UNIVERSITEIT GENT

FACULTY OF MEDICINE

Genes involved in human growth and skeletal development

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klinisch ondersteunende sector

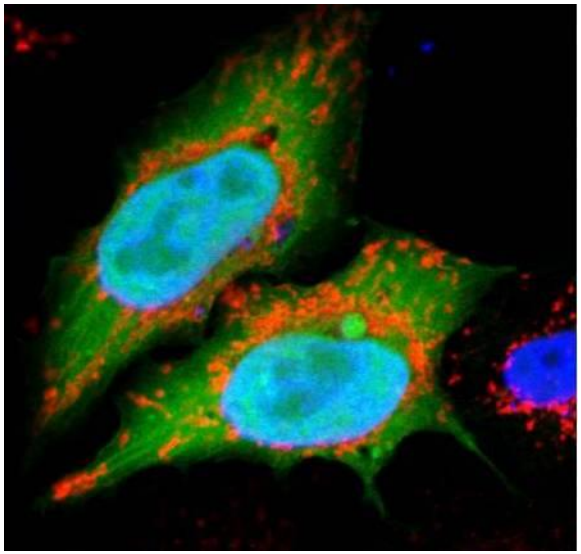
Universitair Ziekenhuis Gent

Center for Medical Genetics

Skeletal development

migration

mitosis



differentiation

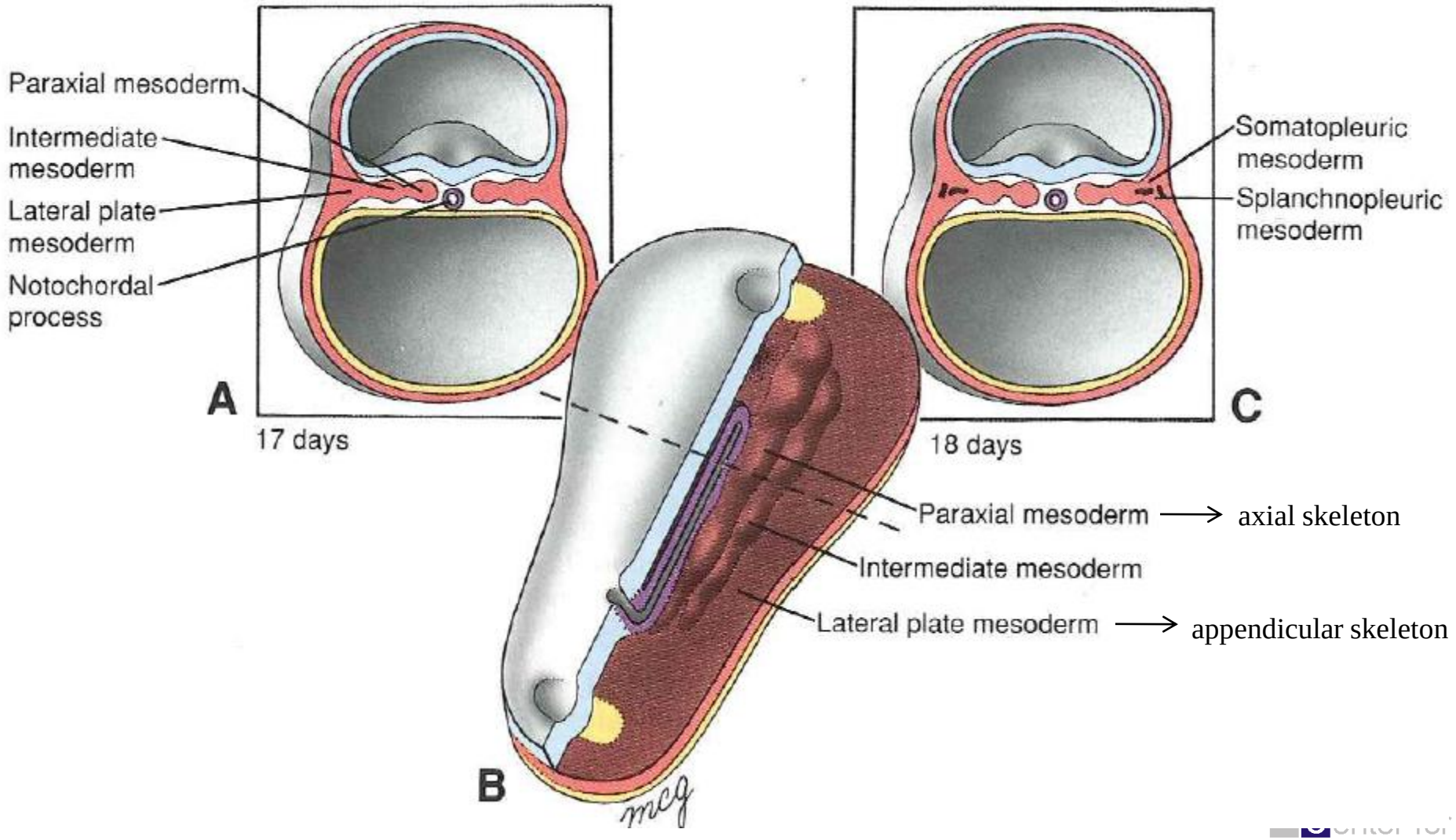
apoptosis

MANY GENES

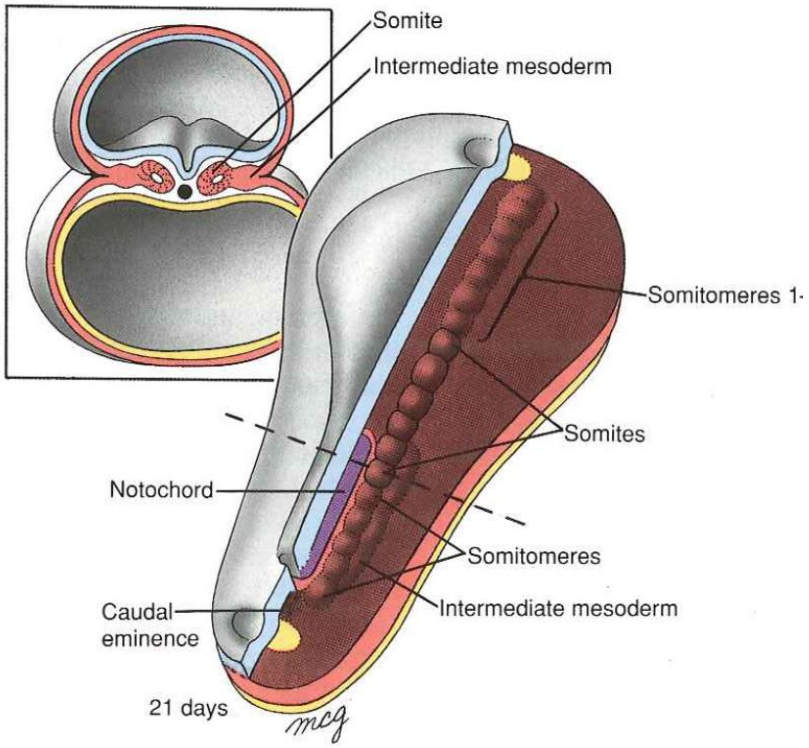
Identification of genes involved in growth and skeletal development

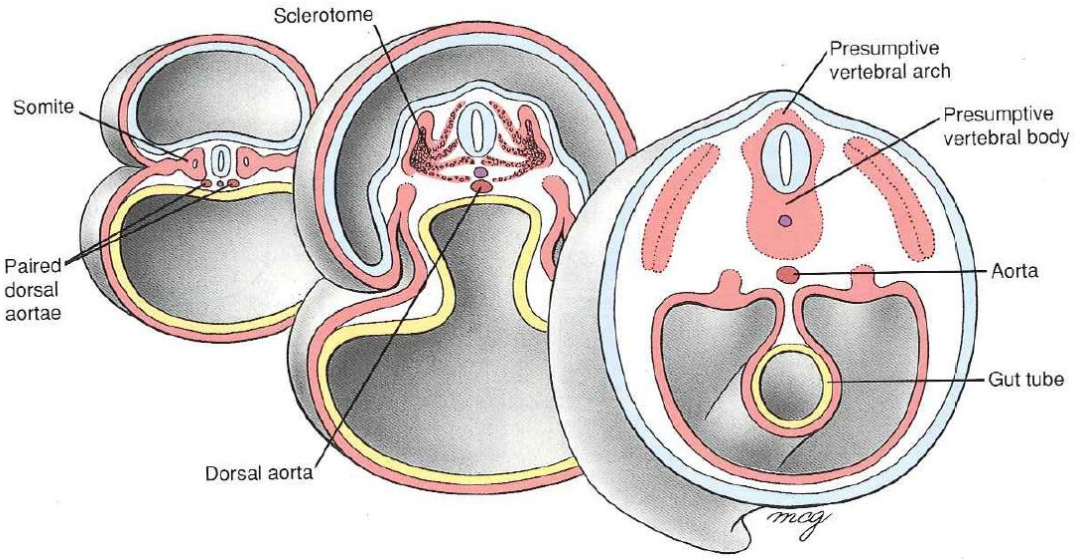
- study of animal models
- study of rare genetic disorders
- genome wide association studies

Skeletal development



Somitogenesis





28 days

Human Embryology. William Larsen (ed), Churchill Livingstone, 1993

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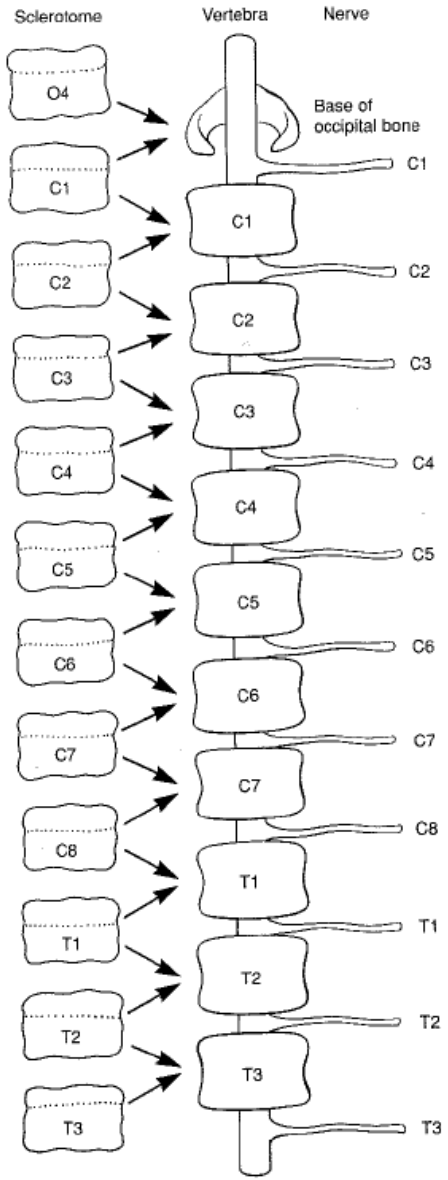
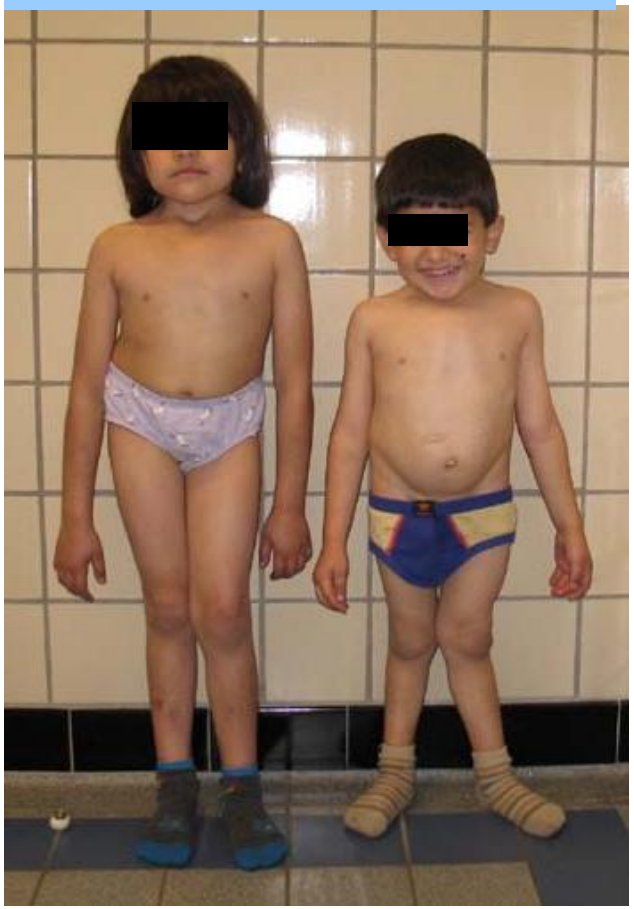
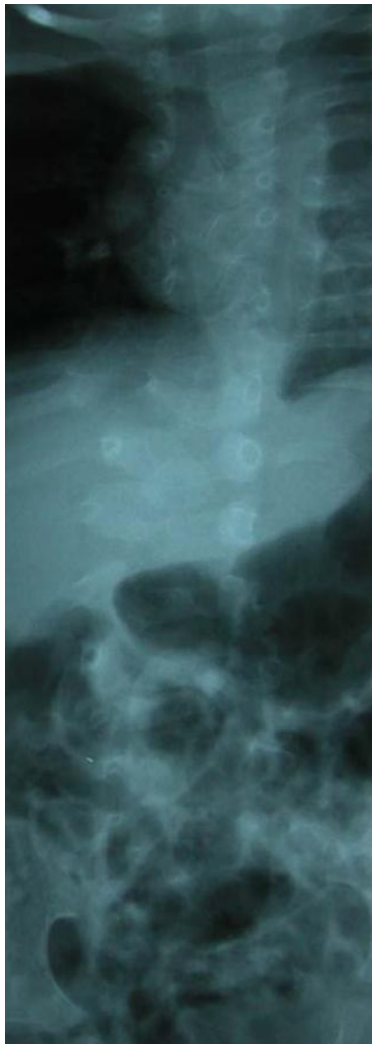
Patterning of axial skeleton – defects in somitogenesis



spondylocostal dysostosis



p.Q360X/p.Q360X in *DLL3*



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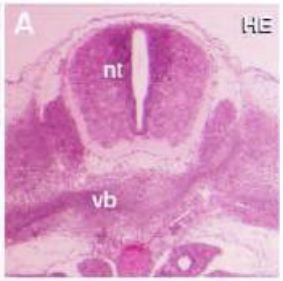
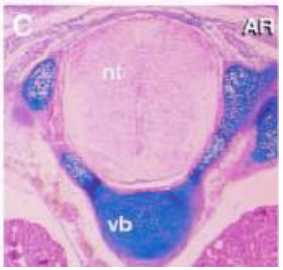
Somitic chondrogenesis





Pax1/Pax9

Shh

BMP2

Sox9

Nkx3-2

?

Runx2

chondroblast

chondrocyte

COL2
COL9
aggrecan
epiphycan

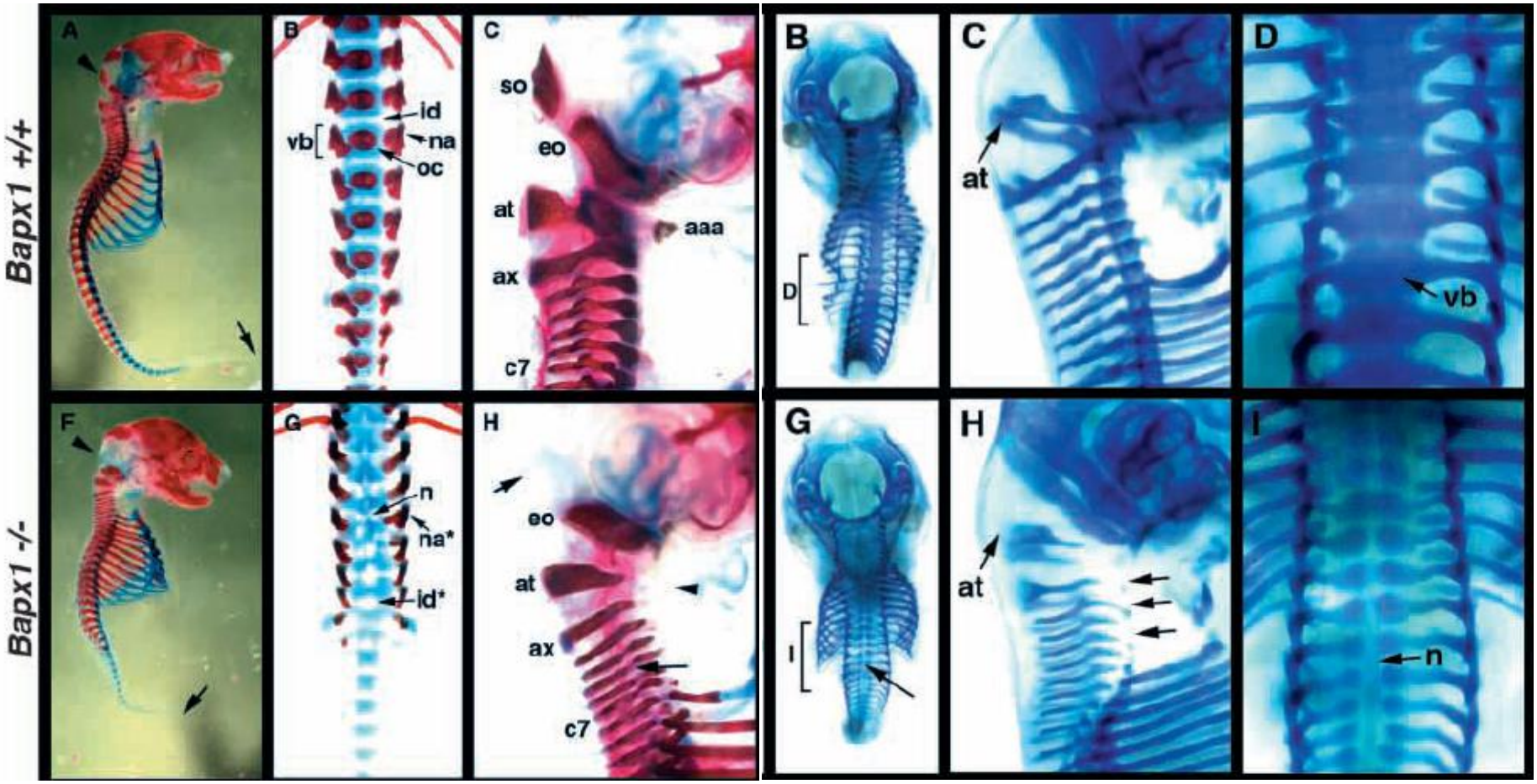
(pre)hypertrophic
chondrocyte

COL10
Ihh

Nkx3-2 (Bapx1) knockout mouse

E18.5 embryos

E14.5 embryos



Tribioli C and Lufkin T. Development 126:5699;1999

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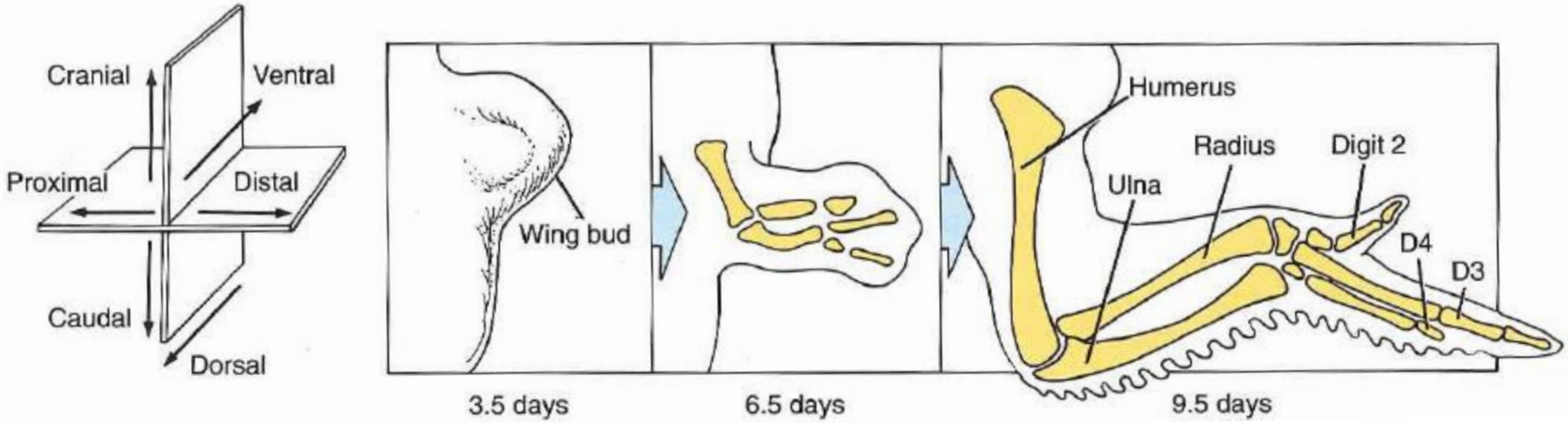
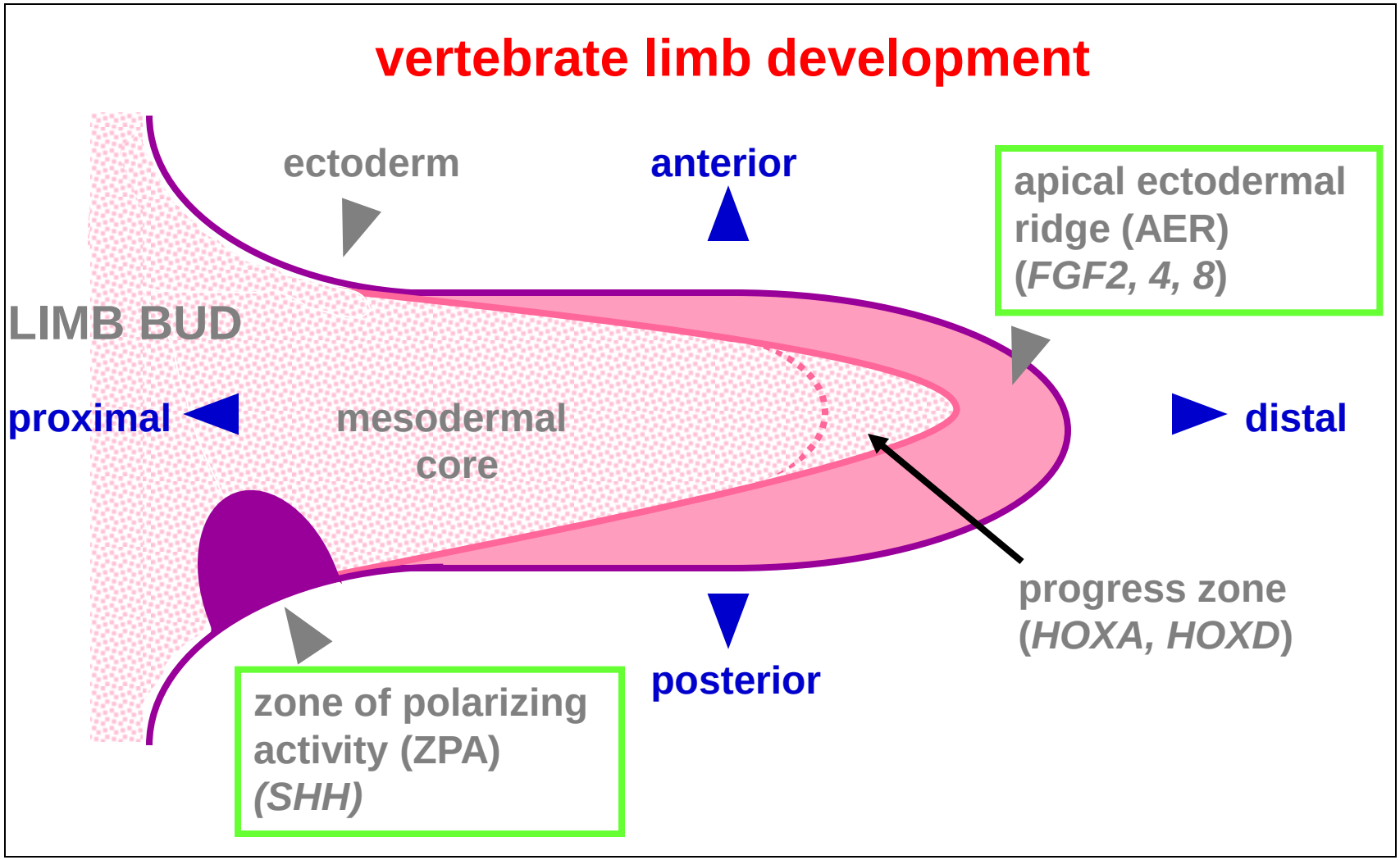
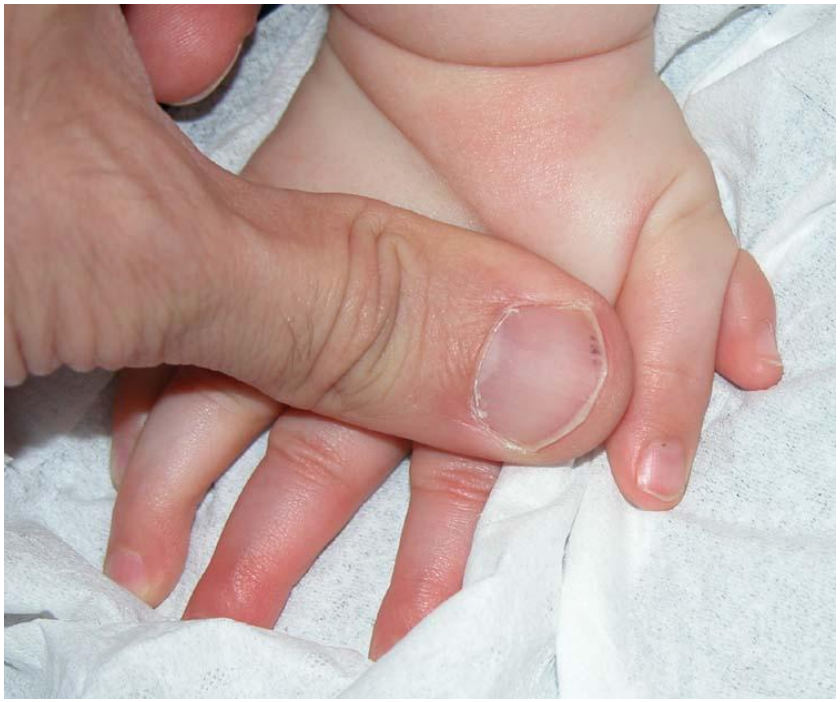
Patterning of limb buds



