Università degli studi di Roma
La Sapienza

Research project simulation

HDAC8 activity on miR-25/miR-181 genes exacerbates phenotype severity in Fibrous Dysplasia

2nd level Master

Stem cells and genome editing (u-stem) In memoriam of Paolo Bianco

2020-2021

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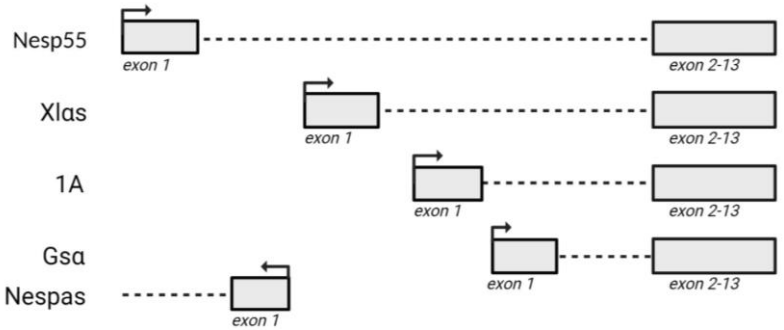
Fibrous Dysplasia a chronic problem in which scar-like tissue grows in place of normal bone. FD shows multiple phenotypes.

etiology

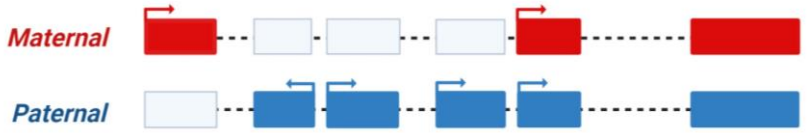
- Mutation in **GNAS** gene
- Post-zigotic mutation

GNAS locus

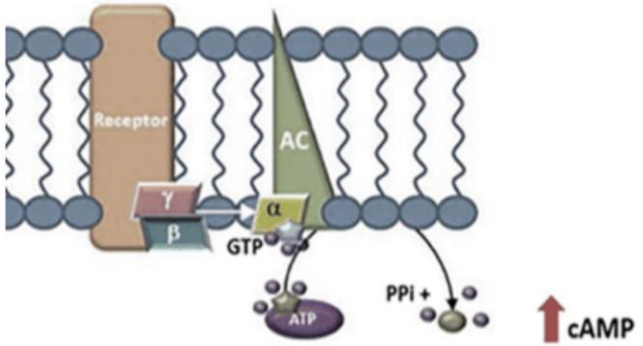
alternative splicing



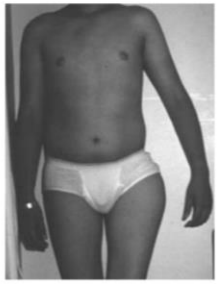
imprinting

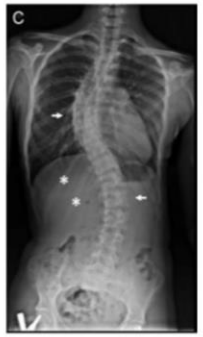


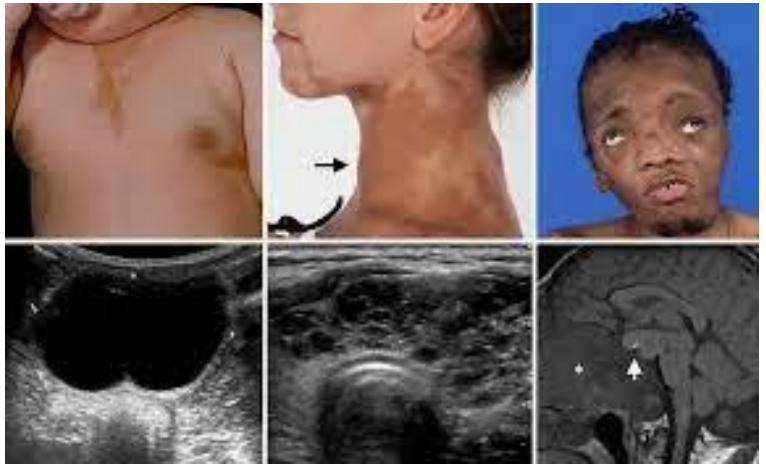
GNAS encodes the α subnit of a G protein



