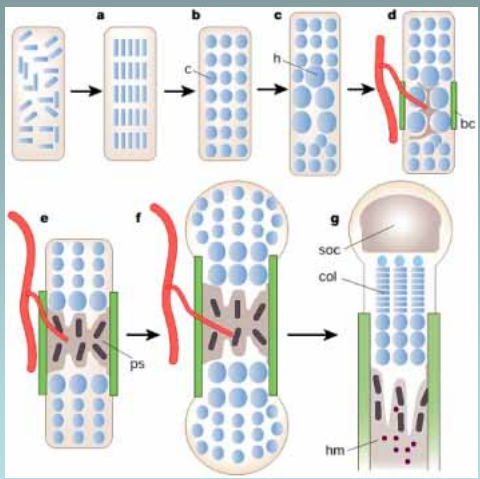
Skeletal dysplasias
Molecular insights

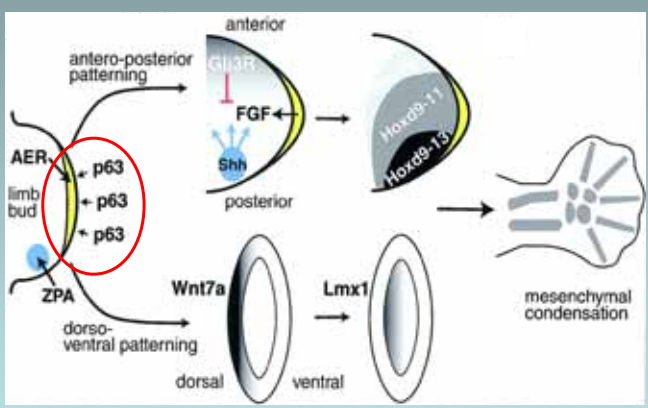
Unravelling the molecular pathogenesis

Endochondral Bone Formation

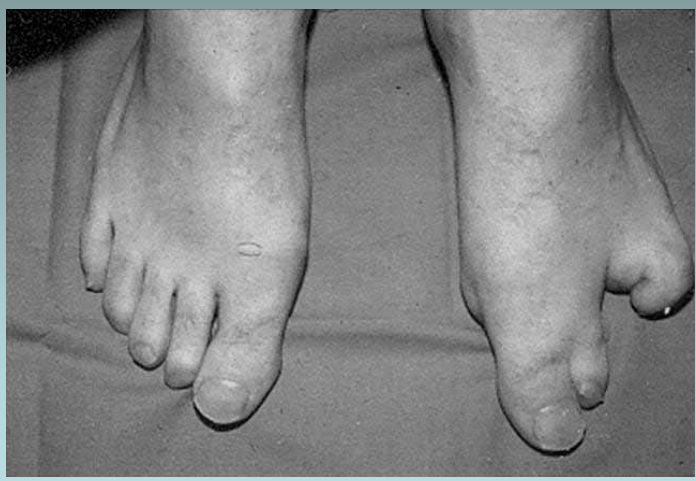
- Limb patterning
- Mesenchymal condensation
- Cartilage differentiation
- Cartilage maturation and hypertrophy
- Vascular invasion and ossification



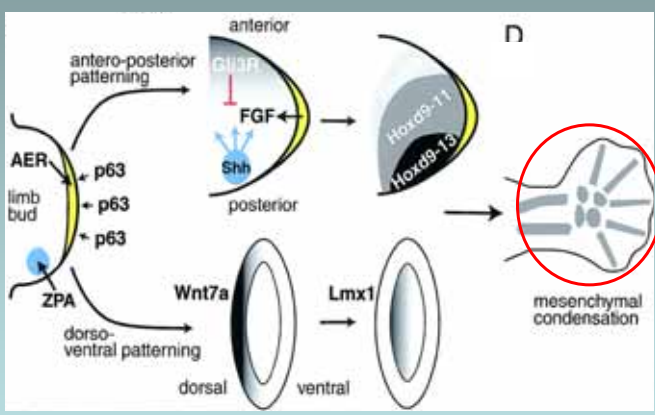
Genetic basis of skeletal diseases



Split hand foot malformation

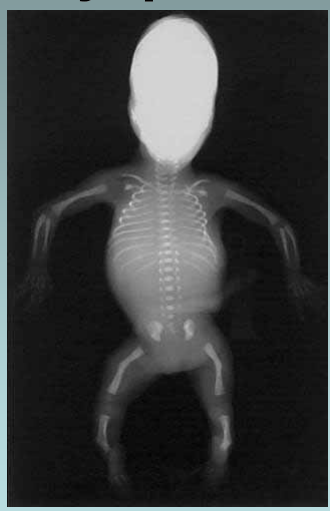


Genetic basis of skeletal diseases

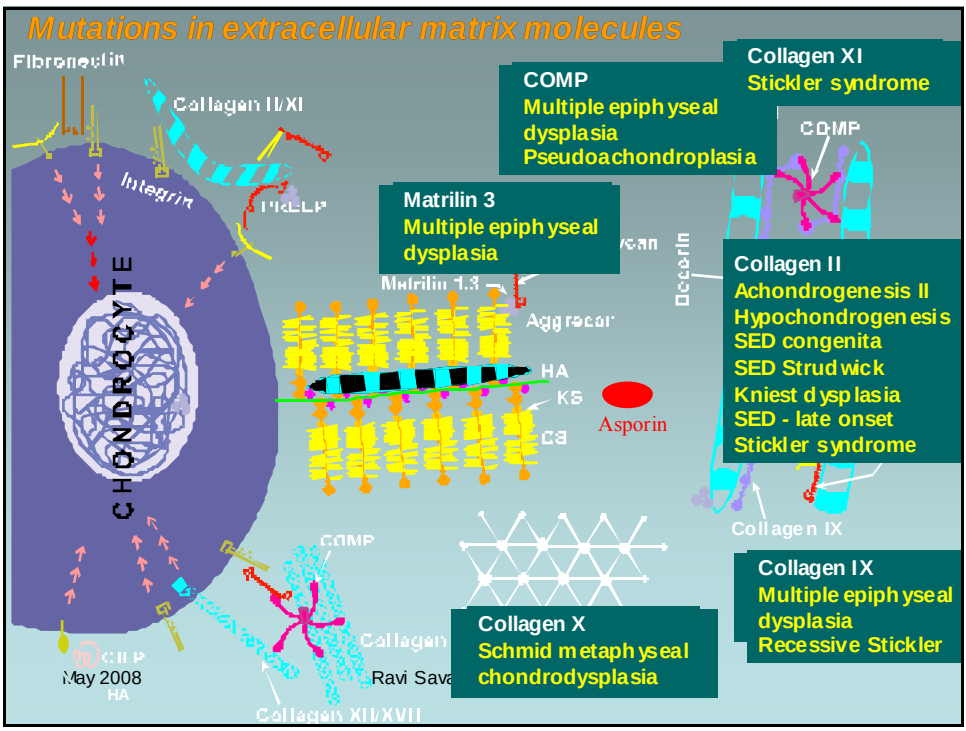


Campomelic dysplasia

- Mutations in Sox-9
- “Master gene” regulating cartilage formation
- Failure in mesenchymal condensation
- Deficiency in cartilage formation

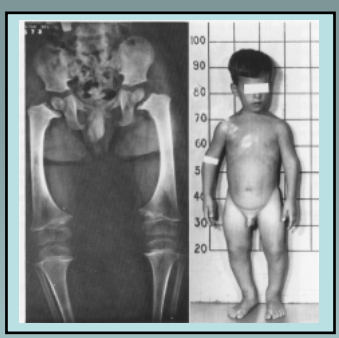


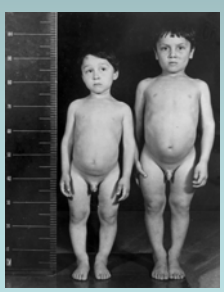
May 2008 Ravi Savarirayan: Bologna



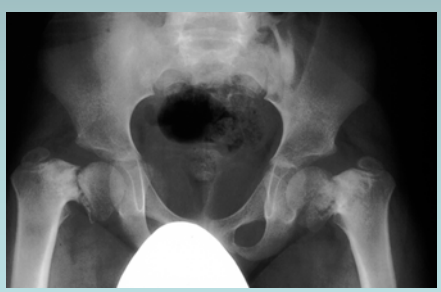
SCHMID METAPHYSEAL CHONDRODYSPLASIA (SMCD, MIM 156500)

- Autosomal dominant bone dysplasia (Schmid, 1949)
- Short stature, bowed legs and waddling gait
- Coxa vara
- Metaphyseal flaring and irregularity
- Variable spinal changes / hand changes





Propositi at age 8 and 6 years. Note short stature and bowed lower limbs.

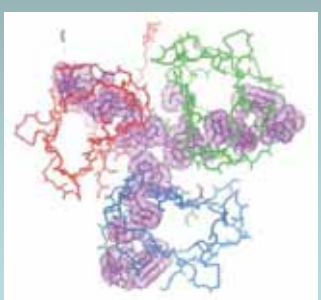


AP pelvis showing metaphyseal irregularity and coxa vara.

Genetic Basis of SMCD

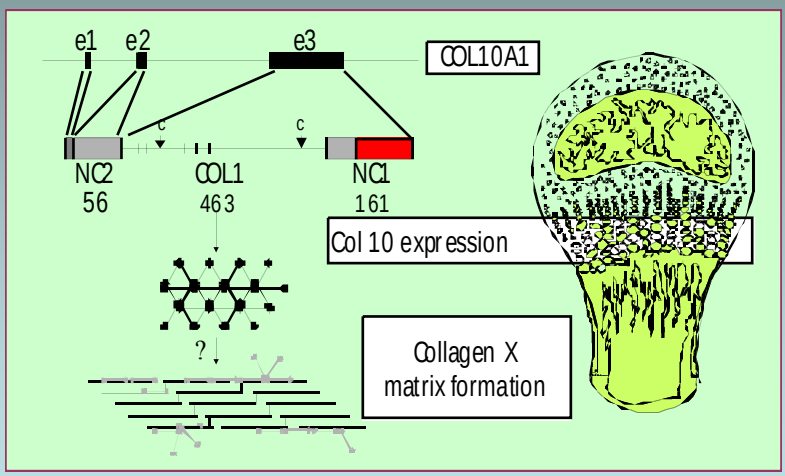
- Due to heterozygous *COL10A1* mutations (Warman et al., 1993)
- 30 missense and nonsense mutations equally represented
- Cluster in C-terminal NC1 trimerization domain
- Effects of missense mutations studied lead to disruption of collagen X trimer formation and intracellular degradation

SMCD missense mutations

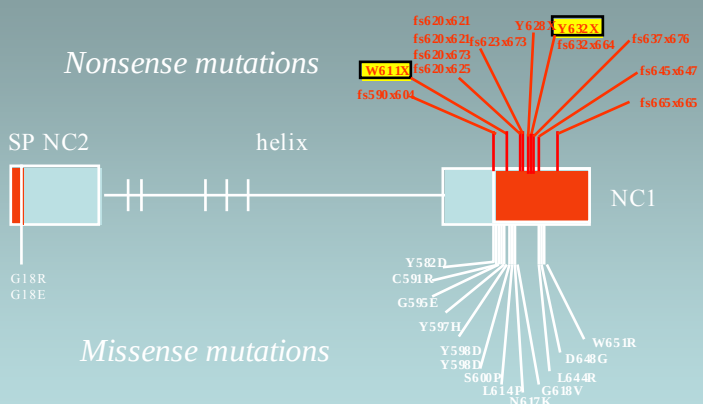


- Mutations **alter 3D structure** of the NC1 trimerization domain
- **Prevent assembly**
- **Prevent secretion**
  - **proteasomal degradation**
- **Functional haploinsufficiency**

