UK FOP patient ACVR1 mutations and their consequences

James T Triffitt

Botnar Research Centre

University of Oxford

SARAH CAMERON

Dr Roger Smith

Sarah Cameron Research Fund

Oxford Thinking

The Campaign for the University of Oxford

UNIVERSITY OF OXFORD

Medical Sciences Division

Supporting research into Fibrodysplasia Ossificans Progressiva (FOP)

‘University of Oxford FOP Research Fund’

fopaction+

Fibrodysplasia Ossificans Progressiva

http://www.fopaction.co.uk/

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*Professor James Triffitt
*Dr Roger Smith
*Professor Paul Wordsworth

UK FOP PATIENTS

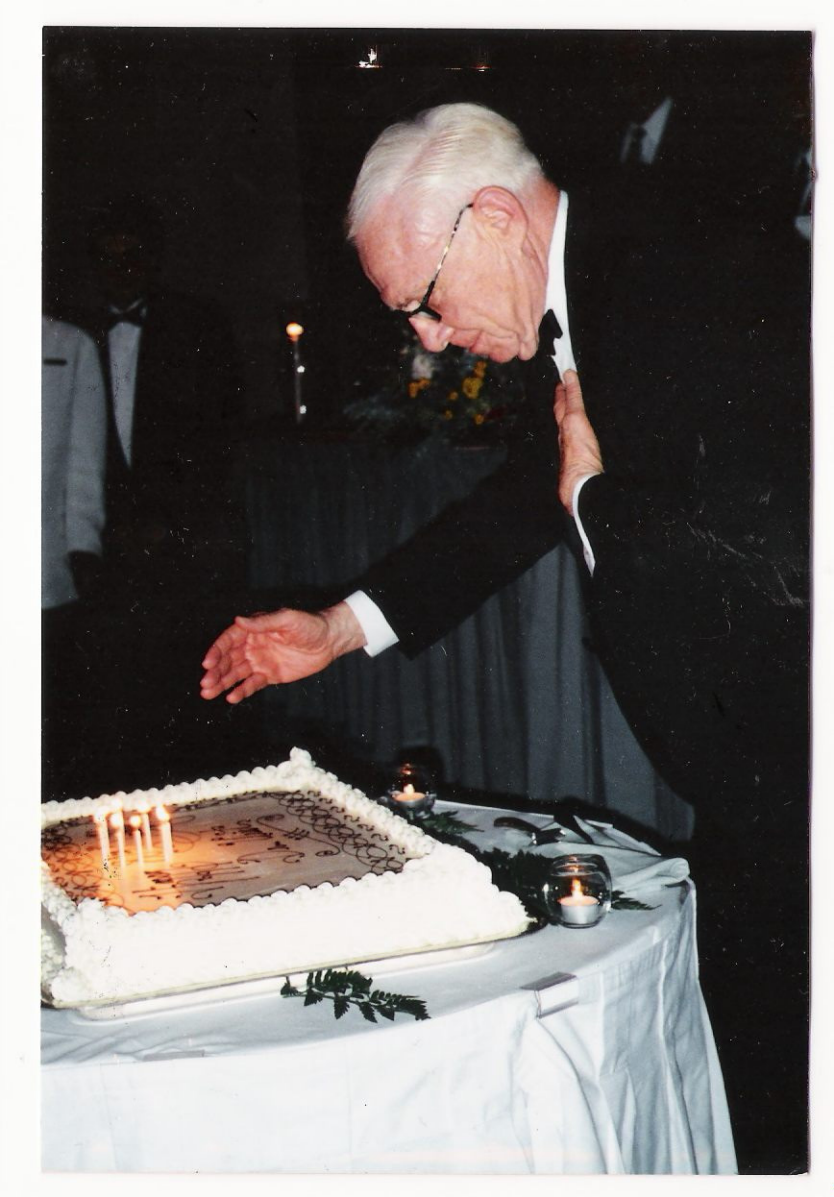
- There are fewer than 40 affected individuals in the UK
- Support for research into FOP is limited to fund-raising by patients, families and friends
- Little support from public funding agencies

Study of Rare Diseases

- However, rare diseases provide fundamental knowledge to understand pathogenesis of other more common disorders

- Further knowledge of the underlying mechanisms that result in FOP may not only improve management of this rare disease but may also throw light on pathogenesis of many disorders that result in altered bone formation from whatever cause.

MARSHALL R. URIST 1994



BONE MORPHOGENETIC PROTEIN

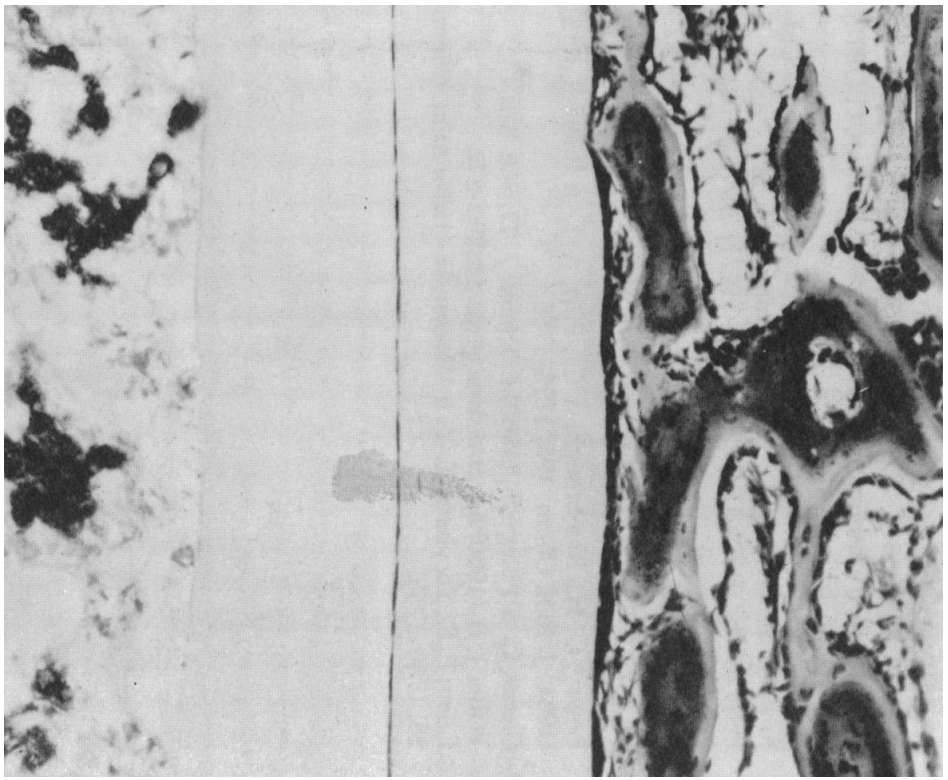
Urist, MR. Bone formation by autoinduction. Science. 1965