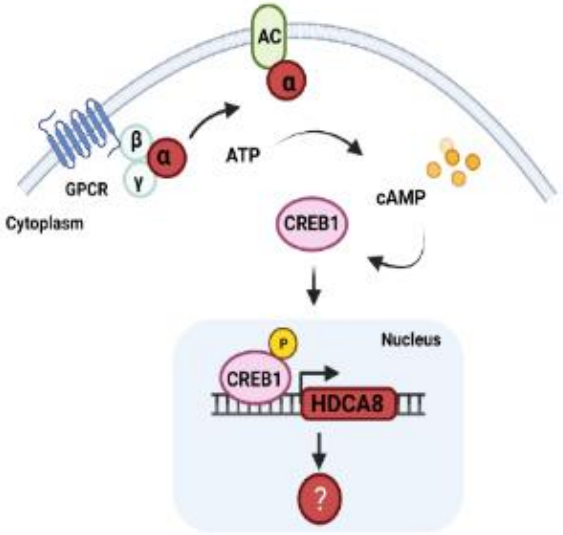
clinical signs







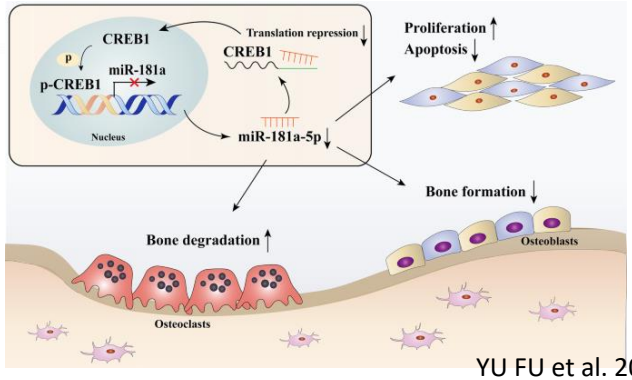
HDAC and FD



Xiao et al. 2021

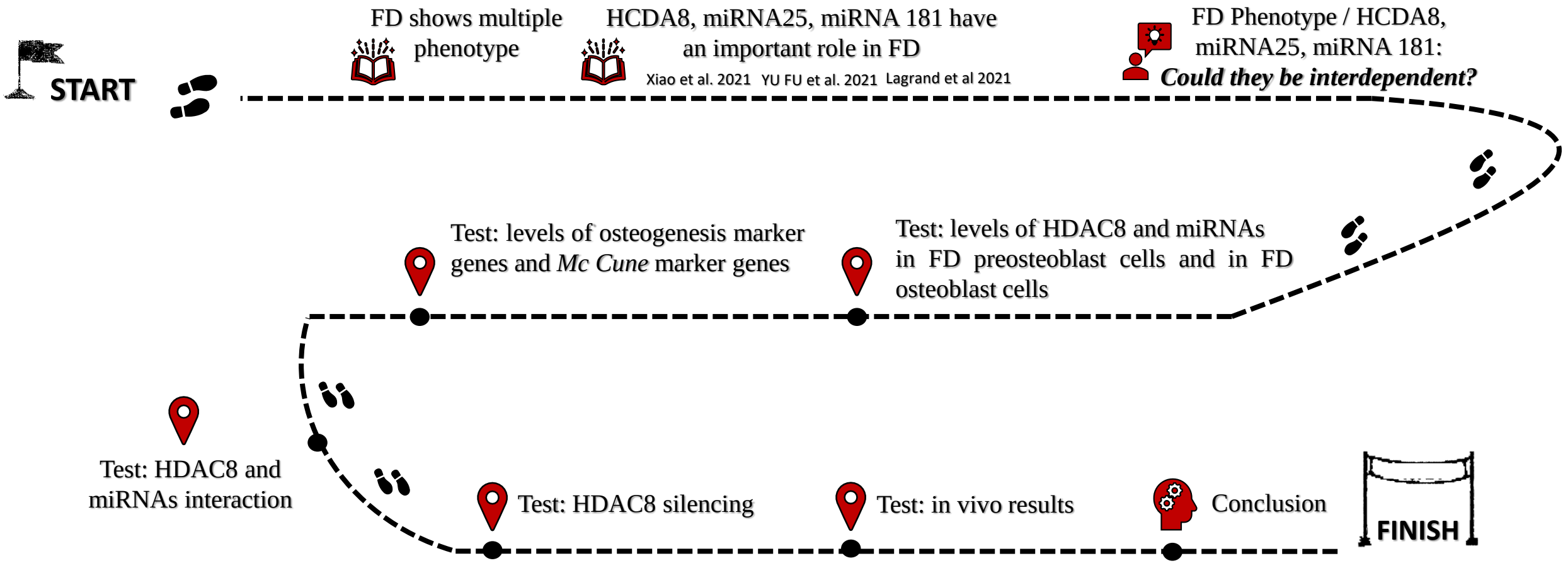
miRNAs and FD

Legrand et al. 2021



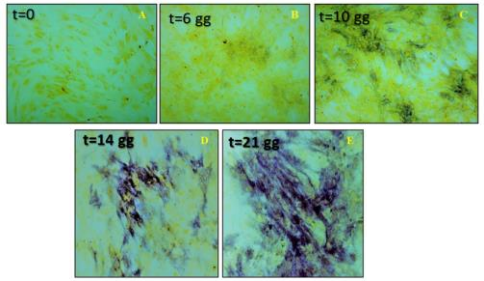
YU FU et al. 2021

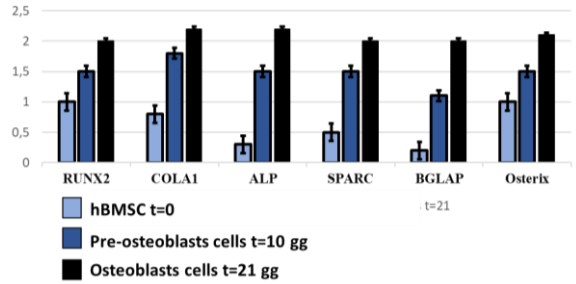
Aim of the project

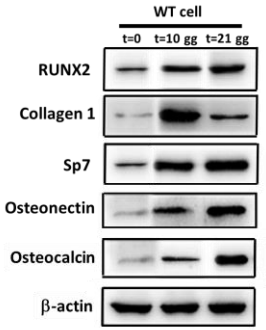


In different osteogenesis stage FD cells have different levels of HDAC8 and miRNAs

