5° MEETING F.O.P. 5th F.O.P. CONFERENCE

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Inactivating *GNAS* Mutations and Heterotopic Ossification in POH

Mutazioni inattivanti di *GNAS* e ossificazione eterotopica nella POH

Robert J. Pignolo, M.D., Ph.D.

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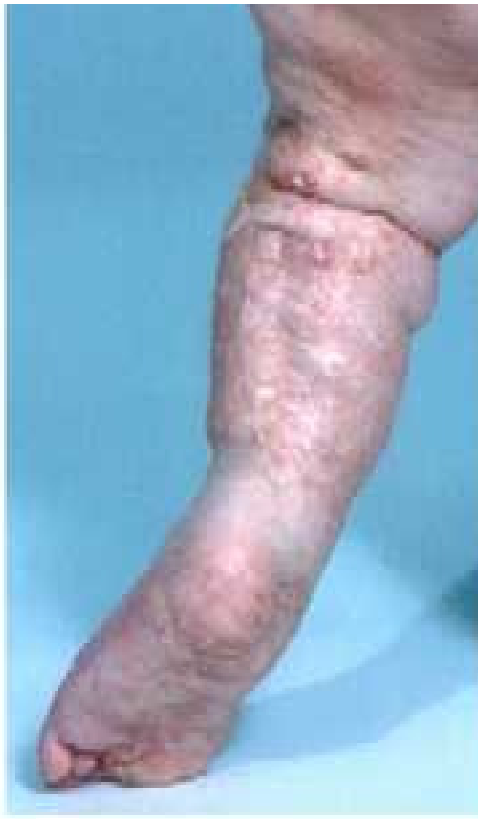
Inactivating *GNAS* Mutations and Heterotopic Ossification in POH

- POH in the spectrum of *GNAS*-based inactivation disorders
- Mosaicism in POH
- Mechanisms of heterotopic ossification in POH-like disorders



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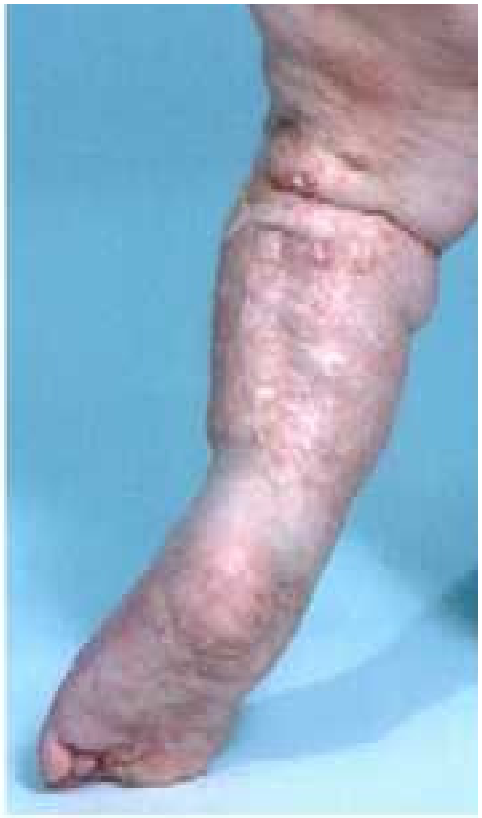

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Progressive osseous heteroplasia (POH; OMIM #166350)

- Rare genetic condition of progressive heterotopic bone formation
- Defined clinically by cutaneous ossification that progresses to involve deep connective tissues including skeletal muscle and fascia [Kaplan et al., 1994]
- Most cases are caused by heterozygous inactivating germline mutations in the *GNAS* gene that encodes the alpha subunit of the G-stimulatory protein of adenylyl cyclase [Shore et al., 2002]

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Clinical characteristics of POH and other *GNAS*-based disorders of HO

Diagnosis	*n*	Superficial HO	Deep HO[a]	> 2 AHO Features[b]	PTH Resistance[c]
POH	52	+	+	-	-
POH/AHO	6	+	+	+[d]	-
POH/PHP1a/1c	5	+	+	+[d]	+[d]
Osteoma cutis	26	+	-[d]	-	-
AHO	10	+	-[d]	+[d]	-
PHP 1a/1c	12	+	-[d]	+[e]	+[d]

All (+) or (no (-) patients within the diagnostic category displayed the indicated characteristic.

[a]Deep HO refers to the extension of superficial (dermal) HO to deep tissues.

[b]AHO features included: short statue, obesity, round face, brachydactaly, neurobehavioral abnormalities.

The presence of heterotopic ossification was common to all presentations and excluded here.

[c]An endocrine evaluation included a survey of calcium, phosphorus, TSH, and intact PTH blood levels.

Two POH patients had endocrine abnormalities: one had a high TSH, and another had high calcium and phosphate levels.

One POH/AHO patient had a high phosphate level.

All POH/PHP 1a/1c patients had PTH resistance or PTH and thyroid hormone resistance.

Differences found to be statistically significant when compared to POH are [d] $p < 0.0001$, [e] $p = 0{,}0002$.

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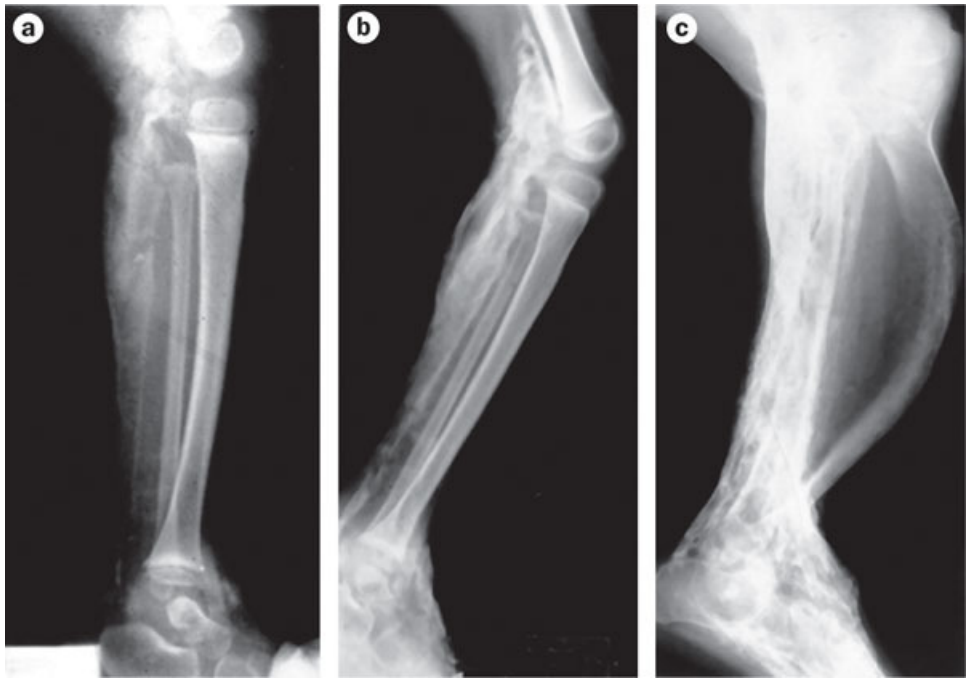
Progression of HO in POH



18 months 30 months 8 years

Kaplan, F. S. ***et al. J. Bone Joint Surg.* 76**, 425–436 (1994)

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AHO features among patients with POH & other *GNAS*-based disorders of HO