“Under the influence of bone morphogenetic protein (BMP), perivascular mesenchymal-type cells (pericytes) differentiate into cartilage and woven bone.” Urist et al Proc. Natl. Acad. Sci. USA, Vol. 81, pp. 371-375, January 1984

Experimental myositis ossificans: cartilage and bone formation in muscle in response to a diffusible bone matrix-derived morphogen.

M R Urist, M Nakagawa, N Nakata, H Nogami

Arch Pathol Lab Med. 1978 Jun ;102 (6):312-6



DISCOVERY OF THE FOP GENE

A Dramatic, Essentially International, Collaborative Discovery

A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva

Nature Genetics 38: 525-527, 2006

Eileen M. Shore, Meiqi Xu, George J. Feldman, David A.Fenstermacher, Tae-Joon Cho, In Ho Choi, J. Michael Connor, Patricia Delai, David L. Glaser, Martine LeMerrer, Rolf Morhart, John G. Rogers, Roger Smith, James T. Triffitt, J. Andoni Urtizberea, Michael Zasloff, Matthew A. Brown, Frederick S. Kaplan



Consortium Group Members

Philadelphia, Washington DC, USA.
Oxford, Glasgow, UK
Seoul, Republic of Korea.
São Paulo, Brazil.
Paris, France.
Garmisch-Partenkirchen, Germany.
Melbourne, Queensland, Australia.
Hendaye, France.

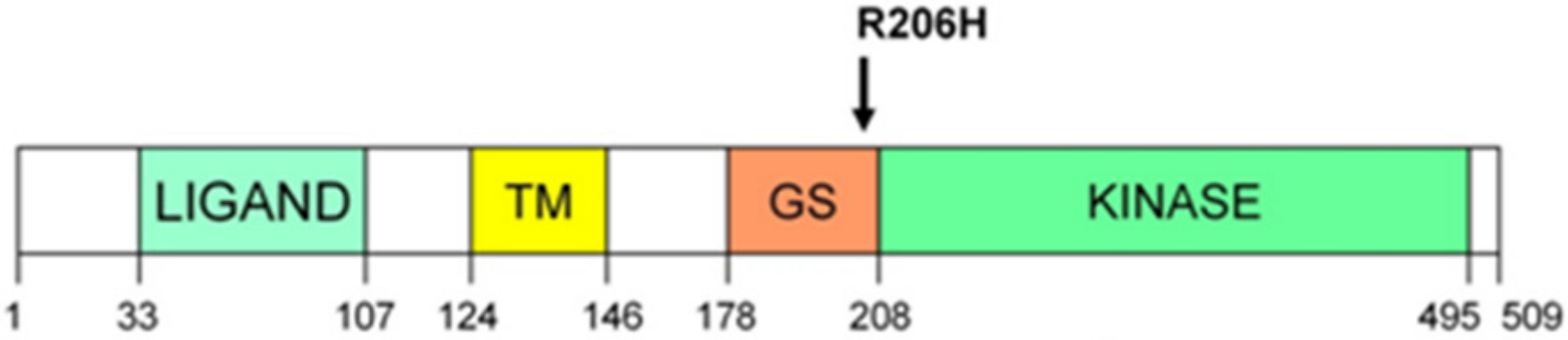
FOP Gene

- Discovery the ACVR1 gene on Chr 2q23-24 harboured FOP mutation in most patients Shore et al (FOP Consortium, Nat Gen. 2006)

FOP Gene

- FOP most commonly arises from an activating *ACVR1* exon 6 mutation (c.617G>A). Substitutes arginine to histidine (R206H)

- Encodes the glycine/serine (GS)-rich domain of the BMP I receptor, which diminishes interaction with the negative regulator FKBP12 (FK506 Binding Protein 12kDA)



FOP Gene

- Many groups screened all clinically diagnosed FOP patients for ACVR1 exon 6 mutation c.617G>A (p.R206H) mutation.

- UK Cohort of 18 available patients - one UK family included in Consortium patient group. However, 2 separate patients did not show exon 6 ACVR1 p.R206H mutation (sequence analysis and RFLP).

- Later an additional patient was found to also have a unique mutation.