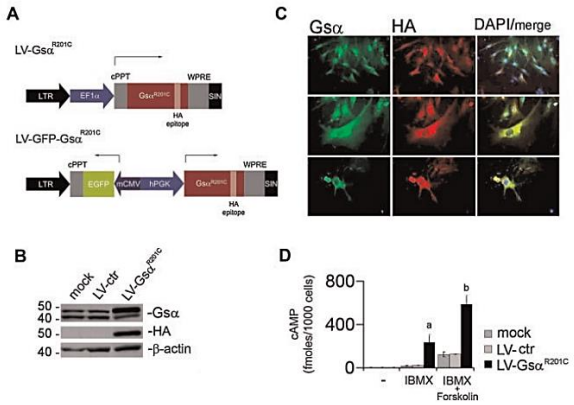
Osteogenic differentiation



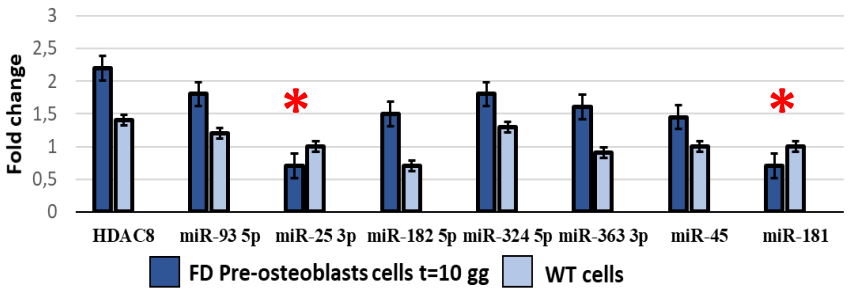


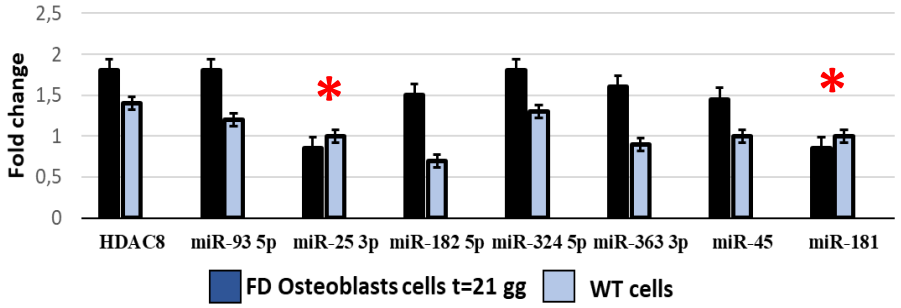


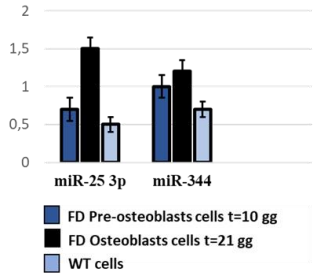
GsaR201C mutation in BMSCs t=10gg e t=21gg

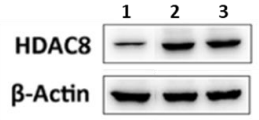


HDAC8 and miRNAs levels in FD pre-osteoblasts cells and in FD osteoblasts cells









1. WT cells
2. Pre-osteoblasts FD cells
3. Osteoblasts FD cells

FD Pre-osteoblast
Higher level of HDAC8
Lower level of miRNA 25
miRNA 181

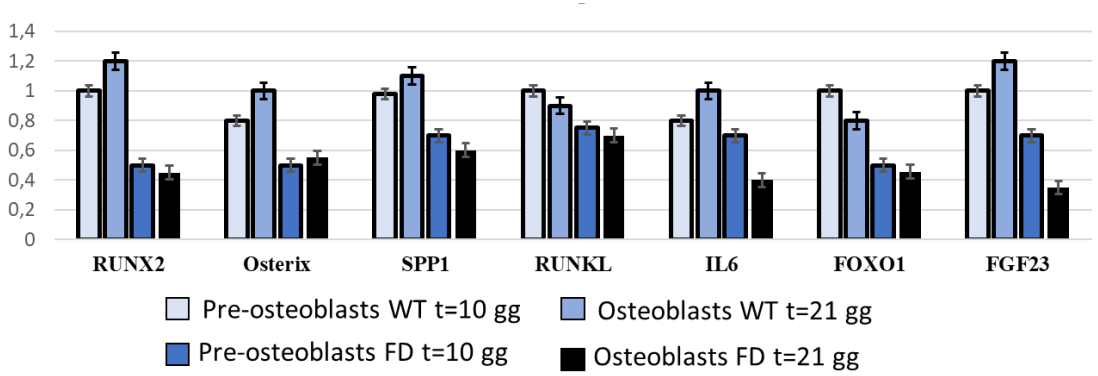
FD Osteoblast
High level of HDAC8
Low level of miRNA 25
miRNA 181

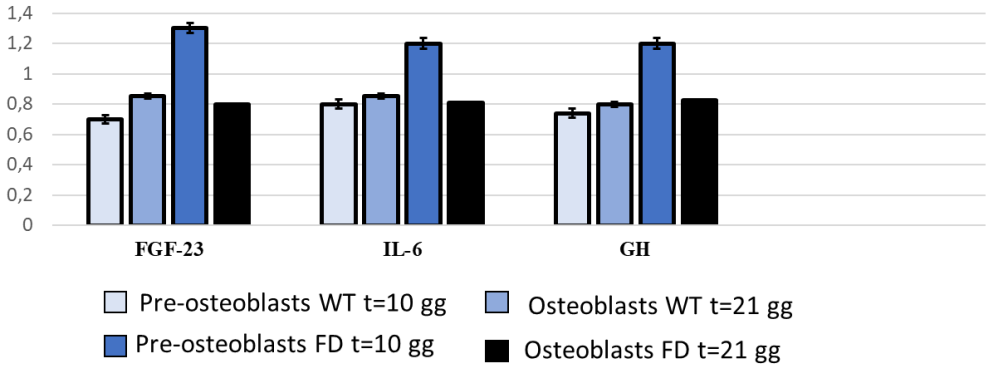
HDAC8 – miRNA 25– miRNA 181

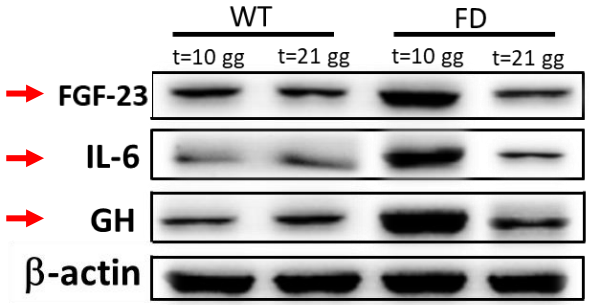
Are they important in ostegenesis?