Diagnosis	Average no. of AHO features per patient (+/- SD)	Short Stature (%)	Obesity (%)	Round face (%)	Brachydactaly (%)	Mental retardation (%)
POH	0.31 (0.61)	7.7	0.0	3.8	15.4	3.8
POH/AHO	2.7 (0.5)	66.7	33.3	66.7	83.3	16.6
POH/ PHP1a/1c	2.2 (1.5)	80.0	40.0	20.0	40.0	40.0
Osteoma cutis	0.0 (0.0)	0.0	0.0	0.0	0.0	0.0
AHO	2.7 (1.9)	50.0	40.0	50.0	80.0	20.0
PHP1a/1c	2.6 (1.4)	50.0	58.3	66.7	66.7	8.3

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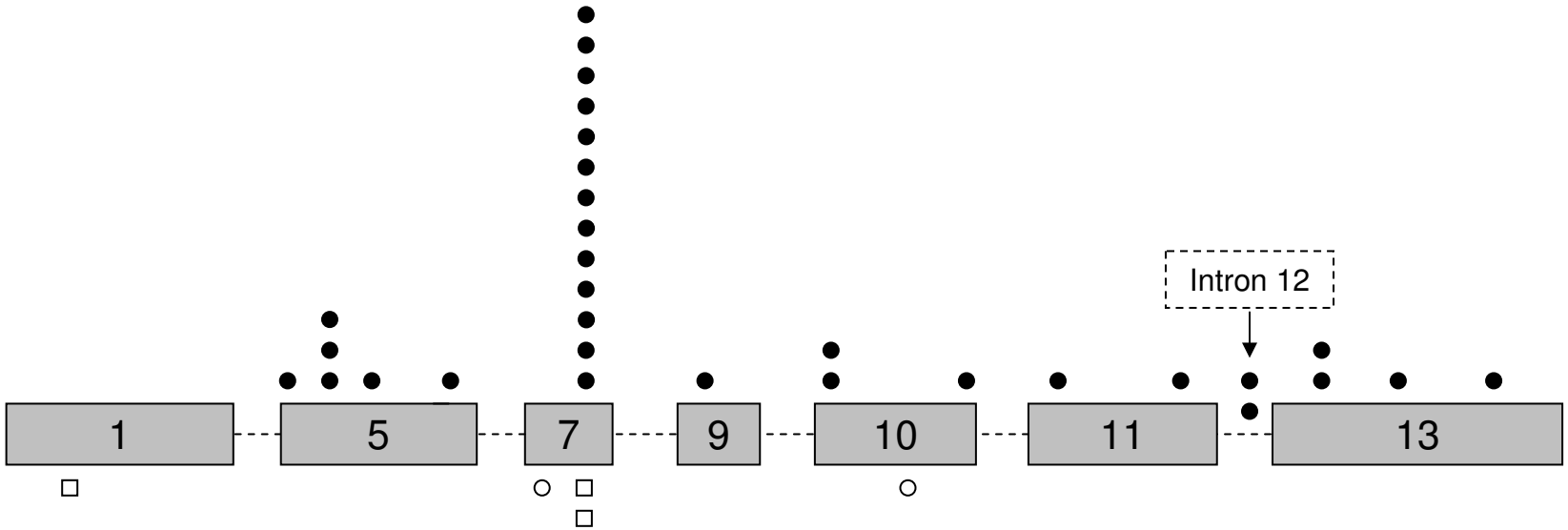
Distribution of *GNAS* mutations in POH & other conditions of progressive HO



- Analysis on 48/52 POH patients
- GNAS mutations in 64%
- 4bp deletion c.565-8 in 13 cases
- No genotype-phenotype correlations

● POH
□ POH/AHO
○ POH/ PHP 1a/1c

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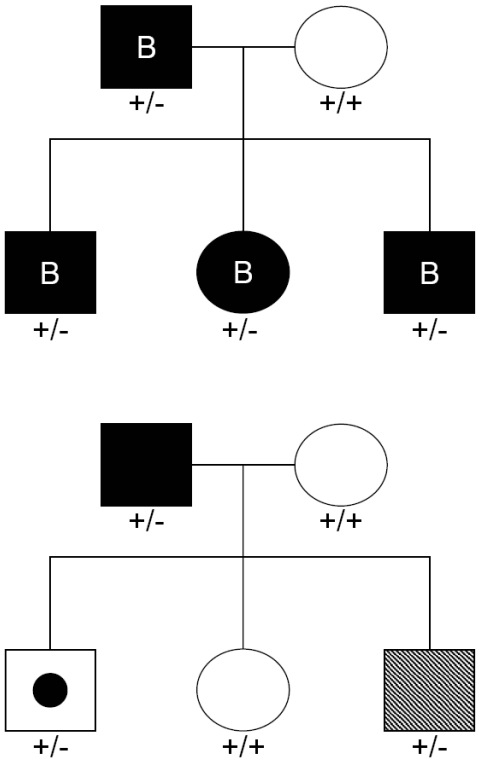
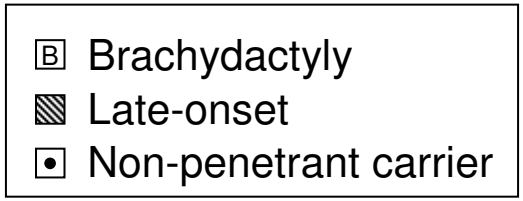
Phenotypic variability of POH





Phenotypic variation in 2 families
with exon 7, c.565-8, 4bp deletion

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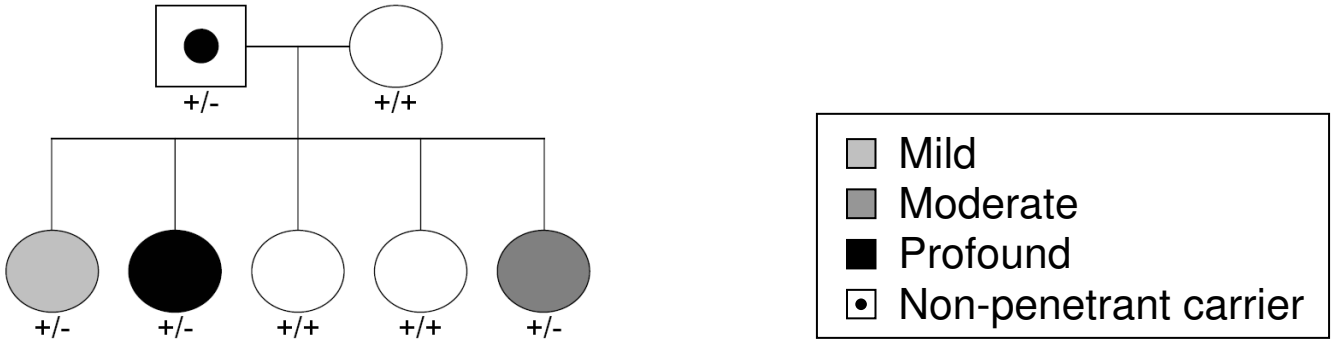
Phenotypic variability of POH