COL10A1



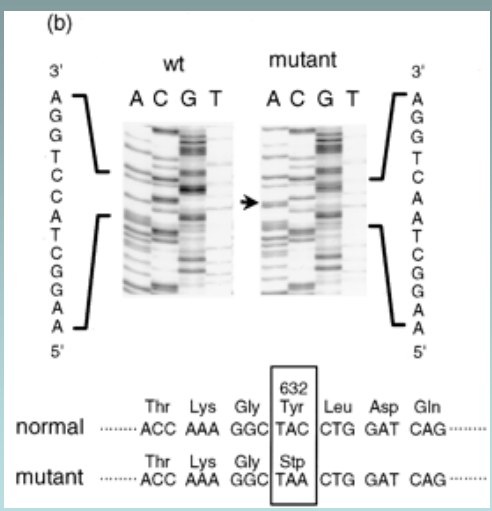
SMCD COL10A1 mutations



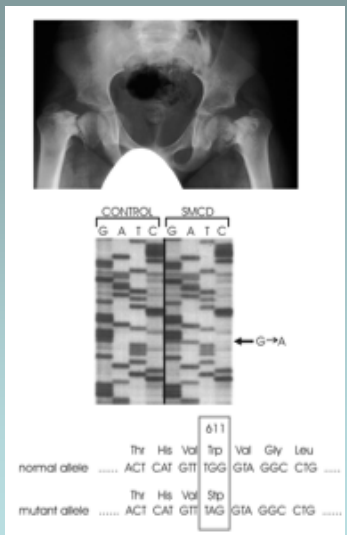
SMCD NONSENSE MUTATIONS

- Two patients diagnosed with classic SMCD
  - Cartilage tissue and cells, bone cells and lymphoblasts available in both cases
- Nonsense mutations determined
  - **Y632X and W611X**
- **What is the pathogenetic consequence of these mutations?**
  - **production of a stable, truncated collagen X protein (dominant negative)?**
  - **reduced COLX mRNA (haploinsufficiency) by nonsense mediated mRNA decay?**

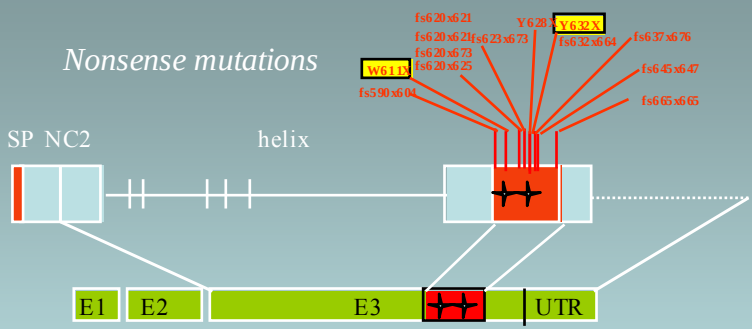
Y632X



W611X



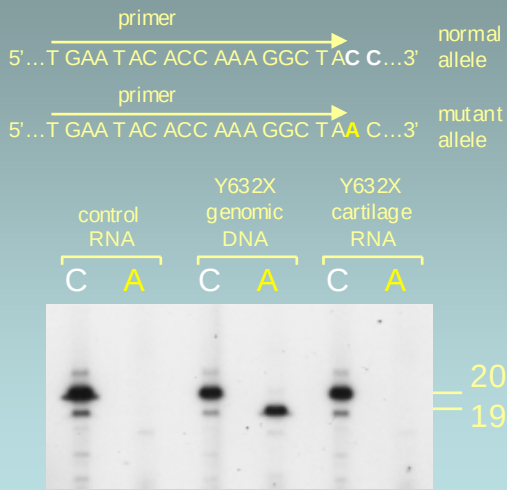
COL10A1 Nonsense Mutations



- Both nonsense mutations are located in the NC1 (last exon)
- No NMD predicted - truncated proteins with dominant negative effects?

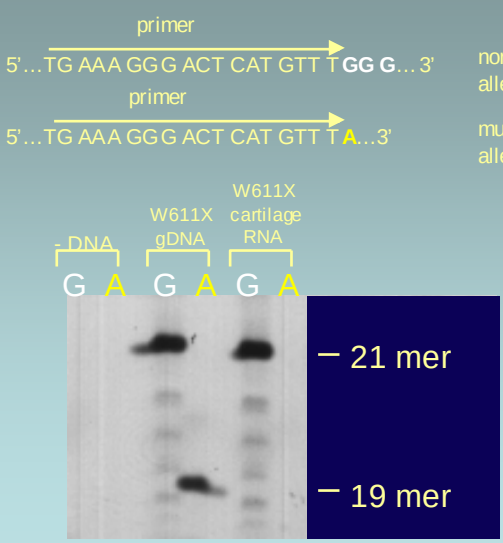
Mutant & wt mRNA in SMCD Y632X

Y632X

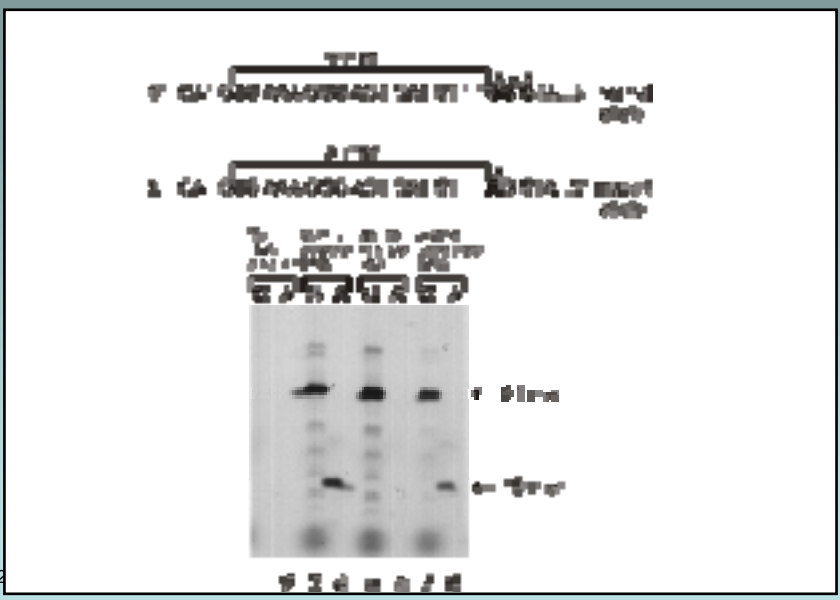


Mutant & wt mRNA in SMCD W611X

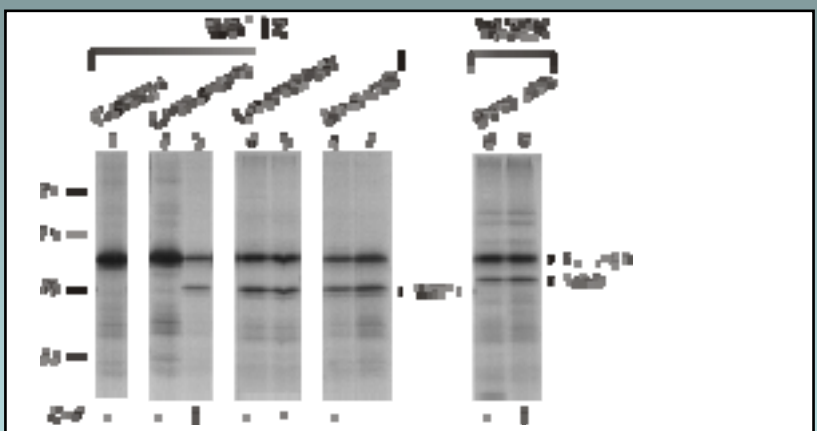
W611X



Tissue specificity of NMD (W611X)



Tissue specificity of NMD



- **Cycloheximide (CHX) inhibits NMD**
- **Confirmation of NMD by protein truncation test**

Conclusions

- Effect of these two nonsense mutations in *COL10A1* result in cartilage cell-specific NMD and haploinsufficiency of *COLX* in growth plate
- Contrasting results observed from other tissues such as blood lymphoblasts and bone cells where no NMD
- These data suggest cell-specific RNA surveillance mechanisms in cartilage
- Possible wider implications to other diseases where nonsense mutations common and pathogenetic mechanisms inferred from data on blood or other tissues
- Challenges dogma on NMD when nonsense mutation located in last exon of a gene

References

- ***Chan, Weng, Graham, Sillence, and Bateman,***

  J Clin Invest 101:1490-1499 (1998)

- ***Bateman, Freddi, Nattrass, and Savarirayan,***

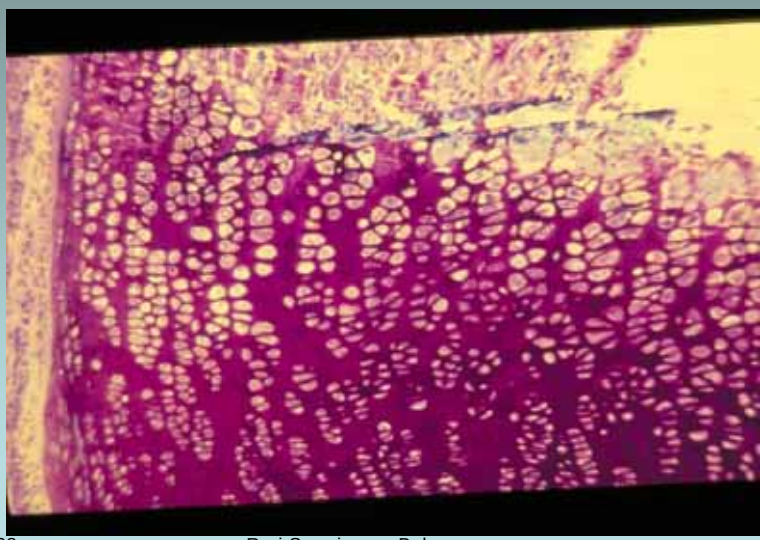
  Hum Mol Genet 12:217-225 (2003)

- ***Bateman, Wilson, Freddi, Lamandé, and Savarirayan***

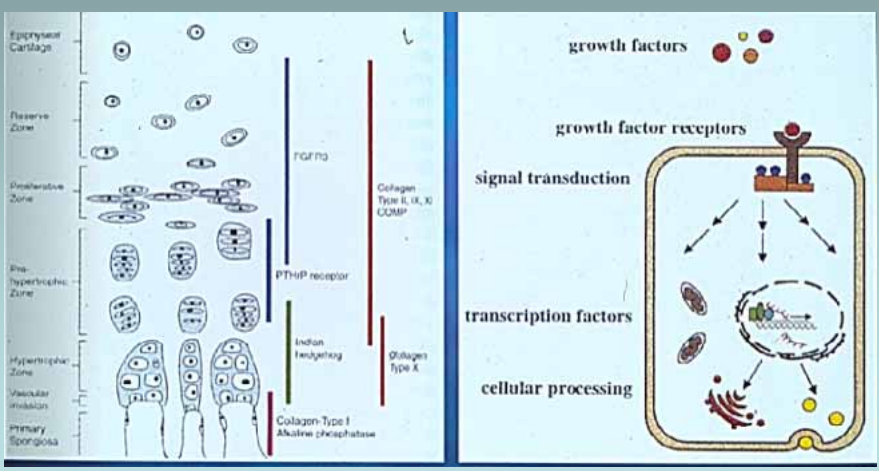
  Hum Mutation 23:396-382 (2004)

  Hum Mutation 25:525-534 (2005)

NORMAL GROWTH PLATE



GROWTH PLATE BIOLOGY

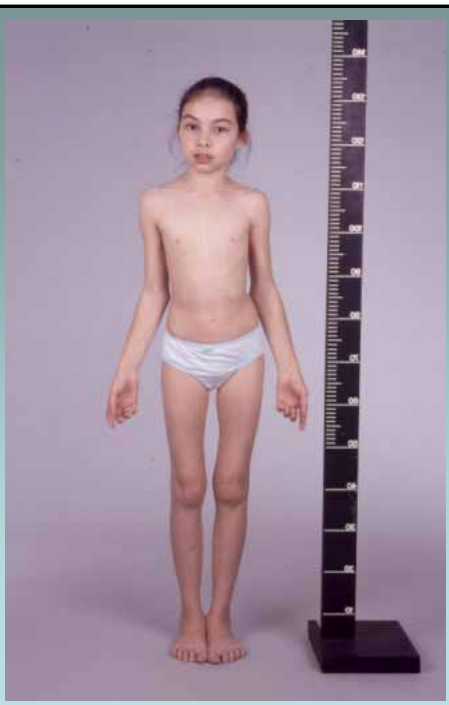


Molecular classification of skeletal dysplasias

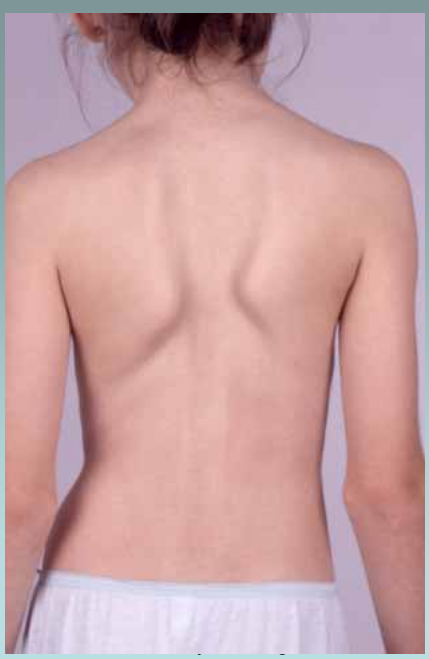
Structural cartilage proteins	Collagen type II	Kniest, Stickler, achondrogenesis 2
Cartilage metabolic pathways	Diastrophic dysplasia transporter	DTD, ATO 2, ACH1B, rMED
Local cartilage growth regulators	FGFR3	Achondroplasia, hypochondroplasia, TD
Transcription factors	CDMP-1	BDC, Grebe dysplasia
Cell membrane proteins	WISP3	PPD
Tumour suppressor genes	EXT 1, 2	Multiple exostoses
Signal transduction mechanisms	TGFβ-1, ROR2	Diaphyseal dysplasia, BDB