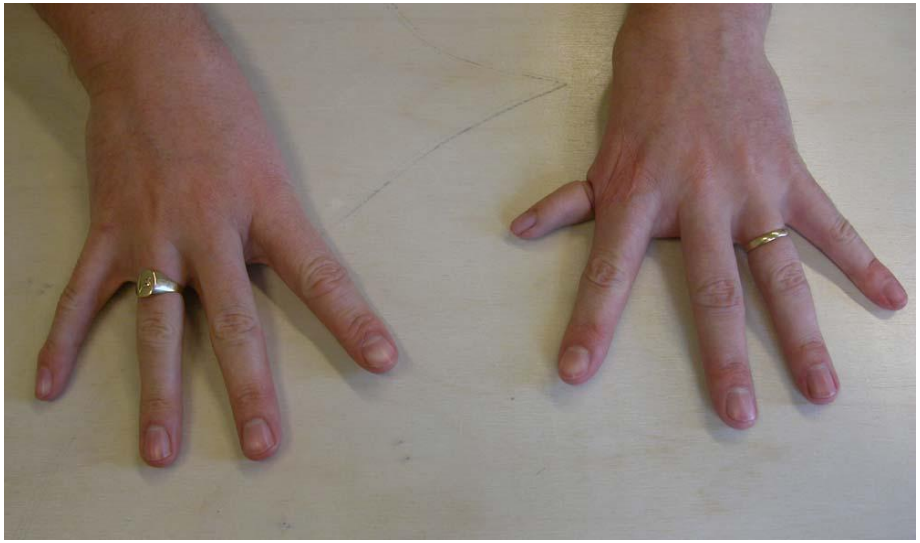
Patterning of limb buds



Disorders affecting skeletal patterning

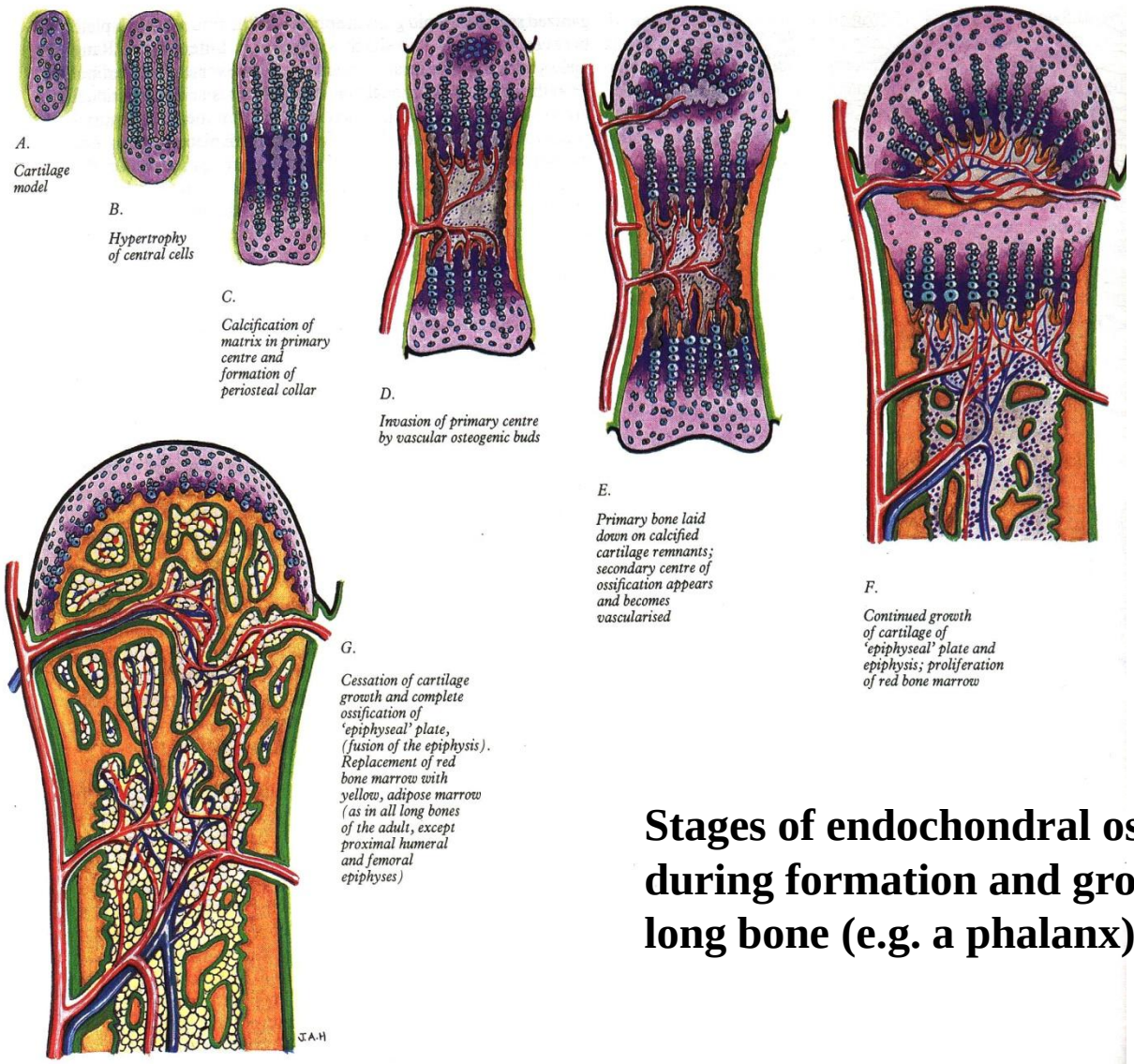


Townes-Brock syndrome (*SALL1*)



Holt-Oram syndrome (*TBX5*)

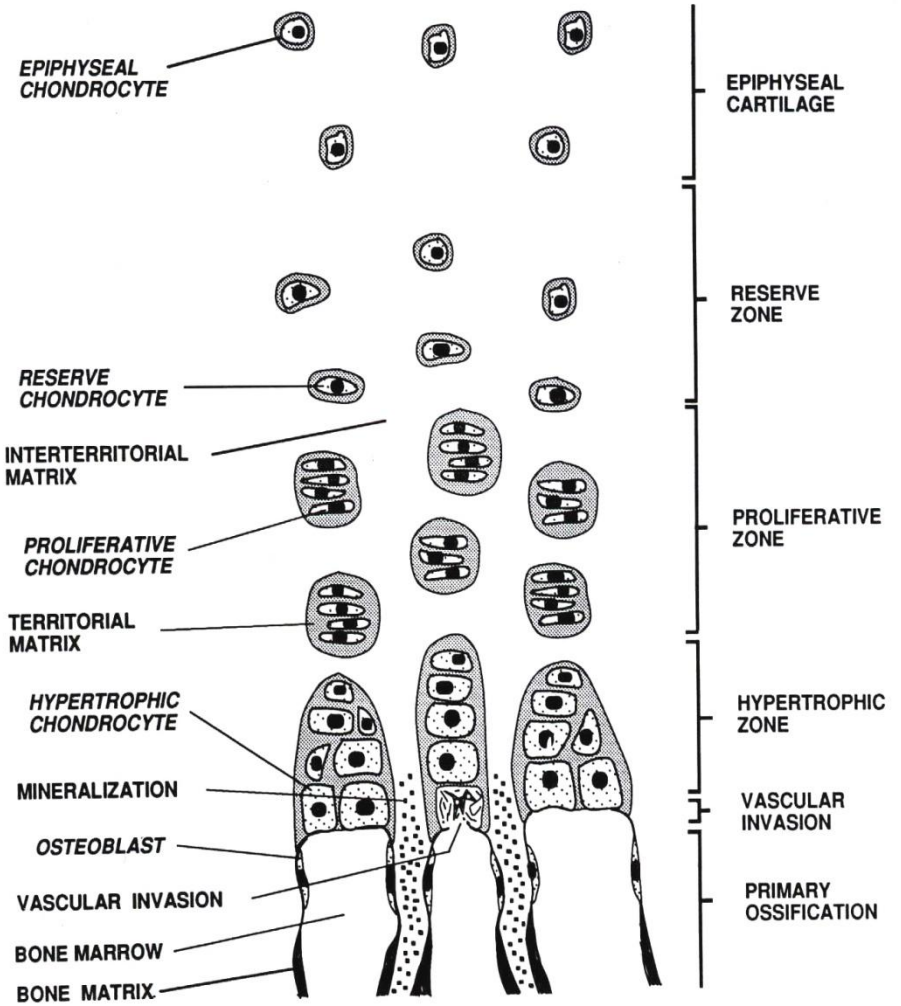
Endochondral ossification



Stages of endochondral ossification during formation and growth of a long bone (e.g. a phalanx)

Adapted from Williams PL et al. Gray's Anatomy. Edinburgh: Churchill Livingstone, 1993

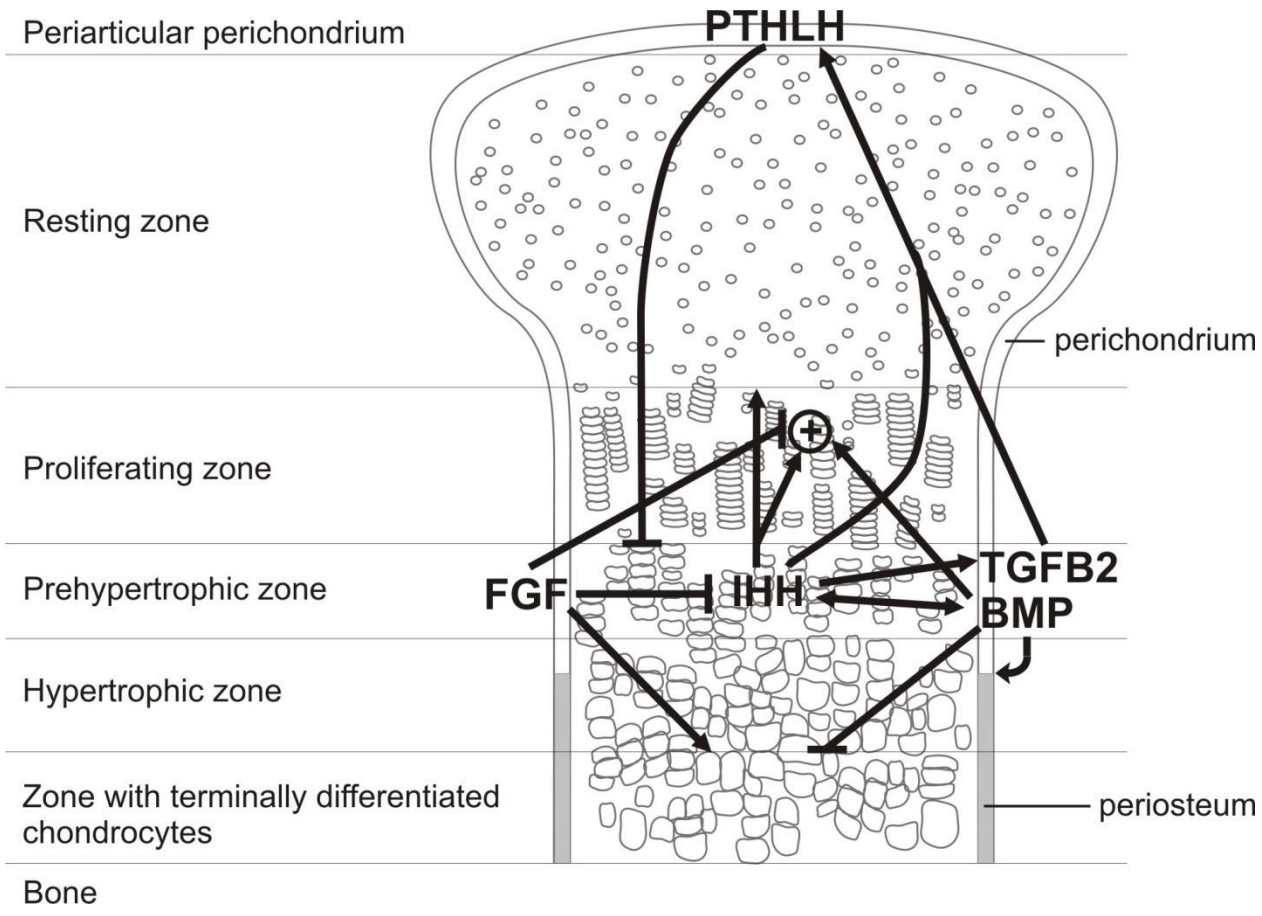
Growth plate



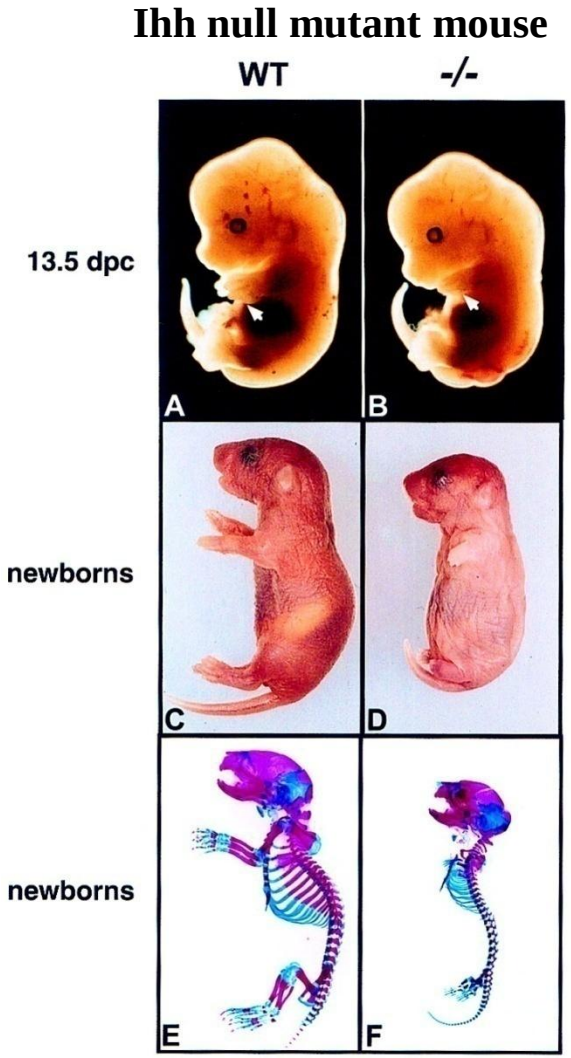
proliferation/differentiation balance

integrity of extracellular matrix

The Indian Hedgehog protein (IHH)



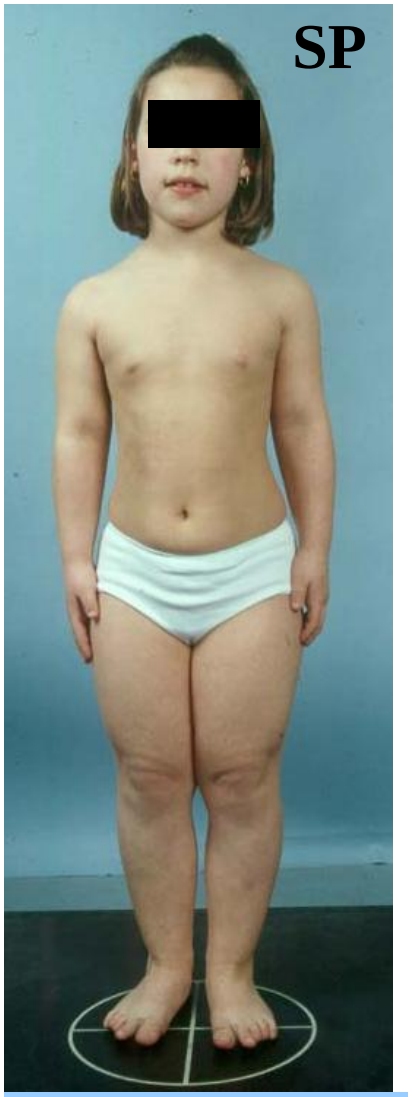
Indian Hedgehog knockout mouse



St-Jacques B et al. Genes Dev 1999;13:2072

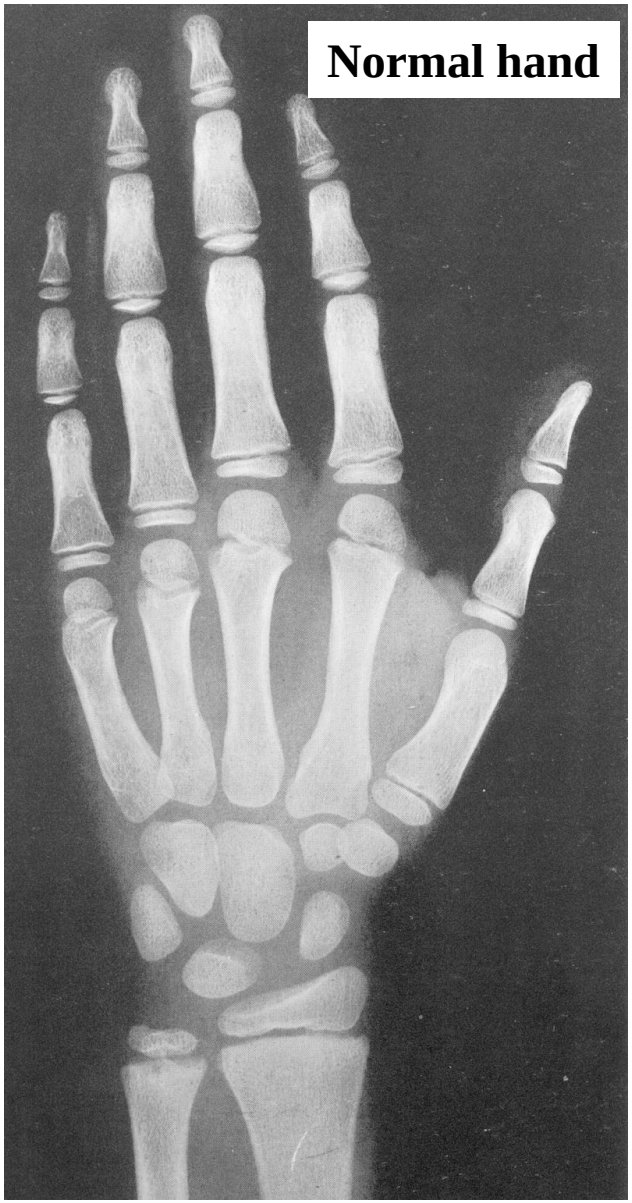
The role of IHH in human growth

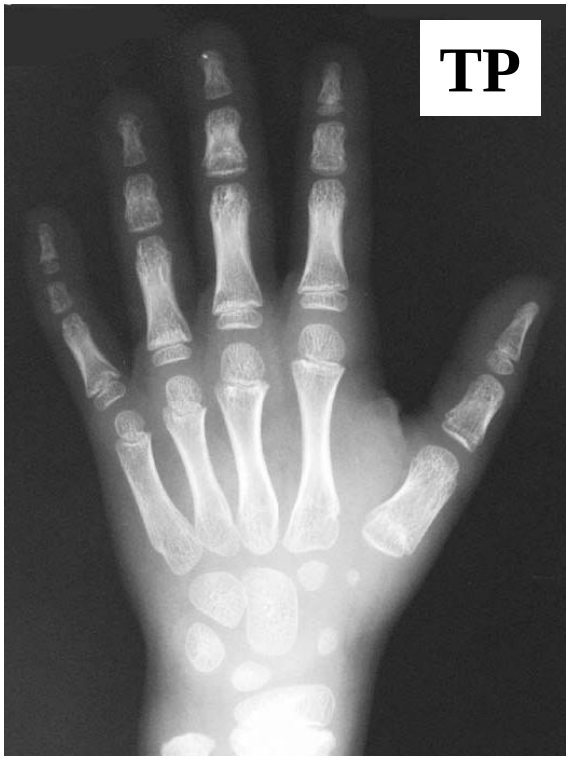
Multiple epiphyseal dysplasia ?

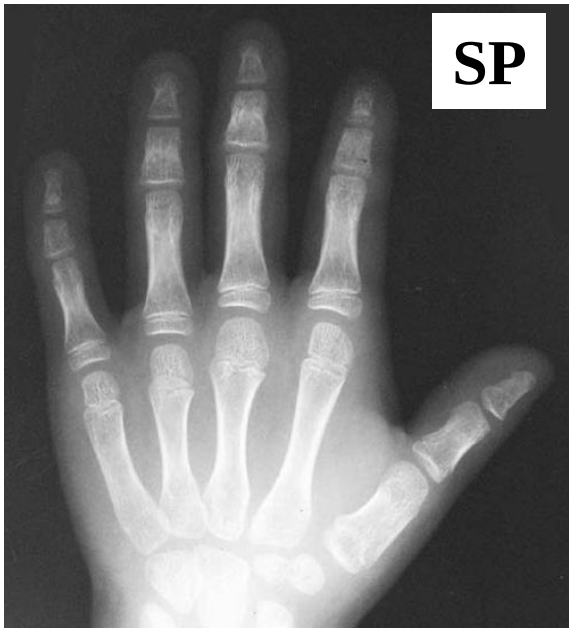


Hypochondroplasia ?