Variability of severity, exon 10, c.725 del C

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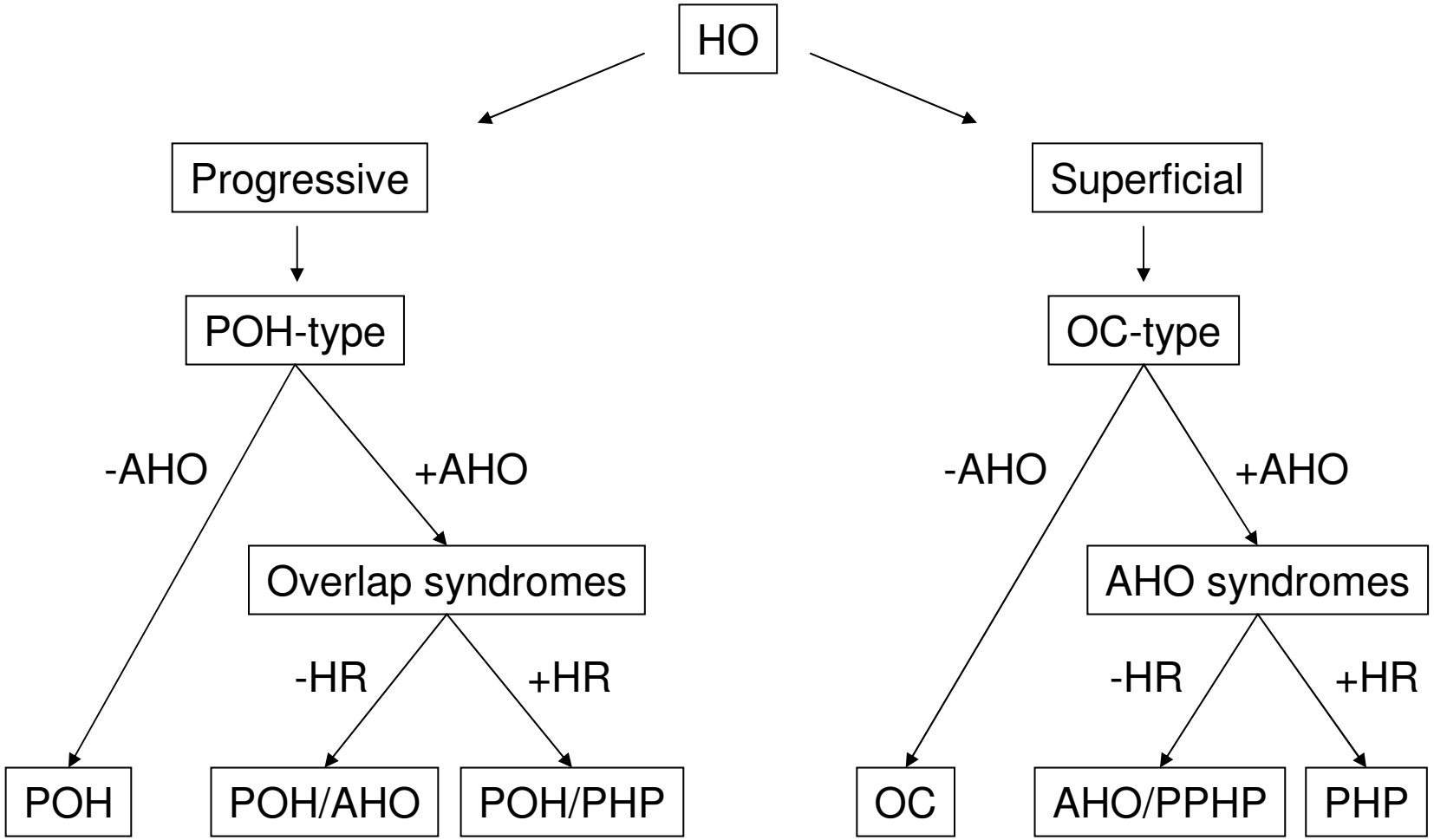
Differential diagnosis of *GNAS*-based HO disorders



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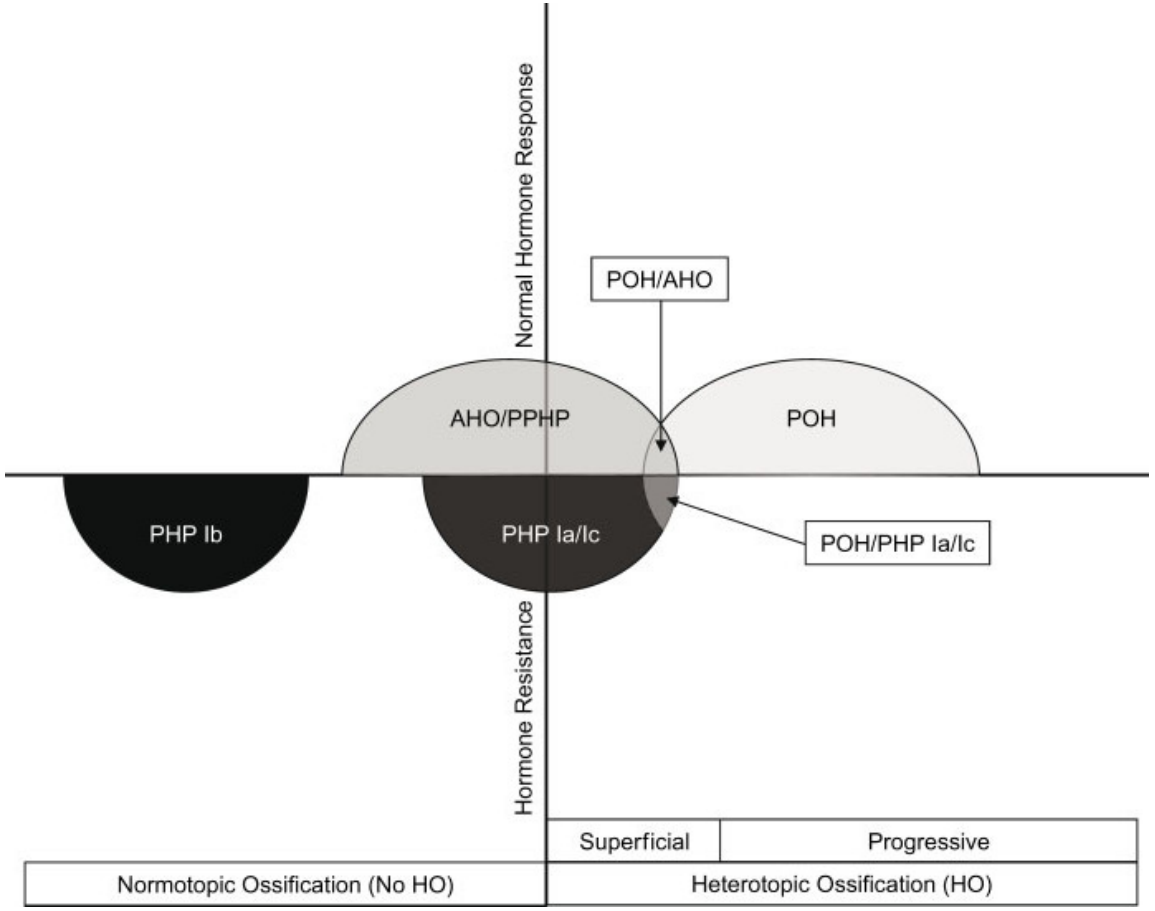
Spectrum of POH and other *GNAS*-based conditions of HO