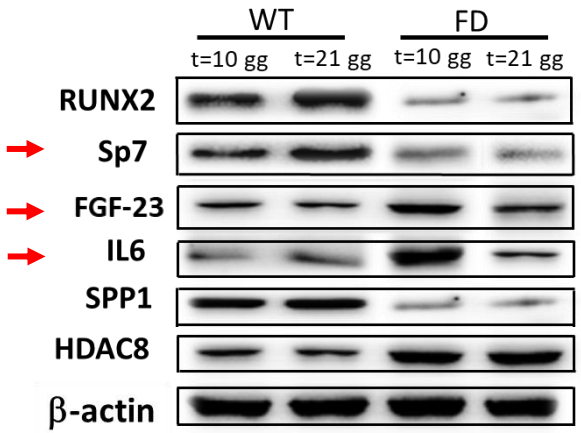
Are they important in FD phenotype?

In FD cells osteogenesis marker genes are altered and in pre-osteoblasts there are some *Mc Cune Albright* marker genes









FD Pre-osteoblasts
Different level of osteogenesis marker
High level FGF23
High level IL- 6
High level GH

FD osteoblasts
Different level of osteogenesis marker
Low level FGF23
Low level IL- 6
Low level GH

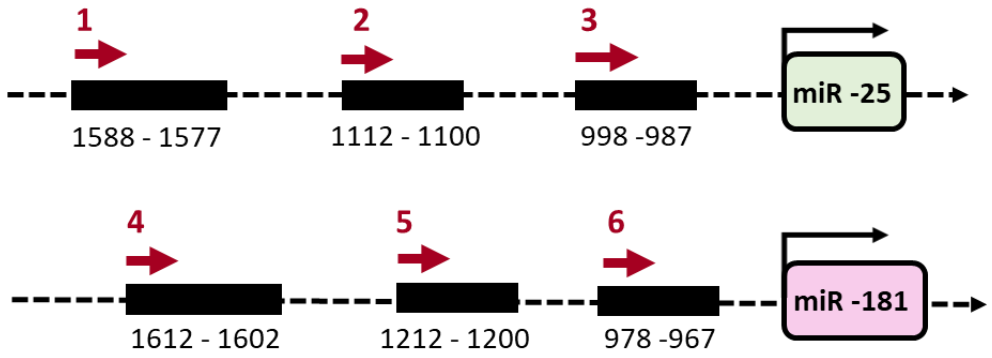
In FD preosteoblasts cells there is a more severe phenotype

Could it be due to an interaction between
HDAC8 and miRNAs?

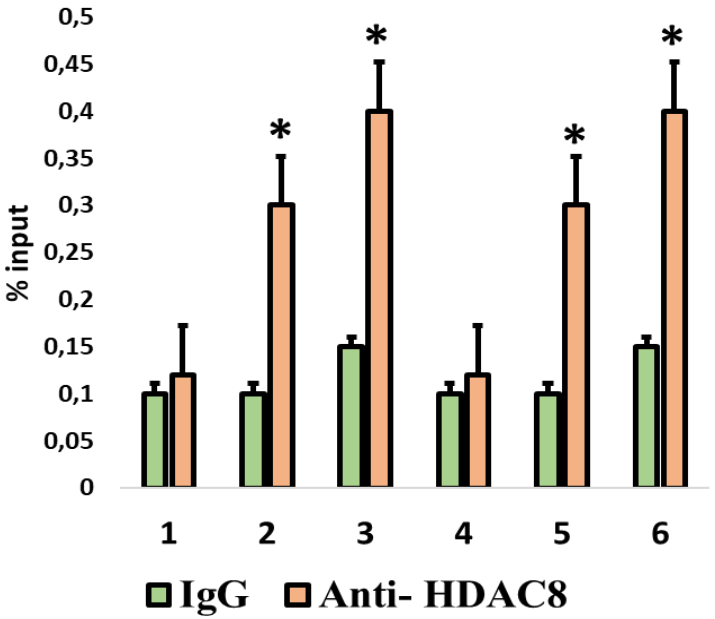
could miRNAs be target genes of HDAC8?

HDAC8 interacts with miRNA-25 and miRNA-181

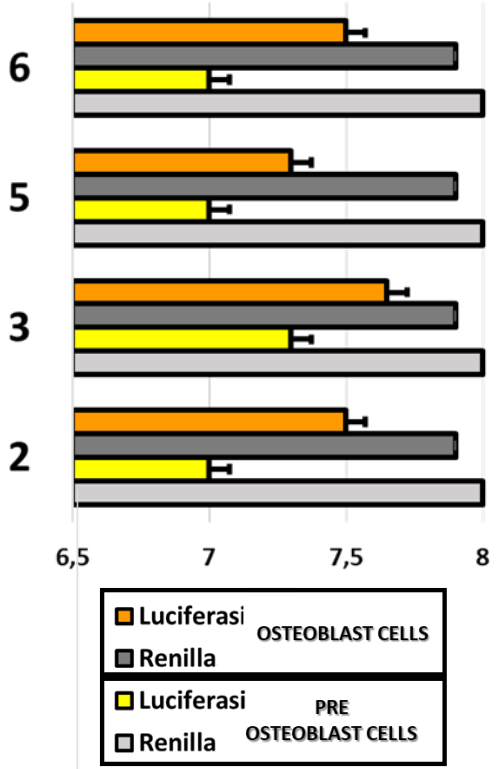
The six primer to cover the human miR-25/ mi-181 promoter region