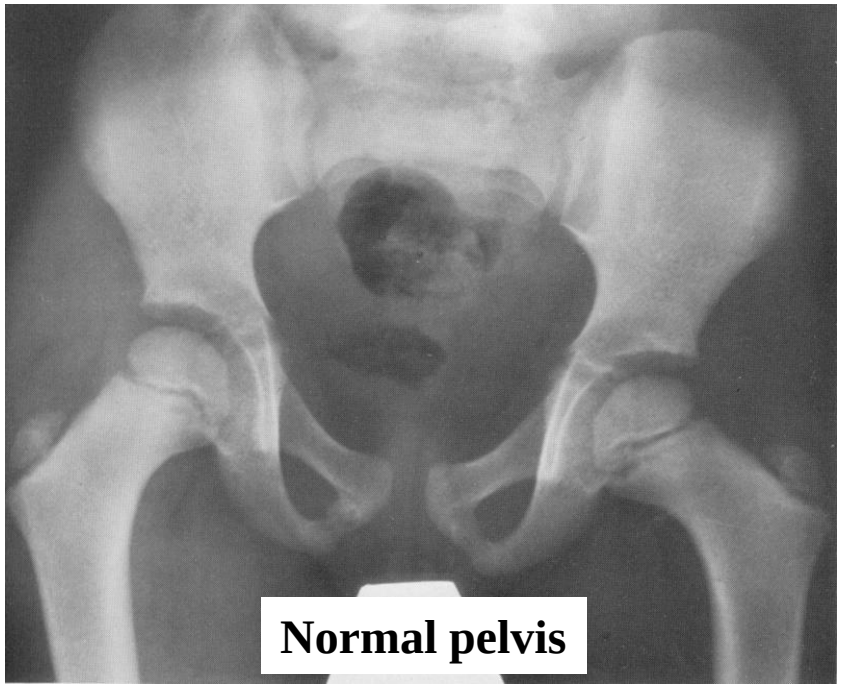
The role of IHH in human growth

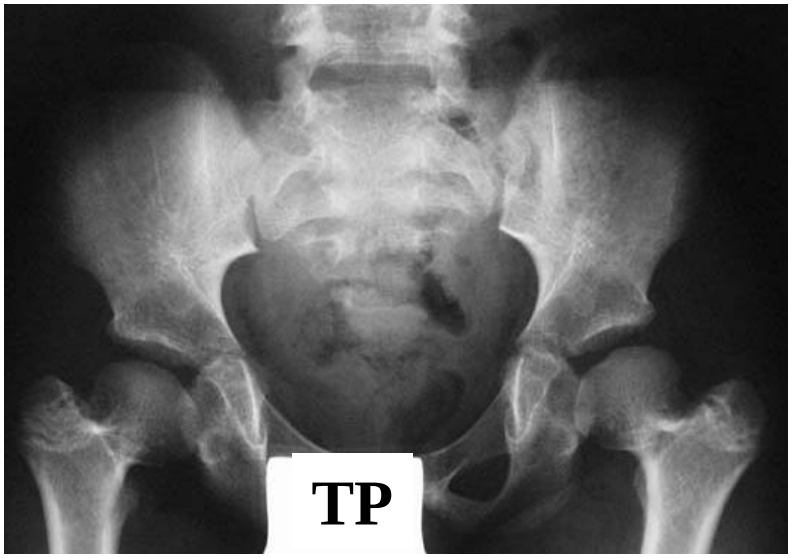


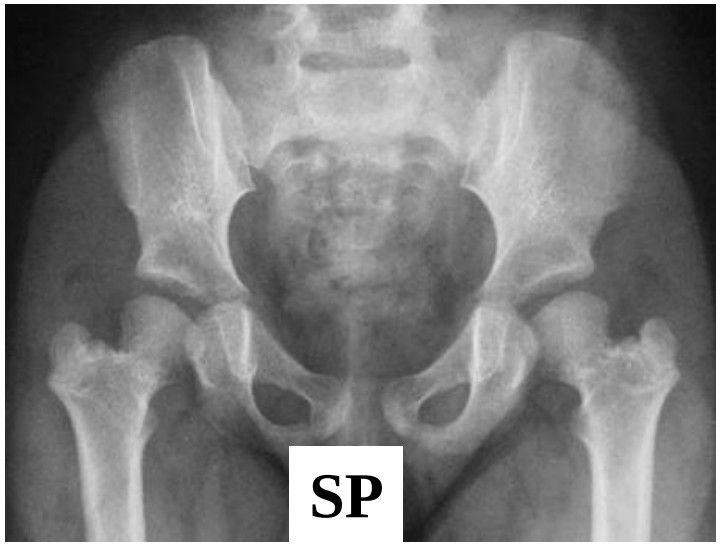




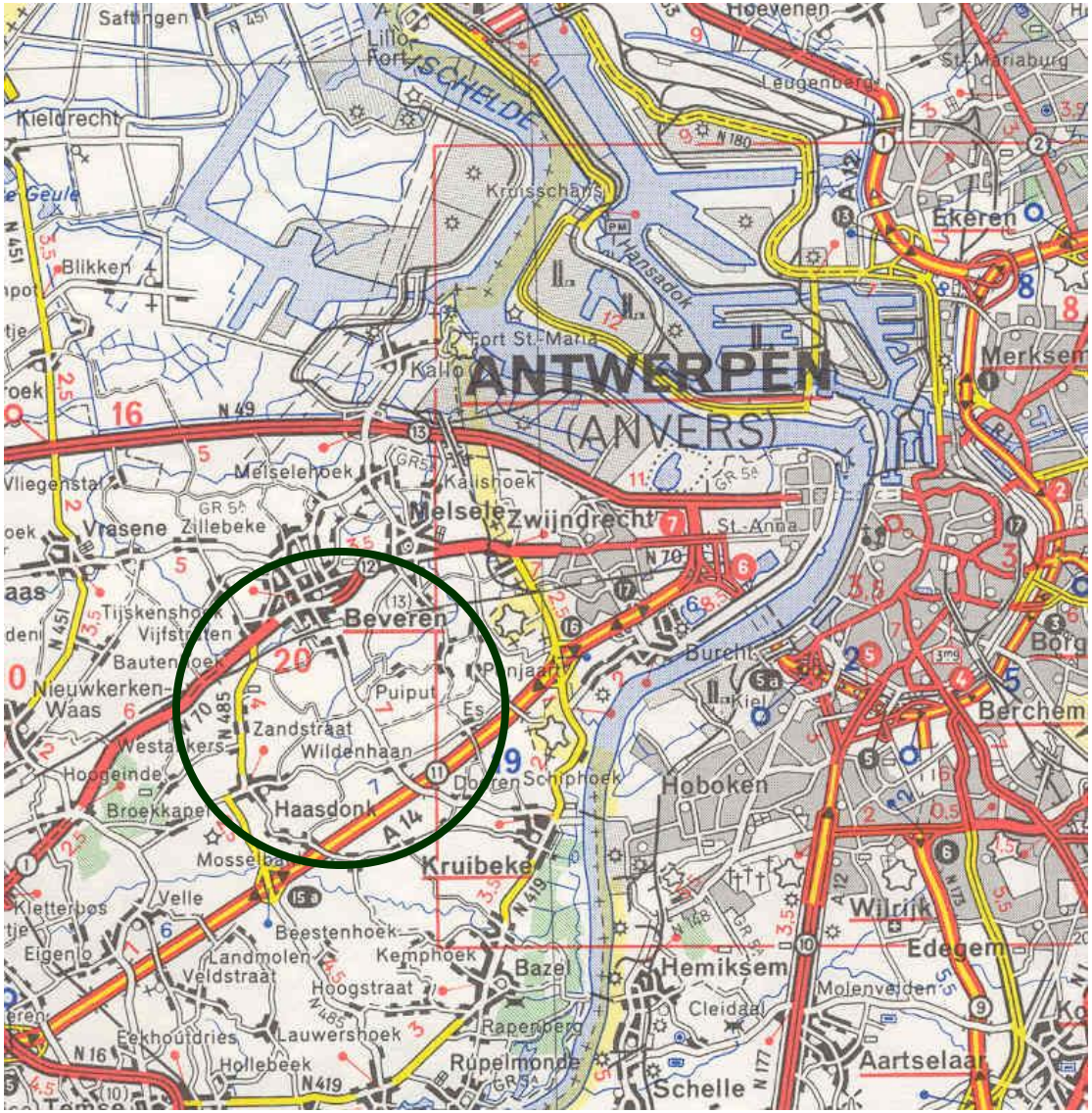
The role of IHH in human growth



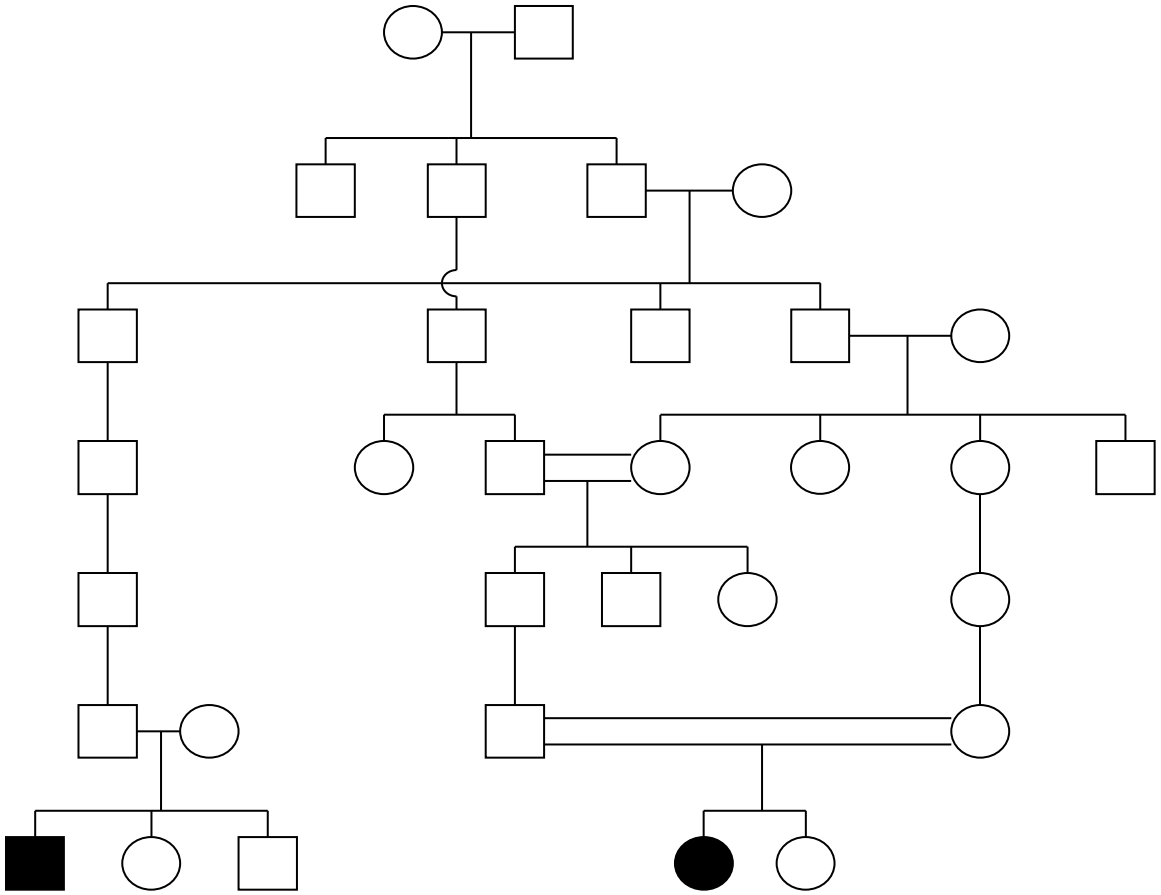




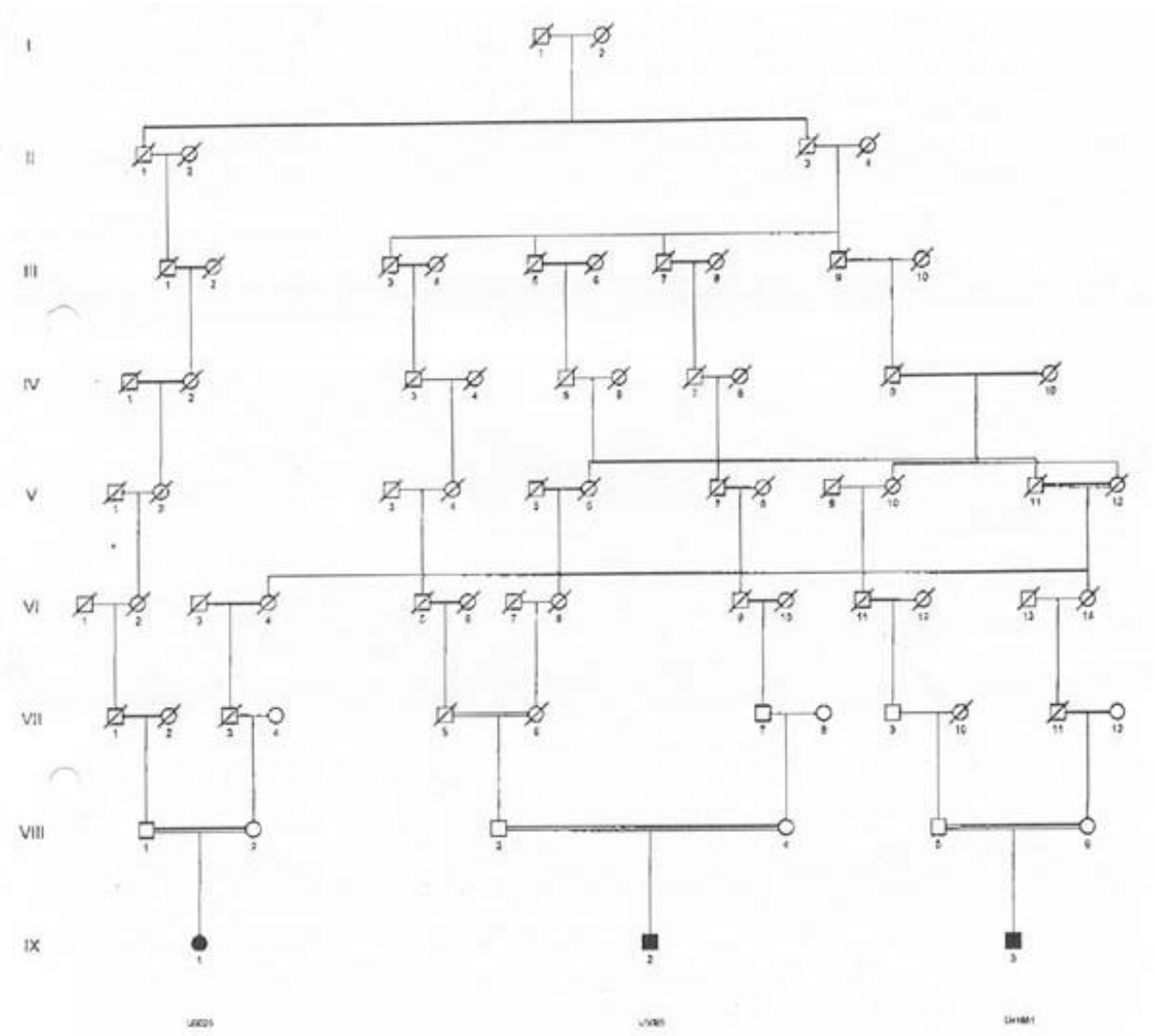
The role of IHH in human growth

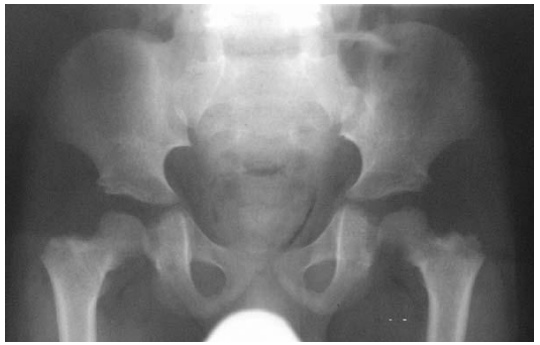


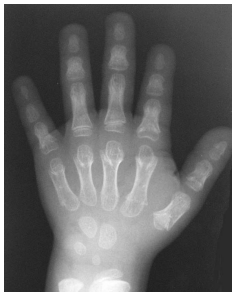
The role of IHH in human growth



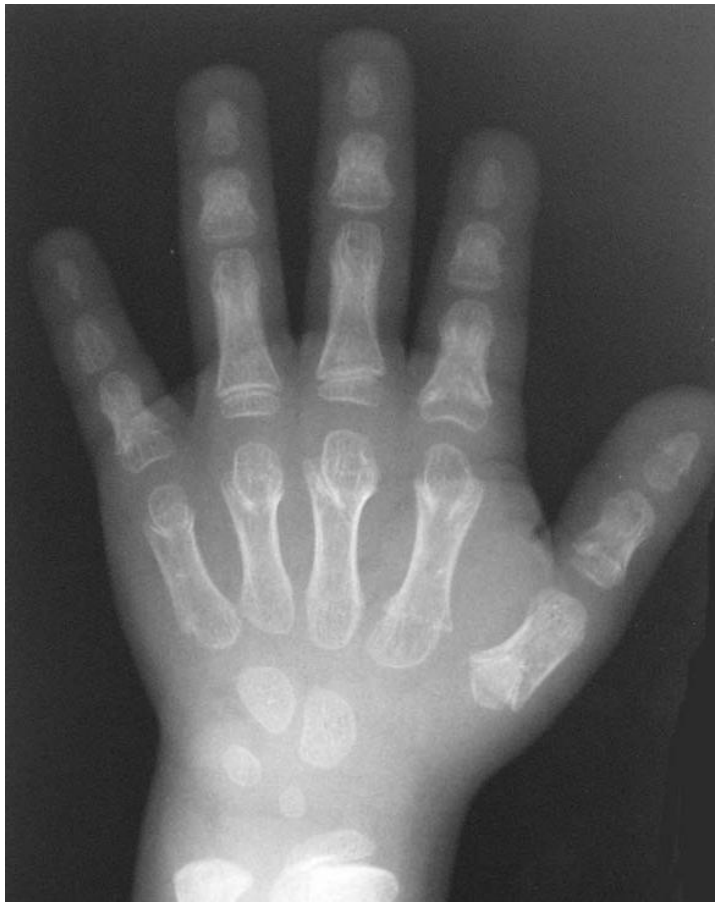
The role of IHH in human growth

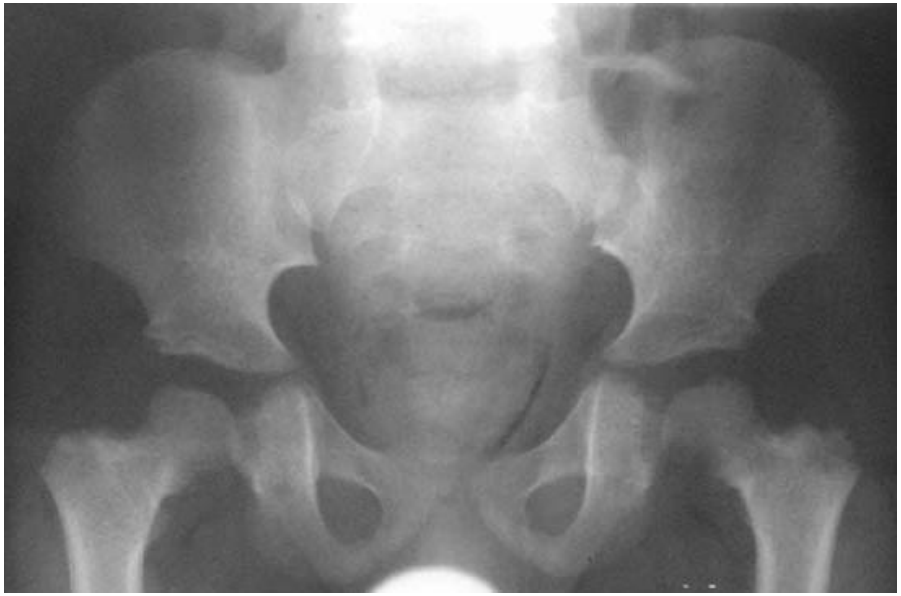






Acrocapitofemoral dysplasia

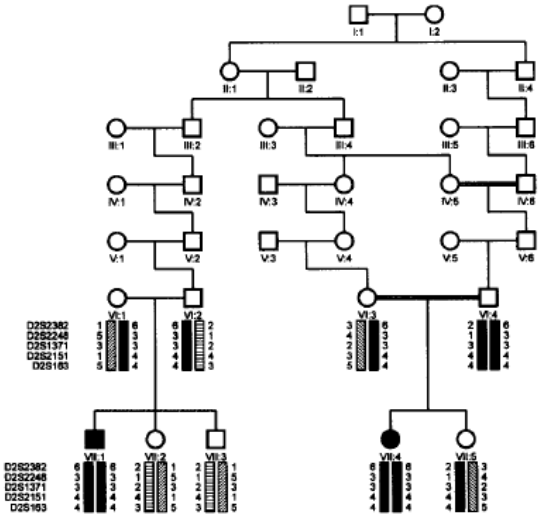




ACROCAPITOFEMORAL DYSPLASIA

Mortier GR et al. J Med Genet 2003;40:201

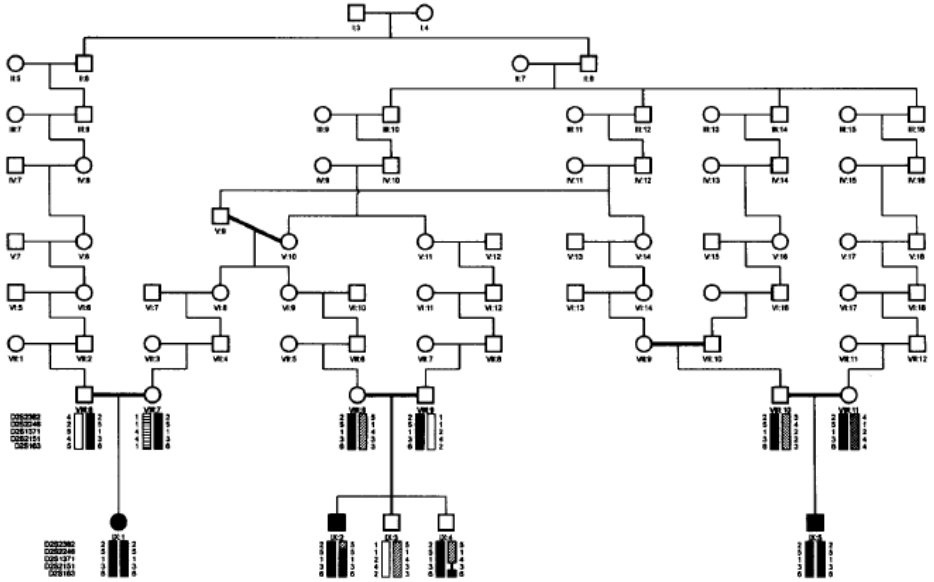
Homozygosity mapping



Two-Point LOD Scores for Linkage of the ACFD Locus to Chromosome 2q35-q36

		Combined LOD Score at $\theta =$					Family 1		Family 2		Families 1 and 2	
Marker	Position[a]	.00	.01	.05	.10	.30	Z_{max}	θ	Z_{max}	θ	Z_{max}	θ
D2S2382	213.49	$-\infty$	7.01	6.81	5.96	2.03	3.24	0	3.89	.05	7.09	.02
D2S2248	214.71	8.02	7.80	6.92	5.789	1.66	1.90	0	6.12	0	8.02	0
D2S1371	215.25	6.32	6.11	5.28	4.26	.97	1.40	0	4.92	0	6.32	0
D2S2151	218.45	5.02	4.85	4.17	3.34	.74	.63	0	4.40	0	5.02	0
D2S163	218.45	$-\infty$	10.85	10.62	9.72	4.98	2.02	0	8.89	.01	10.92	.02

[a] The position is given in centiMorgans according to the Marshfield map.