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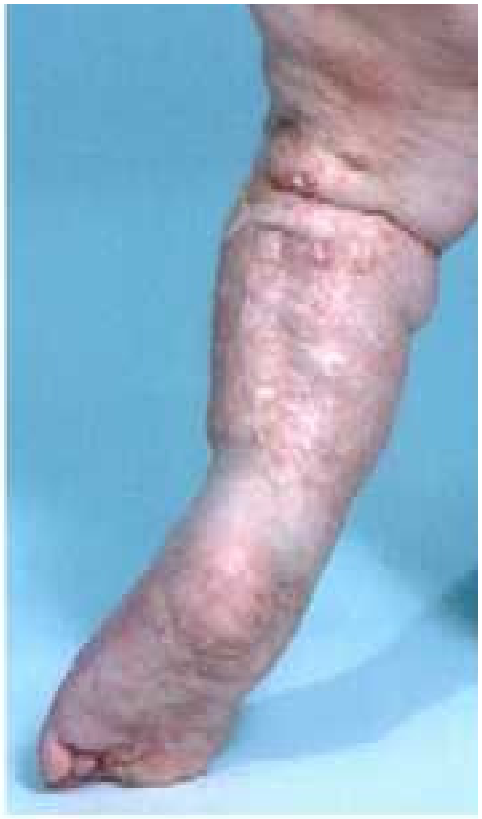

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Mosaicism

- Described in many monogenic skin disorders and occurs in various patterns, with or without lateralization [Frank et al., 2007]
- May also occur as cutaneous periarticular ossification in meloreostosis related to involvement of a corresponding myotome [Murray et al., 1979]
- At least two genotypically different cell populations derived from a single zygote [Happle, 1995], which result from mutation(s) during development, and are retained by a finite number of cells in the postnatal state.

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Mosaicism in POH

- 12 consecutive individuals with severe POH who had lost the mobility of at least 1 joint
- Distribution of lesions recorded using clinical, radiographic, and photographic documentation
- Extent and lateralization of HO was scored as the number of involved body areas
- HO involvement was also plotted onto body maps

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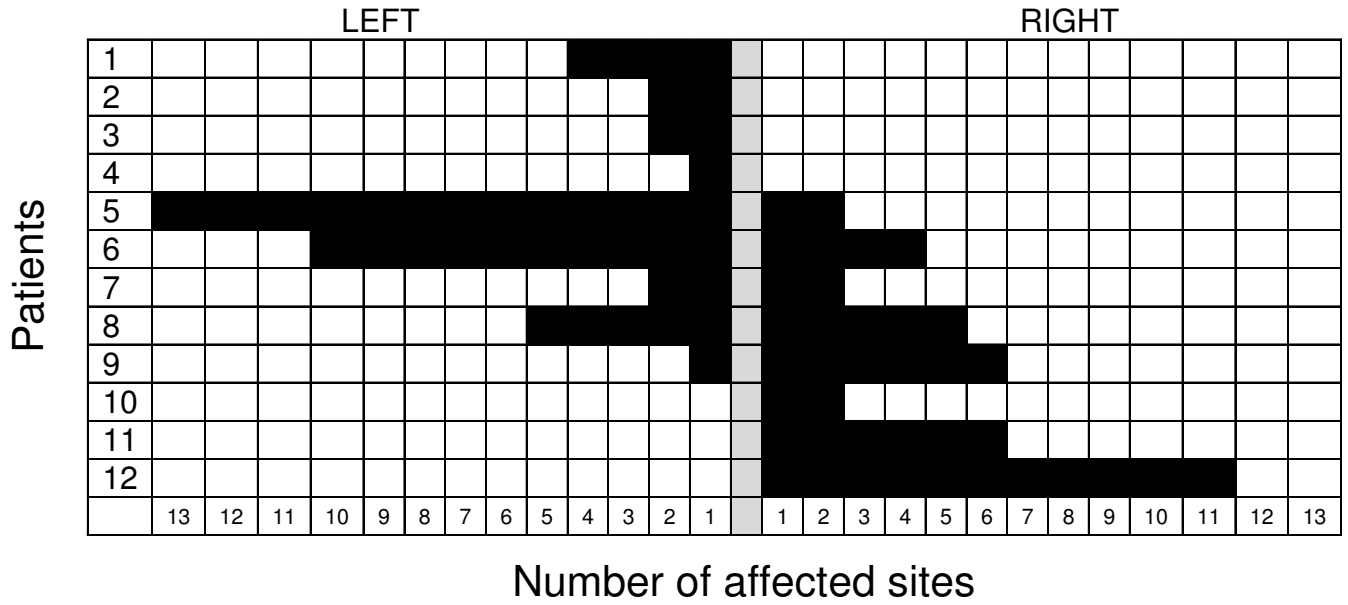
Lateralization of POH lesions



- 83% had a lesion bias toward one side or the other
- 58% (7/12) showed exclusive lateralization

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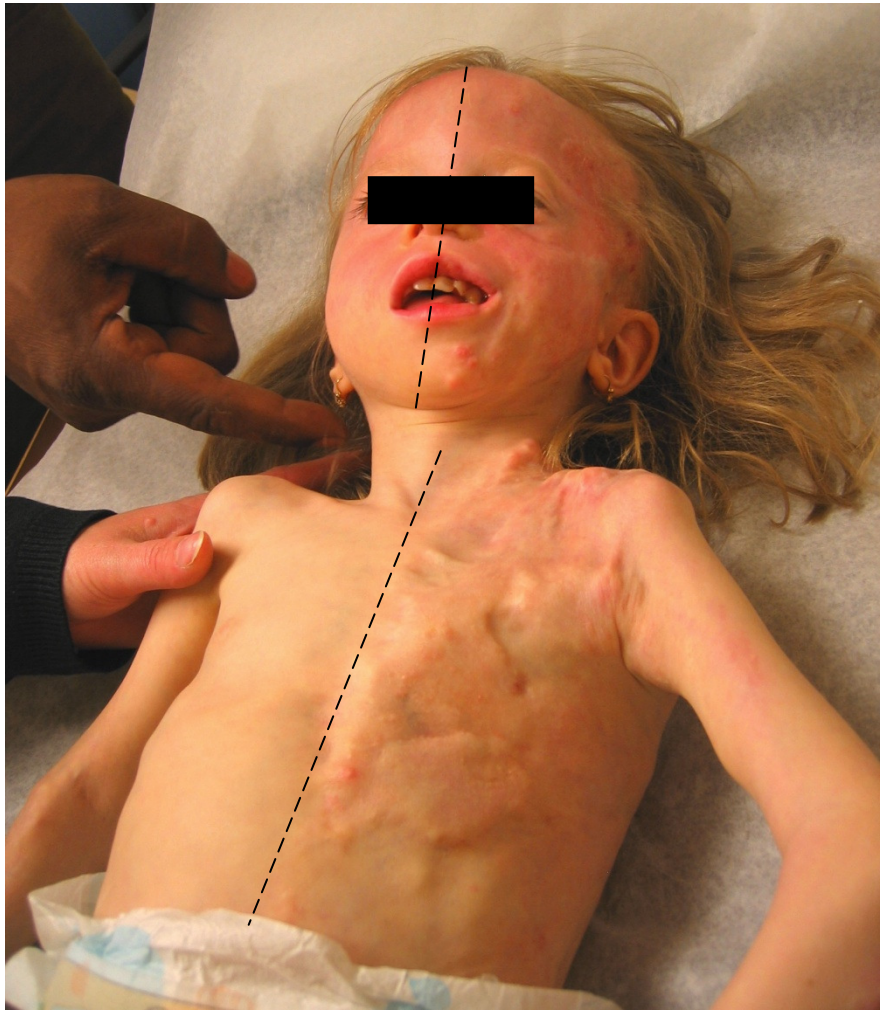
Preferential sidedness in POH