Methods

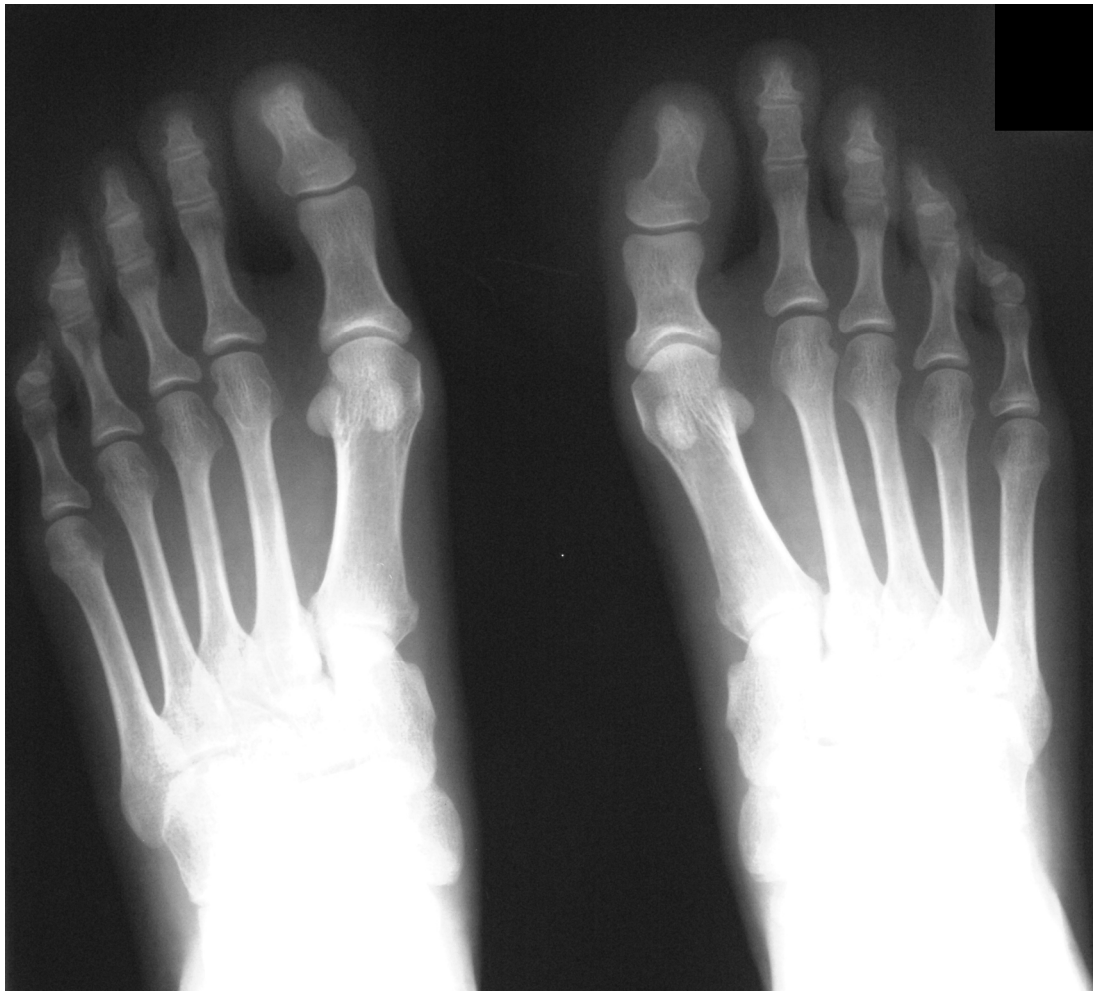
- DNA extraction from blood
- *ACVR1* exon 6 was amplified
- DNA was sequenced bi-directionally

  (Big Dye 3, automated sequencer ABI 3100, Applied Biosystems)
- Subsequently primers were designed for all 11 exons of *ACVR1*
- 100 healthy controls were also screened

Patient 1

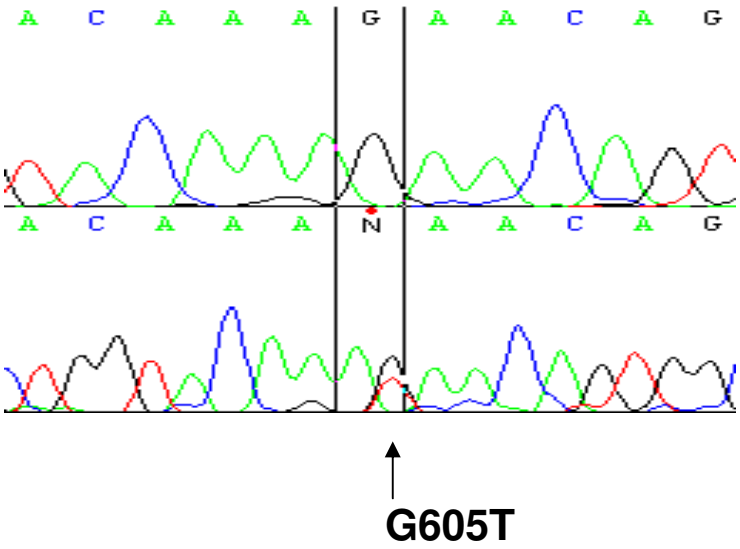
- A 23 year old female
- Diagnosed with FOP in 2003 aged 14 years.
- First presentation was painful bony lump right scapula after a fall.
- Subsequently developed multiple tender bony swellings.
- Detection of unilateral right great toe abnormality-other normal.
- Steroid treatment caused symptomatic improvement during flares, but did not alter the course of disease.
- Patient continued to have frequent flares of the condition with increased inflammatory lesions over her shoulder joints, neck, and jaw, with neck fusion occurring within 6 months of presentation. There was no response to steroids or intravenous pamidronate.
- Right shoulder fixed in internal rotation. Fixed flexion deformities of both elbows were present and lumbar spinal movements restricted.

Patient 1

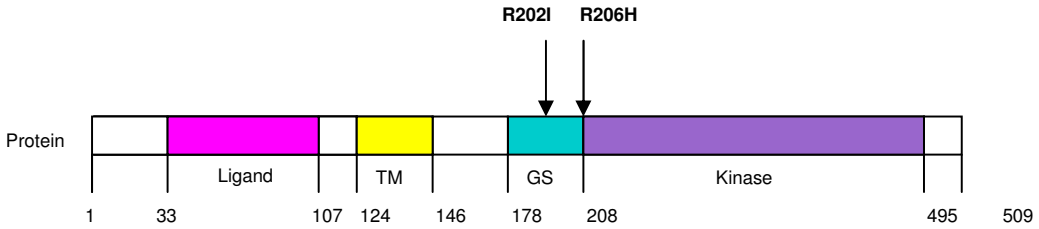


Radiographs of feet

Patient 1



Electropherogram data from sequence analysis of *ACVR1* gene in Patient 1. c.605G>T identified in exon 6 of *ACVR1* gene and corresponds to mutation of arginine to isoleucine at position 202 (p.R202I) in the ACVR1 protein.