Hellemans J et al. Am J Hum Genet 2003;72:1040

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Candidate gene approach

217968292	217982124	SLC11A1	+	sv ev hm seq mm	C	2q35	solute carrier family 11 (proton-coupled divalent metal ion transp
217985853	217992038	NLI-IF	+	sv ev - seq mm	C	2q35	nuclear LIM interactor-interacting factor
218005220	218036527	VIL1	+	sv ev hm seq mm	C	2q35-q36	villin 1
218039349	218154461	KIAA1594	-	sv ev - seq mm	C	2q35	KIAA1594 protein
218154765	218182530	ROCD1	+	sv ev hm seq mm	C	2q35	RCD1 required for cell differentiation1 homolog (S. pombe)
218193542	218223279	PLCD4	+	sv ev hm seq mm	C	2q35	phospholipase C, delta 4
218224017	218245730	ZNF142	-	sv ev - seq mm	C	2q34-q35	zinc finger protein 142 (clone pHZ-49)
218245772	218249540	BCS1L	+	sv ev hm seq mm	C	2q33	BCS1-like (yeast)
218249963	218258156	RNF25	-	sv ev hm seq mm	C	2q35	ring finger protein 25
218258167	218288811	STK36	+	sv ev hm seq mm	C	2q35	serine/threonine kinase 36 (fused homolog, Drosophila)
218296992	218341511	KIAA0173	+	sv ev - seq mm	C	2p24.3-p24.1	KIAA0173 gene product
218367835	218401390	CYP27A1	+	sv ev hm seq mm	C	2q33-qter	cytochrome P450, subfamily XXVIIA (steroid 27-hydroxylase, ce
218406259	218408617	LOC285181	+	sv ev - seq mm	I	2q35	hypothetical gene supported by AK093857
218408481	218417886	PRKAG3	-	sv ev - seq mm	C	2q35	protein kinase, AMP-activated, gamma 3 non-catalytic subunit
218445920	218460325	WNT6	+	sv ev hm seq mm	C	2q35	wingless-type MMTV integration site family, member 6
218466629	218480025	WNT10A	+	sv ev hm seq mm	C	2q35	wingless-type MMTV integration site family, member 10A
218539497	218540049	LOC130623	-	sv ev - seq mm	PE	2q35	similar to keratin 8; Keratin-8 [Homo sapiens]
218545747	218547741	CDK5R2	+	sv ev hm seq mm	C	2q35	cyclin-dependent kinase 5, regulatory subunit 2 (p39)
218562380	218564017	LOC151300	+	sv ev - seq mm	I	2q35	LOC151300
218567183	218571753	HSRNAFEV	-	sv ev - seq mm	C	2q36	FEV protein
218576286	218579501	CRYBA2	-	sv ev hm seq mm	C	2q34-q36	crystallin, beta A2
218588950	218595227	LOC255101	-	sv ev - seq mm	I	2q35	similar to hypothetical protein [Homo sapiens]
218604767	218605799	LOC200760	-	sv ev - seq mm	E	2q35	LOC200760
218617352	218627619	MGC35338	-	sv ev - seq mm	C	2q35	hypothetical protein MGC35338
218640521	218646282	IHH	-	sv ev hm seq mm	C	2q33-q35	Indian hedgehog homolog (Drosophila)
218661422	218747484	FLJ12610	-	sv ev - seq mm	C	2q35	hypothetical protein FLJ12610

start	stop	symbol	orient.	links	evidence	cyto.	full name
218661422	218756153	FLJ31168	-	sv ev - seq mm	C	2q35	hypothetical protein FLJ31168
218757994	218764265	CGI-57	-	sv ev - seq mm	C	2q35	hypothetical protein CGI-57
218764328	218770601	MGC3035	+	sv ev - seq mm	C	2q35	hypothetical protein MGC3035
218792889	218795744	LOC130617	+	sv ev - seq mm	C	2q35	hypothetical protein BC018415
218795868	218805150	ABCB6	-	sv ev hm seq mm	C	2q36	ATP-binding cassette, sub-family B (MDR/TAP), member 6
218805477	218815728	FLJ22169	-	sv ev - seq mm	C	2q36.1	hypothetical protein FLJ22169
218815916	218822764	FLJ10415	+	sv ev hm seq mm	C	2q36.1	hypothetical protein FLJ10415
218822706	218831574	MGC10771	-	sv ev hm seq mm	C	2q36.1	hypothetical protein MGC10771
218831597	218835001	STK16	+	sv ev hm seq mm	C	2q34-q37	serine/threonine kinase 16
218835807	218864317	TUBA1	-	sv ev - seq mm	C	2q36.1	tubulin, alpha 1 (testis specific)

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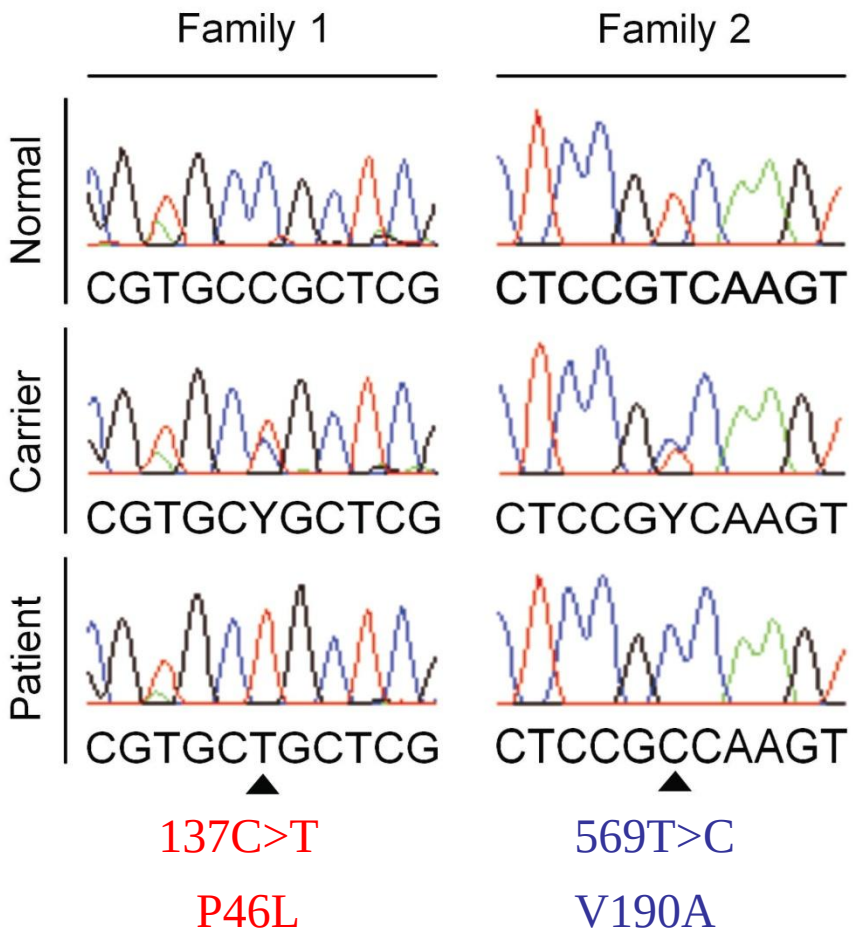
Mutations in IHH cause ACFD



The Indian Hedgehog gene

P46L

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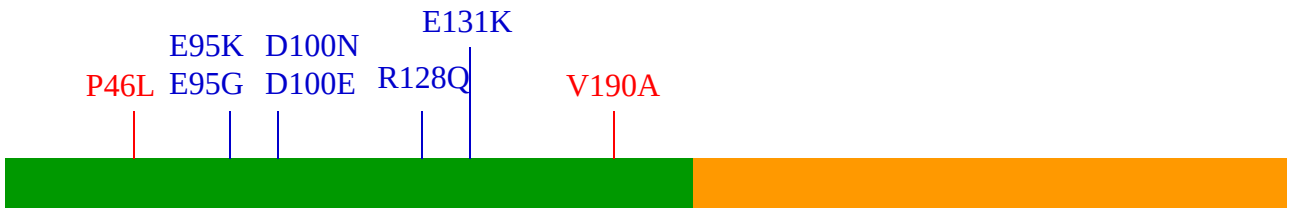
                                                                                                  *     *
HH Drome)    CGPGRG-LG- -RHRARN-LY PLVLKQTIPN LSEYTNSASG PLEGVIRRDS PKFKDLVPNY NRDILFRDEE GTGADGLMSK RCKEKLNVLA
IHH Mouse)   .....V.V.S R.RPP.K..V .AY..FS.. VP.K.LG... RY..K.A.S. ER..E.T... .P..I.K... N....R..TQ ...DR..S..
IHH Brare)   .......Y.. K.RTP.K..T .AY..FS.. VA.K.LG... RY..KVTPS. ER..E.T... .P..I.K... N....RM.TQ ...D...S..
DHH Mouse)   ......PV.. R.RYV.KQ.V .LY..FV.S MP.R.LG... .A..RVT.G. ER.R..... .P..I.K... NS...R..TE ....RV.A..
DHH2 Xenla)  ......PV.G R.RYM.R..V .LY..FV.. VP.K.LG... KS..K...G. ER.IK..... .P..I.K... N....R..TE ...DRV.A..
TWHH Brare)  .......Y.. K.RHPKK..T .AY..F... VA.K.LG... KY..K.T.N. ER..E.I... .P..I.K... N.N..R..T. ...D...S..
AmphiHH)     ....GR.F.. R.RHP.K..T .F.Y..QM.A V..N.FG... LFN.R.T... ER.HT.KQ.F .T..I.K... K....RF.TQ ...D...A..