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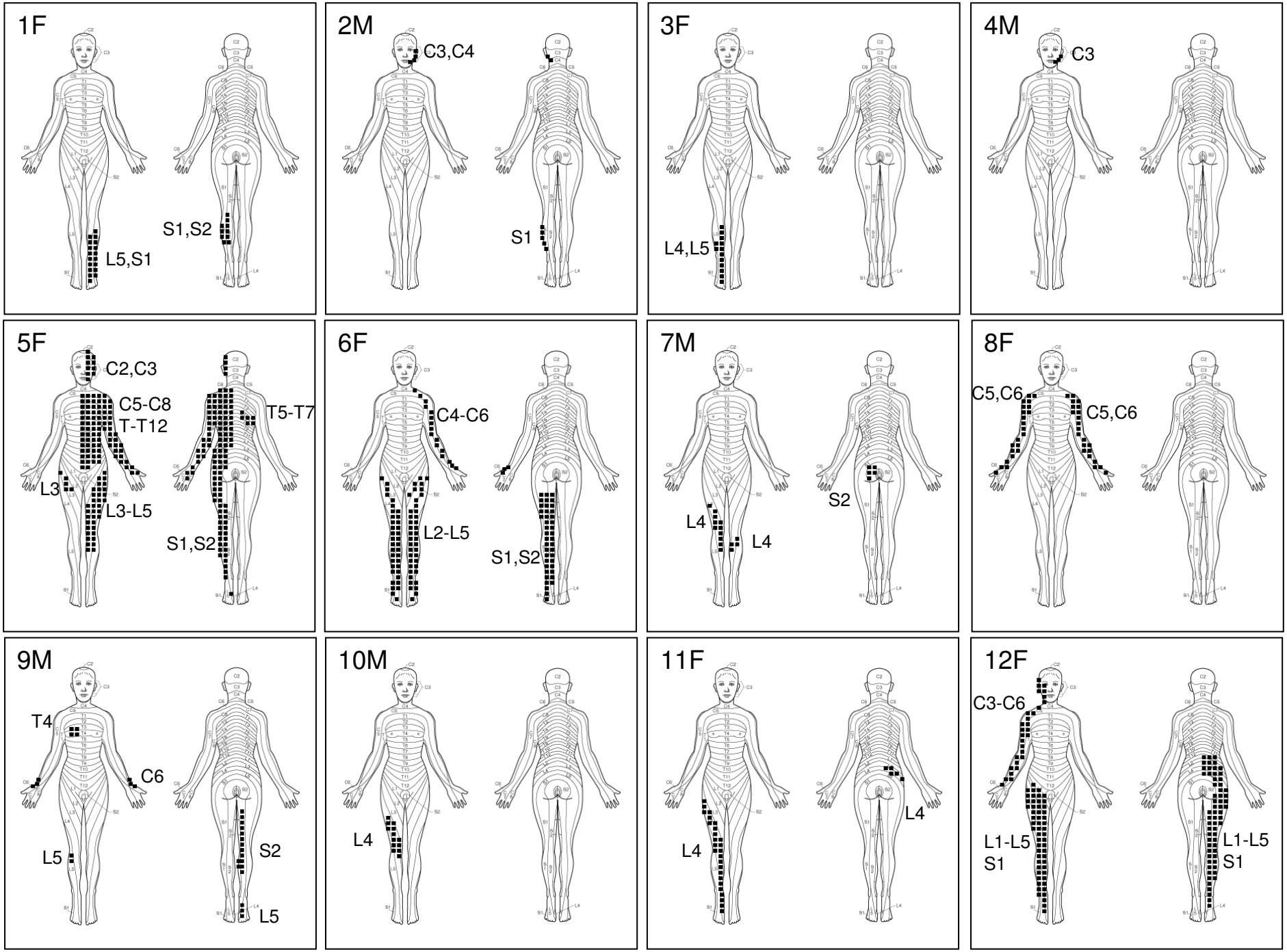
1F
L5,S1
S1,S2
2M
C3,C4
S1
3F
L4,L5
4M
C3
5F
C2,C3
C5-C8
T-T12
L3
L3-L5
T5-T7
S1,S2
6F
C4-C6
L2-L5
S1,S2
7M
L4
L4
S2
8F
C5,C6
C5,C6
9M
T4
C6
L5
S2
L5
10M
L4
11F
L4
L4
12F
C3-C6
L1-L5
S1
L1-L5
S1

Implications for mosaicism in POH

- Involvement of an early mesenchymal progenitor cell, neural crest-derived precursor, or niche cell with stem cell interaction
- Presence of somatic mutations or random inactivation of the second *GNAS* allele in somitic progenitor cells
- Possibility of revertant mosaicism in uninvolved dermatomyotomes, or in patients with *GNAS* mutations and no apparent or very limited disease

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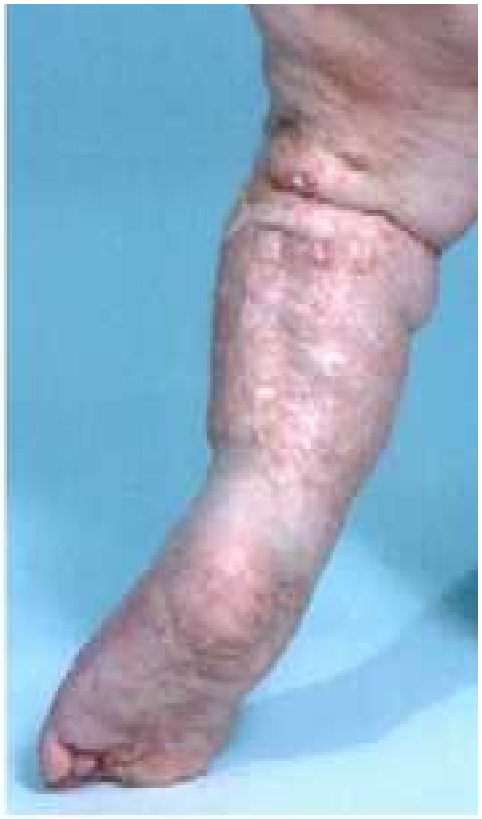

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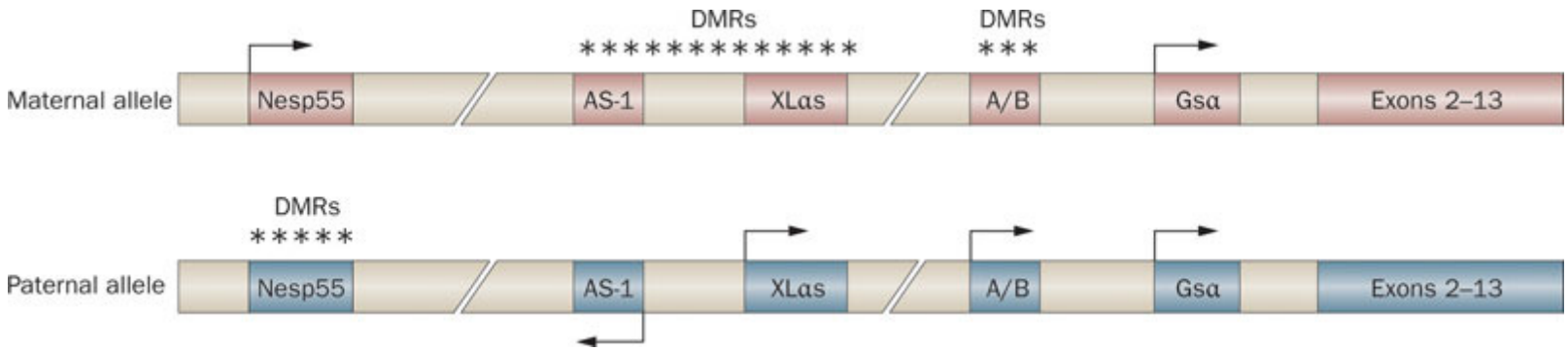
GNAS encodes multiple transcripts