1. Disorders of cartilage metabolic pathways

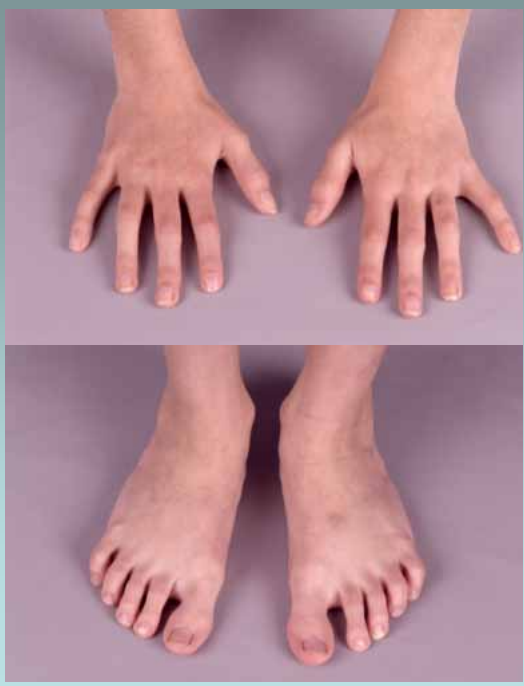
- Sulfate transport within chondrocytes



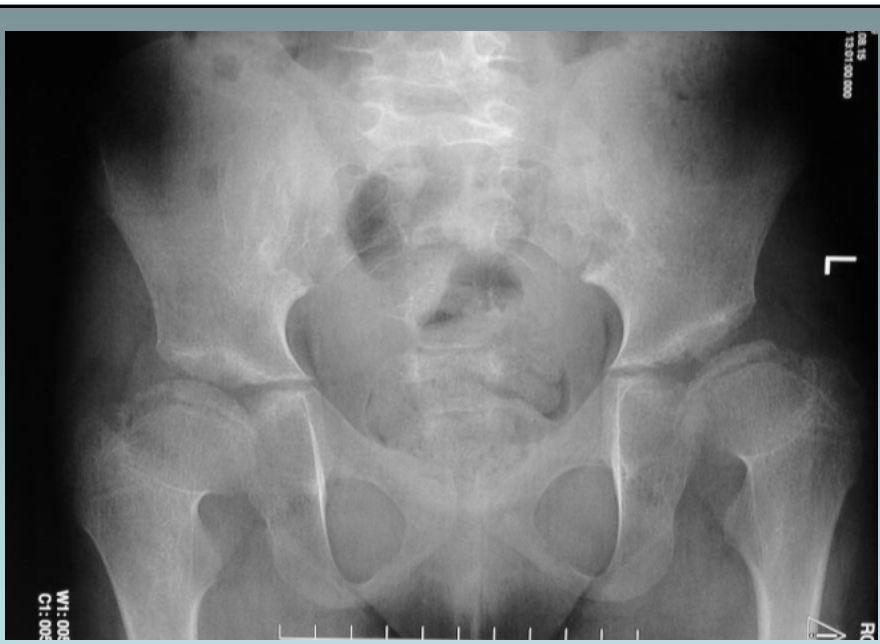
May 2008

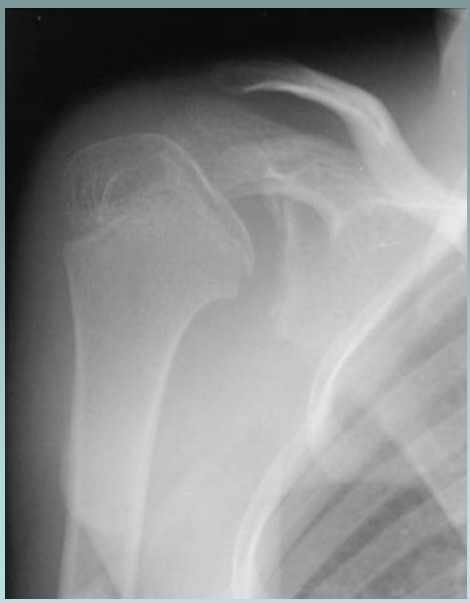


May 2008

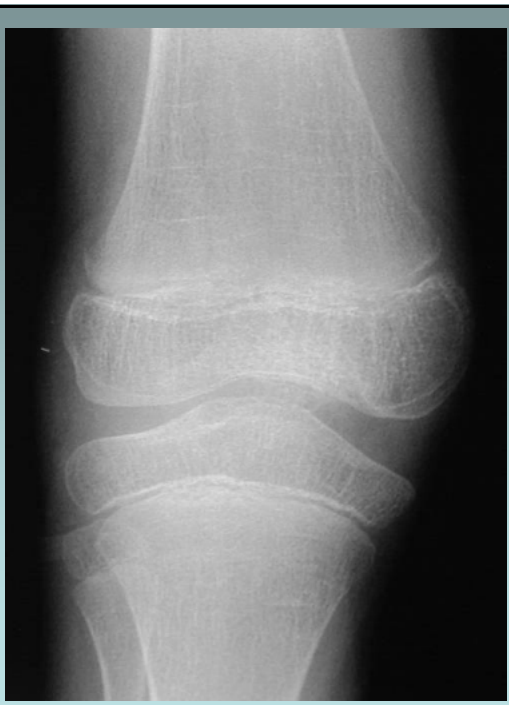


May 2008

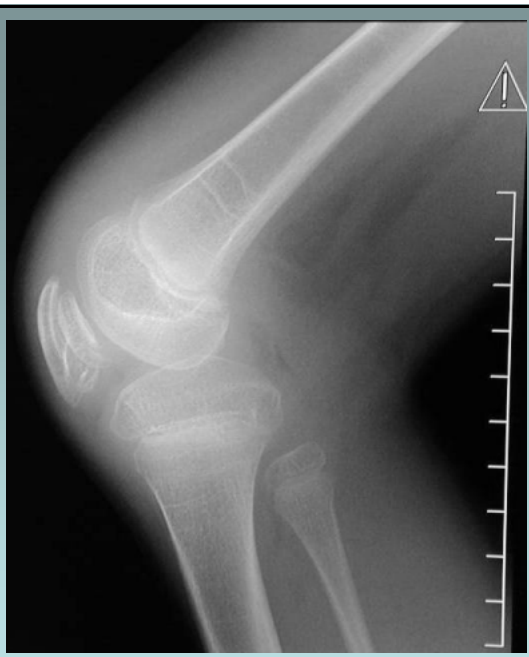


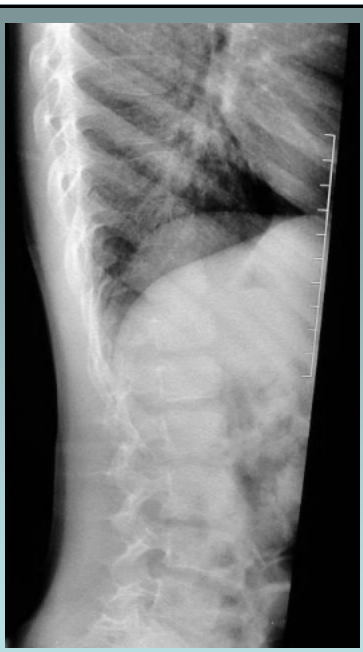


May 2008 Ravi Savarirayan: Bologna



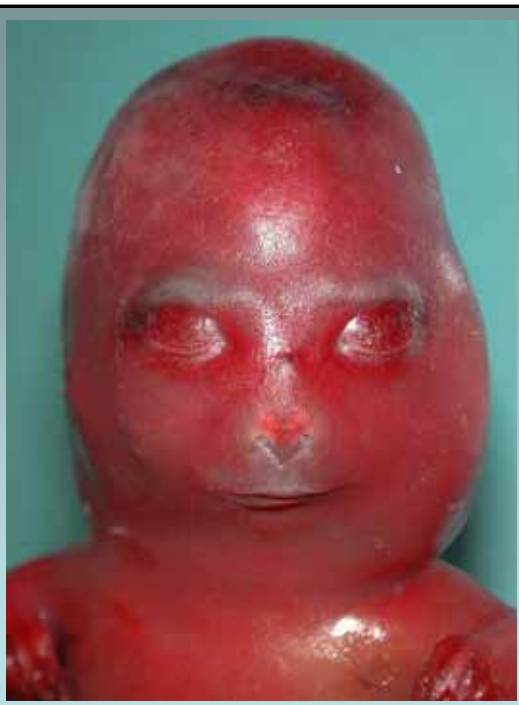
May 2008

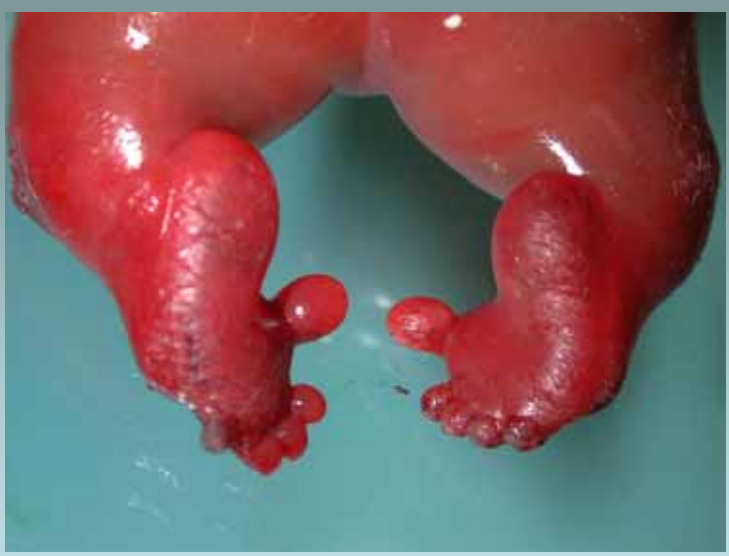


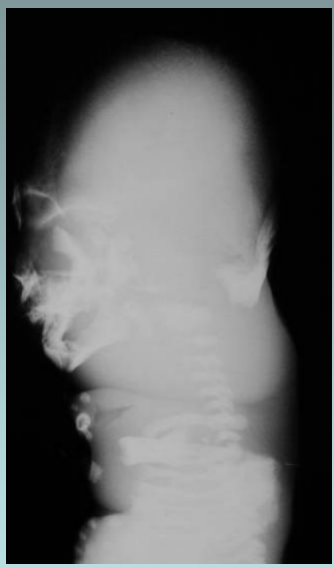


Recessive MED

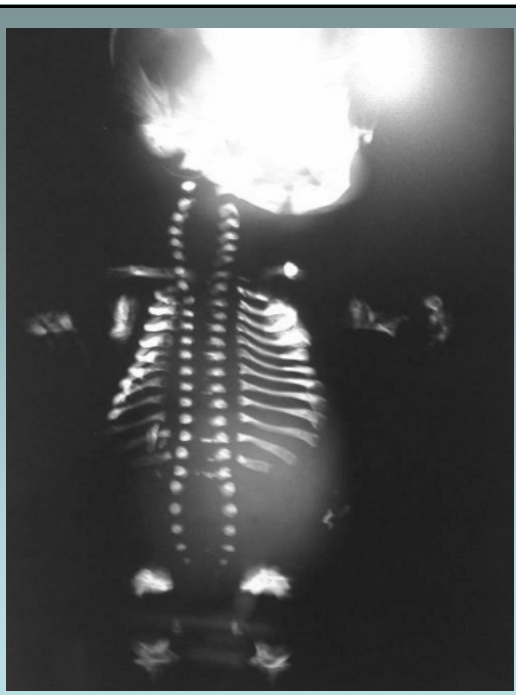
- Homozygous (mild) mutations of DTDST
- In vitro data suggest sulfate transport almost normal with this mutation
- C653S homozygous change in DTDST
- Counselling implications
- Clinical data-waddling gait, patella dislocations