                         *
HH Drome)    YSVMNEWPGI RLLVTESWDE DYHHGQESLH YEGRAVTIAT SDRDQSKYGM LARLAVEAGF DWVSYVSRRH IYCSVKSDSS ISSHVHG
IHH Mouse)   I....Q...V K.R...G... .G..SE.... ......D.T. ....RN...L .......... ...Y.E.KA. VH.....EH. AAAKTG.
IHH Brare)   I....L...V ..R...G... .GL.SE.... ......D.T. ....RN..R. .......... ...Y.E.KG. VH.....EH. VAAKTG.
DHH Mouse)   IA...M...V ..R...G... .G..A.D... .....LD.T. ....RN...L .......... ...Y.E..N. .HV...A.N. LAVRAG.
DHH2 Xenla)  I....M...L K.R...G... .G..AHD... .....LD.T. ....RN.... .......... ...Y.E.KA. .HV..NT.N. LGVRSG.
TWHH Brare)  I....H...V K.R...G... .G..LE.... ......D.T. ....K..... .S........ ...Y.E.KA. .H....AEN. VAAKSG.
AmphiHH)     I....Q.E.V K.R...G... .GF.TE.... ......D.T. ....RT.... .......... ...Y.E.KA. .H....AE.D TTATQG.
```

V190A



BDA1 mutations

ACFD mutations

N-terminal signaling domain

C-terminal autoprocessing domain

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Homozygous mutations

IHH

Indian hedgehog

Acrocapitofemoral dysplasia

(AR)

Heterozygous mutations

IHH

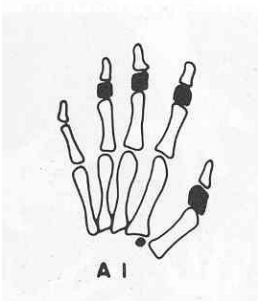
Indian hedgehog

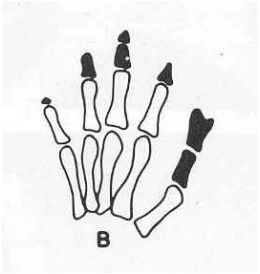
brachydactyly A1

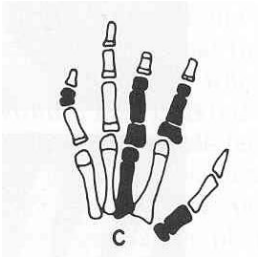
(AD)

Heterozygous mutations

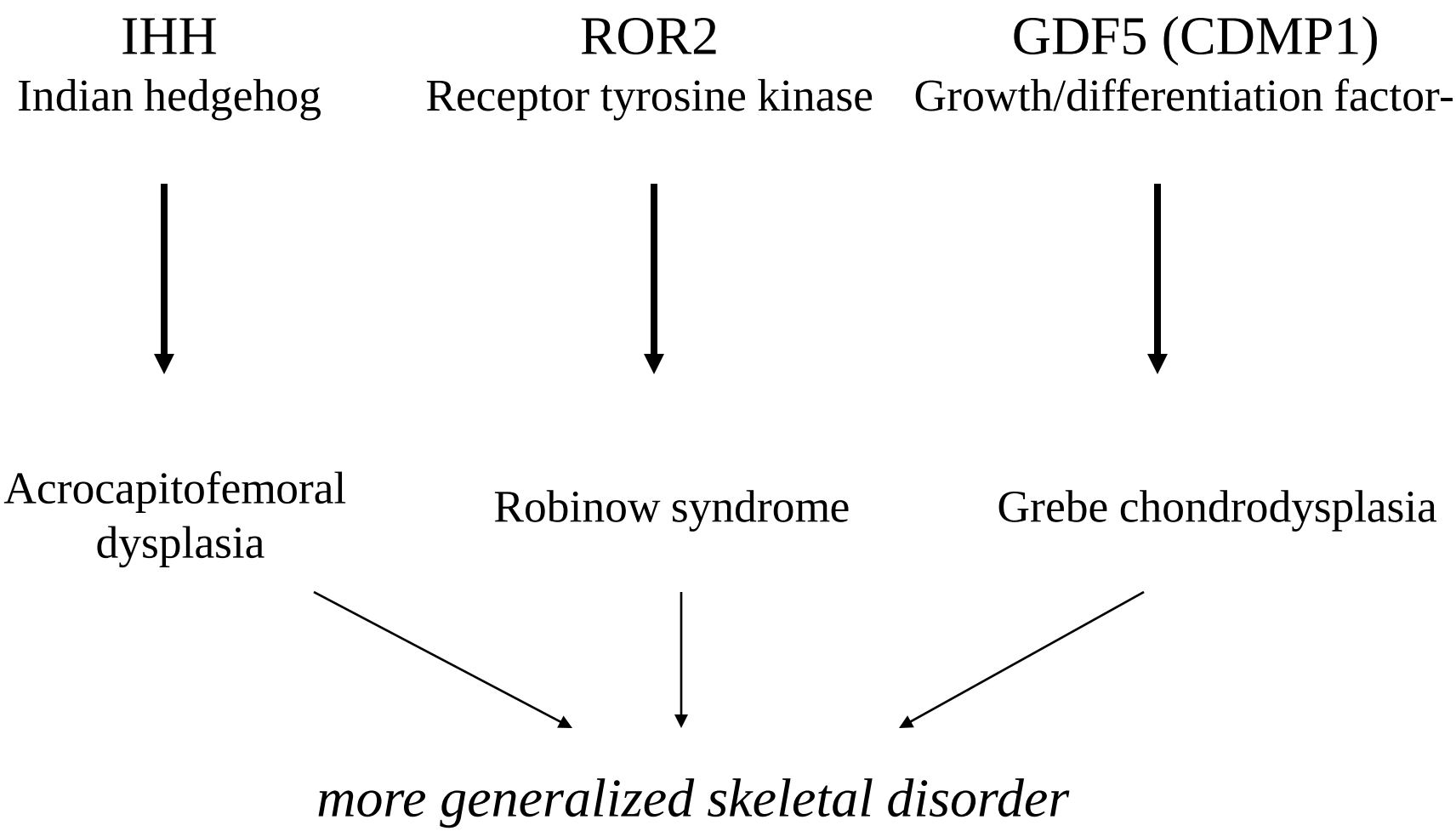
IHH	ROR2	GDF5 (CDMP1)
Indian hedgehog	Receptor tyrosine kinase	Growth/differentiation factor-5
↓	↓	↓
brachydactyly A1	brachydactyly B	brachydactyly C



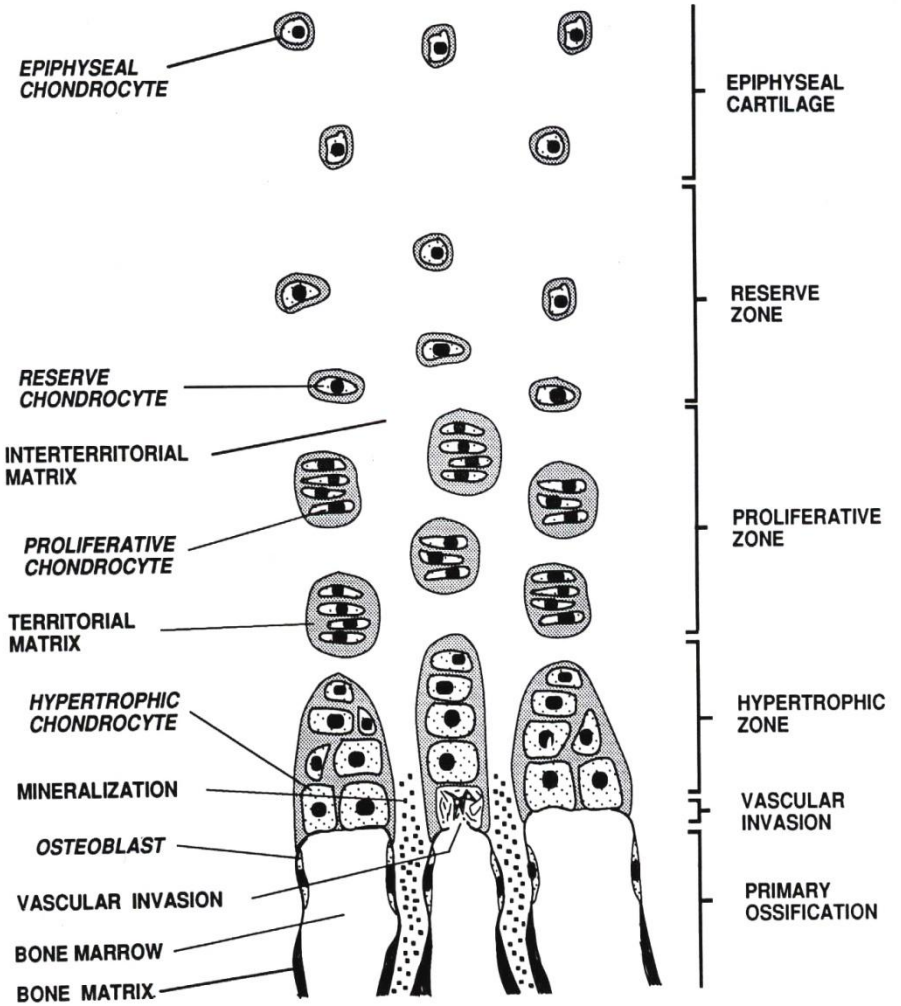




Homozygous mutations



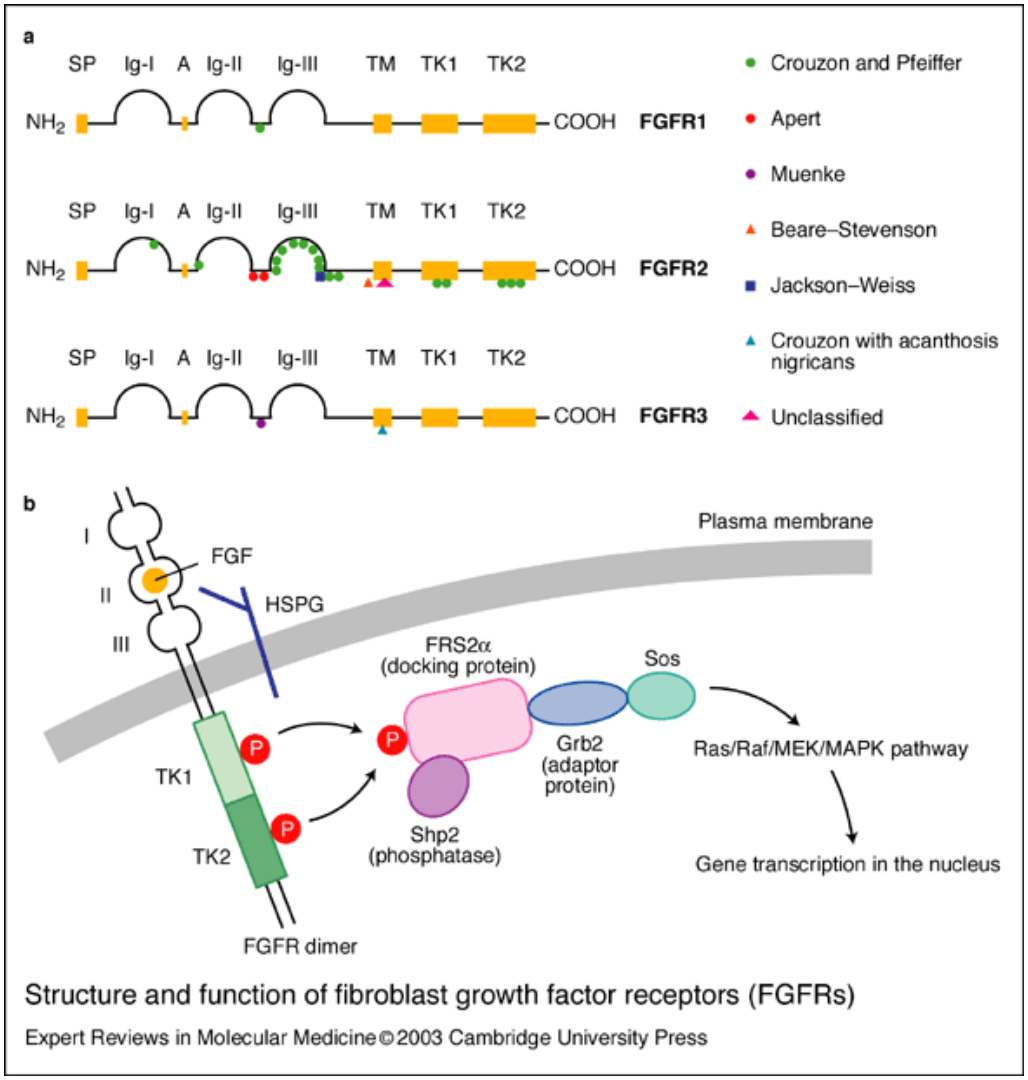
Growth plate



proliferation/differentiation balance

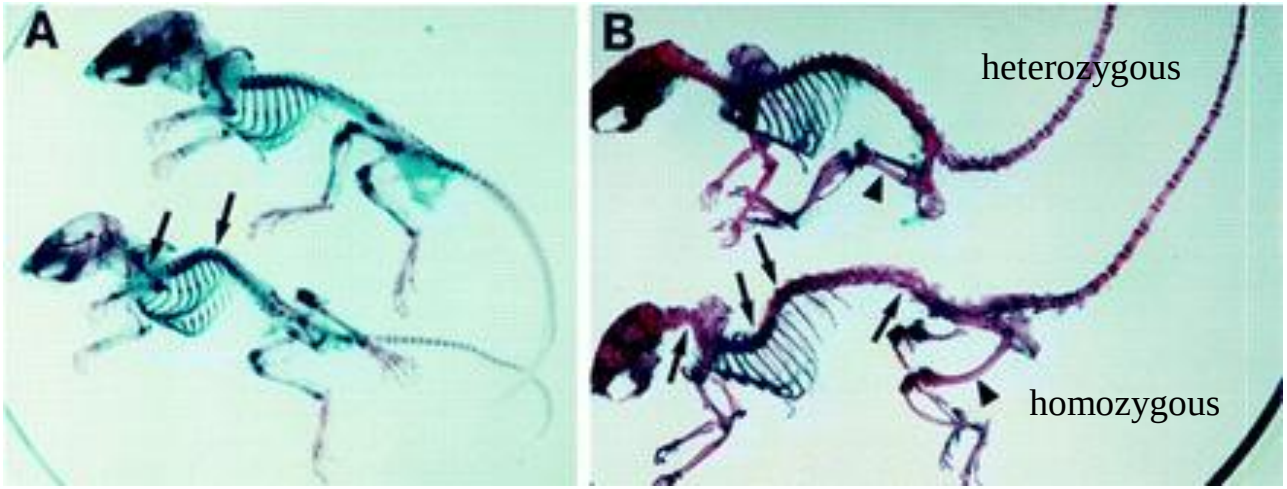
integrity of extracellular matrix

Fibroblast growth factor receptors



Structure and function of fibroblast growth factor receptors (FGFRs)

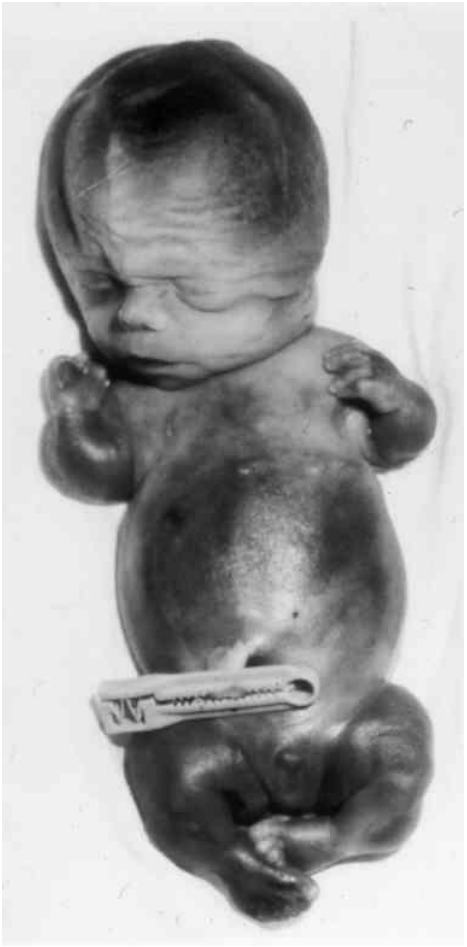
Negative regulator of endochondral ossification



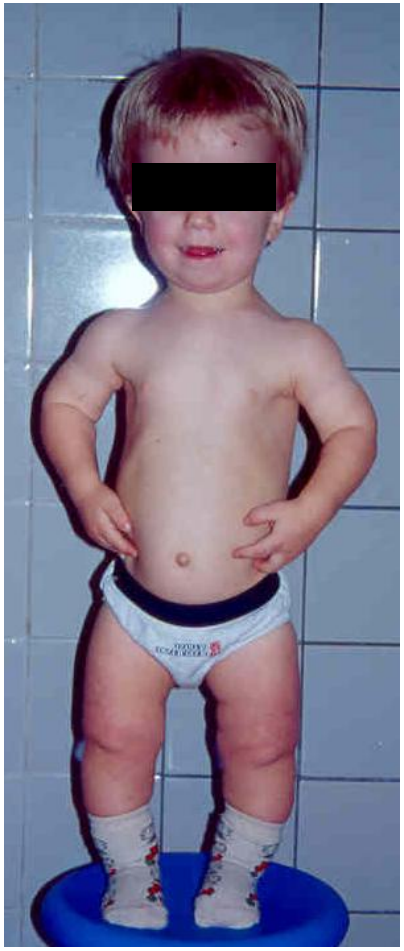
Overgrowth of axial and appendicular skeleton in FGFR3-deficient mice

Deng C et al. Cell 1996 84:911-921

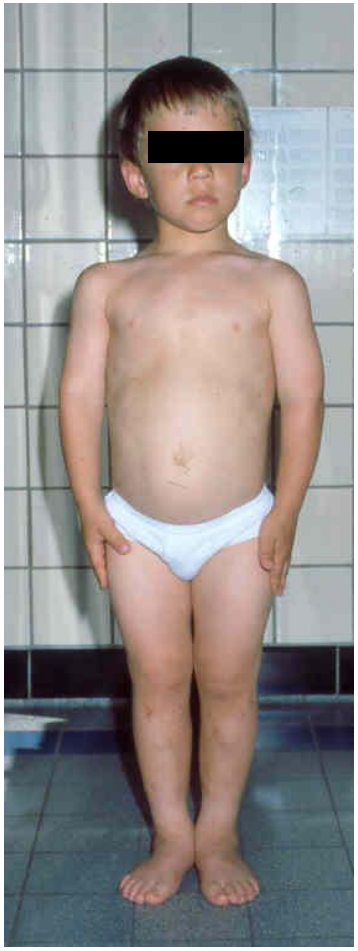
FGFR3 disorders



thanatophoric dysplasia



achondroplasia



hypochondroplasia

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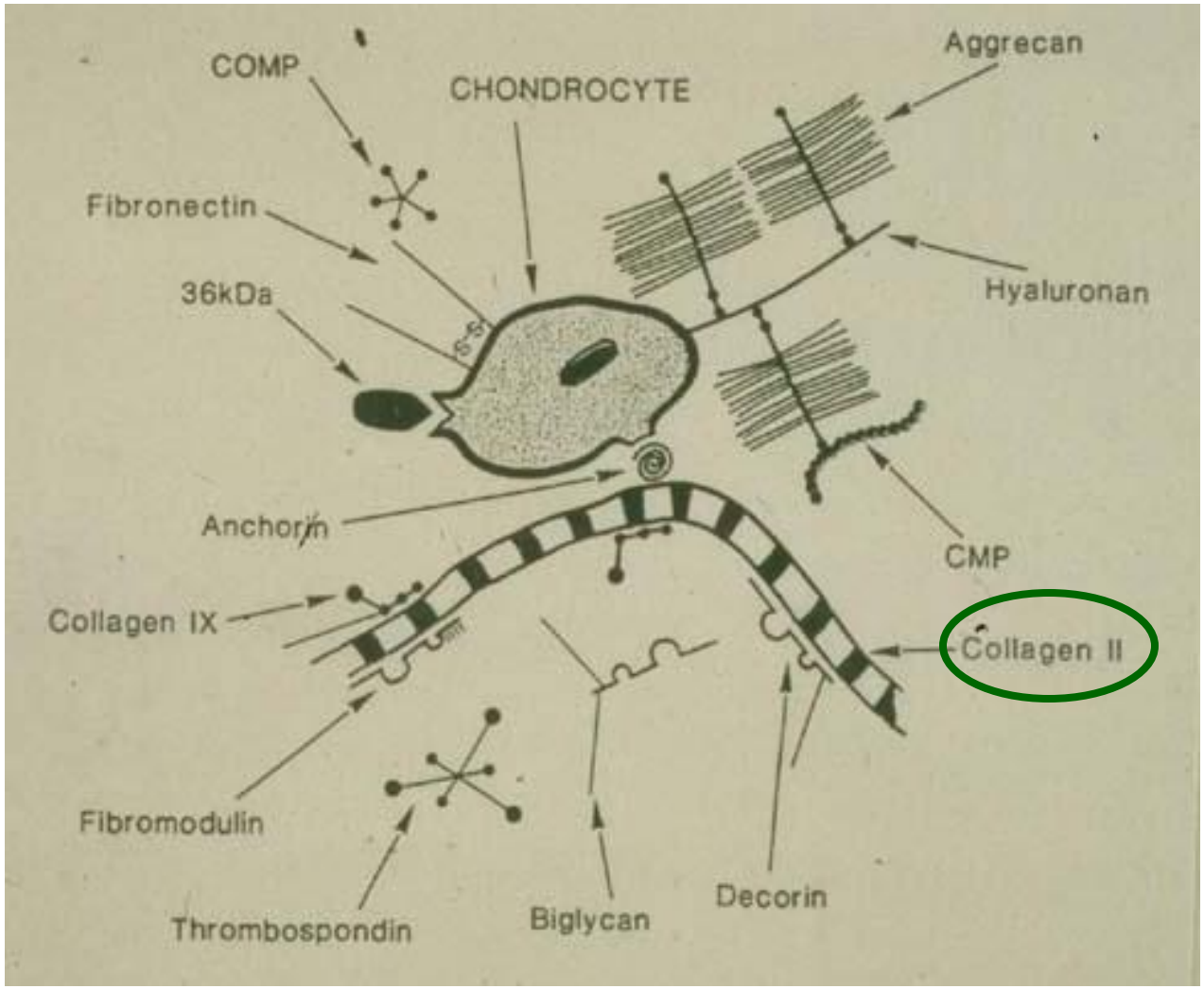
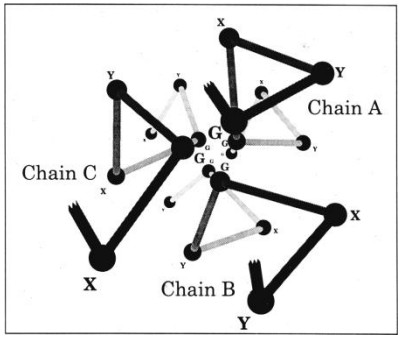
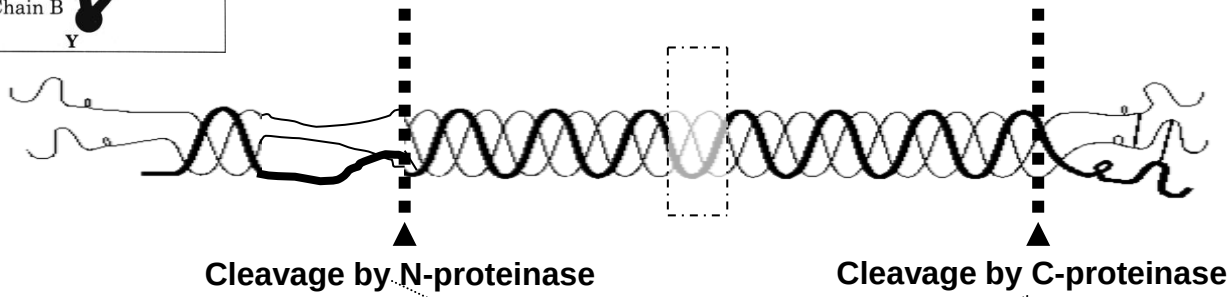
Cartilage extracellular matrix



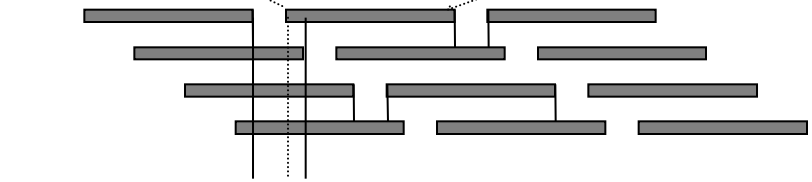
Fibrillar collagens



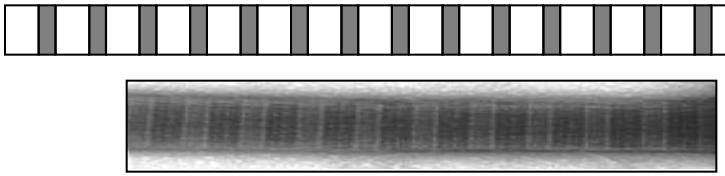
AMINO ACID SEQUENCE



MOLECULE
Type II procollagen



MOLECULAR PACKING

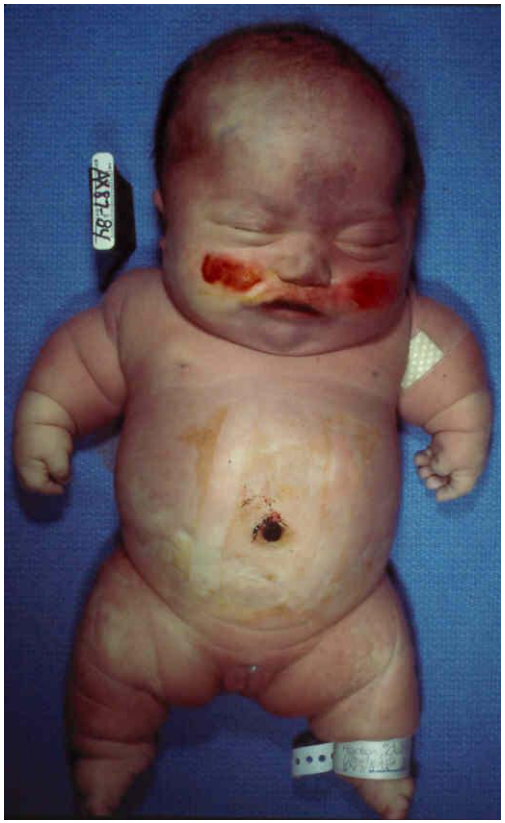


COLLAGEN FIBRIL

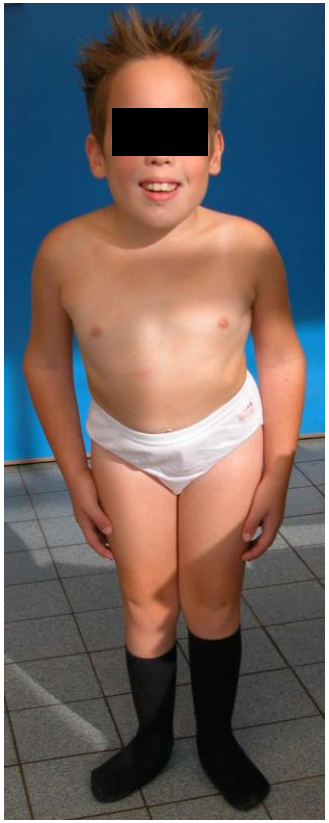
Great potential for errors

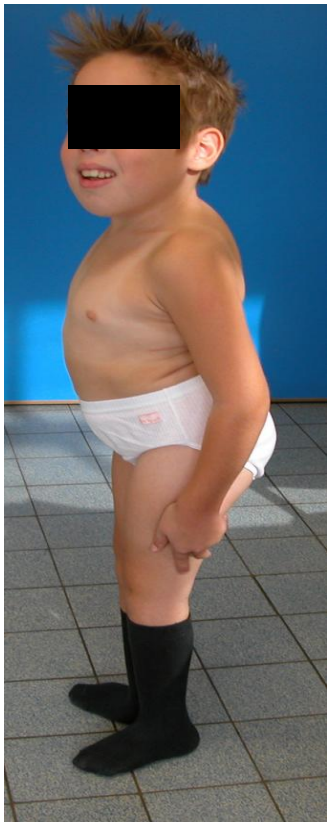
Type II collagen disorders

Lethal conditions



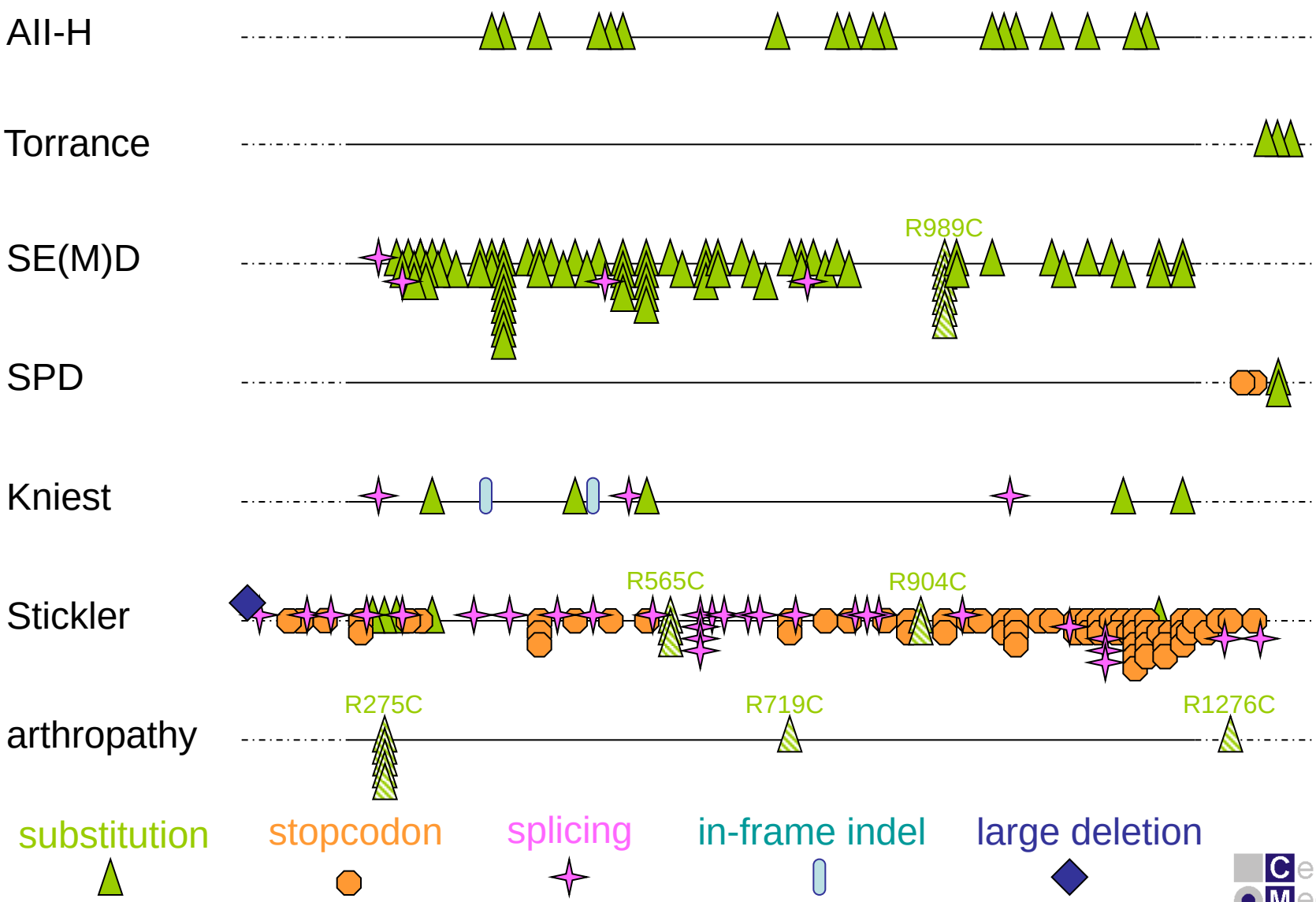
Short-trunk dwarfism





Osteoarthrosis

Genotype-phenotype correlations in type II collagen disorders