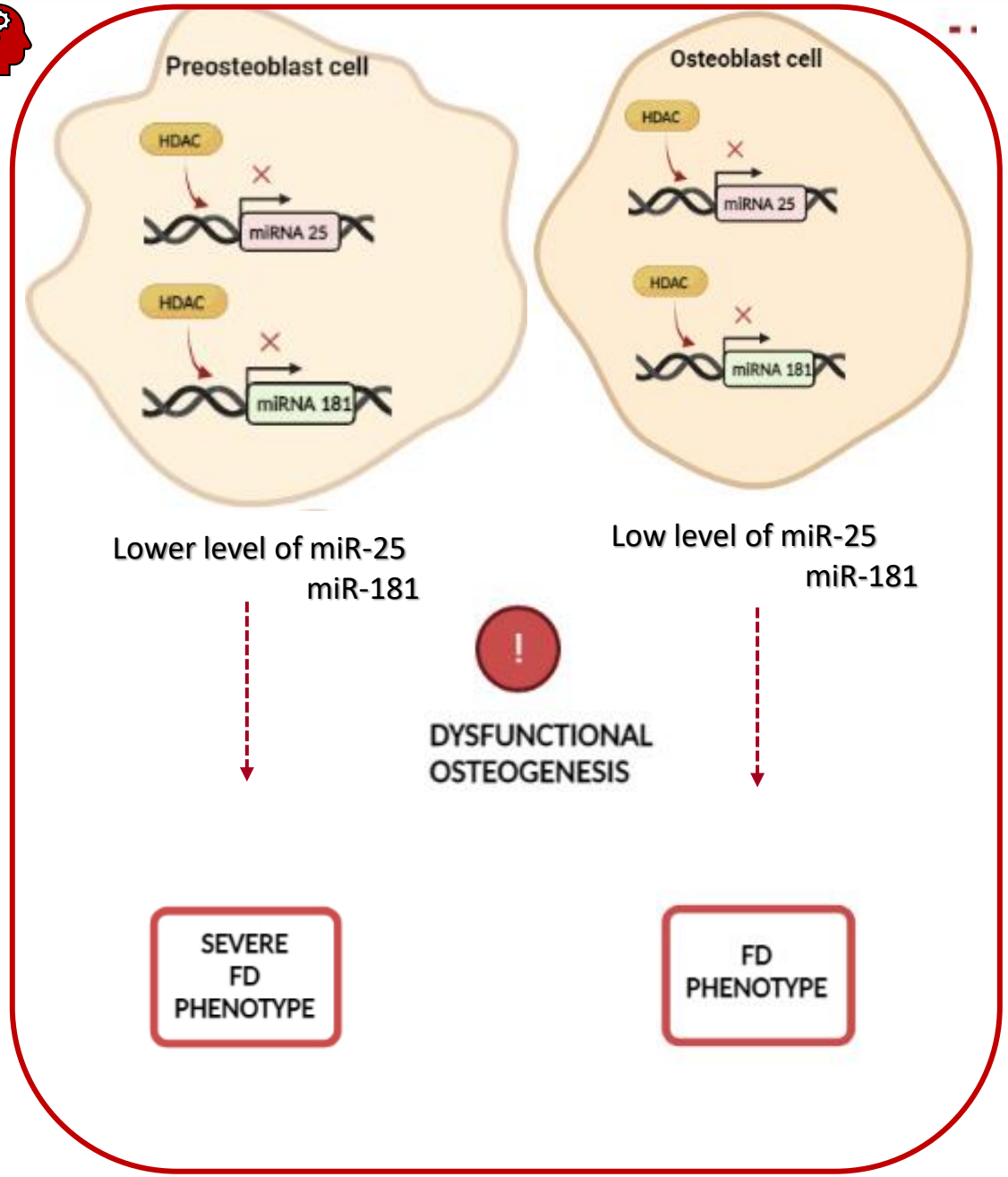


ChIP assay



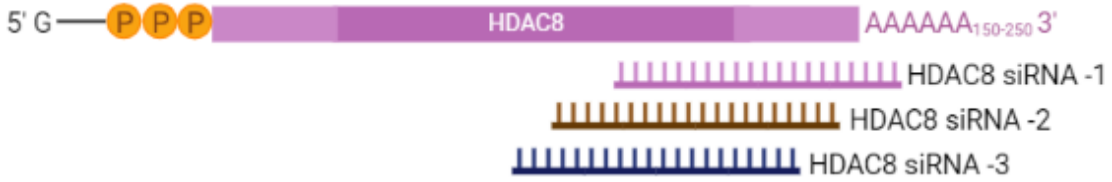
Luciferase assay

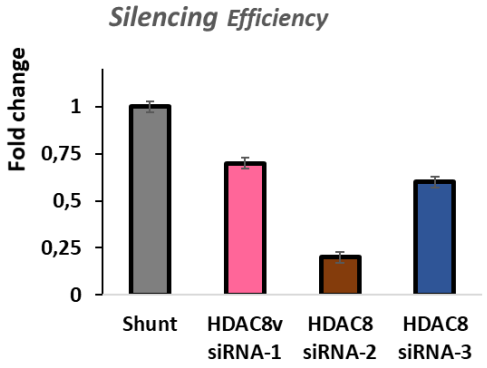


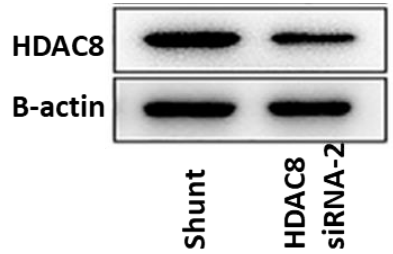


HDAC8 silencing rescues expression of miRNAs and osteogenic markers