ACVR1 PROTEIN

UK ACVR1 FOP MUTATIONS

Patient 2

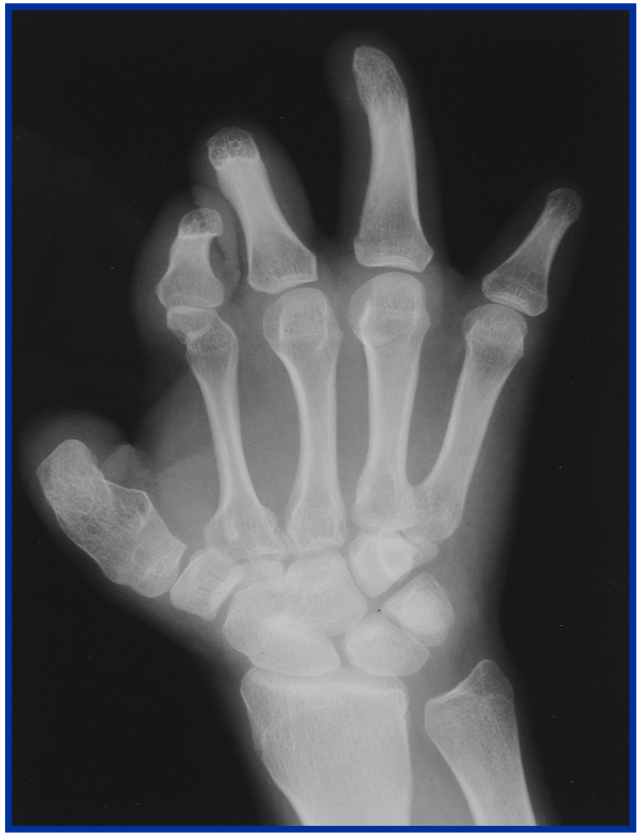
•55 year old female FOP diagnosed in 1956.
•Severe reduction deformities in all digits at birth.
•First presentation with lumps, usually painful, on the occiput.
•At 6 years had stiff spine and shoulders.
•At 14 years both elbows and right hip showed ectopic ossification.
•At 18 years left hip showed ossification.
•At 20 years jaw showed ossification after dental extraction.
•At 26 years presented complete spinal fixation, shoulders fixed in adduction, elbows fixed in flexion, hips restricted, fixed in slight flexion, jaw gape was 0.3cm.

Patient treated with disodium etidronate (EHDP) from 18 years to 22 years. Normal ECG. Appendicectomy at 24 years-normal scar. Mild questionable mental retardation. Diffuse scalp hair thinning since 14 years.

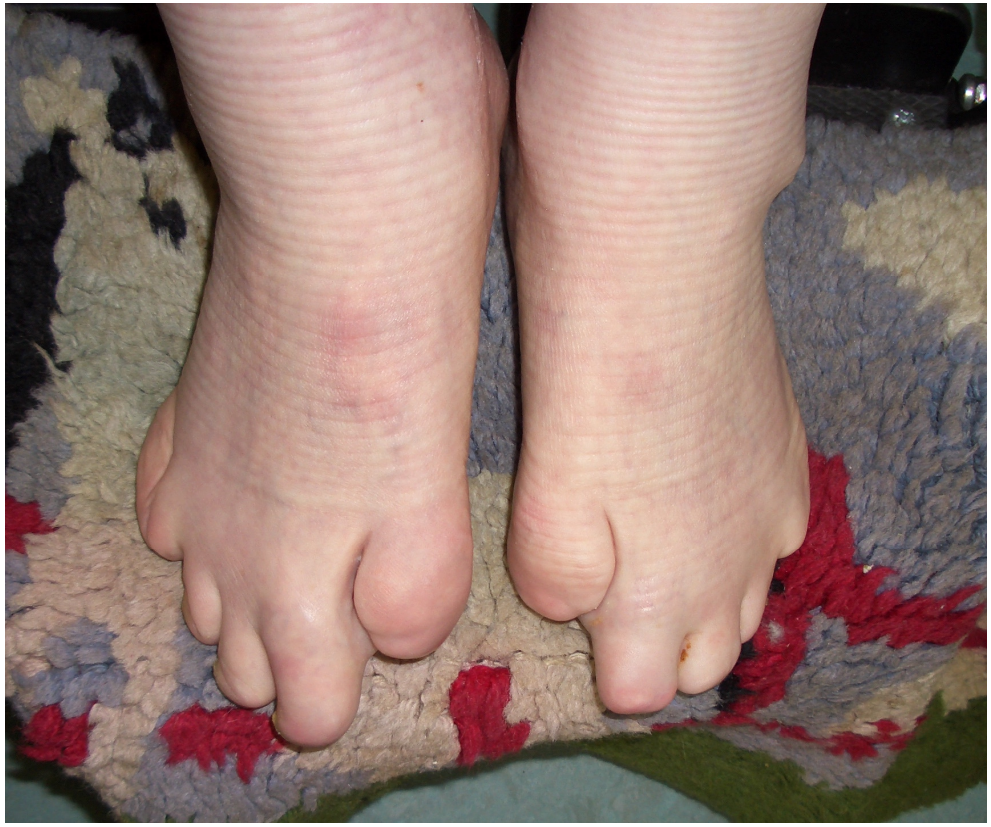
•Presented clinical features outside 'typical' FOP
Smith et al (1976)

Connor and Smith (1982)

