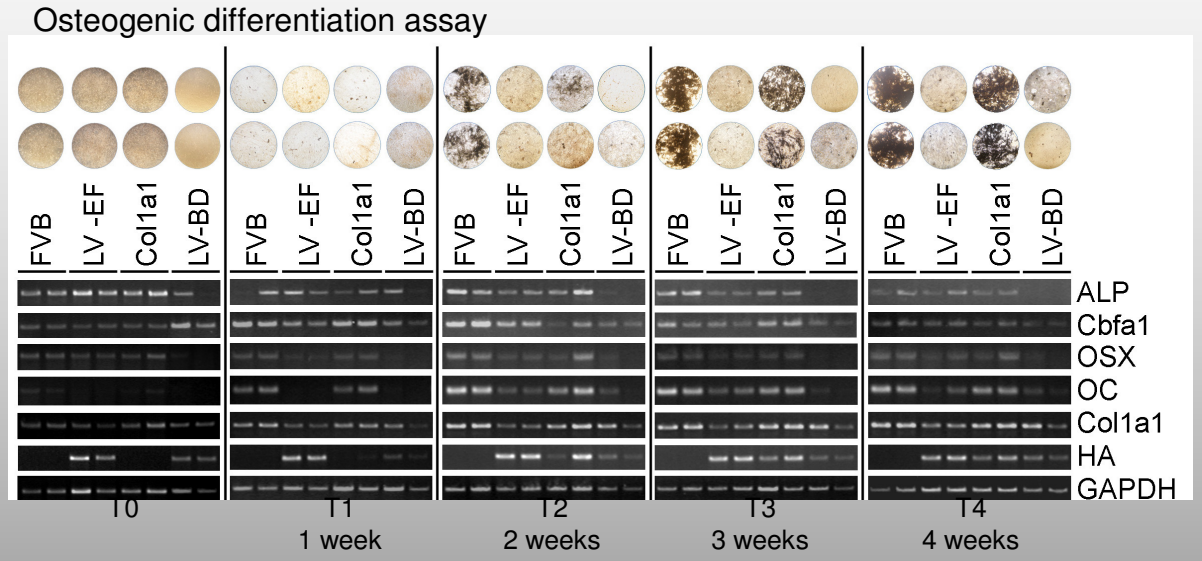
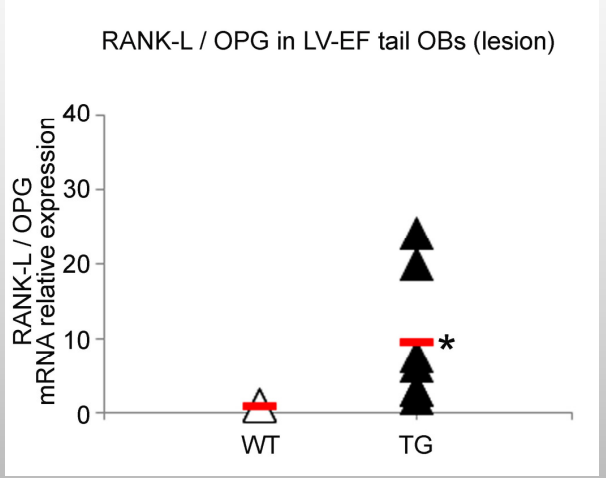
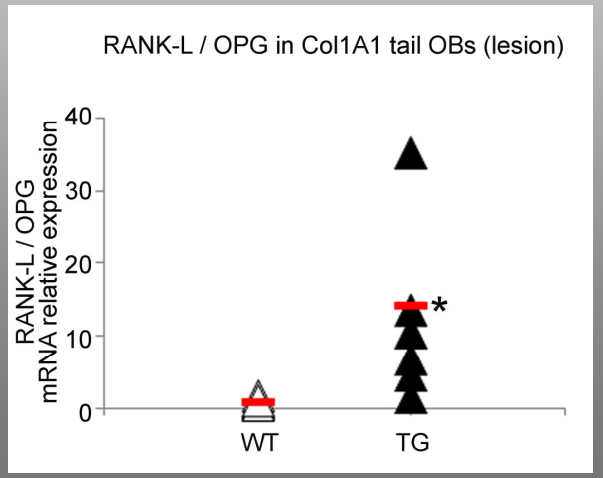