Arg to Cys mutations in the COL2A1 gene

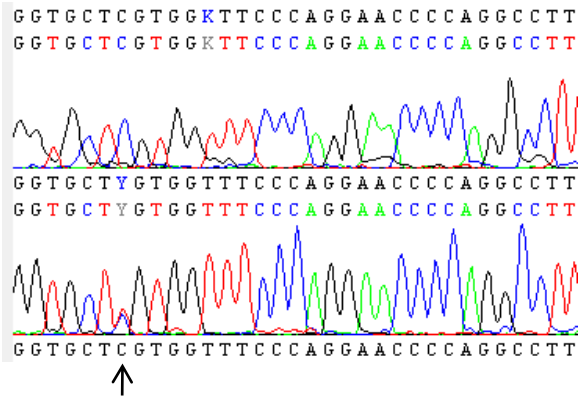
European Journal of Human Genetics (2007), 1–7

www.nature.com/ejhg

ARTICLE

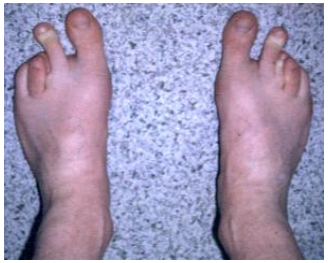
Czech dysplasia metatarsal type: another type II collagen disorder

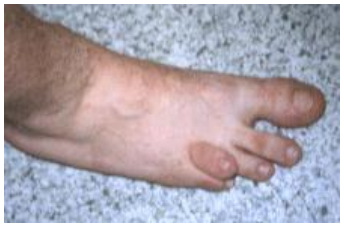
Kristien P Hoornaert[1], Ivo Marik[2], Kazimierz Kozlowski[3], Trevor Cole[4], Martine Le Merrer[5], Jules G Leroy[1], Paul J Coucke[1], David Sillence[6] and Geert R Mortier*,[1]

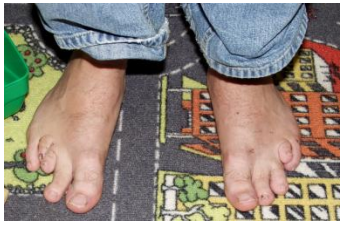


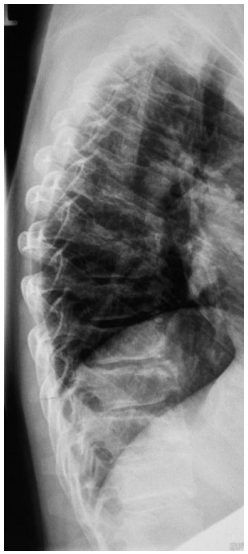
p.Arg275Cys

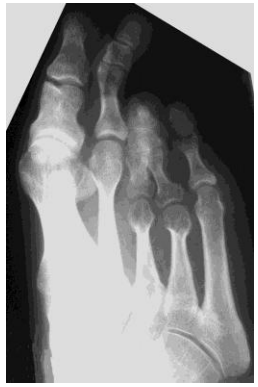
- normal stature
- early-onset arthropathy
- short (postaxial) toes
- absence of ocular/orofacial anomalies
- RX: spondylo-arthropathy











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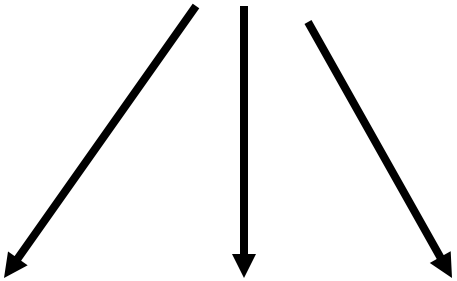
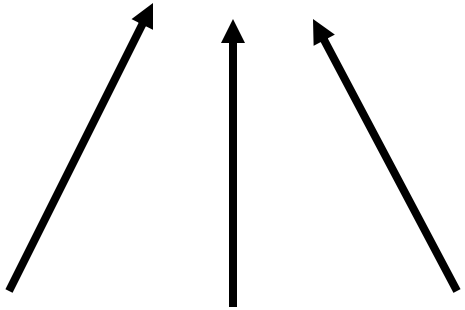
one gene



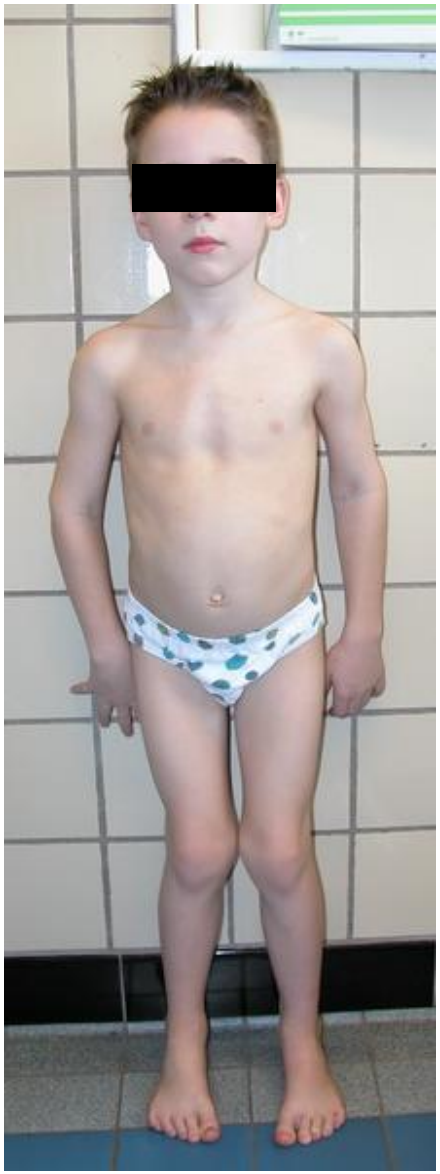
several phenotypes

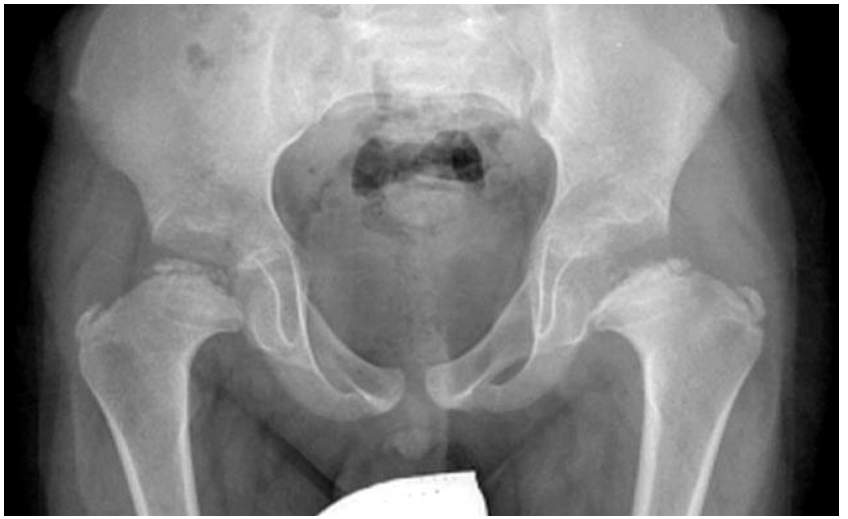
one phenotype

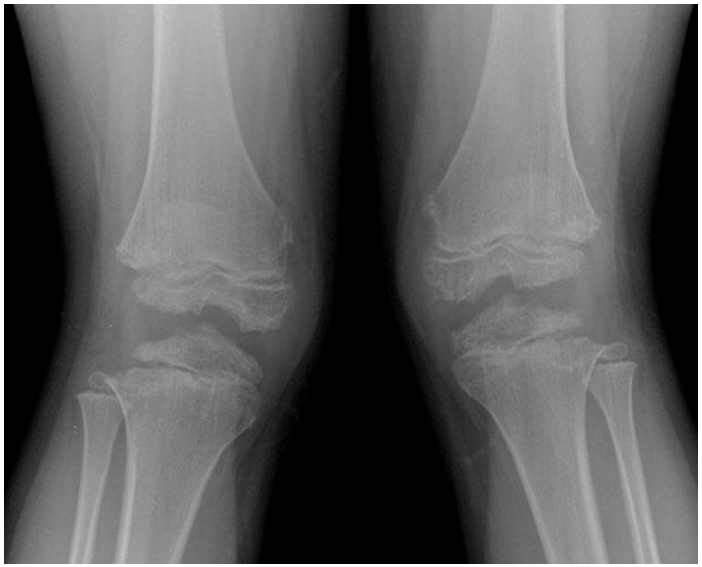


several genes

Multiple epiphyseal dysplasia

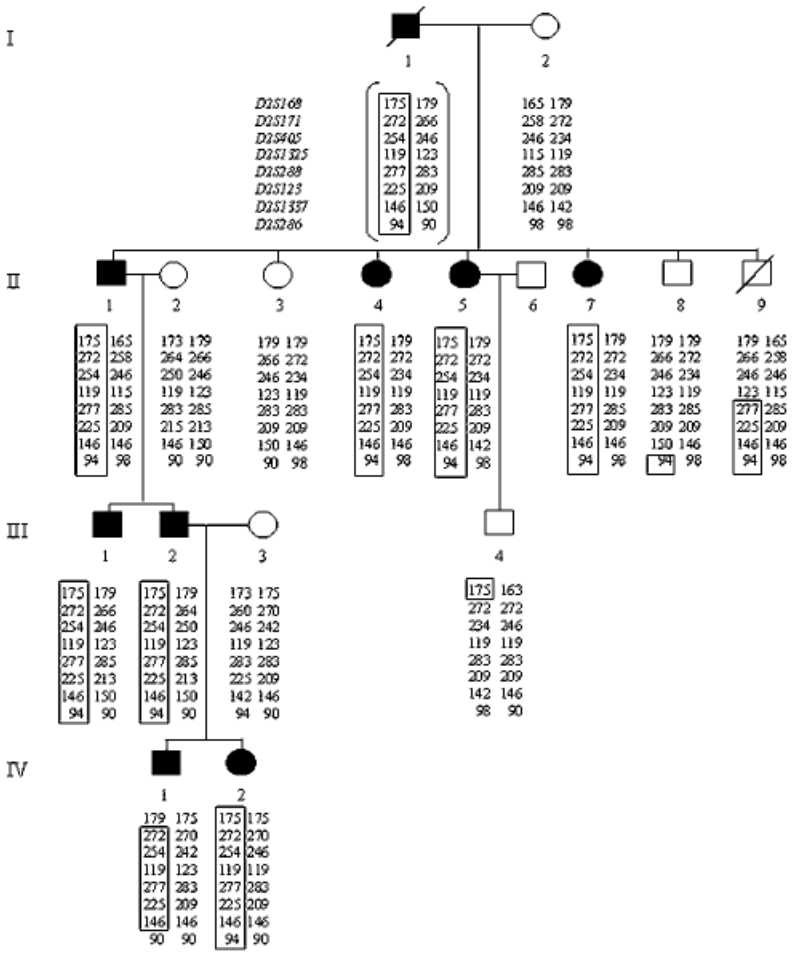


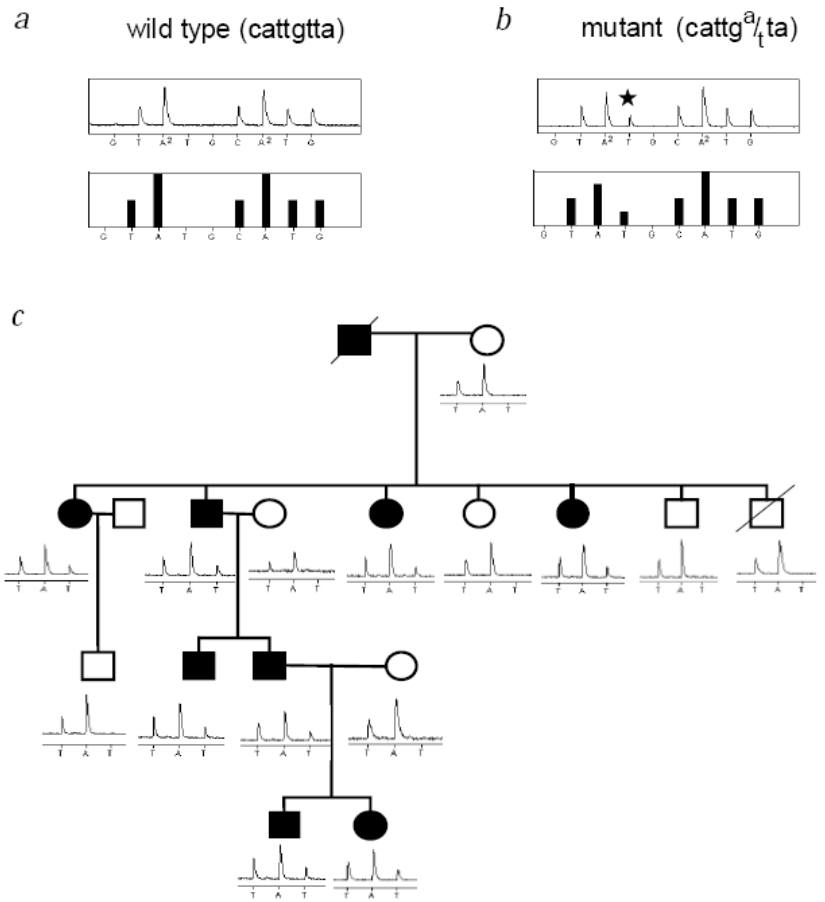




Multiple epiphyseal dysplasia

Mutations in the region encoding the von Willebrand factor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia





Mortier GR et al. Eur J Hum Genet 2001;9:606

Chapman KL et al. Nat Genet 2001;28:393

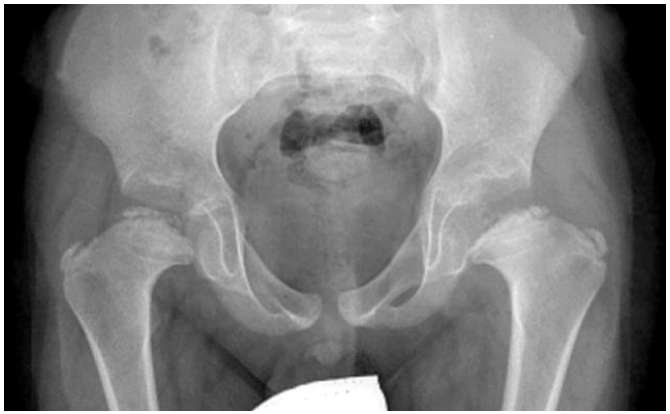
Center for Medical Genetics

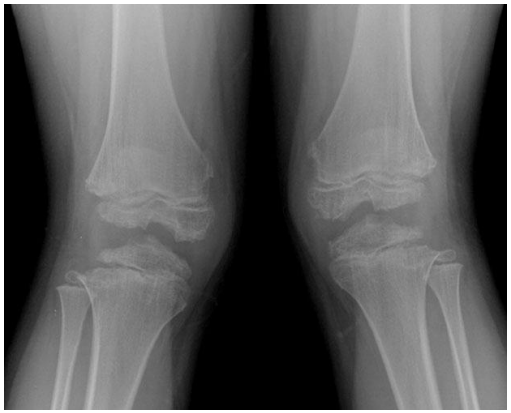
Multiple epiphyseal dysplasia

COL9A1

COL9A2

COL9A3

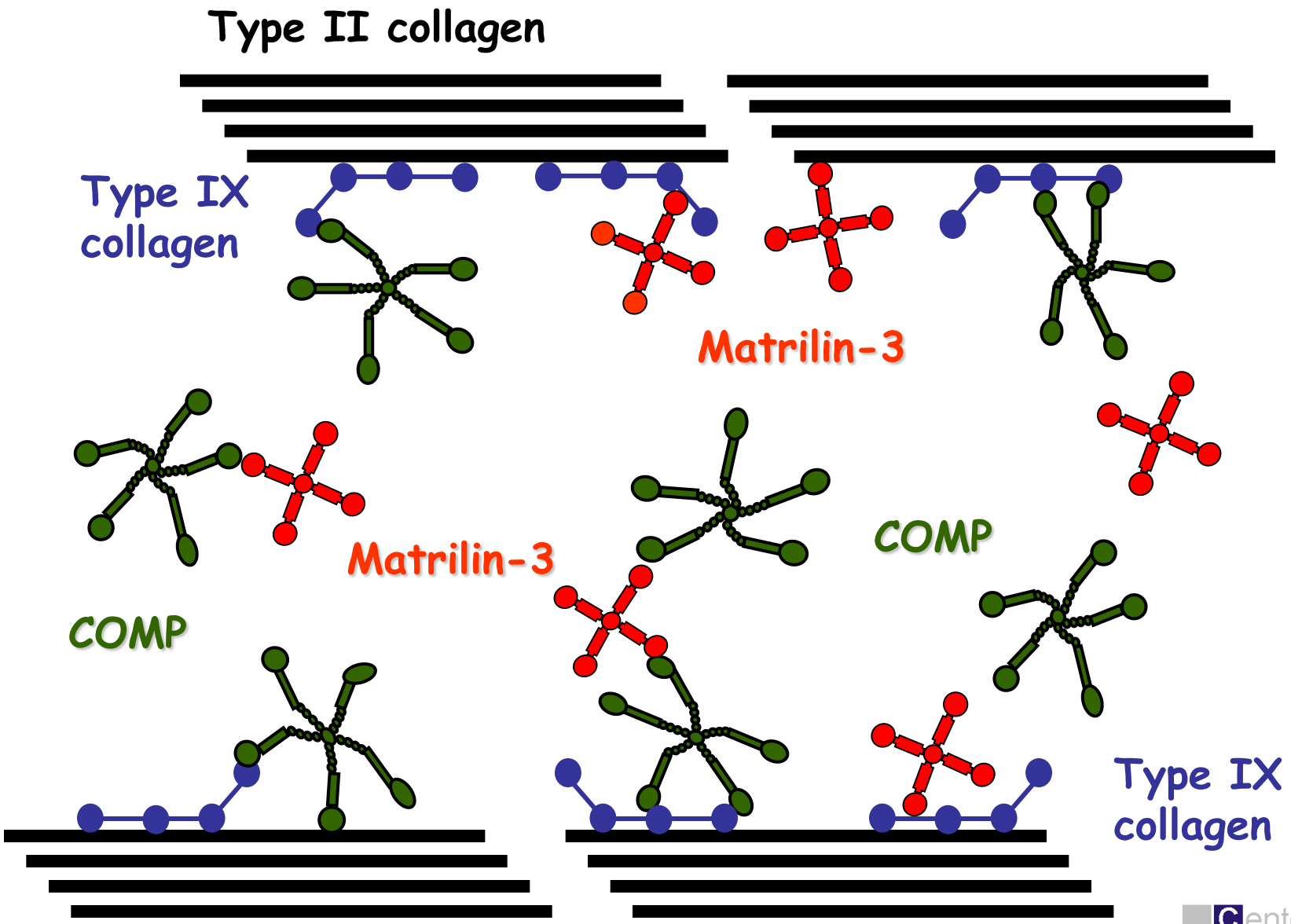




COMP

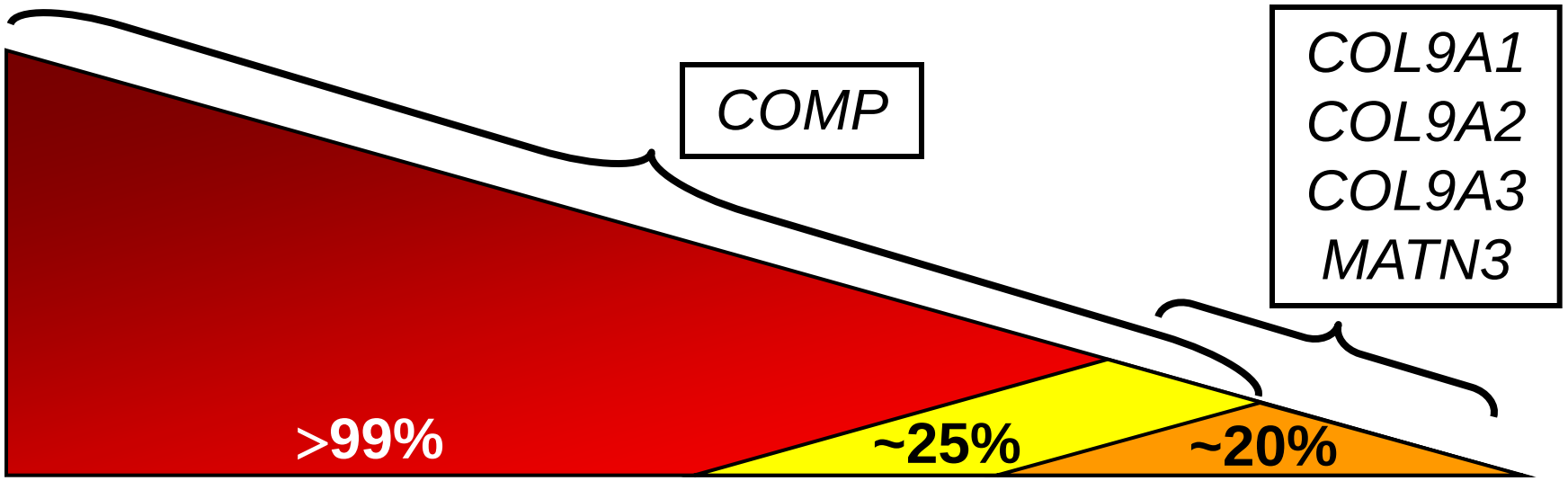
MATN3

Multiple epiphyseal dysplasia

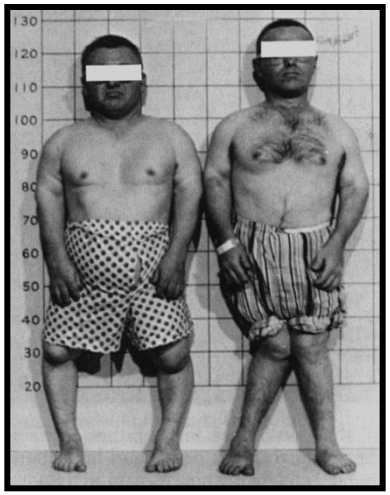


Courtesy of M Briggs - Manchester

PSACH-MED disease spectrum



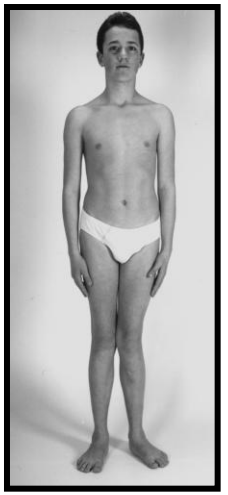
Severe PSACH



Mild PSACH

Severe MED

Mild MED



Courtesy of M Briggs - Manchester

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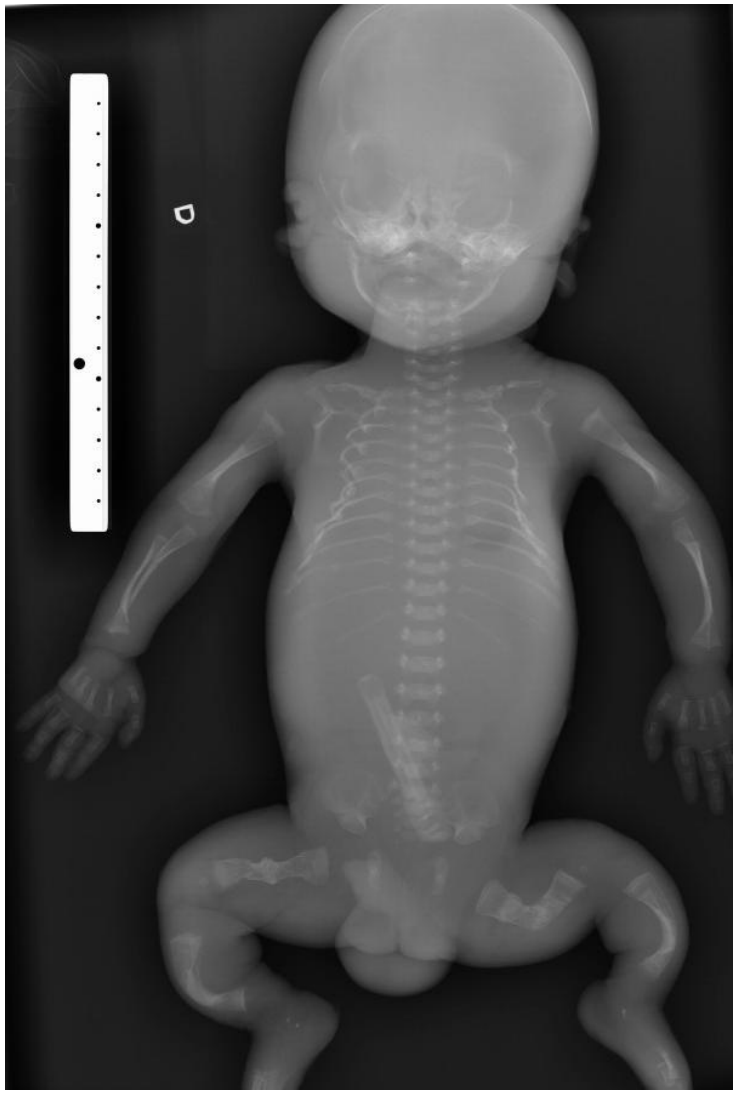
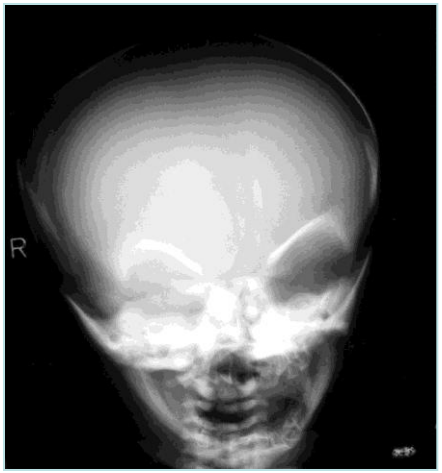
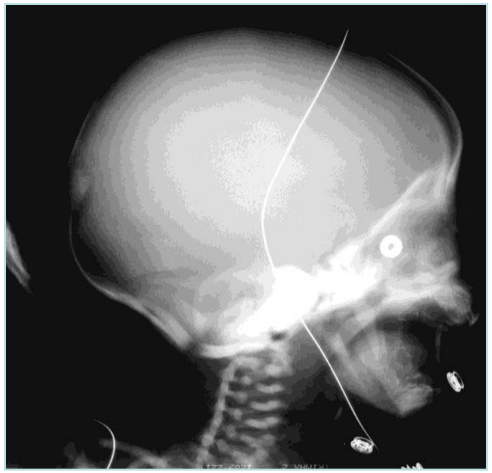
Disorders affecting bone homeostasis

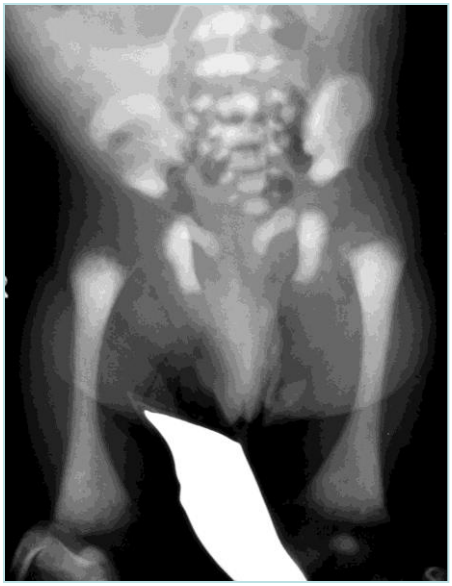
OSTEOGENESIS IMPERFECTA

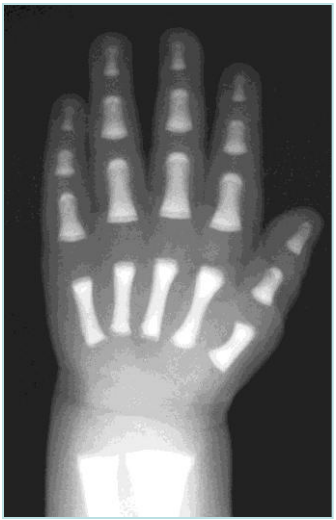


OSTEOPETROSIS

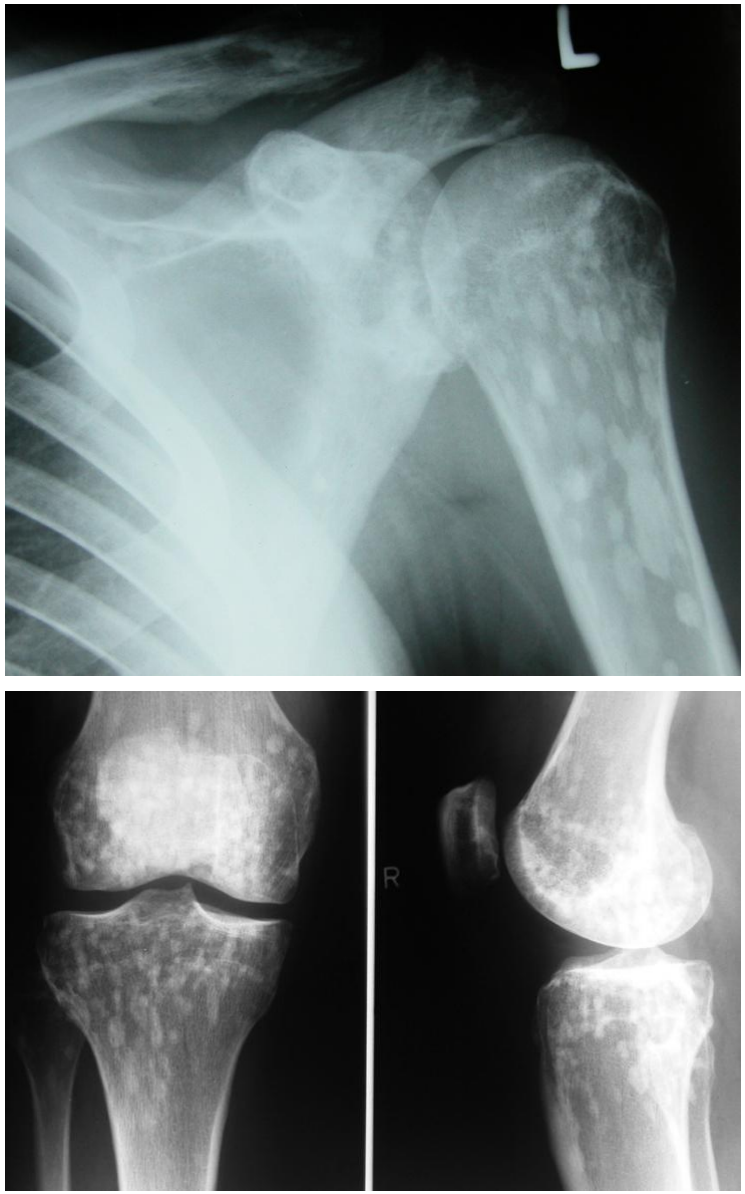








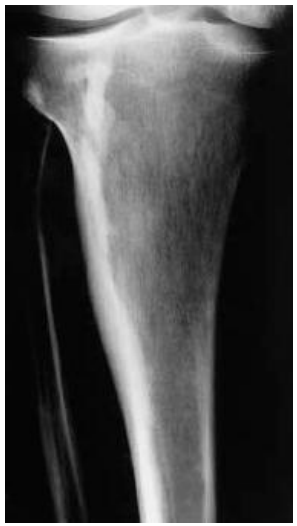
Osteopoikilosis

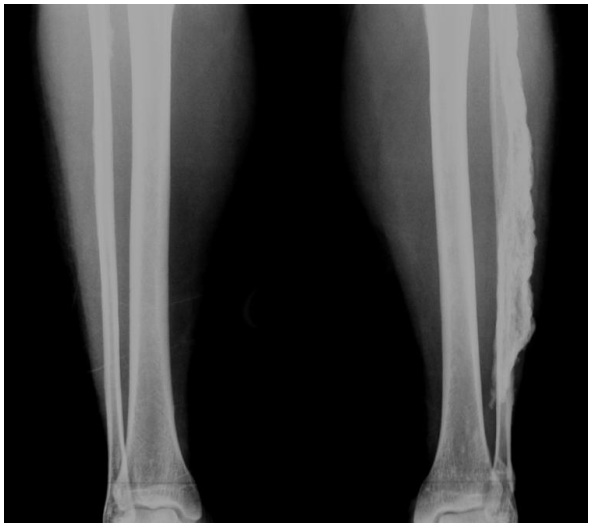


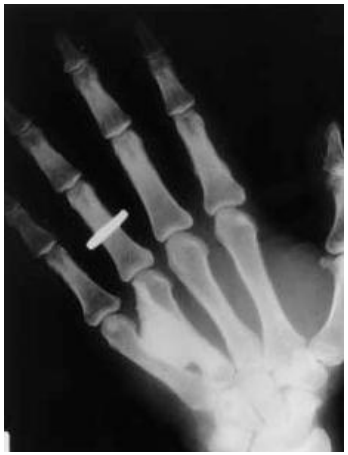
- benign condition
- autosomal dominant
- hyperostotic spots
- isolated or in association with other skin/bone lesions

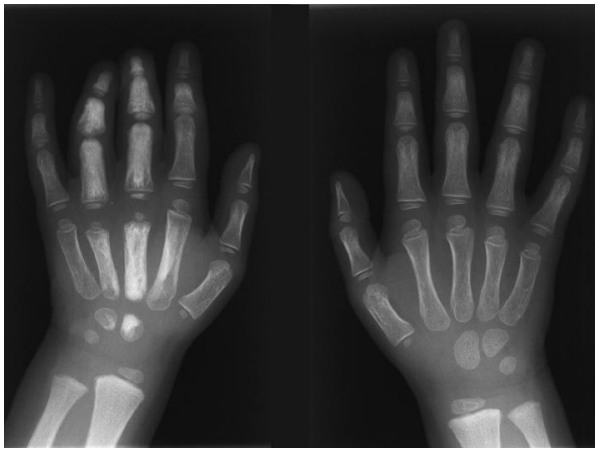
  (Buschke-Ollendorff syndrome)

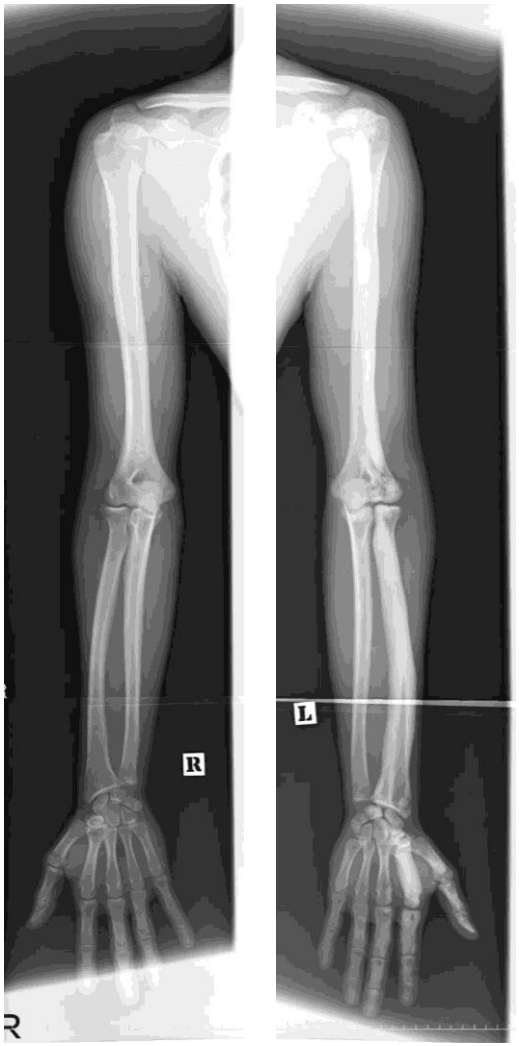
Melorheostosis



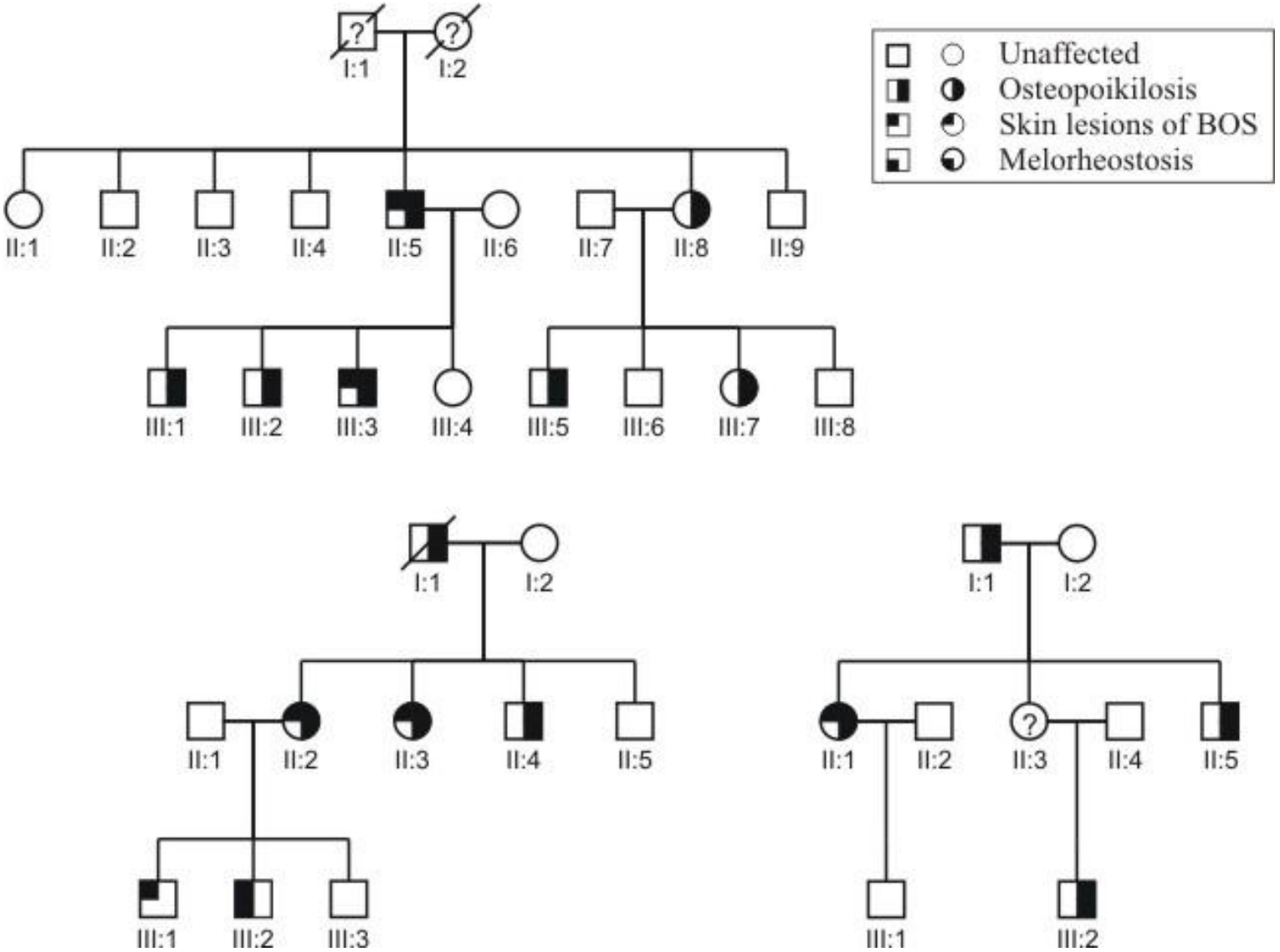




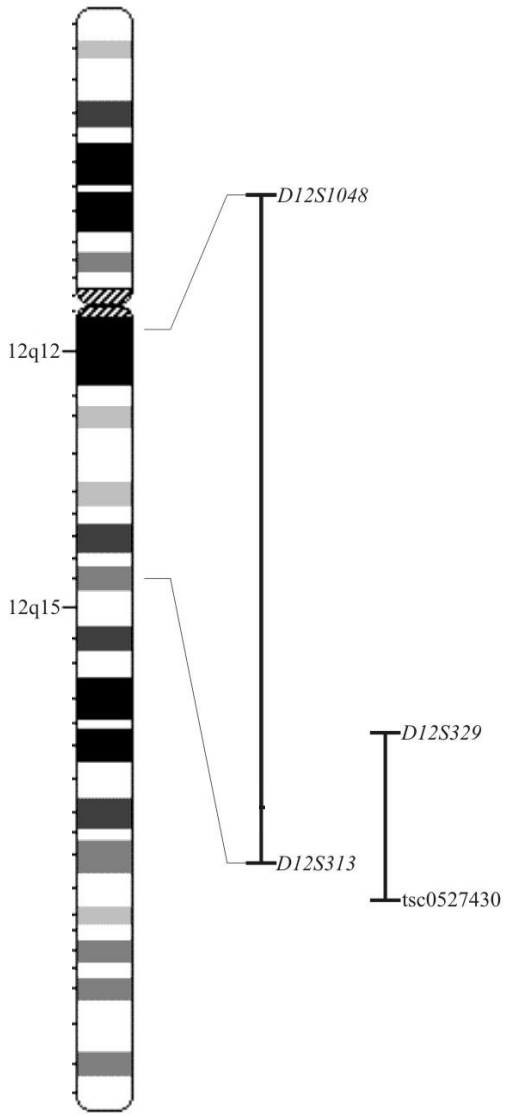




Linkage analysis and candidate gene approach