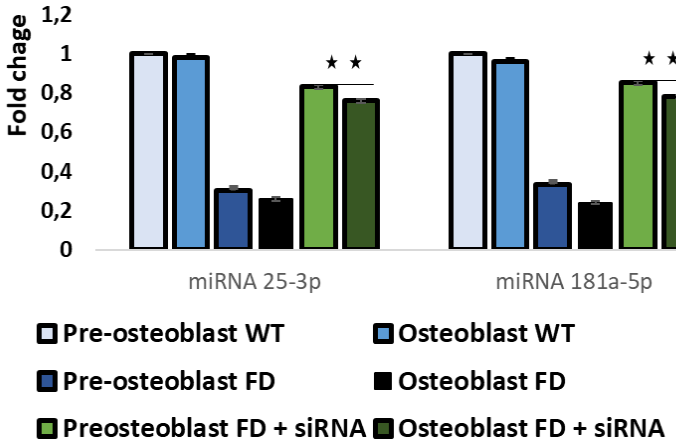
HDAC8 siRNAs efficencing test

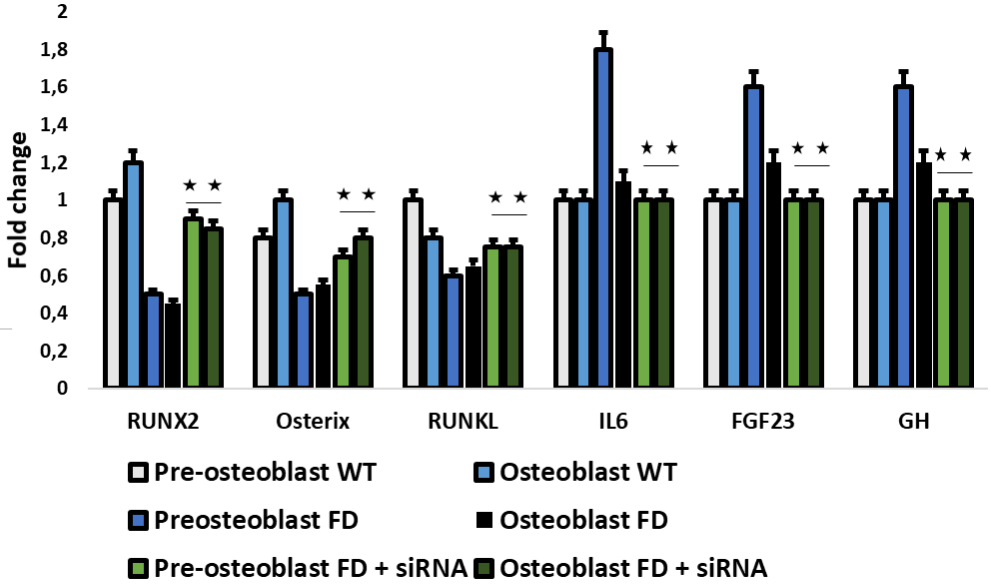


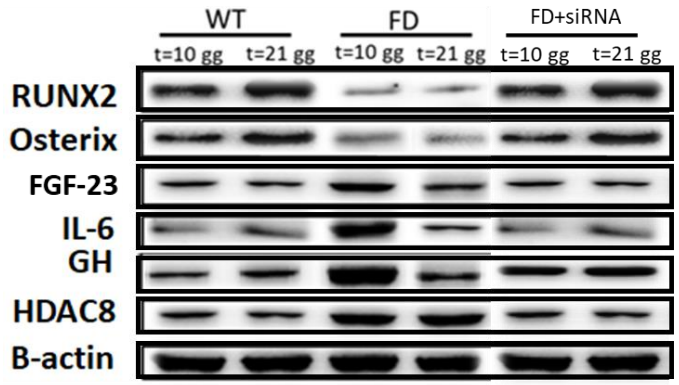




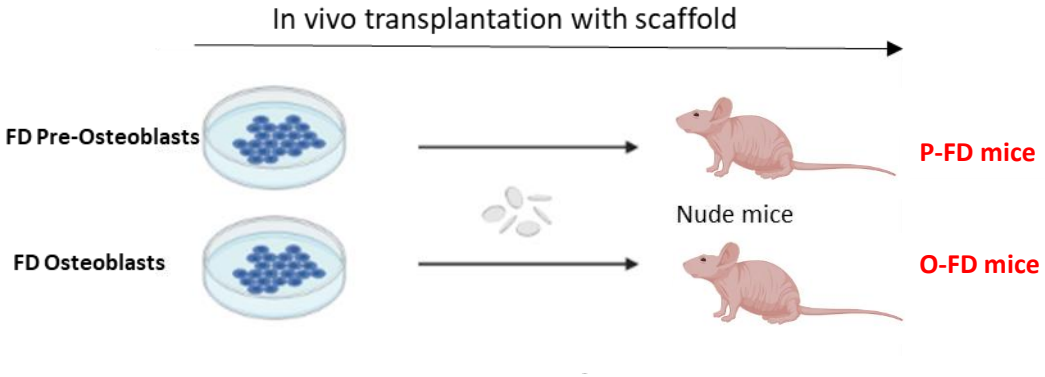
Effect of HDAC8 silencing on osteogenic and *Mc Cune Albright* marker genes