Patient 2



Radiographic image of hand

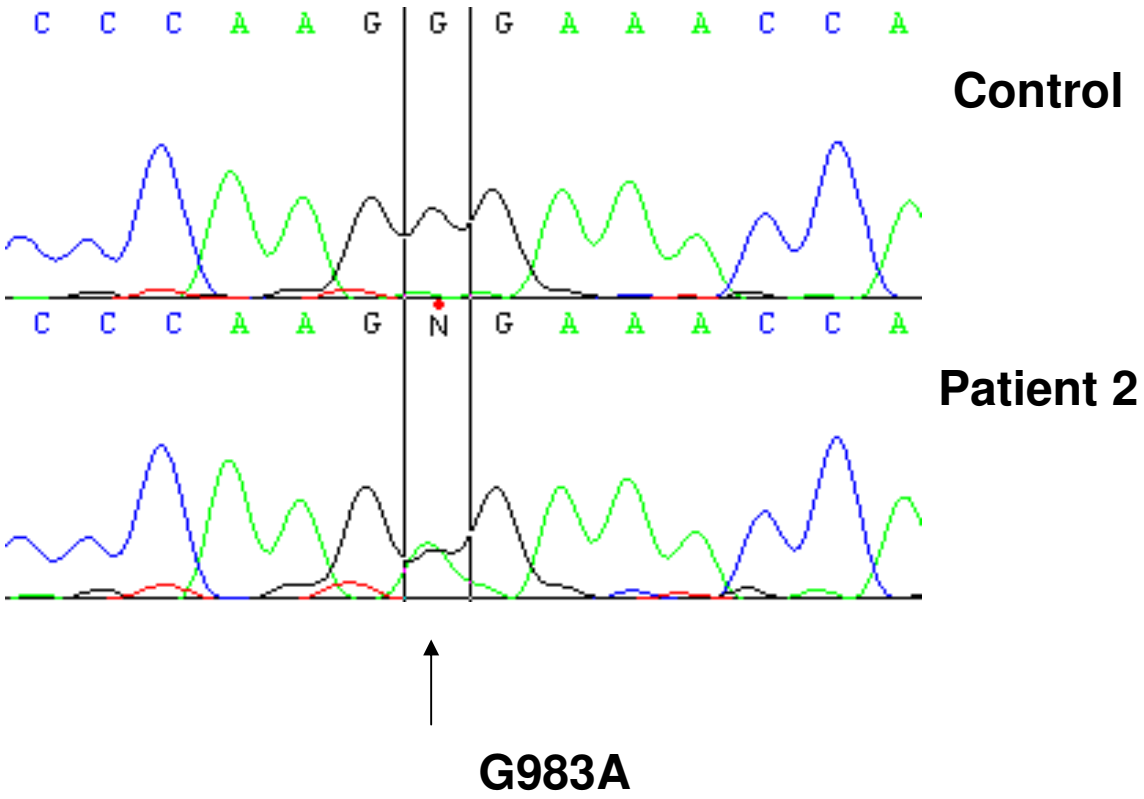


Photograph of feet

All digits severely shortened.

Hand radiograph shows the absence of the distal and intermediate phalanges and some shortening of the proximal phalanges

Patient 2



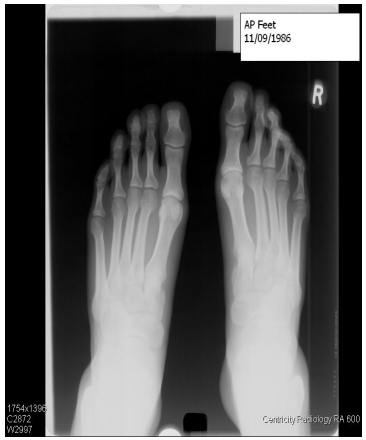
Electropherogram data from the sequence analysis of the *ACVR1* gene in patient 2. c.983G>A identified in exon 8 of *ACVR1* and corresponds to amino acid mutation from glycine to glutamate at position 328 (p.G328E) in kinase domain of ACVR1 protein.

UK ACVR1 FOP MUTATIONS

Patient 3

Clinical Case: 45 year old woman

No family history of musculoskeletal disease–unremarkable childhood. Bilateral 5th finger camptodactyly, but no other toe or finger abnormalities



Age 21 – Motorbike accident

- 2 months later trismus & surgical release of
- medial pterygoid muscle

- Postop inflammation threatened airway
- prompting tracheostomy until swelling subsided

- 3 weeks after right thigh intramuscular analgesia had been given;
  - tender soft tissue swelling (18x13cms)
  - Bone scan: marked up-take in thigh soft tissue
  - Restricted knee flexion, gradually alleviated by massage

Gregson CL et al. Bone 2011

Patient 3

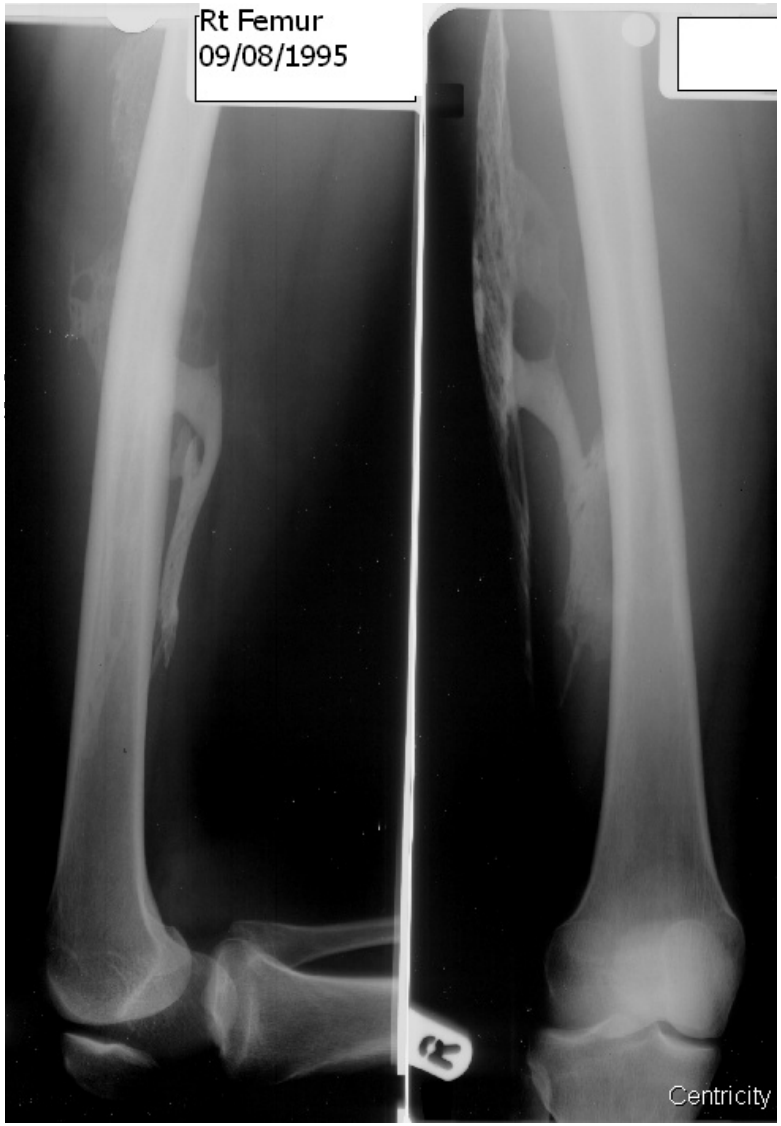
Age 31, 38 weeks pregnant

Emergency caesarean section with spinal anaesthetic

Intramuscular left thigh injections

- Tender inflammation in left thigh
- Swelling and pain persisted
- Restricted hip & knee movement