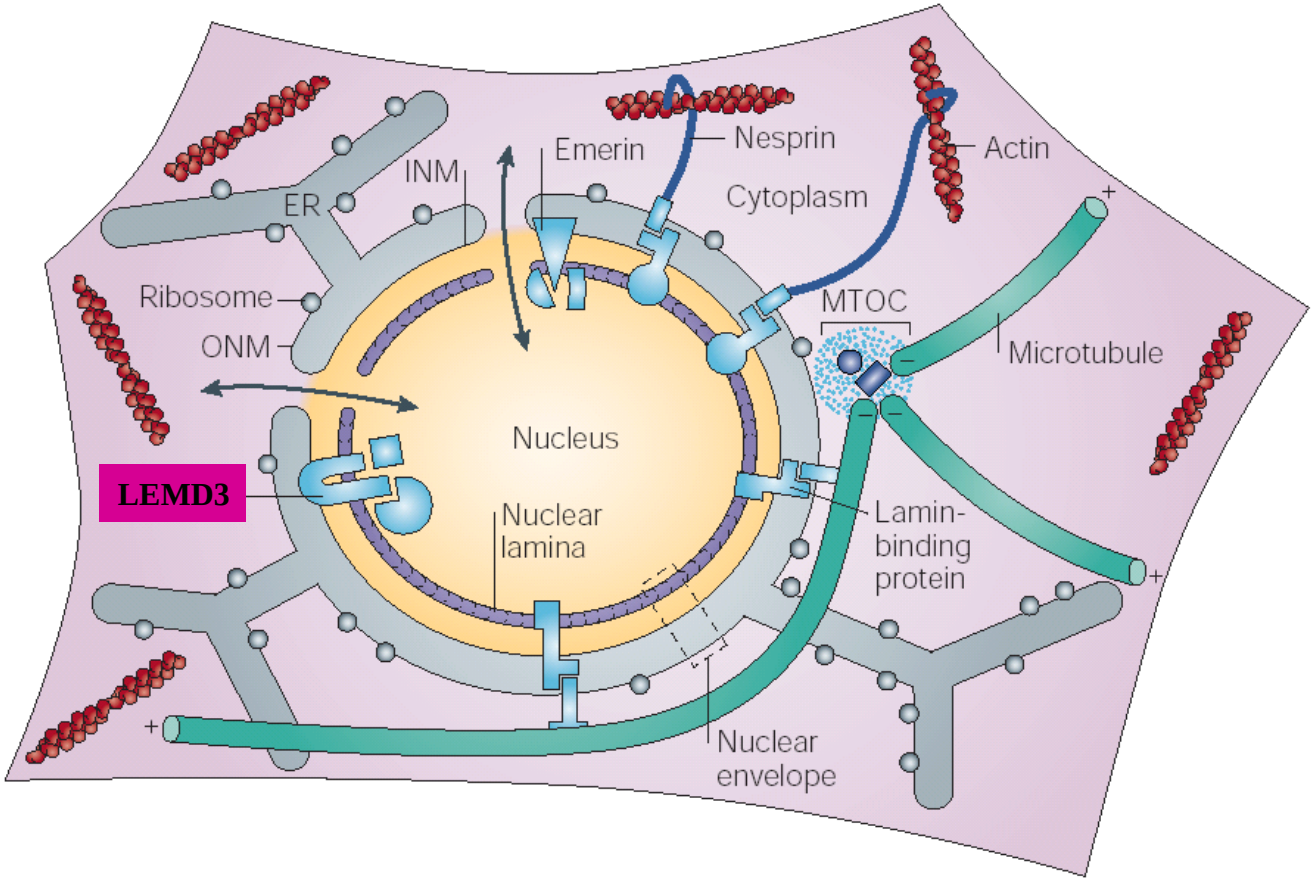
Gene mapping



- Genome wide linkage analysis
- Linkage for two markers on 12q
- Region: D12S1048 – D12S1663
- Combined maximum two-point LOD score of 6.691
- Identification of microdeletion between D12S329 and tsc0527430
- Region of interest:
  - 3.07Mb
  - 23 genes
- Two candidate genes:
  - *WIF1*
  - *LEMD3*

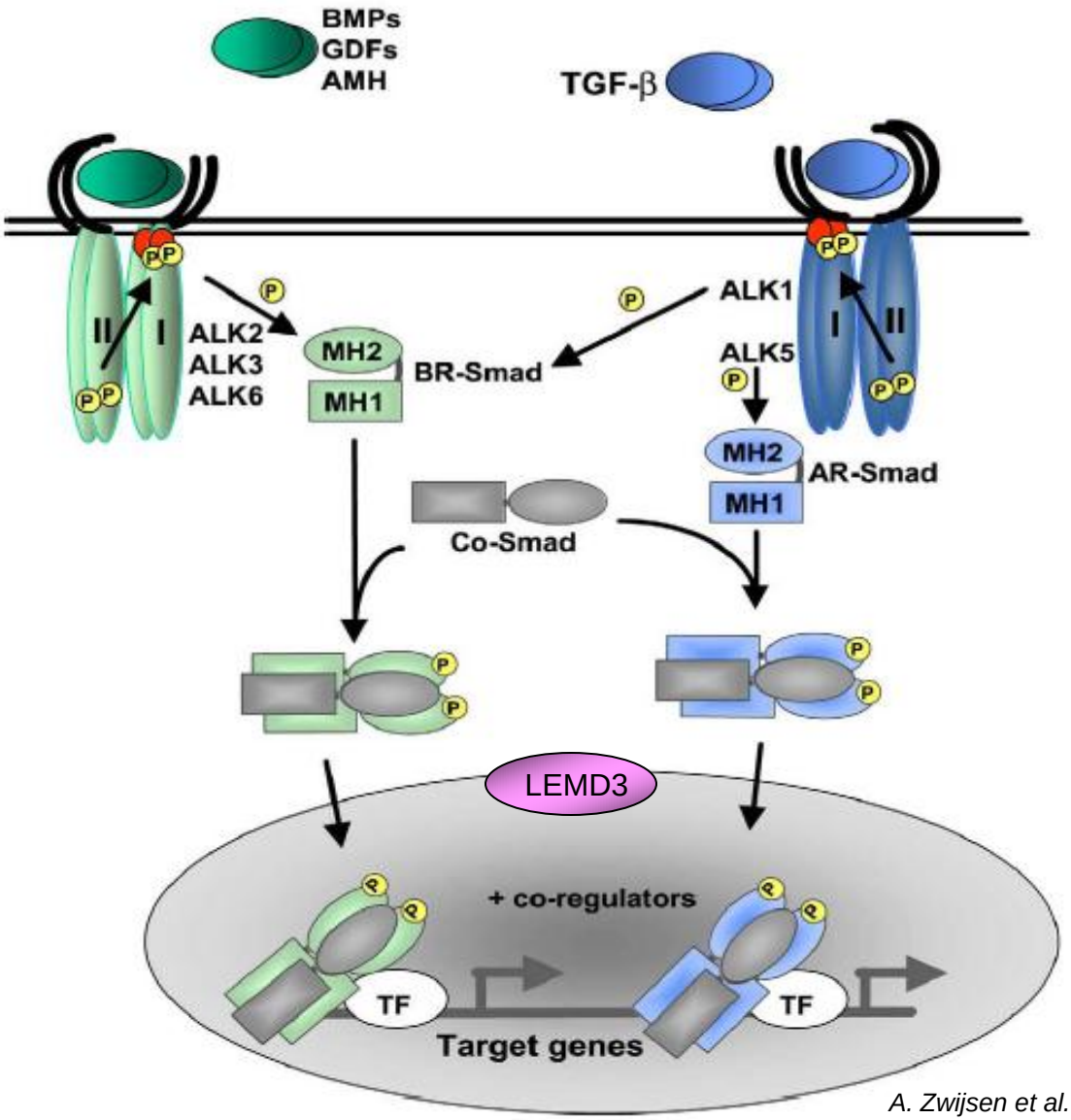
Hellemans J et al. Nat Genet 2004;36:1213

LEMD3: integral protein of the inner nuclear membrane



Adapted from Gruenbaum Y et al. Nature Rev Mol Cell Biol 6,21,2005

Mutations in LEMD3 cause osteopoikilosis



Modulation of the pathway

Antagonists

Pseudoreceptors

Scaffold/anchoring proteins

Trafficking/degradation proteins

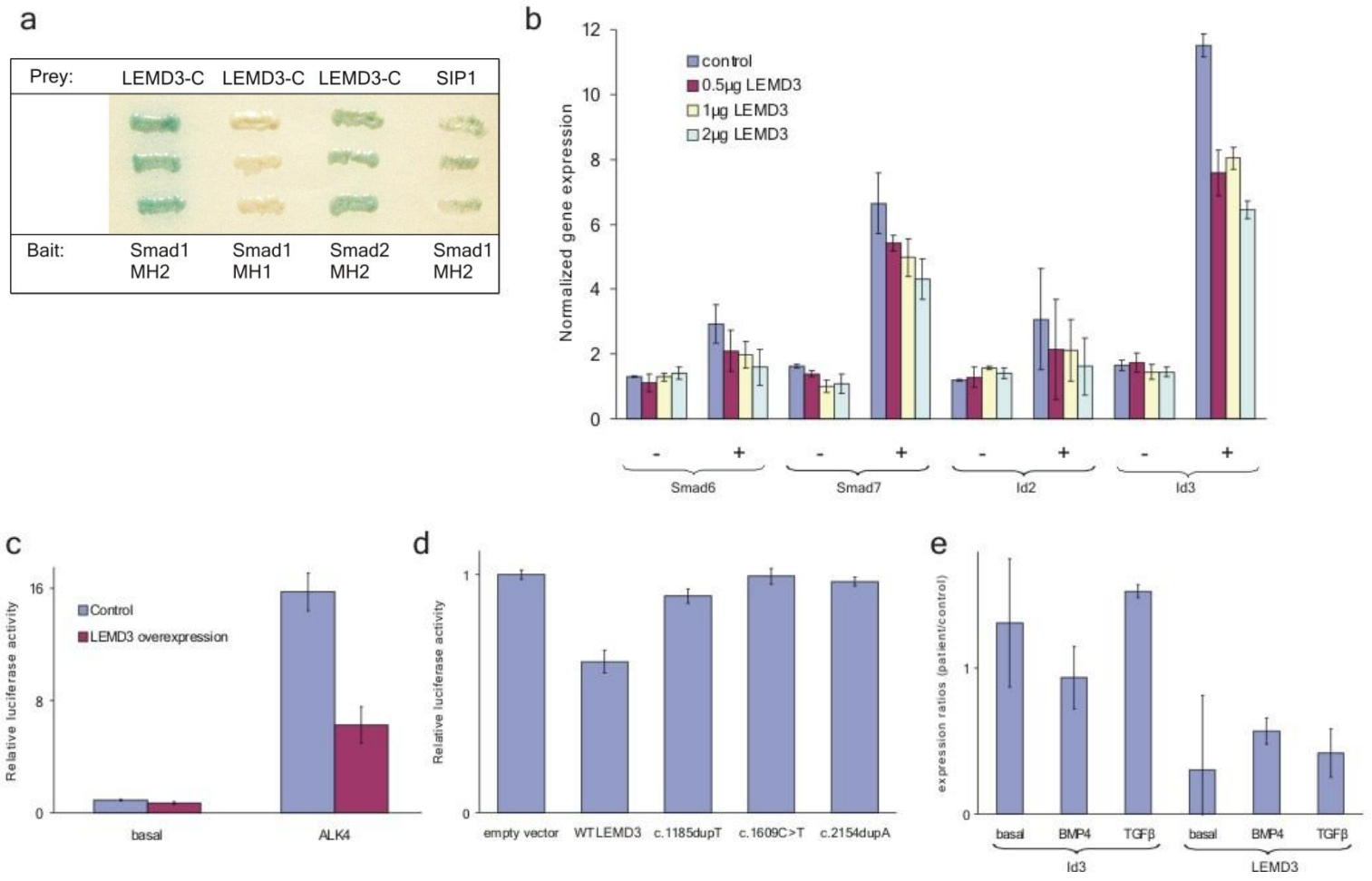
Oligomerization (I-Smads)

Nuclear membrane proteins

Transcriptional regulators

A. Zwijsen et al. FEBS Letters 546 (2003) 133-139

LEMD3 in BMP/TGFβ signaling



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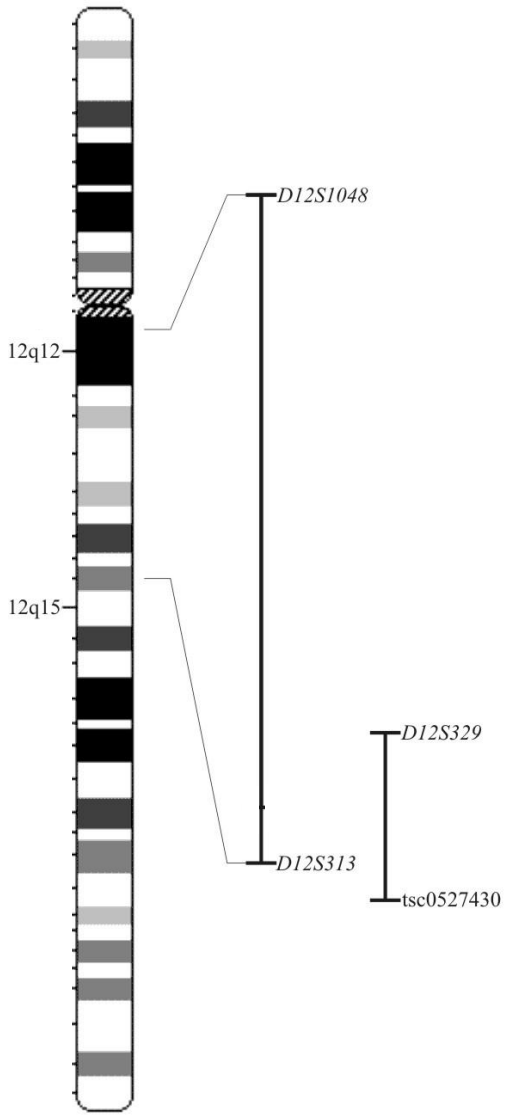
Gene mapping



- Genome wide linkage analysis
- Linkage for two markers on 12q
- Region: D12S1048 – D12S1663
- Combined maximum two-point LOD score of 6.691
- Identification of microdeletion between D12S329 and tsc0527430
- Region of interest:
  - 3.07Mb
  - 23 genes
- Two candidate genes:
  - *WIF1*
  - *LEMD3*

Hellemans J et al. Nat Genet 2004;36:1213

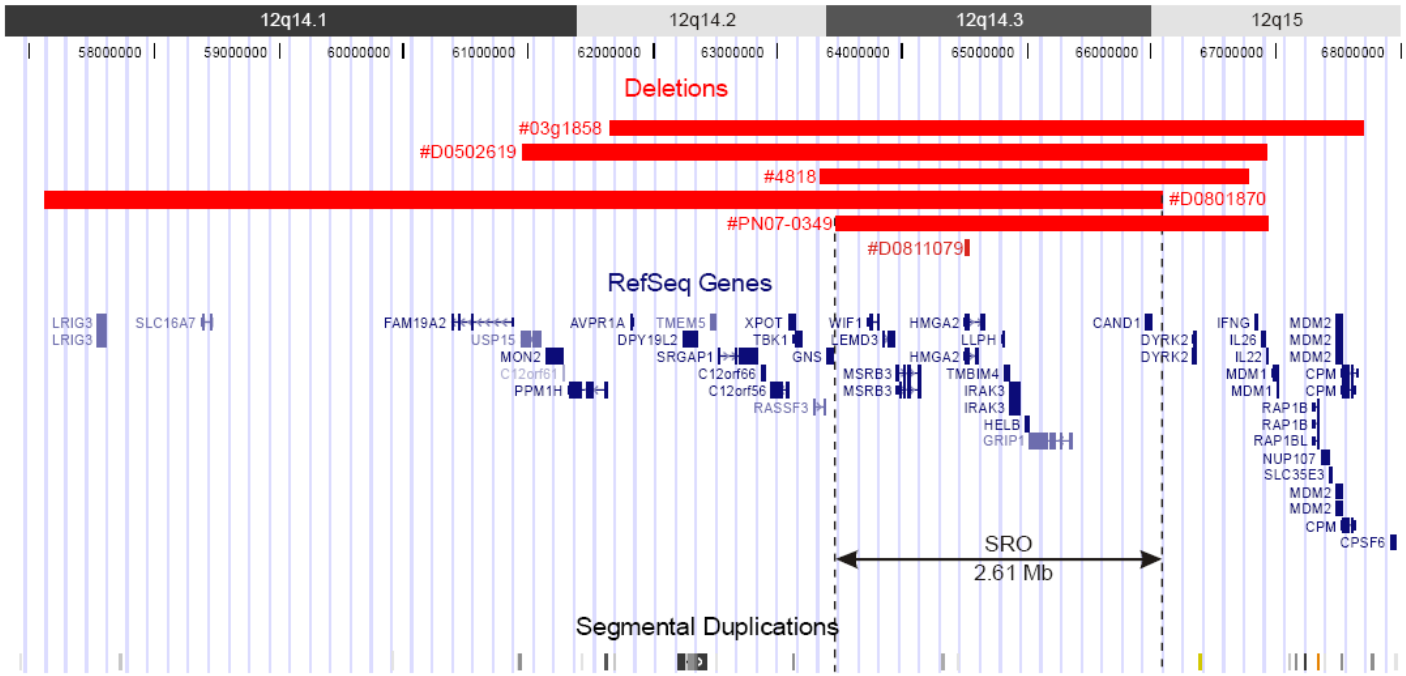
12q14 microdeletion syndrome

SHORT REPORT

Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14

Björn Menten, Karen Buysse, Farah Zahir, Jan Hellemans, Sara J Hamilton, Teresa Costa, Carrie Fagerstrom, George Anadiotis, Daniel Kingsbury, Barbara C McGillivray, Marco A Marra, Jan M Friedman, Frank Speleman, Geert Mortier

J Med Genet 2007;**44**:264–268. doi: 10.1136/jmg.2006.047860



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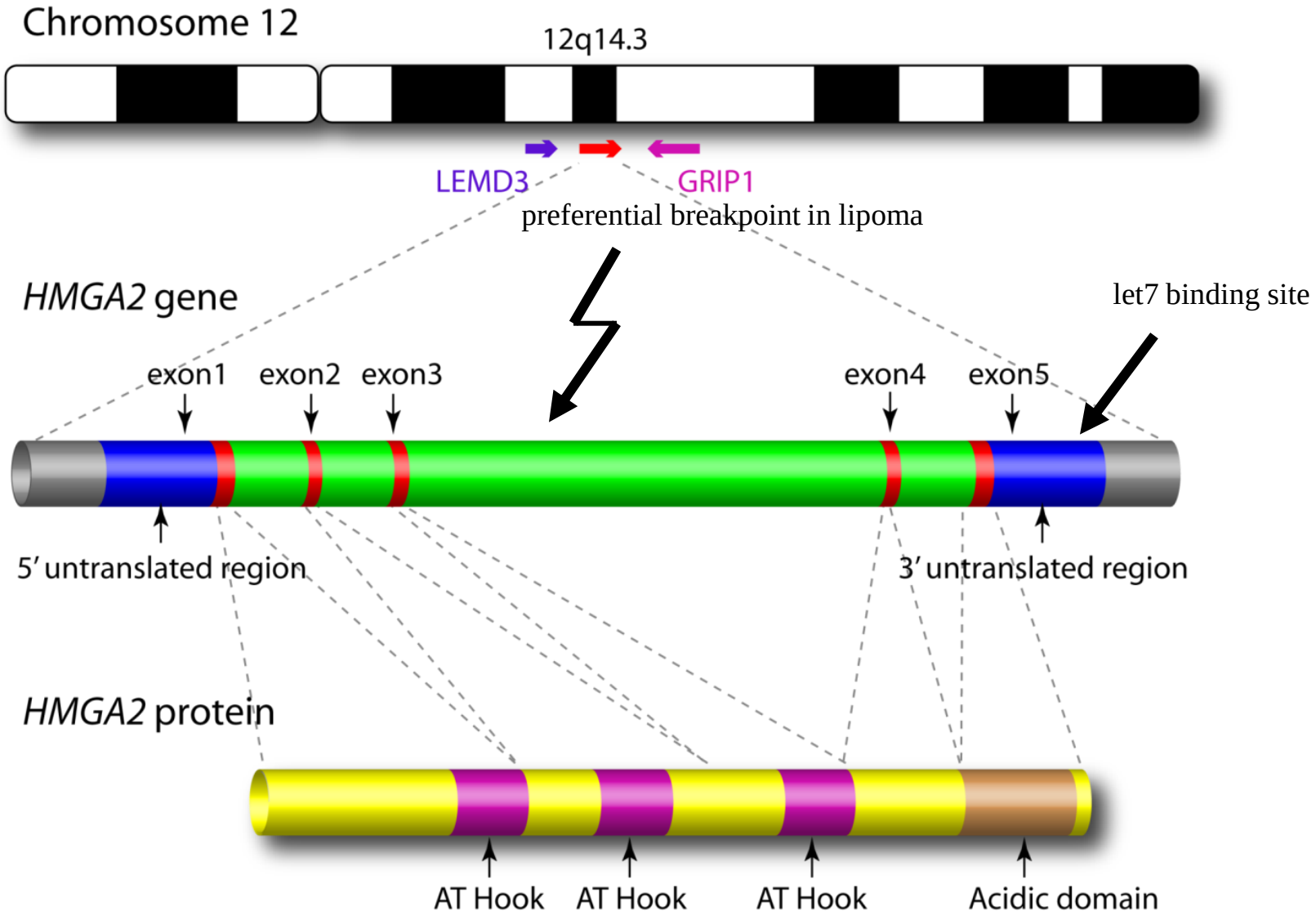
The HMGA2 gene



Mayr et al. (2007) Science 315, 1576

HMGA2 knockout mouse

Zhou et al. (1995) *Nature* 376(6543):771-4

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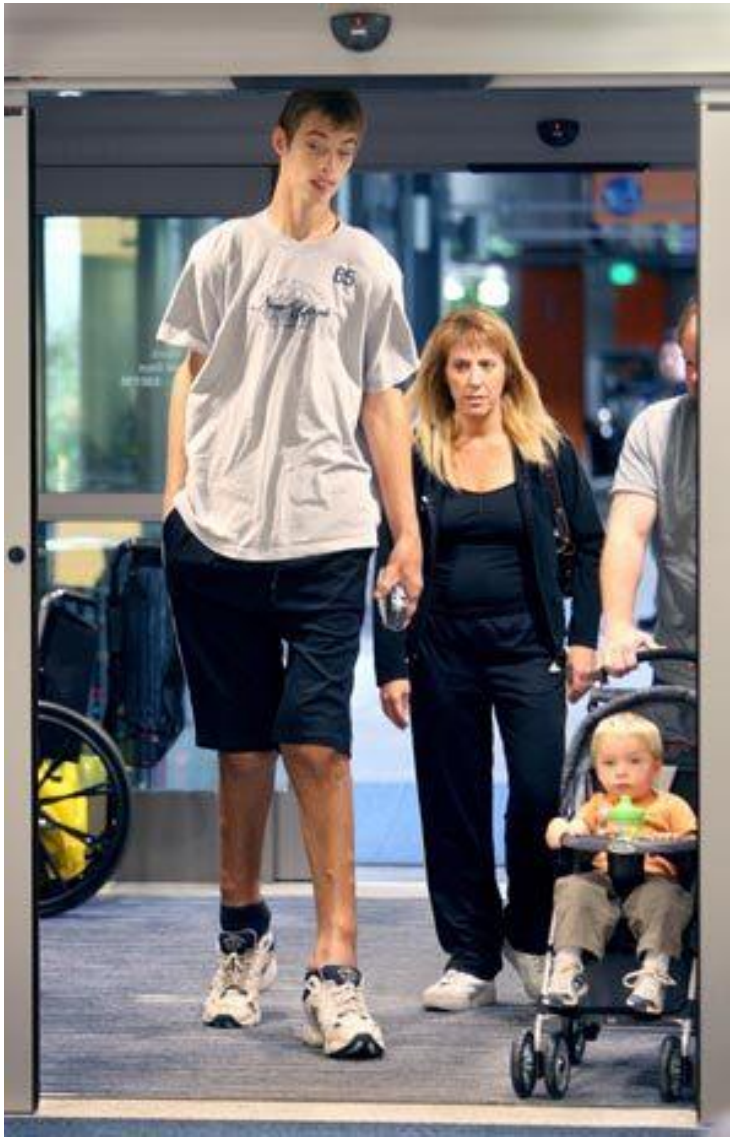
Chromosomal inversion disrupting HMGA2



Height: 220 cm!

LETTERS

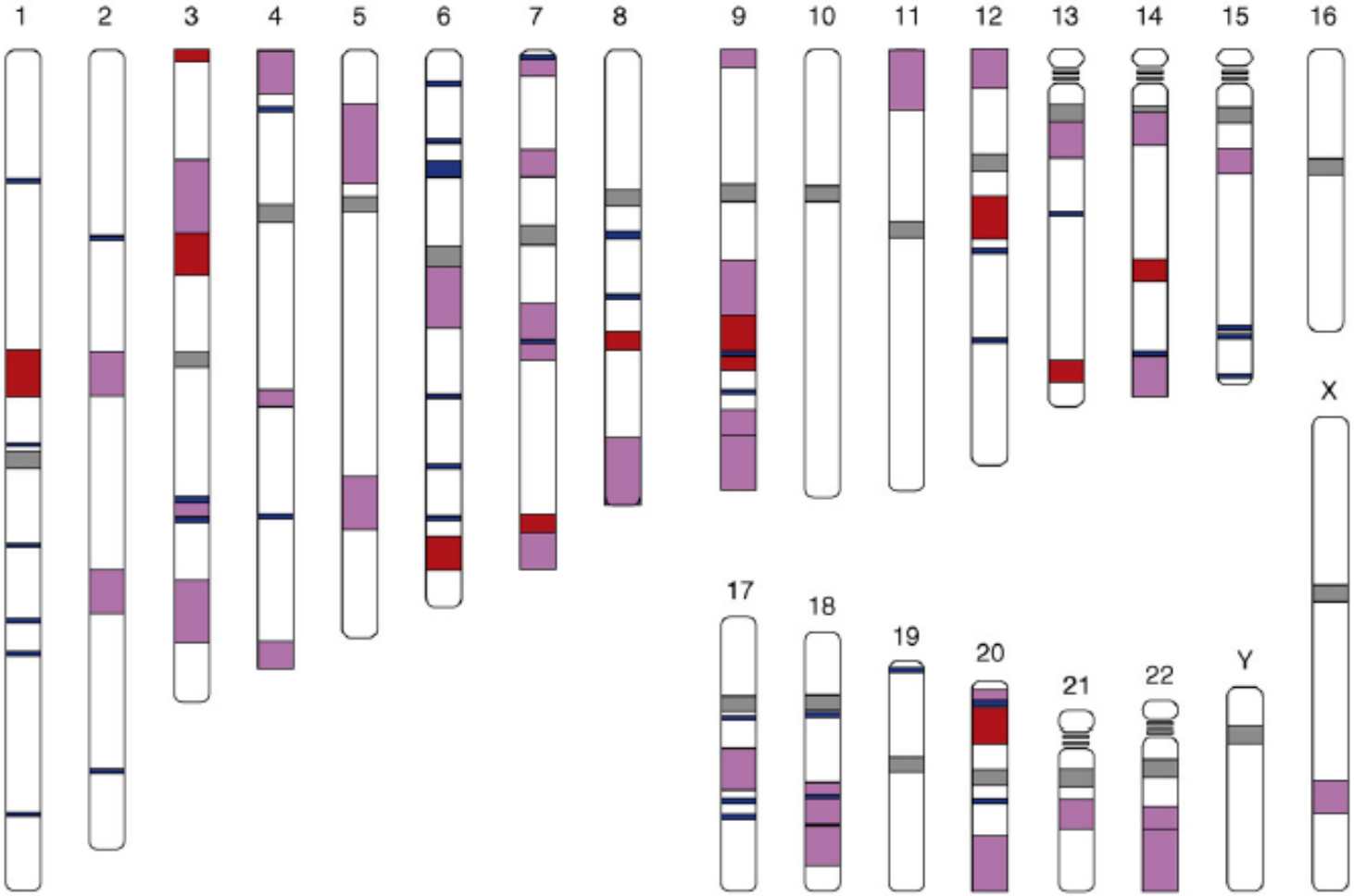
nature genetics

A common variant of *HMGA2* is associated with adult and childhood height in the general population

Michael N Weedon[1,2,21], Guillaume Lettre[3,4,21], Rachel M Freathy[1,2,21], Cecilia M Lindgren[5,6,21], Benjamin F Voight[3,7], John R B Perry[1,2], Katherine S Elliott[5], Rachel Hackett[3], Candace Guiducci[3], Beverley Shields[2], Eleftheria Zeggini[5], Hana Lango[1,2], Valeriya Lyssenko[8,9], Nicholas J Timpson[5,10], Noel P Burtt[3], Nigel W Rayner[6], Richa Saxena[3,7,11], Kristin Ardlie[3], Jonathan H Tobias[12], Andrew R Ness[13], Susan M Ring[14], Colin N A Palmer[15], Andrew D Morris[16], Leena Peltonen[3,17,18], Veikko Salomaa[19], The Diabetes Genetics Initiative, The Wellcome Trust Case Control Consortium, George Davey Smith[10], Leif C Groop[8,9], Andrew T Hattersley[1,2], Mark I McCarthy[5,6,21], Joel N Hirschhorn[3,4,20,21] & Timothy M Frayling[1,2,21]