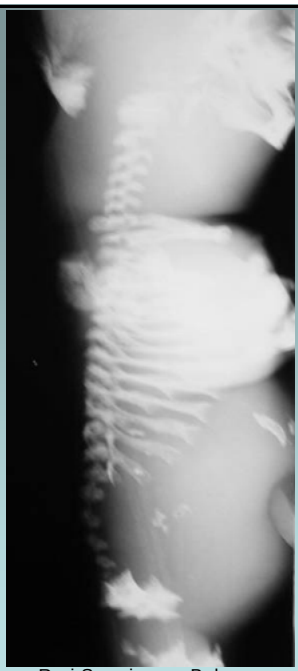




May 2008 Ravi Savarirayan: Bologna



May 2008



Achondrogenesis 1B

- Most severe end of the DTDST spectrum
- Severe DTDST mutations (homozygous null)
- Invariably lethal

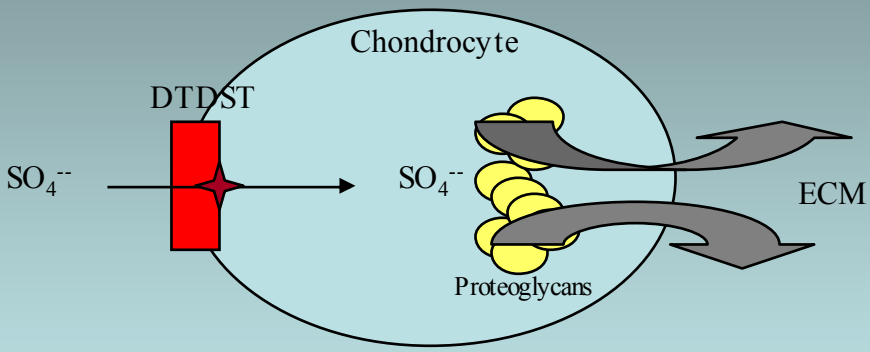
DTDST DISORDER SPECTRUM

- Wide spectrum (correlation with amount of impaired sulfate uptake to cell)

Includes:

- Achondrogenesis 1B (Case 2)
- Atelosteogenesis II
- Diastrophic dysplasia
- MED (recessive) (Case 1)

DTDST AT WORK

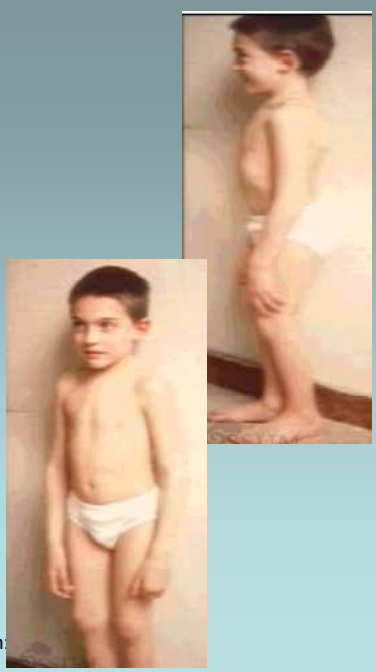


Undersulfation of ECM leads to abnormal chondrogenesis

2. SED TARDA

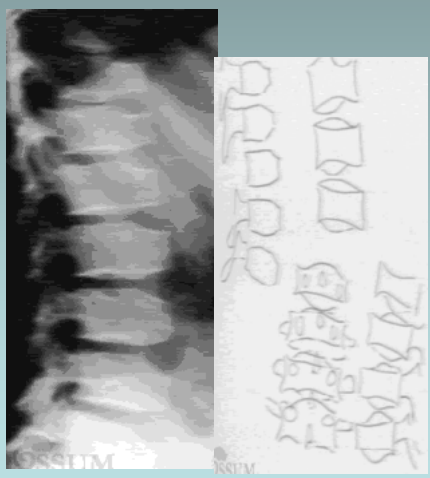
- Short trunk
- Epiphyseal changes
- Late onset
- Boys
- Gene recently identified (SEDL)

(Gedeon et al., NatGenet 1999;22:400-404.)



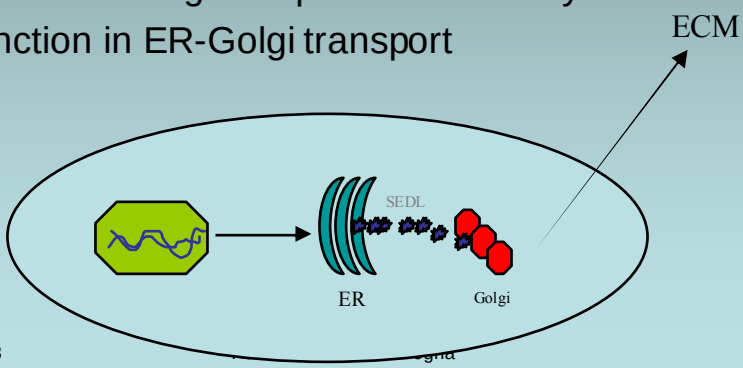
SED TARDA

- Radiology
- Differential diagnoses
- SEDL mutations



SEDL

- novel gene, 140aa protein (*sedlin*)
- widely expressed
- yeast homologue is part of TRAPP system
- function in ER-Golgi transport



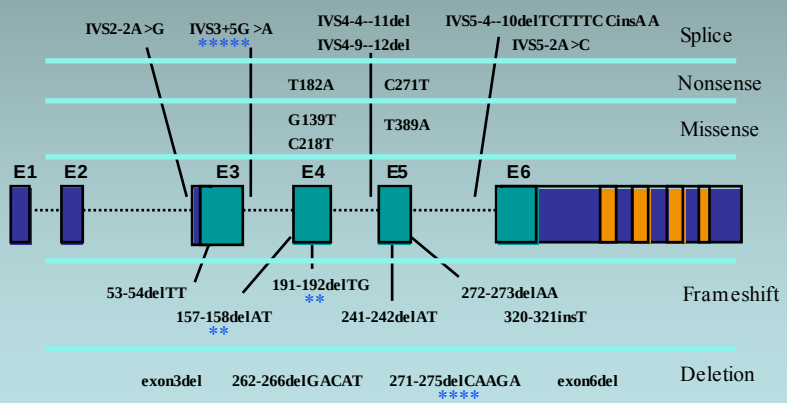
SEDL MUTATIONS

(Gedeon, Savarirayan et al., *Am J Hum Genet 2001*)

- Spectrum of SEDL mutations identified in 30 cases (diverse populations)
- 21 different mutations identified (all types)
- 57% deletions (4 “common” and account for almost half cases)
- NO clear genotype-phenotype correlation
- data suggests that complete, unaltered SEDL product required for normal bone growth

SEDL MUTATIONS

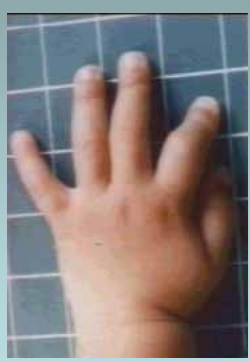
(Gedeon, Savarirayan et al., *Am J Hum Genet*)

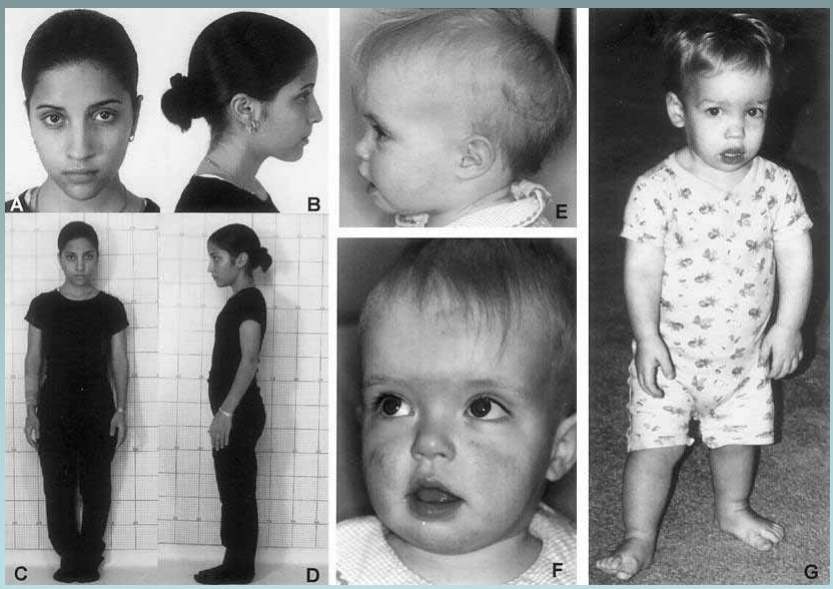


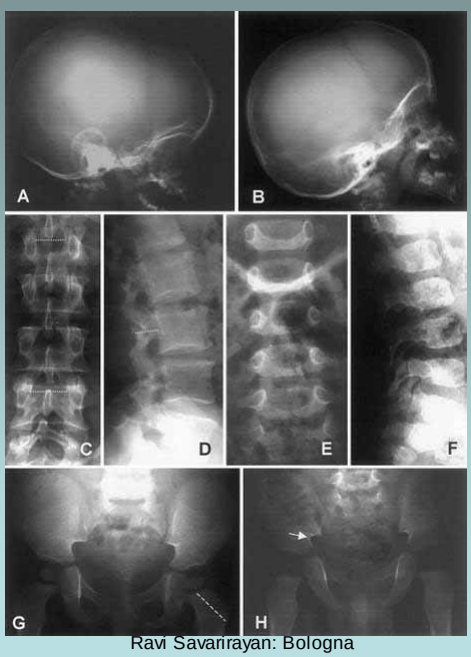
2. Local regulators of cartilage growth

HYPOCHONDROPLASIA

- Clinical
- Radiographic
- Common mutation in 50-60% (FGFR3;N540K)
- Phenotype-Genotype correlation




A
B
C
D
E
F
G


A
B
C
D
E
F
G
H

FIBROBLAST GROWTH FACTOR RECEPTOR GENES

- FGF: 14 in number combine to produce tissue growth and differentiation
- Mediated by FGFRs (4 in number)

FGF RECEPTORS

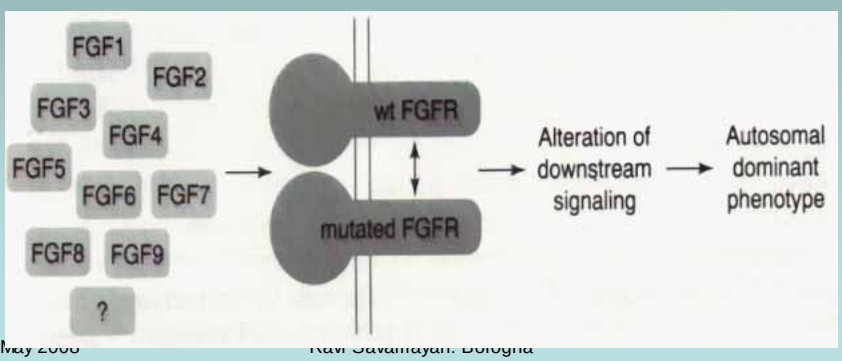
- 4 Different Genes
- Very similar structure
- Dimerise to transmit signals