Shore EM & Kaplan FS Nat Rev Rheumatol 6: 518-527 (2010)

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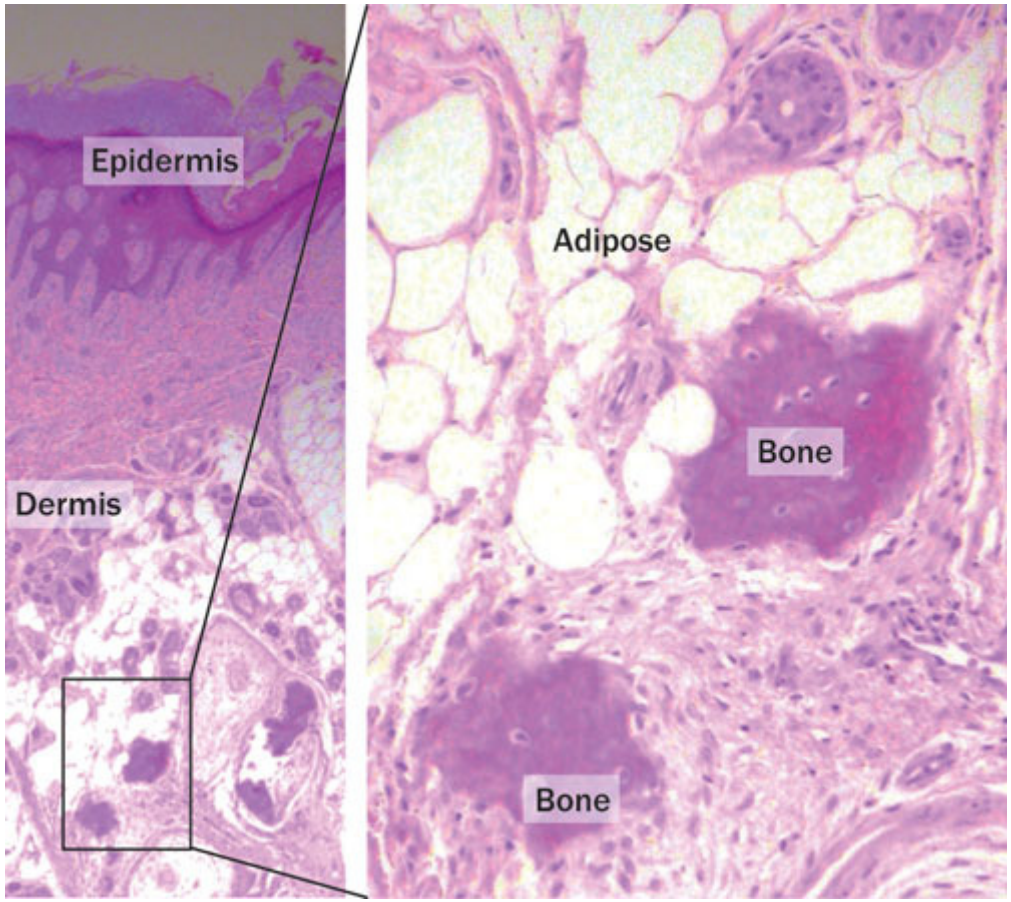
Bone formation in POH



Shore EM & Kaplan FS Nat Rev Rheumatol 6: 518-527 (2010)

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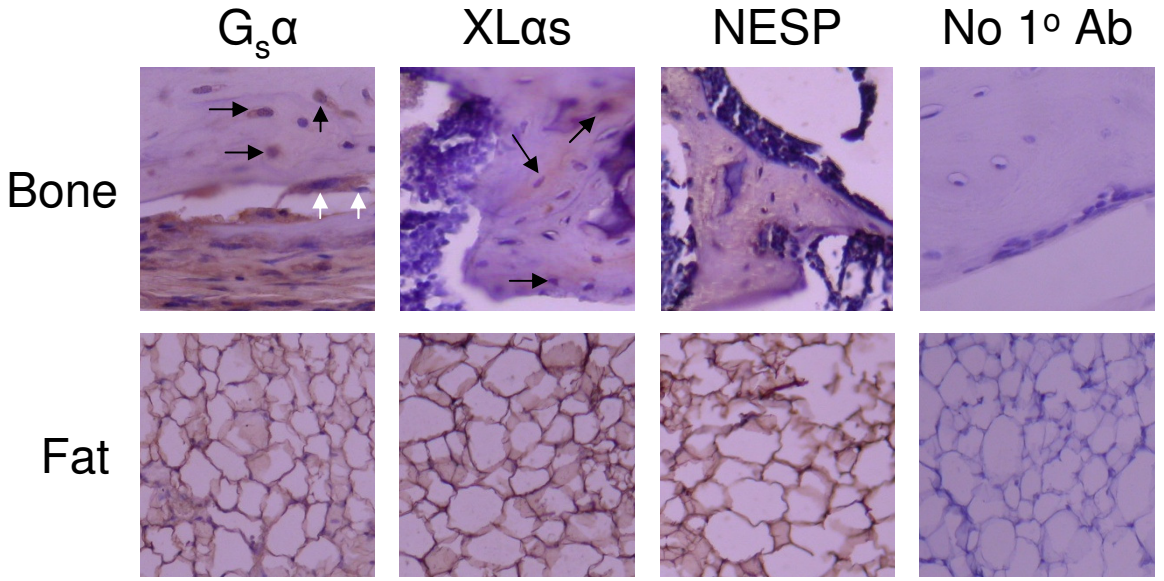
Expression of *Gnas* protein products in bone and fat



Pignolo et al, Submitted (2011)