Nature Genetics 2007;39:1245

Genome-wide association studies



47 loci identified

Weedon & Frayling. Trends in Genetics 2008;24:595-603

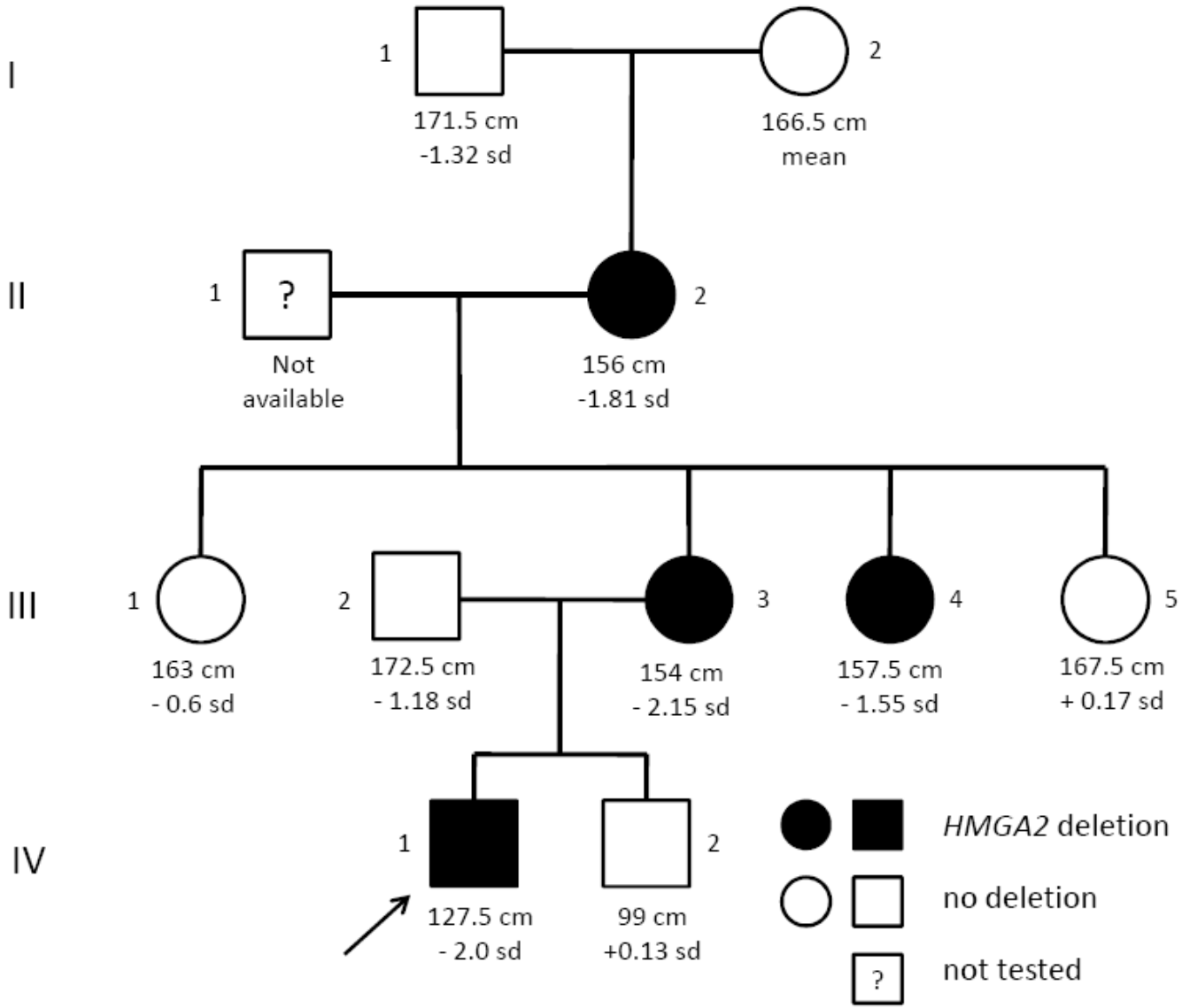
Genome-wide association studies

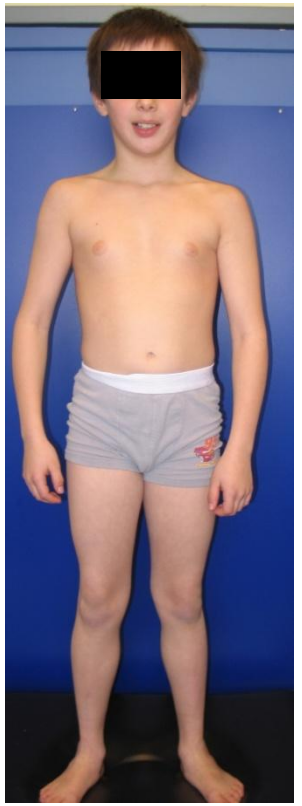
Table 1. Height loci in instances in which there is a strong case for the causality of a specific gene.

Index SNP[a]	Chr	Position (bp)[b]	Recombination region (kb)[c]	N genes[d]	Implicated gene[e]	Human growth or skeletal monogenic syndrome [f]	Mouse knockout causes skeletal or growth defects[g]	Expression evidence
rs6686842	1	41303458	287	3	*SCMH1*		Skeletal defects	
rs6724465	2	219652090	194	6	*IHH*	Brachydactyly	Skeletal defects	
rs10935120	3	135715782	186	3	*ANAPC13*			Yes [29]
rs6440003	3	142576899	299	2	*ZBTB38*			Yes [21]
rs1812175	4	145794294	134	1	*HHIP*		Skeletal defects	
rs12198986	6	7665058	54	1	*BMP6*		Skeletal and growth	
rs1776897	6	34302989	72	2	*HMGA1*		Growth and size	
rs2814993	6	34726871	813	10	*PPARD*		Growth and size	
rs4713858	6	35510763	85	4	*PPARD*		Growth and size	
rs798544	7	2729628	189	2	*GNA12*			Yes [29]
rs2282978	7	92102346	300	5	*CDK6*		Growth and size	Yes [29]
rs10958476	8	57258362	137	2	*PLAG1*		Growth and size	
rs9650315	8	57318152	137	2	*PLAG1*		Growth and size	
rs7846385	8	78322734	162	0	*PXMP3*	Zellweger syndrome	Skeletal defects	
rs10512248	9	97299524	176	1	*PTCH1*	Gorlin syndrome, holoprosencephaly	Skeletal defects	
rs1042725	12	64644614	84	1	*HMGA2*	Tall stature	Growth and size	
rs11107116	12	92502635	47	1	*SOCS2*		Growth and size	
rs8041863	15	87160693	25	1	*ACAN*	Spondyloepiphyseal dysplasia type Kimberley	Skeletal and growth	
rs3760318	17	26271841	226	4	*RNF135*	Overgrowth syndrome		
rs4794665	17	52205328	108	2	*NOG*	Various skeletal defects	Skeletal and growth	
rs757608	17	56852059	65	3	*TBX2*		Skeletal defects	
rs8099594	18	45245158	498	3	*DYM*	Dyggve-Melchior-Clausen		
rs967417	20	6568893	49	0	*BMP2*		Skeletal and growth	
rs6060369	20	33370575	673	13	*GDF5*	Various skeletal defects	Skeletal defects	

Weedon & Frayling. Trends in Genetics 2008;24:595-603

HMGA2 intragenic deletion in proportionate short stature





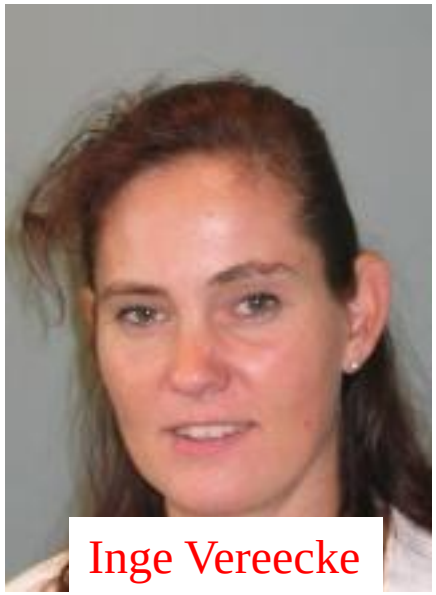
CONCLUSIONS

The study of rare genetic disorders has led to the identification of genes that are important in human growth and development.

These new insights can enhance our understanding of the pathophysiology of more common disorders in the population such as growth disorders, cancer, osteoporosis and osteoarthrosis.

This new knowledge may also unravel the genetic factors that determine variation in human height

Acknowledgments


Inge Vereecke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