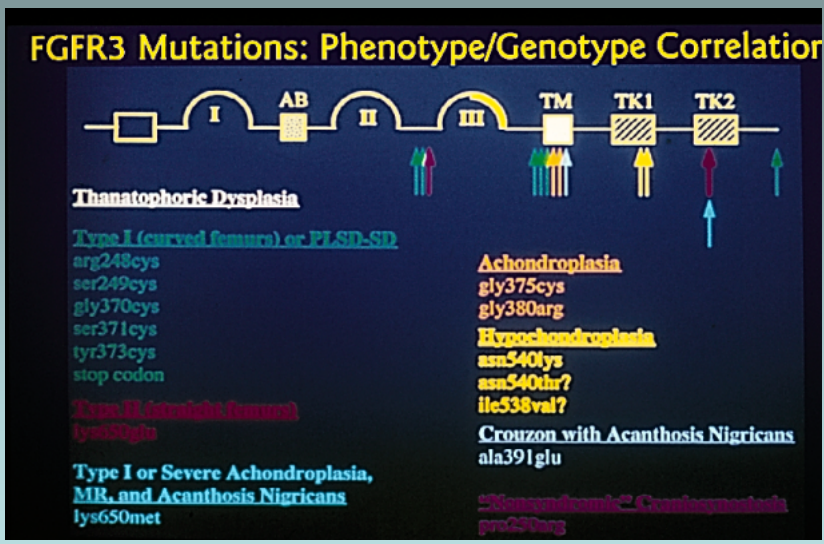
Bellus et al., (1999) Am J Hum Genet

- Studied 90 patients with HCH without common (N540K) mutation
- 2 novel mutations at Lys650 codon in 6 patients
- Significantly milder phenotype clinically and radiographically versus N540K group.
- These lys650 codon mutations compared with TD2 (lys650glu) and SADDAN (lys650met)
- SEVERITY OF PHENOTYPE RELATED TO DEGREE OF LIGAND-INDEPENDENT ACTIVATION OF FGFR3

FGFR SIGNAL TRANSDUCTION

- Mutations that cause phenotype 'activate' this process

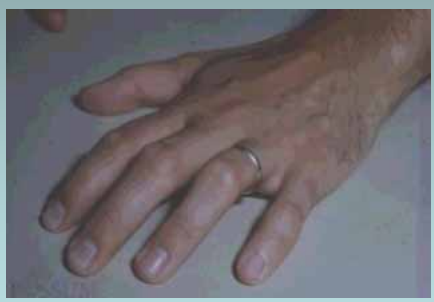




3. Cell membrane proteins

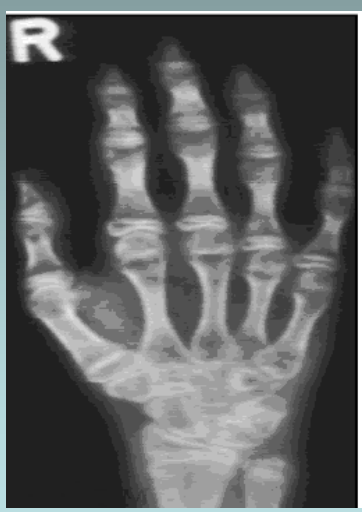
Progressive Pseudorheumatoid Chondrodysplasia (PPD)

- Onset 3-11 years
- SED with progressive arthropathy like juvenile RA
- Short as adults
- No response to anti-rheumatoid drugs

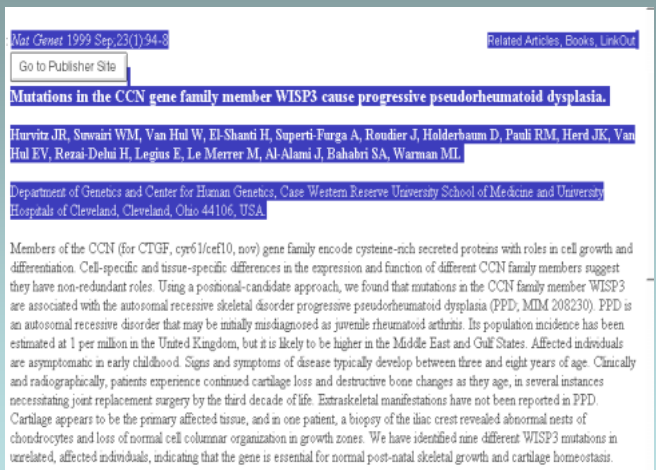


PPD

- Decreased joint spaces
- Widened joints
- Pain in joints/spine
- Mutations in WISP3



PPD GENE



Nat Genet 1999 Sep;23(1):94-8 Related Articles, Books, LinkOut

Go to Publisher Site

Mutations in the CCN gene family member WISP3 cause progressive pseudorheumatoid dysplasia.

Hurvitz JR, Suwairi WM, Van Hul W, El-Shanti H, Superti-Furga A, Roudier J, Holderbaum D, Pauli RM, Herd JK, Van Hul EV, Rezai-Delui H, Legius E, Le Merrer M, Al-Alami J, Bahabri SA, Warman ML

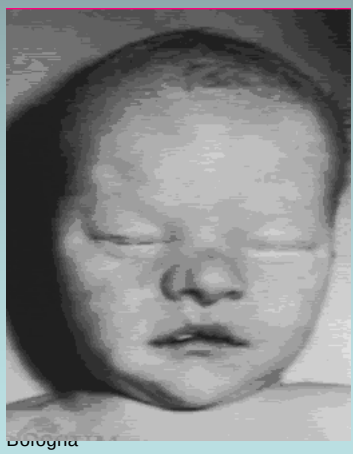
Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio 44106, USA.

Members of the CCN (for CTGF, cyr61/cef10, nov) gene family encode cysteine-rich secreted proteins with roles in cell growth and differentiation. Cell-specific and tissue-specific differences in the expression and function of different CCN family members suggest they have non-redundant roles. Using a positional-candidate approach, we found that mutations in the CCN family member WISP3 are associated with the autosomal recessive skeletal disorder progressive pseudorheumatoid dysplasia (PPD; MIM 208230). PPD is an autosomal recessive disorder that may be initially misdiagnosed as juvenile rheumatoid arthritis. Its population incidence has been estimated at 1 per million in the United Kingdom, but it is likely to be higher in the Middle East and Gulf States. Affected individuals are asymptomatic in early childhood. Signs and symptoms of disease typically develop between three and eight years of age. Clinically and radiographically, patients experience continued cartilage loss and destructive bone changes as they age, in several instances necessitating joint replacement surgery by the third decade of life. Extraskeletal manifestations have not been reported in PPD. Cartilage appears to be the primary affected tissue, and in one patient, a biopsy of the iliac crest revealed abnormal nests of chondrocytes and loss of normal cell columnar organization in growth zones. We have identified nine different WISP3 mutations in unrelated, affected individuals, indicating that the gene is essential for normal post-natal skeletal growth and cartilage homeostasis.

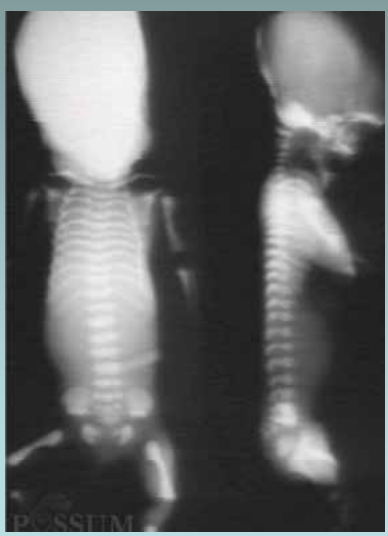
4. Transcription factors

ELLIS-VAN CREVELD SYNDROME

- Autosomal recessive
- Short limbs/ribs
- Postaxial polydactyly
- Dysplastic nails/teeth
- Common atrium (60%)



EvC: Radiology

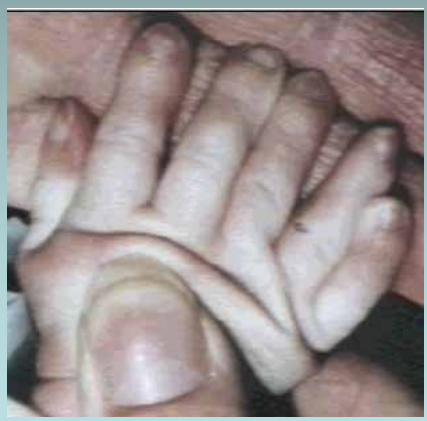


EvC

- Novel gene *EVC*

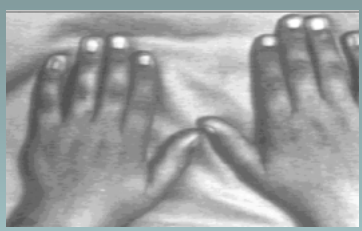
[Nat Genet 24:283 (2000)]

- Expressed in ribs, vertebrae, limbs, atrial septum
- Leucine-zipper gene
- Homozygous mutations

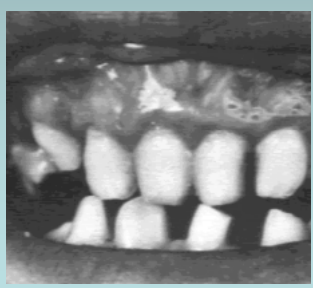


EvC

- Heterozygous manifestations
- Weyers acrodental dysostosis



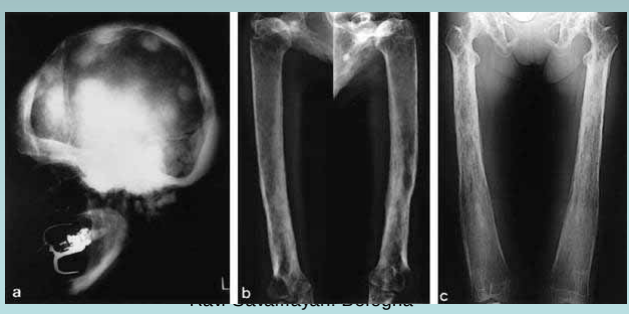
- ?allelic to other short-rib polydactylies



5. Signal transduction molecules

DIAPHYSEAL DYSPLASIA

- Autosomal dominant sclerosing bone dysplasia
- progressive sclerosis of diaphyses/base of skull
- associated proximal myopathy, headache, lethargy



DIAPHYSEAL DYSPLASIA

- mapped to 19q13.1-13.3 recently (Ghadami et al., 2000 Am J Hum Genet)
- mutations found in TGF-ß1 gene (Janssens et al., 2000 Nat Genet)
- Mutations in LAP (keeps TGF-ß1 inactive) and impair this ability
- TGF-ß1appears to stimulate osteoblasts and has role in bone formation-resorption balance

DIAPHYSEAL DYSPLASIA

(Cormier-Daire, Savarirayan et al., Hum Genet 2001)

- characterised TGF-ß1 mutation in 5 families
- genetic homogeneity confirmed
- all located in LAP and ?affect dimerization
- “common” R218C mutation in 50% cases
- marked intra and interfamilial variability
- delayed onset of puberty (mean 18 years) found

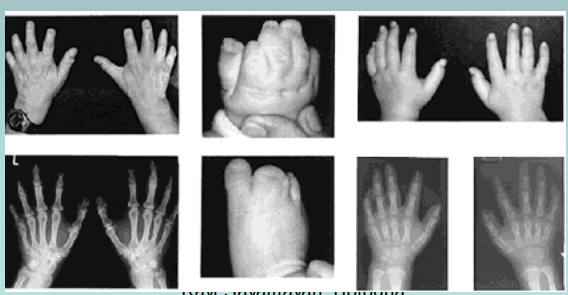
DIAPHYSEAL DYSPLASIA

(Cormier-Daire, Savarirayan et al., Hum Genet)

	Our Series				Kinoshita *et al*		Janssens *et al*		
Ethnic Origin	Portuguese	French	Belgian	Australian	Japanese	European	Israeli	Europe	**TOTAL**
Number of Families	1	2	1	1	7	2	1	5	**20**
R218H	1				2				**3**
R218C		1			4	2	1	2	**10**
C225R		1	1	1	1			1	**5**
Y81H								1	**1**
Leucine Repeat								1	**1**