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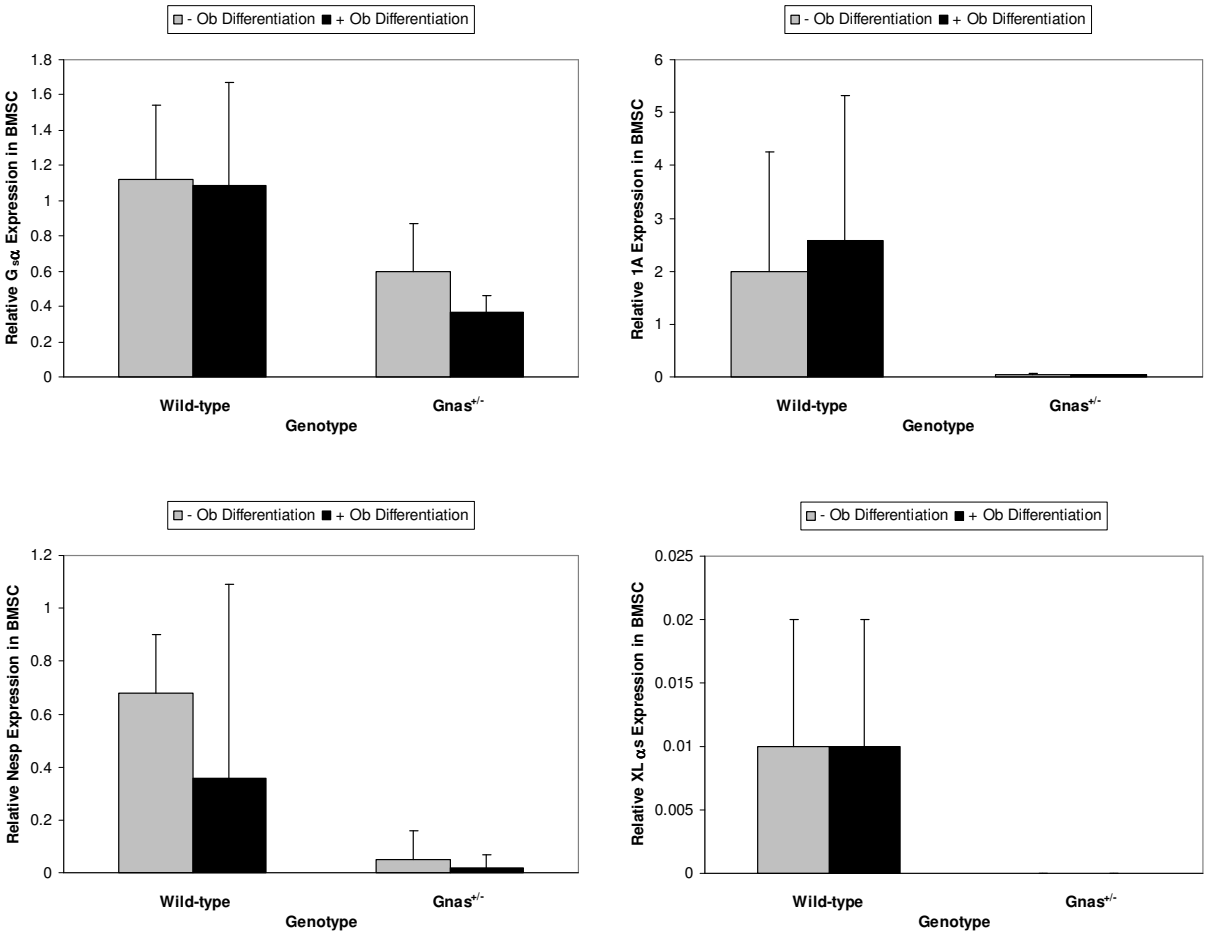
Expression of *Gnas* transcripts in bone marrow stromal cells (BMSCs)



Pignolo et al, Submitted (2011)

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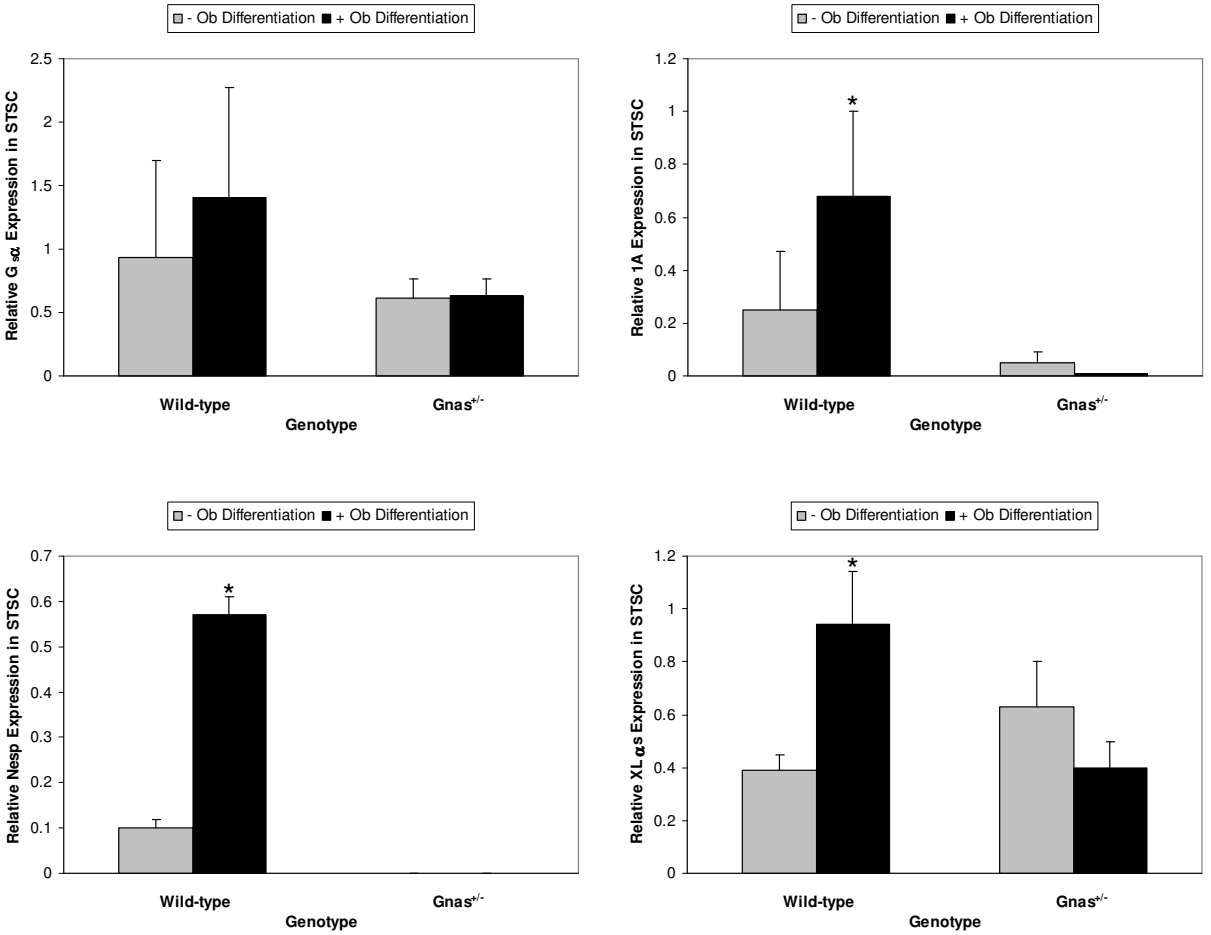
Expression of *Gnas* transcripts in adipocyte soft tissue stromal cells (STSCs)



Pignolo et al, Submitted (2011)

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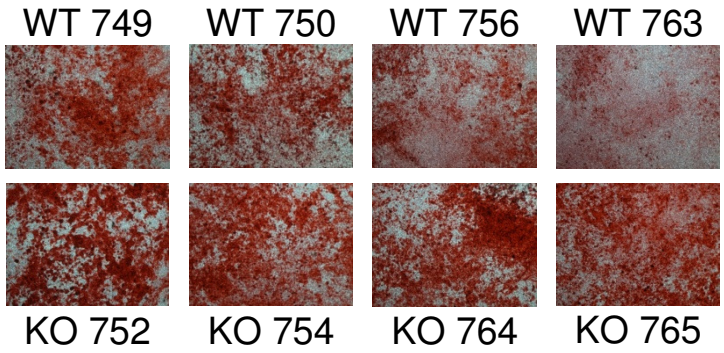
Accelerated osteoblast differentiation in STSCs from *Gnas*$^{+/-}$ mice