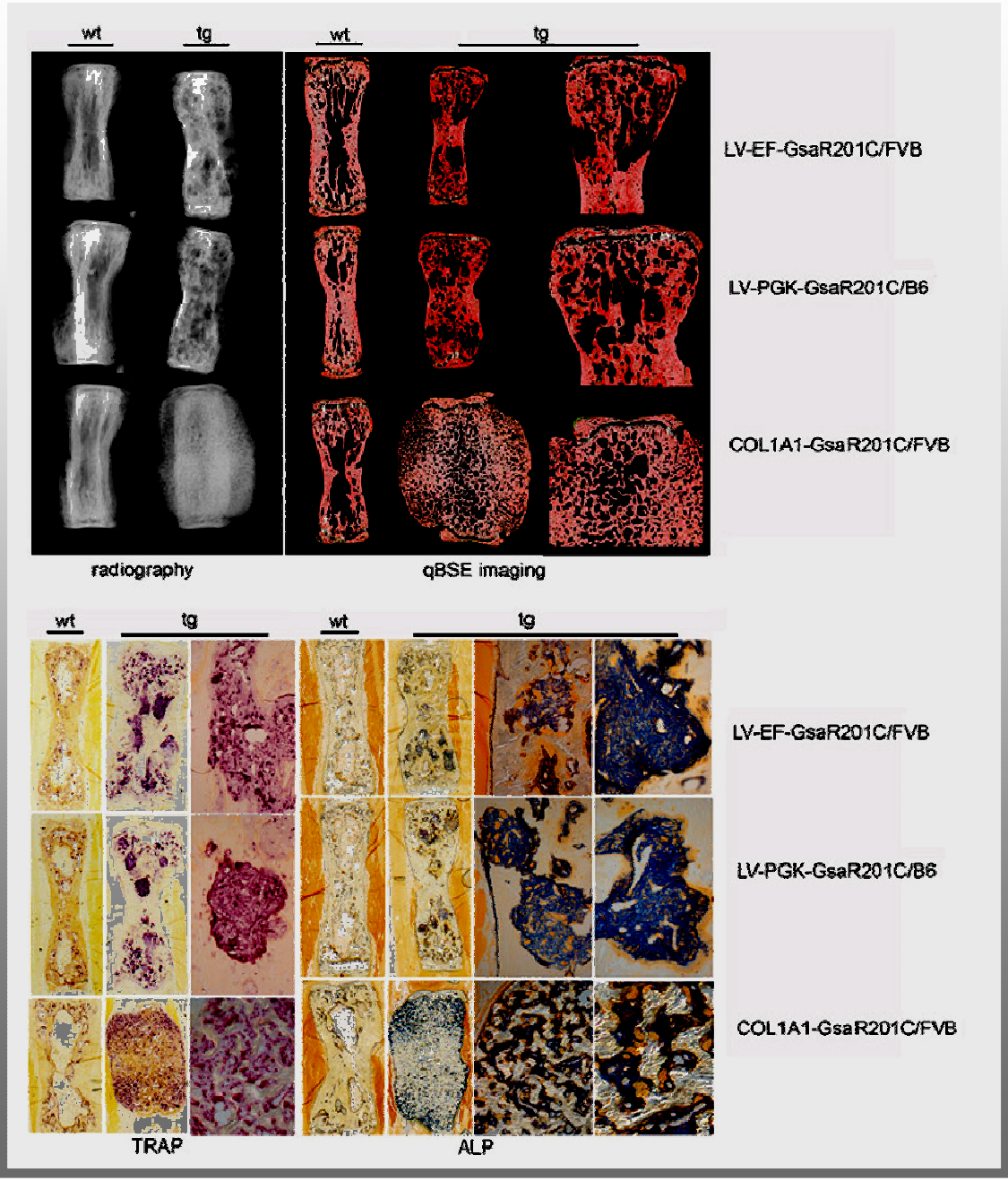
Skeletal progenitors, osteoclastogenesis and FD lesions







Skeletal progenitors, osteoclastogenesis and FD lesions



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