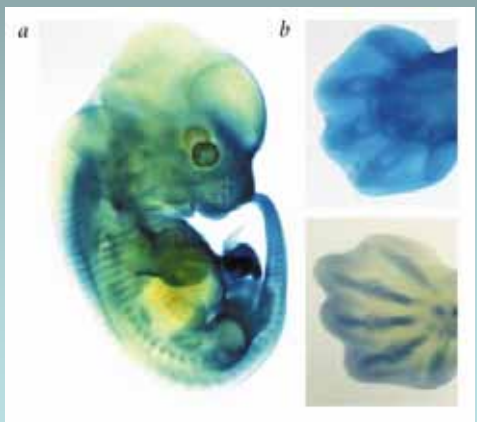
BRACHYDACTYLY, TYPE B

- many recent advances
- brachydactyly type B-mutations in ROR2 (Oldridge et al., Nat Genet 24, 275 – 278 (2000)
- orphan receptor tyrosine kinase



Brachydactyly type B

- ROR2 expression in skeletal anlagen
- homozygous mutations cause recessive Robinow syndrome



Animal Models

Outline

- Phenotype of Dexter cattle
- Pedigree data
- Mapping data
- Candidate gene (*AGC1*)
- Molecular data
- Conclusions

Prize winners at Manchester show in Illustrated London News July 1869

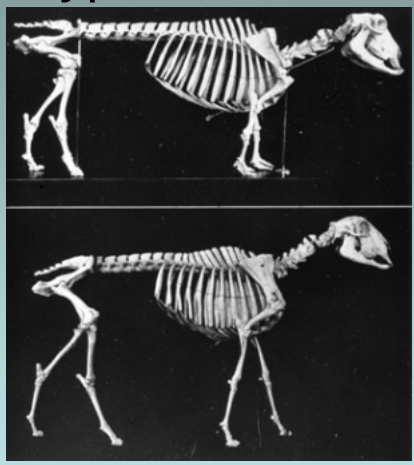
Phenotype

- Long legged Dexter
- Homozygous normal (B/B)

- Short-legged Dexter
- Heterozygous carrier (B/b)

- Dexter "bulldog calf"
- Homozygous affected (b/b)

Heterozygous (short-leg) phenotype



B/b

B/B

- Rhizomelia
- Short long bones (knee-fetlock)
- Vertebral body irregularities

Courtesy of Massey University, New Zealand

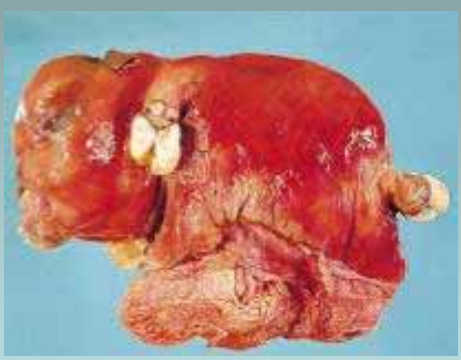
Homozygous "bulldog" phenotype Clinical

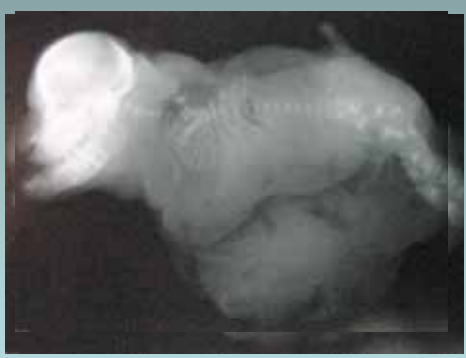
- First documented in 19th century
- Disproportionate dwarfism
- Small body weight
- Short limbs
- Relative macrocephaly
- Retruded muzzle, cleft palate, large tongue
- Ventral abdominal hernia
- Abortion in 7th month of gestation
- *FGFR3* (TM) mutations excluded *(Usha et al, 1997)*

Homozygous “bulldog” phenotype Radiographic

- Micromelia
- Platyspondyly
- Extreme shortening of ribs
- Craniofacial abnormalities, midface hypoplasia and relative prognathism
- Normal ossification, no fractures

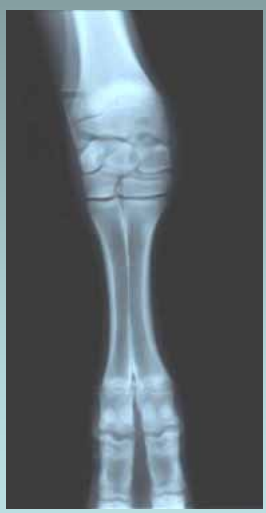
Homozygous “bulldog” phenotype





Limb Phenotype

B/*b* *b*/*b*

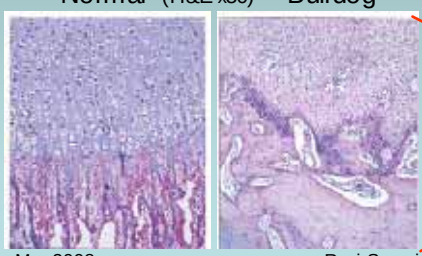


Homozygous “bulldog” phenotype Histological

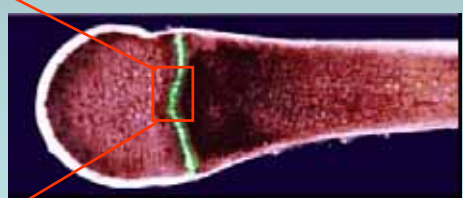
- Very abnormal growth plate with minimal column formation/hypertrophy
- Increased density of small chondrocytes

Harper et al., 1998

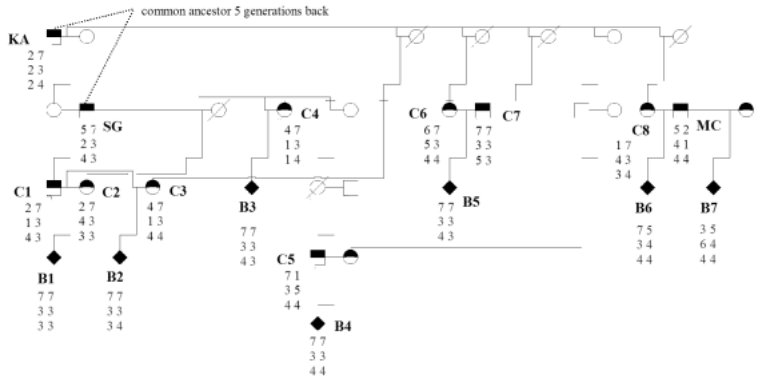
Normal (H&E x50) Bulldog



Growth Plates



Australian Dexter Pedigree



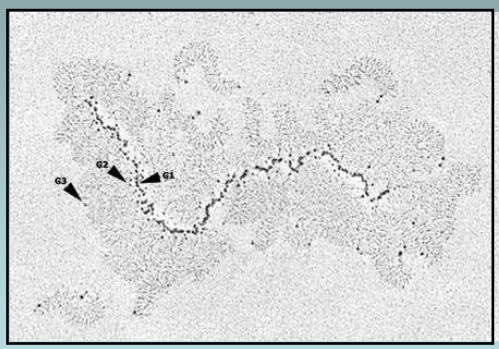
- Half shaded symbols: carriers, Shaded symbols: (B1-B7) bulldog calves

Mapping data

- Homozygosity mapping in Dexter pedigree revealed 5cM region of shared haplotype
- *AGC1* in this mapped region as a positional candidate

Candidate gene: *AGC1*

- Encodes core protein of aggrecan
- Major structural component of articular cartilage ECM
- Mouse, chicken, human chondrodysplasias with *AGC1* mutations
- Good functional candidate



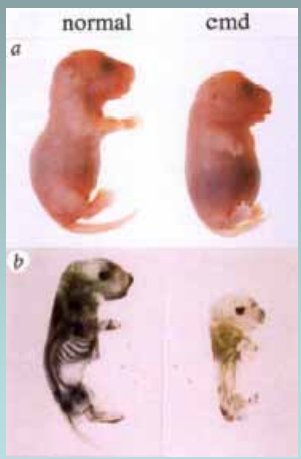
EM: Aggrecan core protein with side chains

Candidate gene: *AGC1*

- Homozygous *AGC1* mutations cause *cmd* phenotype in mice and *nanomelia* in chickens
- Heterozygous *AGC1* mutations cause dwarfism and shortened skeletal elements in mouse and chick and SED Kimberley type (premature arthritis phenotype) in humans
- Polymorphisms VNTR region of *AGC1* associated with hand osteoarthritis in humans

AGC1: murine model (*cmd*)
(Watanabe et al, 1994)

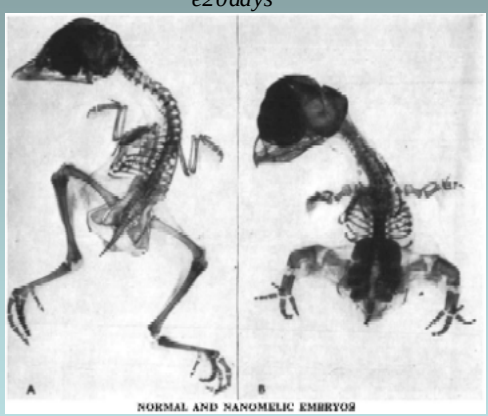
- Dwarf stature
- Short snout
- Cleft palate
- Short limbs/tail
- Homozygous 7bp deletion in *AGC1* leading absence of aggrecan in ER and ECM
- *cmd-bc* mouse



AGC1: fowl model (*nanomelia*)
(Landauer, 1965)

- Large head
- Short maxilla
- Short legs and wings
- Short long bones
- *Homozygous AGC1* mutation in exon 12 with truncated protein (small amount translated

e20days

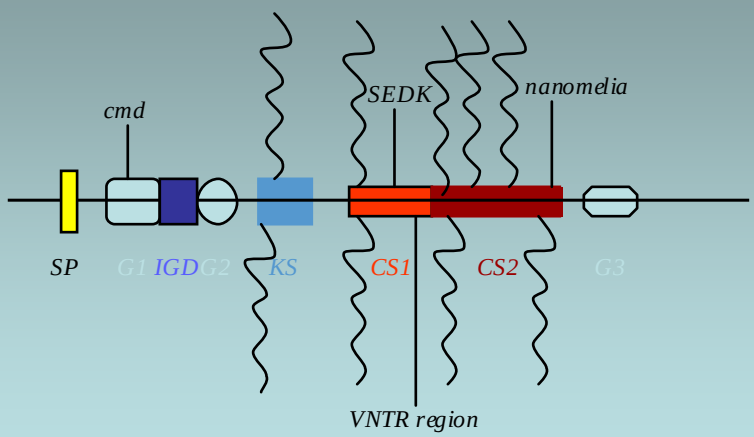


AGC1:human model (SED Kimberley)

(Anderson et al., 1990, Gleghorn et al., 2005)

- Mild AD disorder
- Short stature (mild), stocky build
- Early onset OA weight-bearing joints
- Radiographic vertebral and epiphyseal changes (CFE)
- Heterozygous *AGC1* mutation (1bp insertion) with truncated protein (60% length wild type)
- Pathogenic consequences unclear

AGC1 protein structure



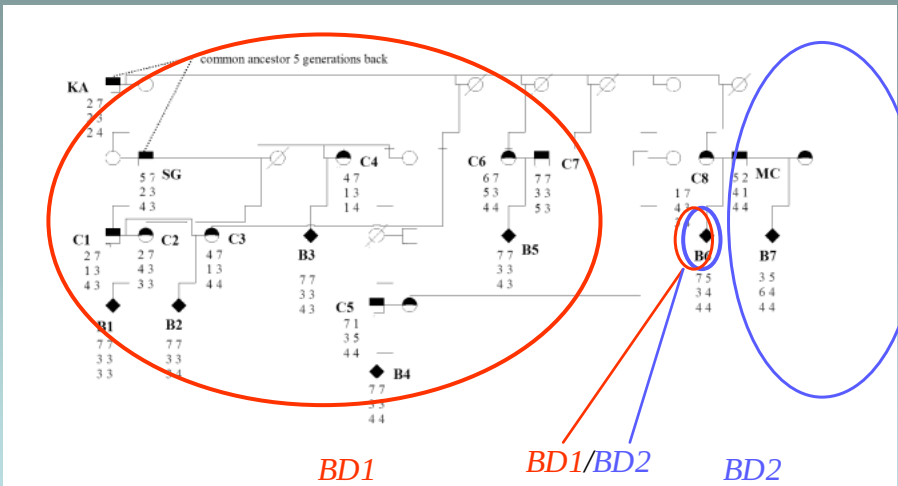
Dexter molecular data

- All coding regions of *AGC1* sequenced (Exons 2-19)
- Homozygous 4bp insertion in exon 11 (termed *BD1*) found in bulldogs B1-B5 (2266_2267ins GGCA)
- Leads to frameshift and premature stop codon in exon 11 and truncated protein (third of wild type length)