A



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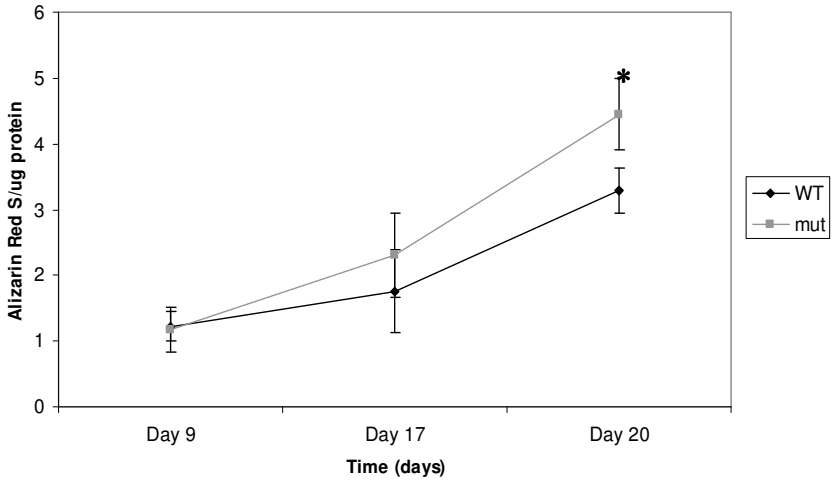

Pignolo et al, Submitted (2011)

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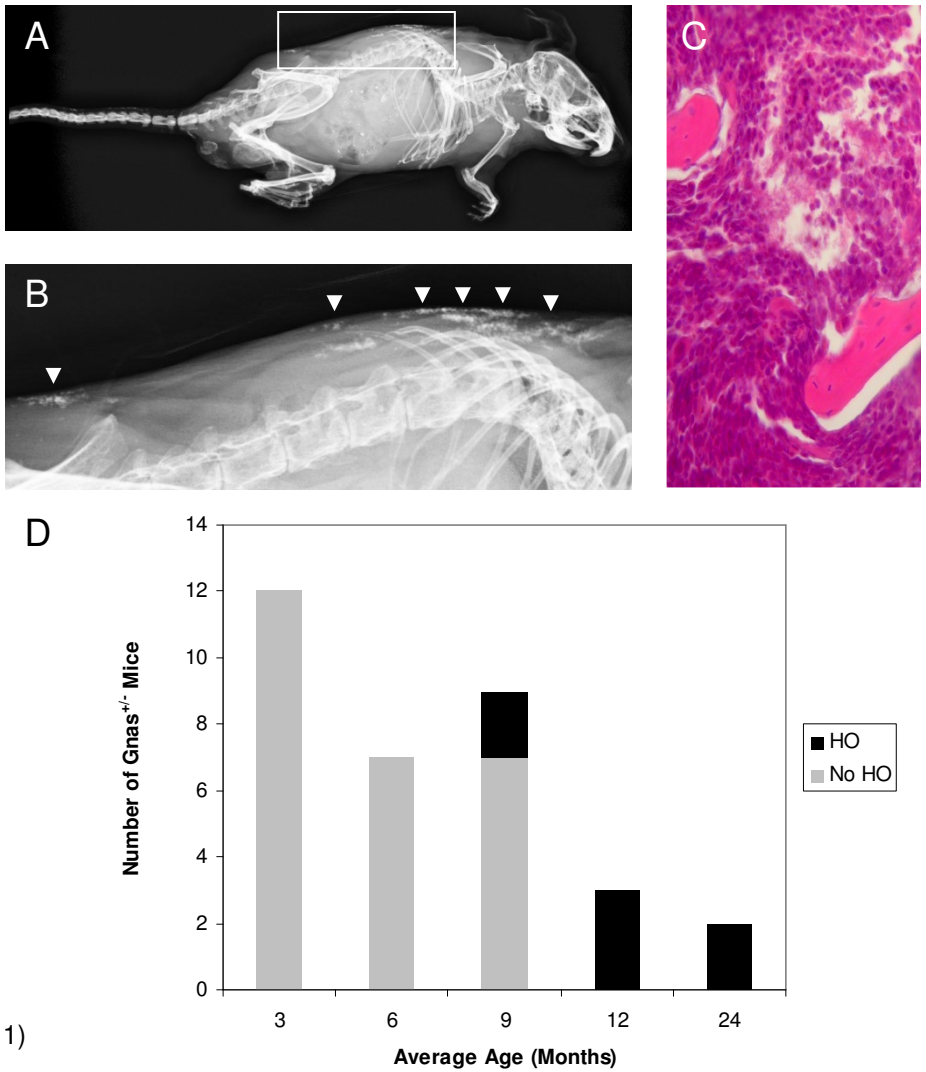
Gnas$^{+/-}$ mice develop HO



Pignolo et al, Submitted (2011)

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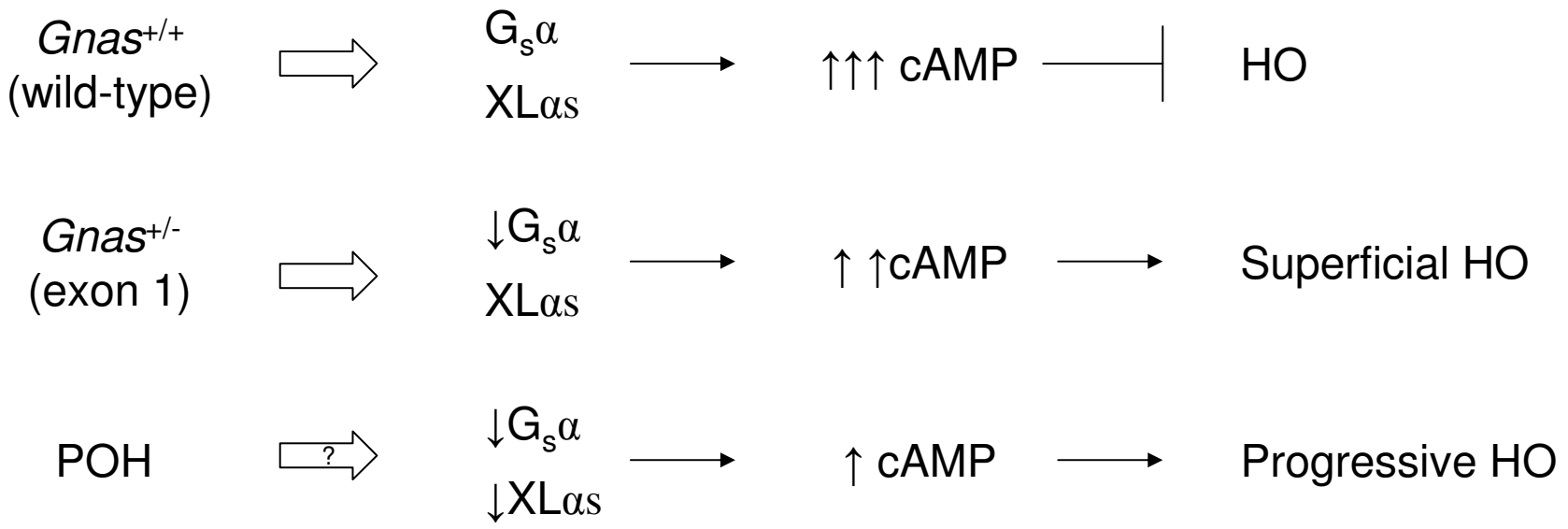
Regulation of bone formation by *GNAS*/*Gnas*



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Summary

- Unlike other *GNAS*-based disorders of HO, POH occurs in the absence of multiple features of AHO or hormone resistance
- POH lesions lateralize in a dermatomyotomal pattern, suggesting either a second inactivating mutation in *GNAS* in a progenitor cell or a de novo mutation in a related gene that normally functions in a GNAS-interacting pathway
- GNAS is a key factor which regulates cell fate decisions in fat-derived stem cells

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