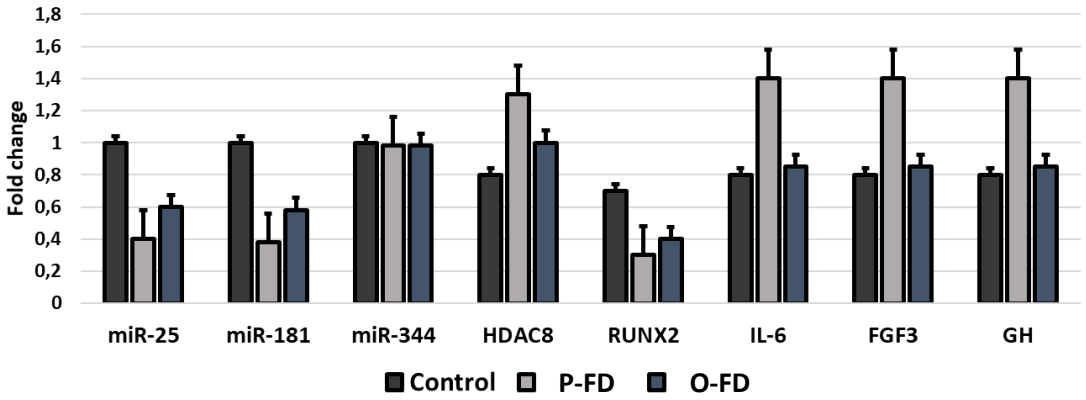


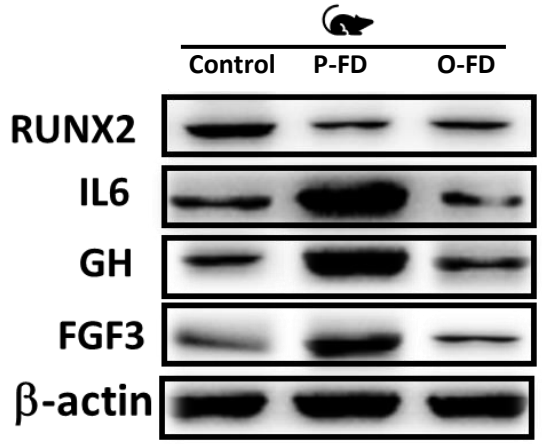


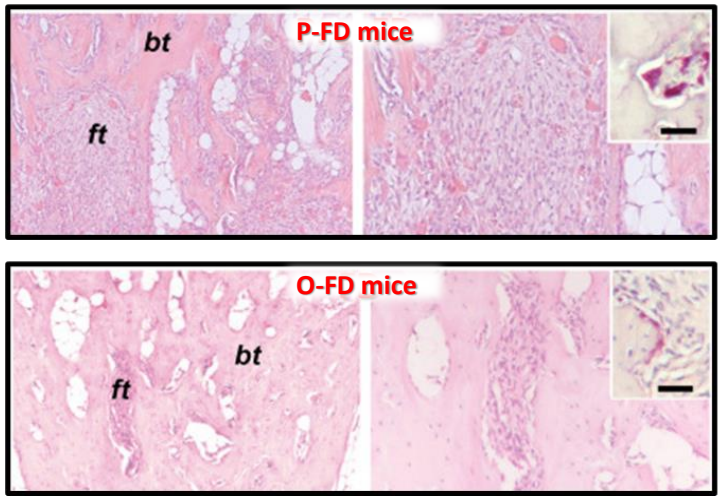


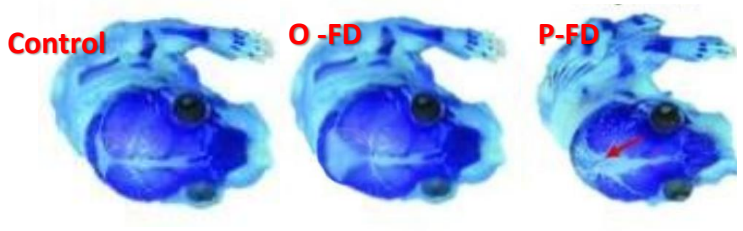
In vivo effects of FD pre-osteoblast/osteoblast cells transplantation in nude mice