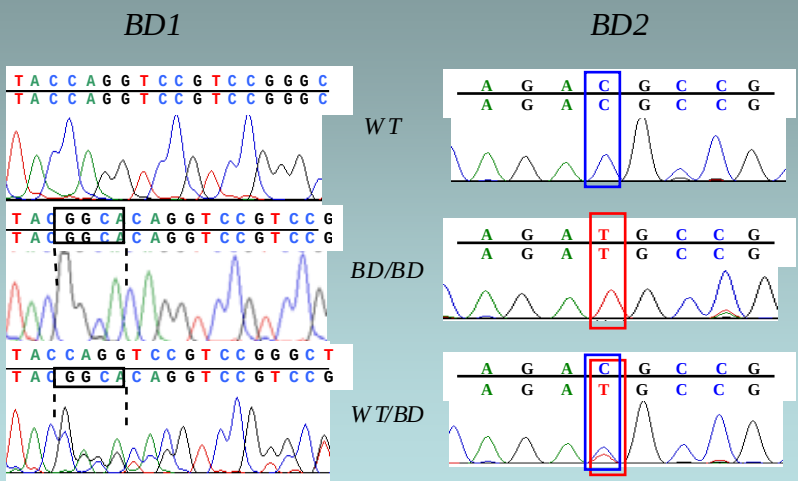
Molecular data

- Second mutation found in bulldog B7 homozygous C>T transition (-198C>T, termed *BD2*), also leads to PTC
- Bulldog B6 compound heterozygote for *BD1/BD2*
- These sequence changes segregated fully with the homozygous and heterozygous phenotypes worldwide
- over 900 animals tested *BD1* 92%; *BD2* 8%

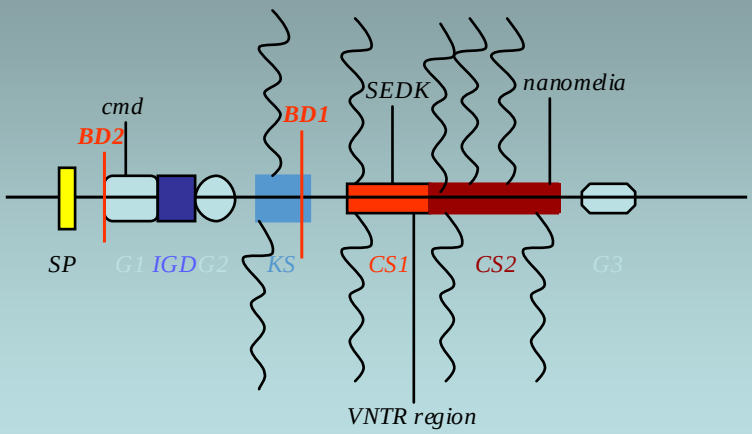
Molecular data



Molecular data

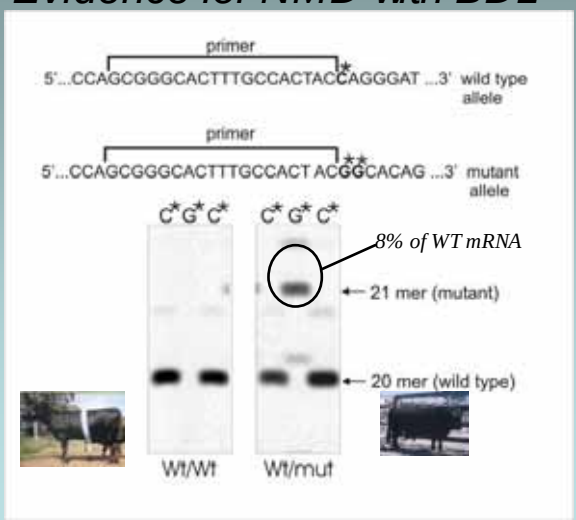


AGC1 protein structure



Primer extension analysis on chondrocyte mRNA

Evidence for NMD with BD1



Conclusions

- *AGC1* defined as gene underlying Dexter cattle chondrodysplasia
- Data suggest pathogenetic mechanism in heterozygous animals may be haploinsufficiency for *AGC1* in growth plate ECM
- Potential use of these animals as model for understanding human chondrodysplasia and arthritis phenotypes

Acknowledgements

In the press
Mammalian Genome

Murdoch Childrens Research Institute

- *John Bateman*
- *Ravi Savarirayan*

REPROGEN

- ***Julie Cavanagh***
- *Imke Tammen*
- *Peter Windsor*
- *Frank Nicholas*
- *Herman Raadsma*

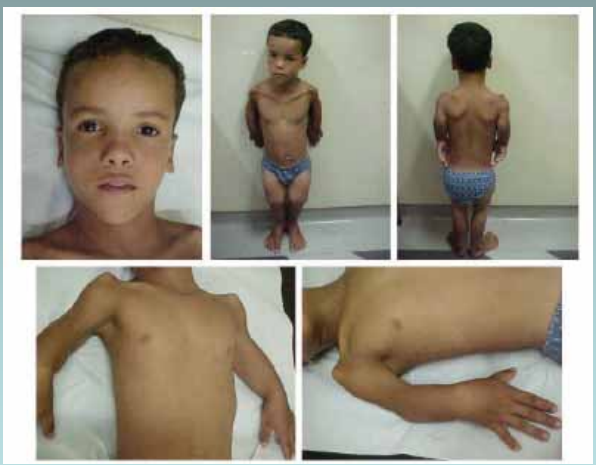
Phenotype

- First described by Maroteaux in 1989 and delinetaed into dominant and recessive forms by Borochowitz 1991.
- *Omo*=humerus (Greek)
- 25 cases reported to date

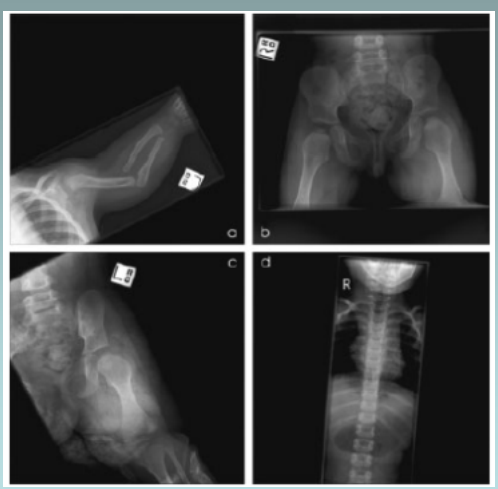
Phenotype

- flat nasal bridge
- Low set ears
- Prominent forehead
- Short long bones (humeri/femora-club like)
- Axial skeleton relatively normal
- Joint limitations
- Cranial deformations
- CHD/pterygia/craniosynostosis

Phenotype

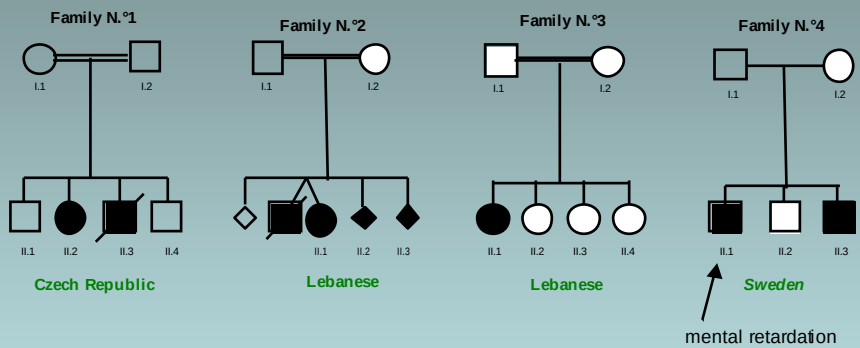


Phenotype



OMODYSPLASIA

Pedigrees and Genetics

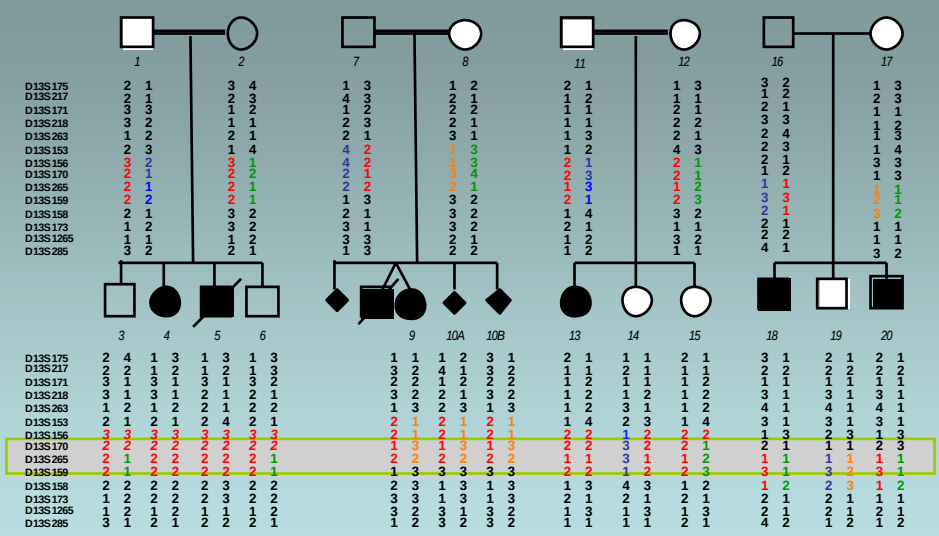


Genetics: autosomal recessive transmission

=> upper and lower limbs are affected in contrast with dominant form in which the lower limbs are normal

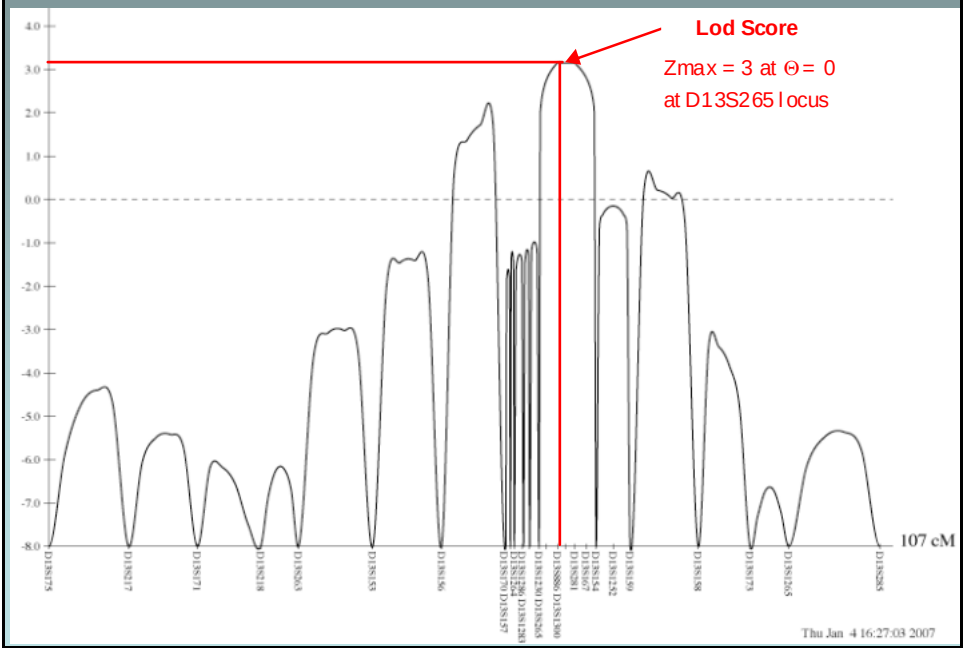
Skeletal phenotype: very homogenous in all affected subjects

Haplotypes of Chromosome 13

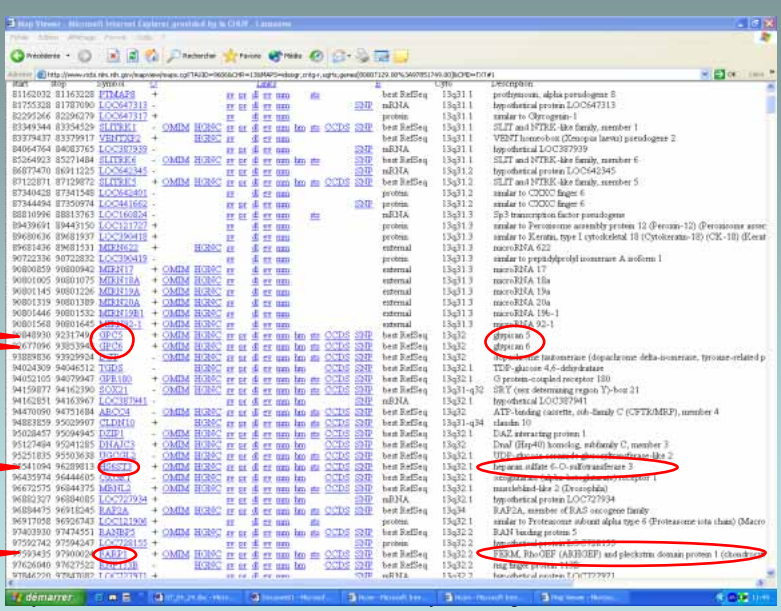


 Candidate Region between D13S170-D13S159 : 13q31.1-q32.2 => 8.3 cM

Locus 13q31.1-q32.2



Genes on the Region 13q31.1-q32.2



33 genes
on the region

Molecular Study of GPC6 gDNA: Results I

Direct Sequencing of **9 exons** and intron-exon boundaries of the of the **GPC6 gene** :

Family	Consanguinity	Location	Nt change	a.a change
N.°1	Yes	Exon 4	c.777_779delC homozygote	p.L260fsX
N.°2	Yes	No mutation found by sequencing analysis for these families		
N.°3	Yes			
N.°4	No	Exon 3	c.700 C>T heterozygote	p.R234X + ?