- No ossification developed in caesarean scar, nor at spinal injection site



Patient 3

Age 42 – Car accident, rear impact, wearing seatbelt

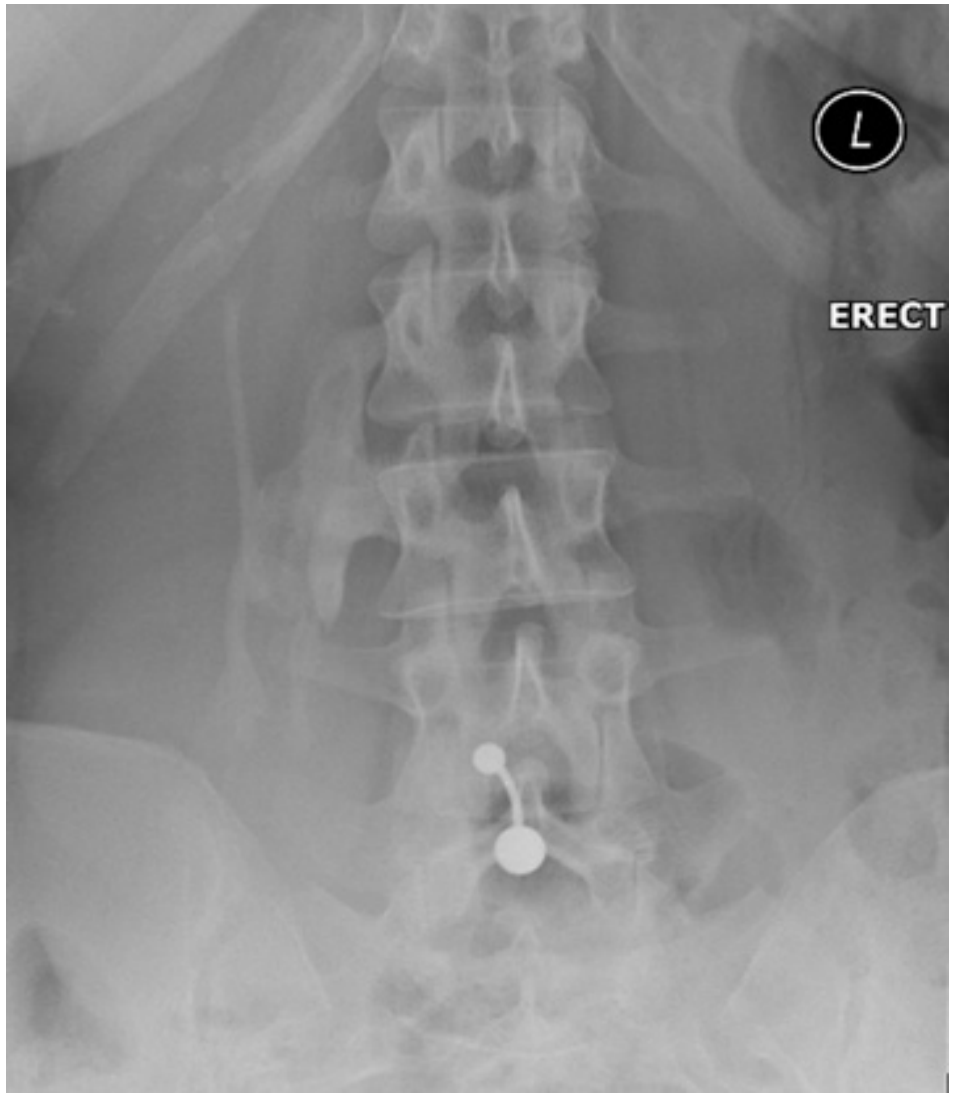
- 1 week later
  - cervical, thoracic & lumbar spine pain
  - partly eases over 6 weeks

  X-rays: cervical spondylosis and suggestion of early ossification between facets joints (not visible 11 years earlier)

- Representation 7 months later;
  - abdominal wall, right flank, shoulder & breast pain
  - CT scan: diffuse swelling without ossification in right abdominal wall

Patient 3

- X-rays 1 year later: ossification of lumbar paraspinal muscles
- USS: ossification in paraspinal muscles, with oedema in paralumbar muscle & right breast



Patient 3

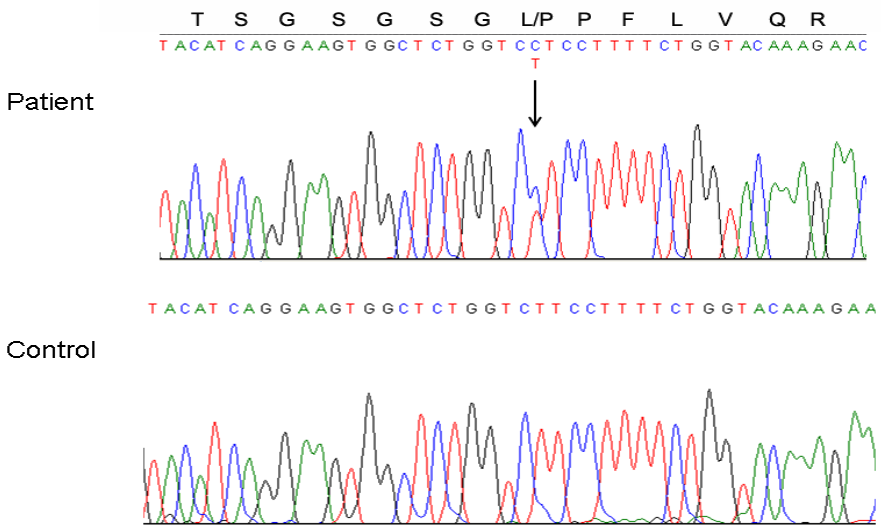
Currently at age 45

Examination

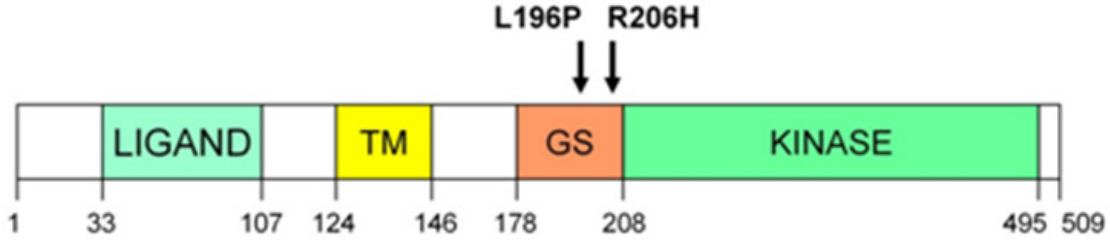
- 2 tattoos, 2 body-piercings, tracheostomy & caesarian scars and abdominal wall all without ossification
- Ossification of submandibular scar
- Non-tender bony masses palpable deep in both thighs & the right lower back
- Following physiotherapy & hydrotherapy, she walks 600m unaided, swims 1500m 3x/week, drives a car, but remains unable to return to work