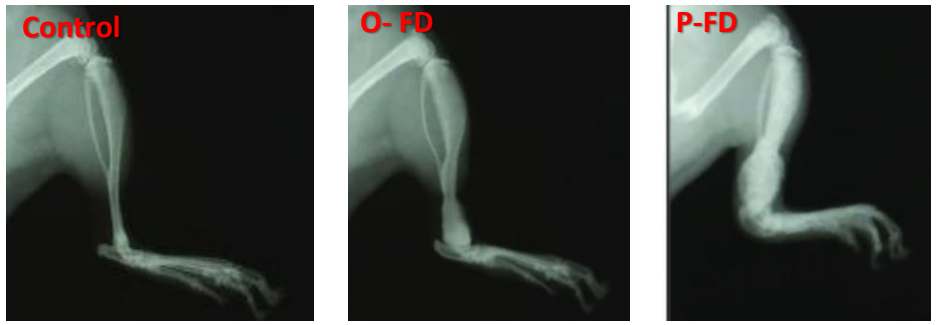






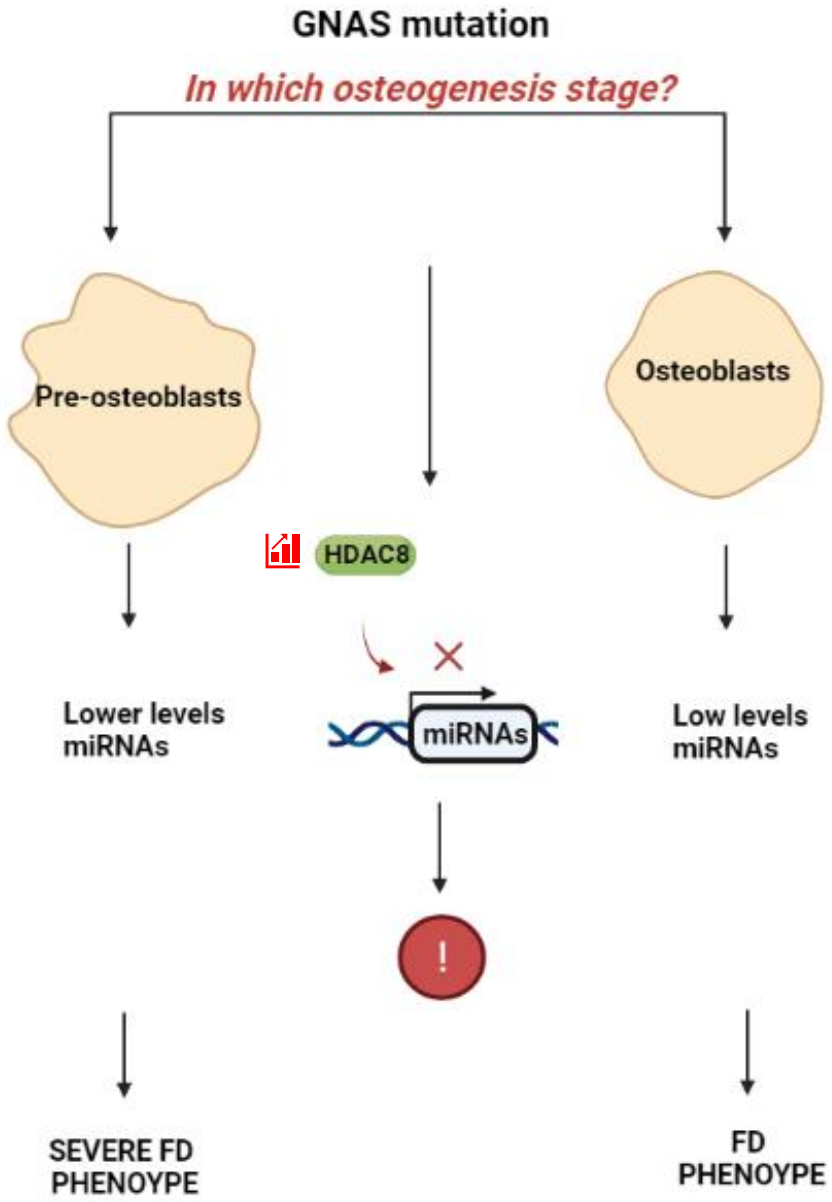






Adapted from Zhao et al 2018

Conclusion



Fibrous Dysplasia

SEVERE PHENOYPE

NO SEVERE PHENOYPE

Why?

It's due to time in which the GNAS mutation occurs

Why?

GNAS mutation increases the HDAC8 levels which regulate some miRNAs levels during osteogenesis

So...

miRNAs have an important role in osteogenesis!

Future perspectives

- Clarify the regulative role of miRNAs in the osteogenesis
- Verify if the knock-down of HDAC8 could rescue the FD phenotype in vivo in an inducible/conditional FD-mouse model (Zhao et al., 2017)
- Evaluate the possible role of HCDAC – miRNAs interaction in other cell types implicated in FD pathology
- Evaluate if the HDAC upregulation affects the expression of other genes
- Generate a *in-vivo* McCune Albright model

Limits and Pitfalls

- Focused on limitated aspects of FD histophatology
- Actual absence of a *in-vivo* McCune Albright model

Timeline: 2 years

Material and costs	€
Bone Marrow Mesenchymal Stem Cells, Frozen 1 million/vial *stemexpress*	817,74
Lentivirus *Santa cruz biotechnology*	445,00
Alkaline Phosphatase detection kit ScienCell Research Laboratories	350,00
Chromatin Immunoprecipitation Kit	565,00
Antibodies	500,00
Primers	480,00
Luciferase Assay System	150,00
Osteogenic differentiation kit	400,00
PhD researchers	≃ 43.200,00
TOT	**≃ 50.000 €**

References

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- **A signature of circulating miRNAs associated with Fibrous Dysplasia of bone: the mirDys study** Mélanie A Legrand,Marjorie Millet,Blandine Merle,Jean-Charles Russeau,Anaelle Hemmendinger,Evelyne Gineyts,Elisabeth Sornay-Rendu,Pawel Szulc,Oliver Borel,Martine Croset,Roland Chapurlat First published June11,2020 https://doi.org/10.1002/jbmr.4111.

- **Constitutive Expression of GsaR201C in Mice Produces a Heritable, Direct Replica of Human Fibrous Dysplasia Bone Pathology and Demonstrates Its Natural History.** I. Saggio, C.Remoli, E. Spica, S.Cersosimo, B.Sacche, P.G. Robey, K. Holmbeck, A. Cumano, A.Boyde, P. Bianco, and M. Riminucci. Journal of Bone and Mineral Research, Vol. 29, No. 11, November (2014), pp 2357–2368 .

- **Expression of an active Gαs mutant in skeletal stem cell is sufficient and necessary for fibrous dyplasia initiation and maintenance** Xuefeng Zhaoa,b, Peng Denga, Ramiro Iglesias-Bartolomec, Panomwat Amornphimolthamb,d, Dana J. Steffenb,e,Yunyun Jinf,g, Alfredo A. Molinolob, Luis Fernandez de Castroh, Diana Ovejeroh, Quan Yuana, Qianming Chena,Xianglong Hana, Ding Baia, Susan S. Taylore,, Yingzi Yangf, Michael T. Collinsh, and J. Silvio Gutkindb, PNAS January 16, 2018 115 (3) E428-E437; first published December 27, 2017.

- **FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate**.Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, White KE,Waguespack S, Gupta A, Hannon T, Econs MJ et al. 2003a

- **Fibrous dysplasia as a stem cell disease.** Riminucci M., Saggio I., Robey P.G., Bianco P. J Bone Miner Res. 2006; 21(Suppl 2),pp 125–131.

- **HDAC8, A Potential Therapeutic Target, Regulates Proliferation and Differentiation of Bone Marrow Stromal Cells in Fibrous Dysplasia** Tao Xiao, Yu Fu, Weiwen Zhu, Rongvao Xu, Ling Xu, Ping Zhang, Yifei Du, Jie Cheng,Hongbing Jiang . STEM CELLS TRANSLATIONAL MEDICINE 2019;8:pp 148–161

- **Osteoclastogenesis in fibrous dysplasia of bone:in situ and in vitro analysis of IL-6 expression.** Bone 33 434–442 Riminucci M, Kuznetsov SA, Cherman N, Corsi A, Bianco P & GehronRobey P 2003b.

- **Skeletal progenitors and the GNAS gene: fibrous dysplasia of bone read through stemcells**. Riminucci M, Gehron Robey P, Saggio I, Bianco P. J Mol Endocrinol. 2010;45(6), pp 355–64.

- **Transfer, analysis, and reversion of the fibrous dysplasia cellular phenotype in human skeletal progenitors.** S. Piersanti, C. Remoli, I. Saggio, A. Funari, S.Michienzi, B. Sacche;, P.G. Robey, M. Riminucci and P. Bianco; Journal of Bone and Mineral Research, Vol. 25, No. 5, May 2010, pp 1103–1116.

- Zhao, X., Deng, P., Iglesias-Bartolome, R., Amornphimoltham, P., Steffen, D. J., Jin, Y., ... & Gutkind, J. S. (2018). **Expression of an active Gαs mutant in skeletal stem cells is sufficient and necessary for fibrous dysplasia initiation and maintenance**. *Proceedings of the National Academy of Sciences*, *115*(3), E428-E437.