I.2 - Mother
II.1 - Affected child
II.3 - Affected child

Study of cDNA by RT-PCR: Results II

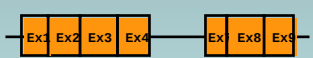
• cDNA GPC6 = 1668 bp (5 fragments)

Family 2
father (I.1)
mother (I.2)

Normal Band (wild type)

Mutated Band : ***Skip* Ex.4**

Family 3
patient (II.2)



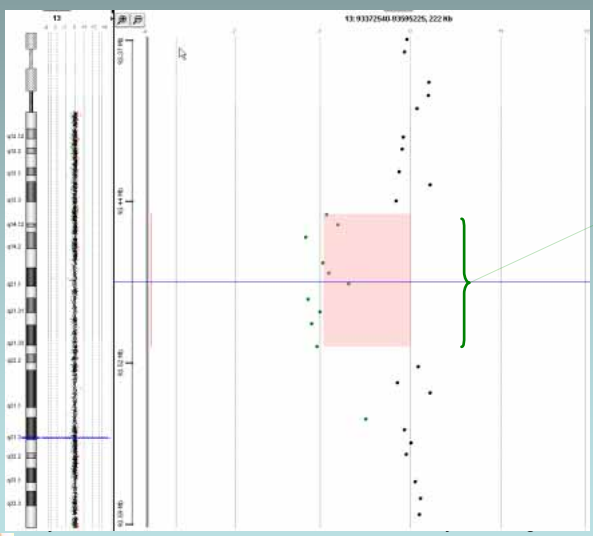
Mutated Band : ***Skip* Ex.5-6**

Mutated Band : ***Skip* Ex.4-5-6**

Large intragenic deletions encompassing one or more GPC6 exons predicting **prematurely truncated proteins**

Array Comparative Genomic Hybridization (aCGH) : Family N.°2

Father **I.1:** Heterozygote **Deletion** encompassing Exon 4

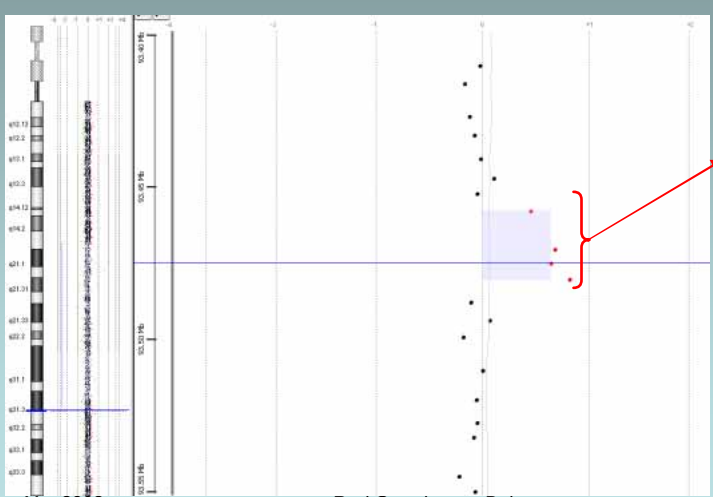


14 deleted probes

Family member	Deletion
I.1 - Father	Heterozygote
I.2 - Mother	Heterozygote
II.1 - Affected child	Homozygote
II.2 - *Foetus*/affected	Homozygote
II.3 - *Foetus*/affected	Homozygote

Array Comparative Genomic Hybridization (aCGH) : Family N.°4

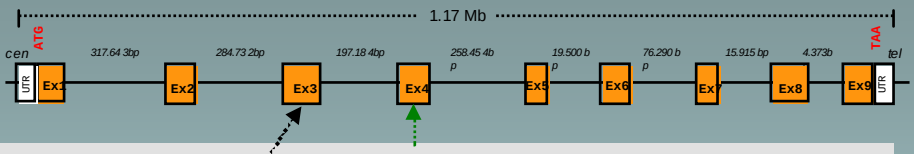
Patient **II.1:** Heterozygote **Duplication** encompassing Exon 4



4 duplicated probes

I.2 - Father
II.1 - Affected child
II.3 - Affected child

Omodysplasia Mutations



Family 1 — **L260fsX** (Homozygote)

Family 2 — Homozygote **deletion** encompassing Ex.4 = 66.49 Kb

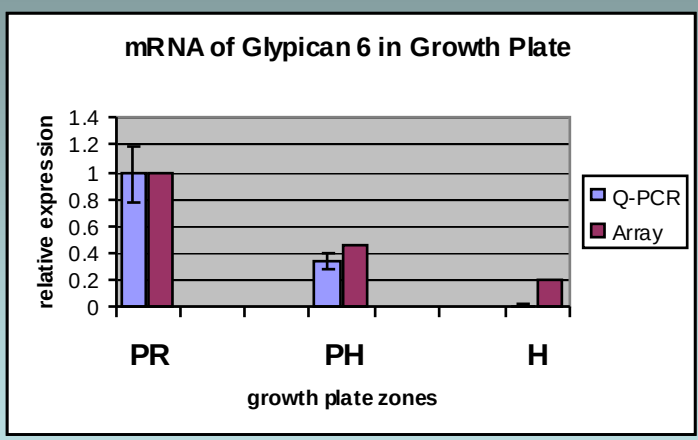
Family 3 — Homozygote **deletion** encompassing Ex.4-5-6 = 95 - 104 Kb

R234X (Heterozygote)

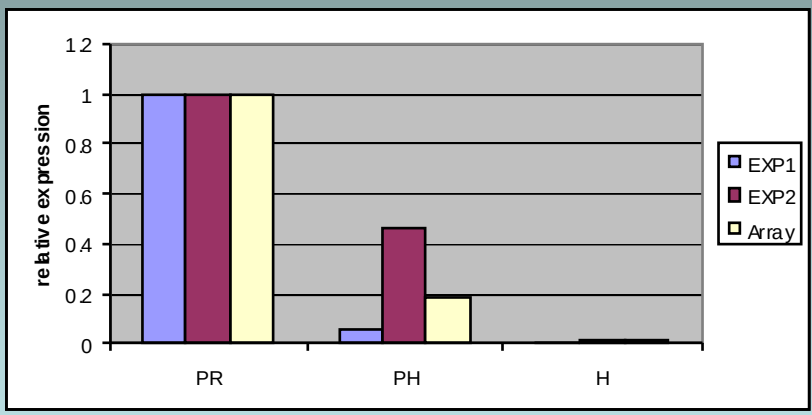
Family 4 — Heterozygote **duplication** encompassing Ex.4 = 21 - 33.4 Kb

Sporadic Case — Homozygote **deletion** encompassing Ex.3 = 248 - 268 Kb

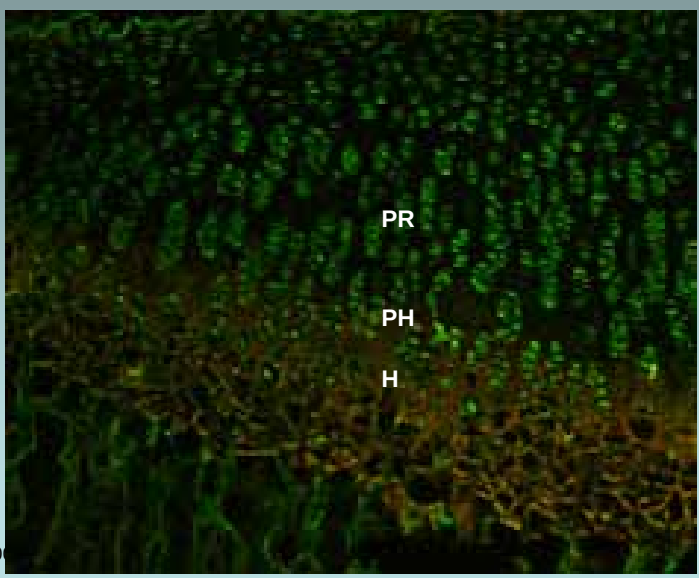
Family 5 — Homozygote **deletion** encompassing Ex.3 = ? Kb



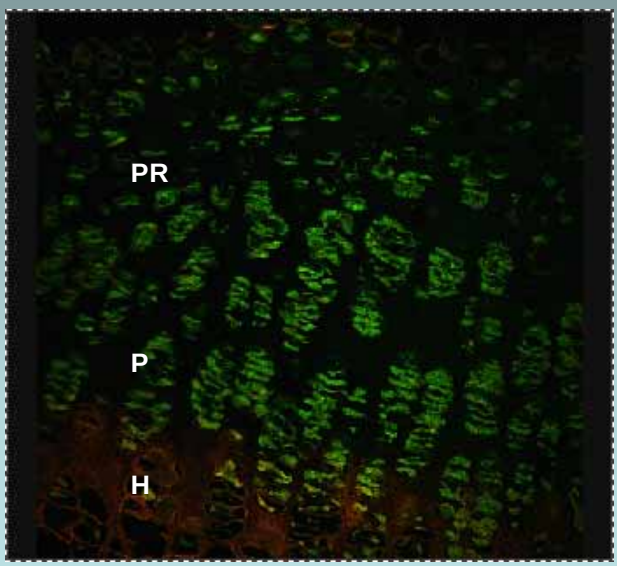
Q-PCR: 2 experiments



Immunofluorescence for Gpc6 and ColX



Immunofluorescence for Gpc6 and ColX



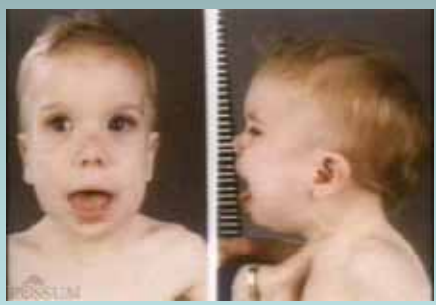
May 2008

Glypicans

- Cell membrane bound HSPG
- GPI anchors
- 14 conserved cysteines in core
- GPC1-6 identified
- Drosophila: 2 glypicans (*dally and dally-like)* - devopmental genes
- Interaction with dpp in fruit fly

Glypicans

- Human disease: SGBS
- X-linked overgrowth syndrome
- MR
- Hernias
- Extra nipples
- CHD
- Rib defetcs
- Cleft palate



Glypican 6

- Co-factor for growth factors (FGFR2)
- Establsih morphogen gradients
- Zebrafish mutant (abn. Gastrulation)

Glypican 6: Future work

- Studies in human growth plate from patients to confirm mouse data
- Animal model
- Limb bud knock down models (P Fairlie)

- Relationship between overgrowth and dwarfism? (GPC 3 vs 6)