Patient 3

Sequencing results



Heterozygous novel c.587T>C mutation in the GS rich domain of *ACVR1* exon 6 which substitutes a conserved leucine with proline at residue 196 in the GS domain of the ACVR1 protein(L196P)



Classic and Atypical FOP Phenotypes

More recently further new mutations in ACVR1 identified and the phenotype reported to vary*

Classic FOP

•Clinical features of FOP - characteristic great toe malformations and progressive heterotopic ossification)

FOP-plus

•Classic defining features of FOP plus one or more atypical features

FOP variants

•Major variations in one or both of the two classic defining features of FOP

****Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. Kaplan et al. Human Mutation 30, 379–390, 2009***

Summary
Patients 1,2,3

Patient 1 (R202I)

23 year old female
Onset of HO and diagnosed with FOP in 2003 aged 14 years.
Unilateral mild right great toe abnormality-other normal.
Continued to have frequent flares of the condition with increased inflammatory lesions over her shoulder joints, neck, and jaw, with neck fusion occurring within 6 months of presentation.

Patient 2 (G328E)

55 year old woman - Severe reduction deformities in all digits at birth - Diffuse scalp hair thinning since 14 years - progressive HO -Mild cognitive impairment

Patient 3 (L196P)

45 year old woman - onset of HO delayed until aged 21-
normal great toes-mild clinical course-preserved reproductive capability-unusually long intervals between traumatic triggers and ossification-mild bilateral 5th finger camptodactaly-early ossification of cervical spine facet joints

The FOP-variant syndrome*

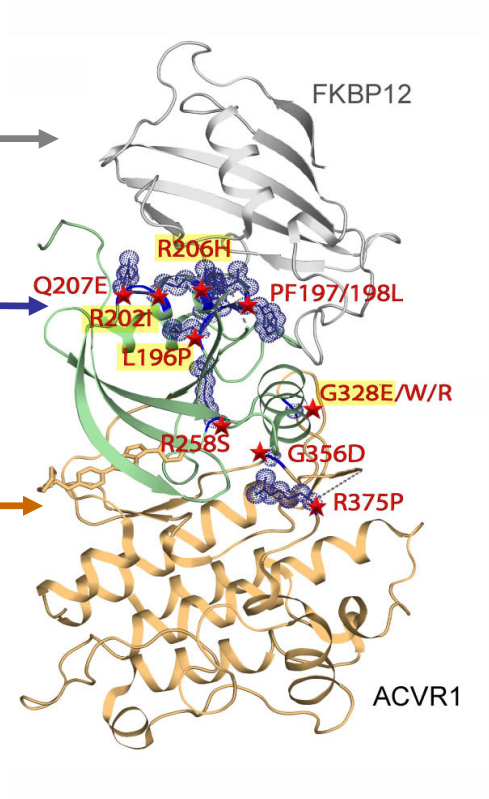
FOP mutations in ACVR1 break bonds that keep the inactive state, leading to weak activation

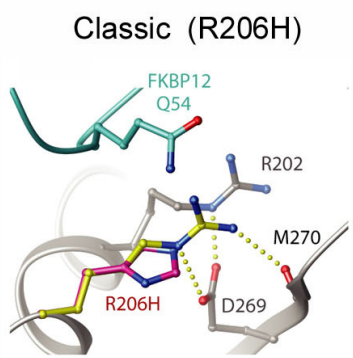
FKBP12 binds the GS domain in the in an inactive state

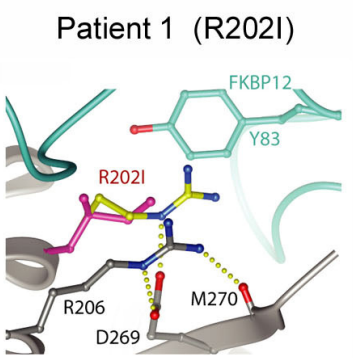
FOP mutation sites

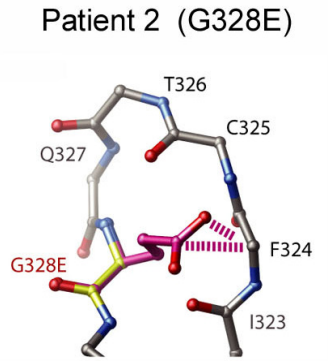
"Dorsomorphin" inhibitor bound to ATP pocket

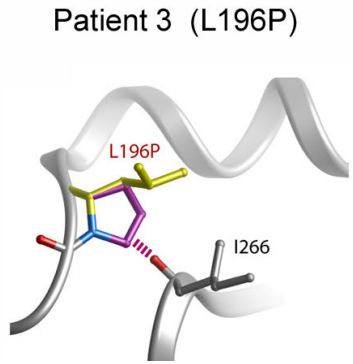
Wild-type kinase locked in inactive conformation











A. Bullock et al. 2011

Acknowledgements

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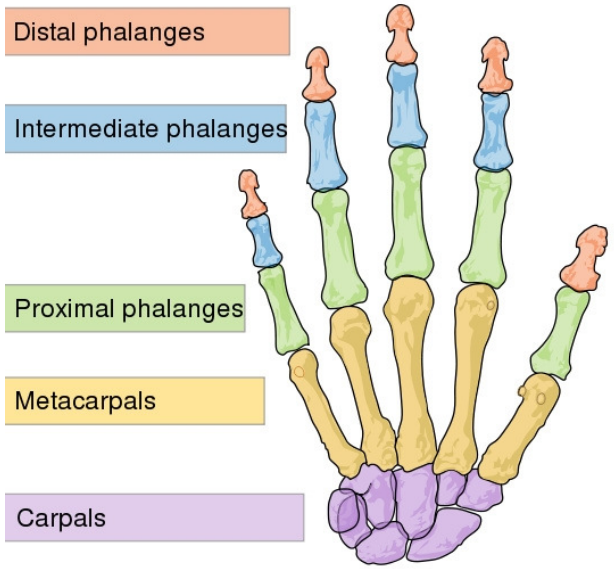
Peter Hollingworth

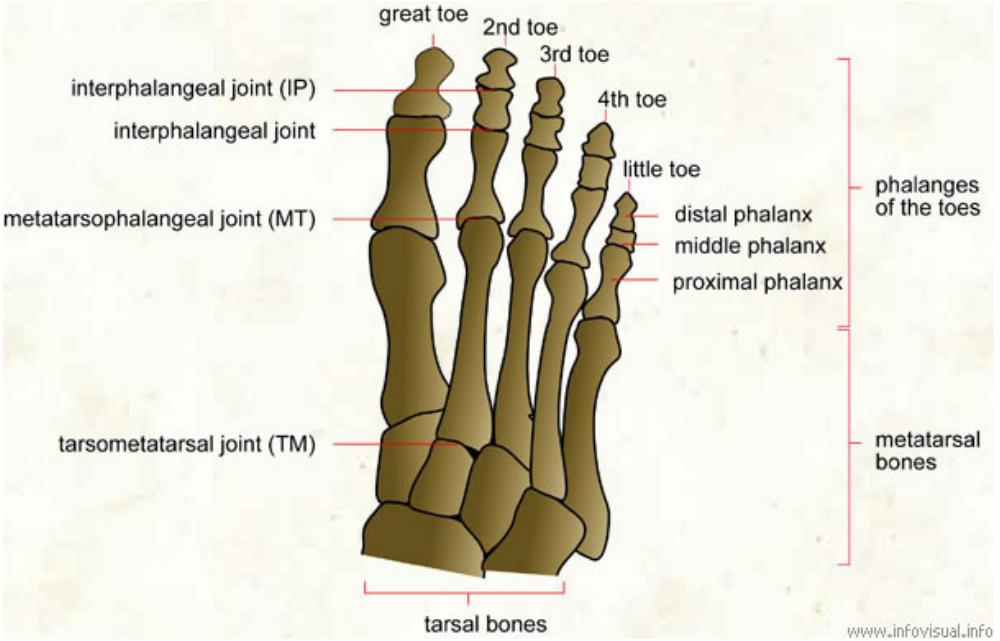
Jon Tobias

fopaction+

Fibrodysplasia Ossificans Progressiva

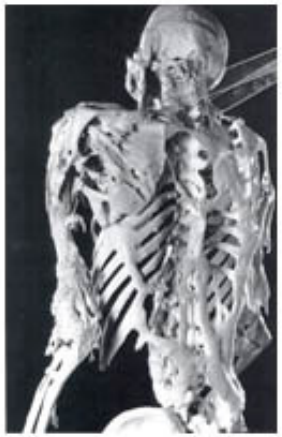
President: Richard Simcox





http://www.wpclipart.com

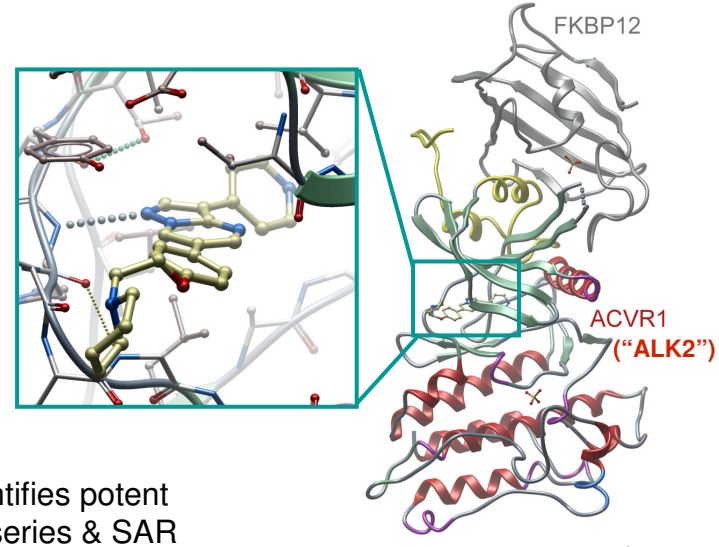
Structure & Inhibition of ACVR1



FOP (Fibrodysplasia Ossificans Progressiva)

Drug target for FOP

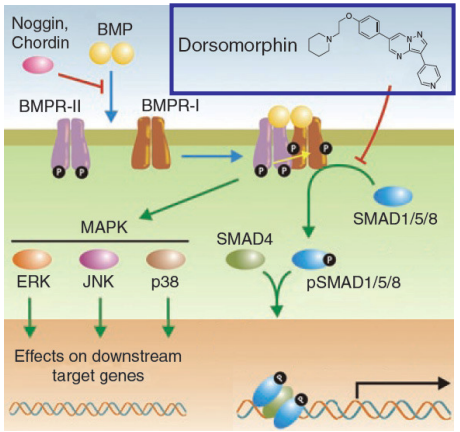
- **Kinase activation** in FOP by R206H mutation
- Muscle turns to bone **(a living statue)**
- No treatment & irreversible
- SGC collaboration with Prof Jim Triffitt
- Found additional R202I and G328E mutations
- SGC structure of ACVR1-FKBP12 complex



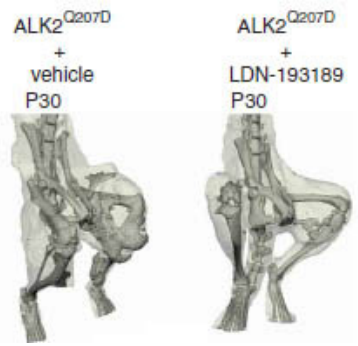
PDB 3H9R (2.35 Å)

Existing chemical probe activity in mouse disease model

SGC identifies potent inhibitor series & SAR (imidazo[1,2-b]pyridazines)



Peterson, Nat Chem Biol. 2008



Nat Medicine 2008