Mechanisms of Limb Formation and Associated Phenotypes

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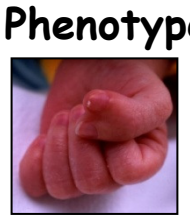


Phenotype

Clinical Genetics

Diagnosis

Genetic Disease

Genetics

Gene Function

Molecular Pathology

Genome

Charité

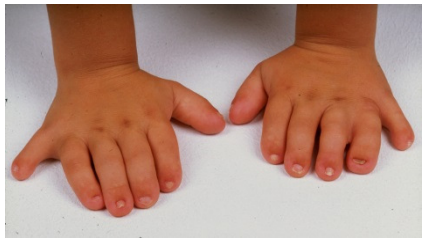
Institute for Medical Genetics

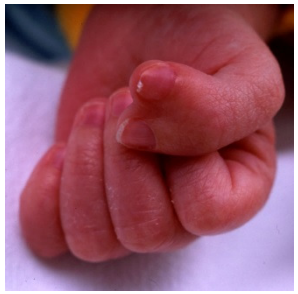
MPIMG

FG Development and Disease

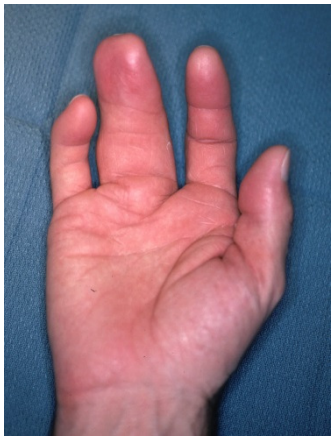
Malformations of the Limbs

polydactyly

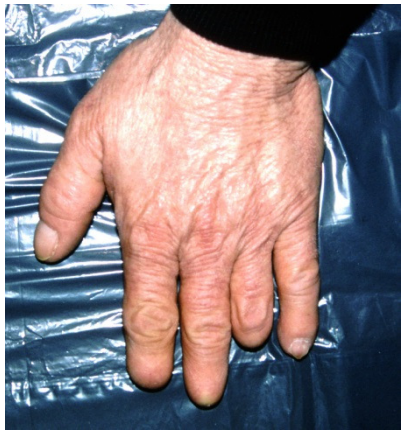




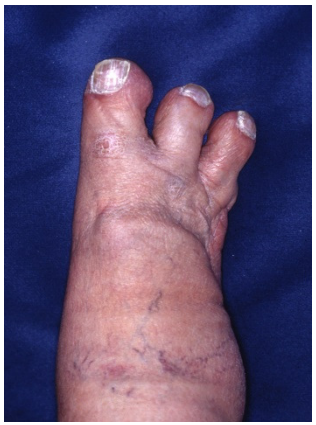
syndactyly



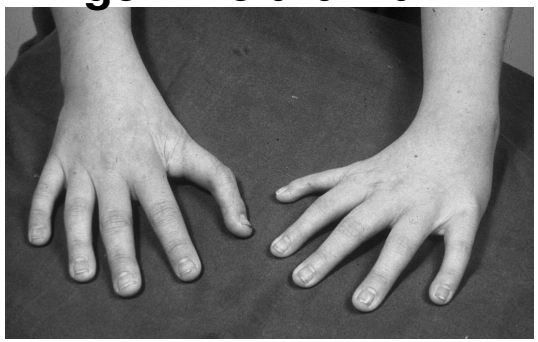
brachydactyly



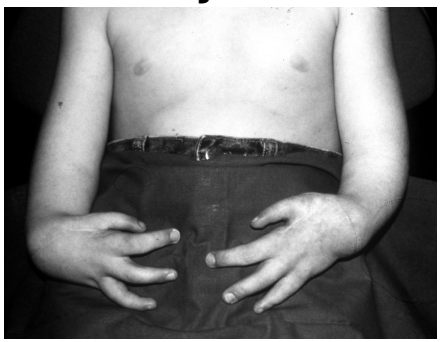
oligodactyly



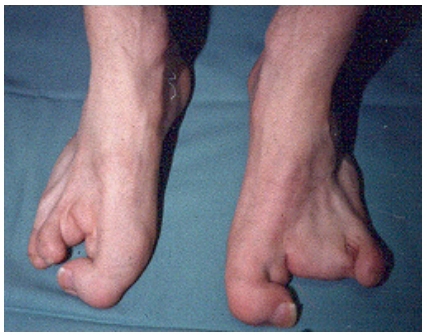
finger-like thumb



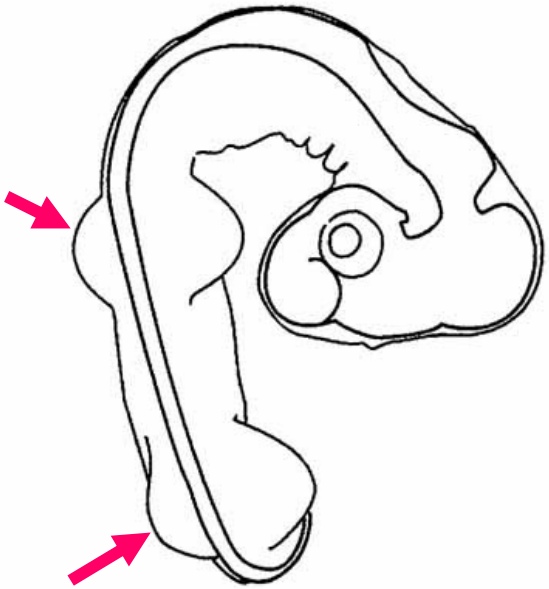
radial ray defect

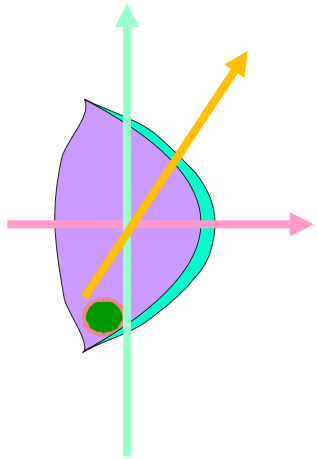


ectrodactyly



Patterning the Limb





Shh

anterior-posterior

Wnt7a
Lmx1
en1

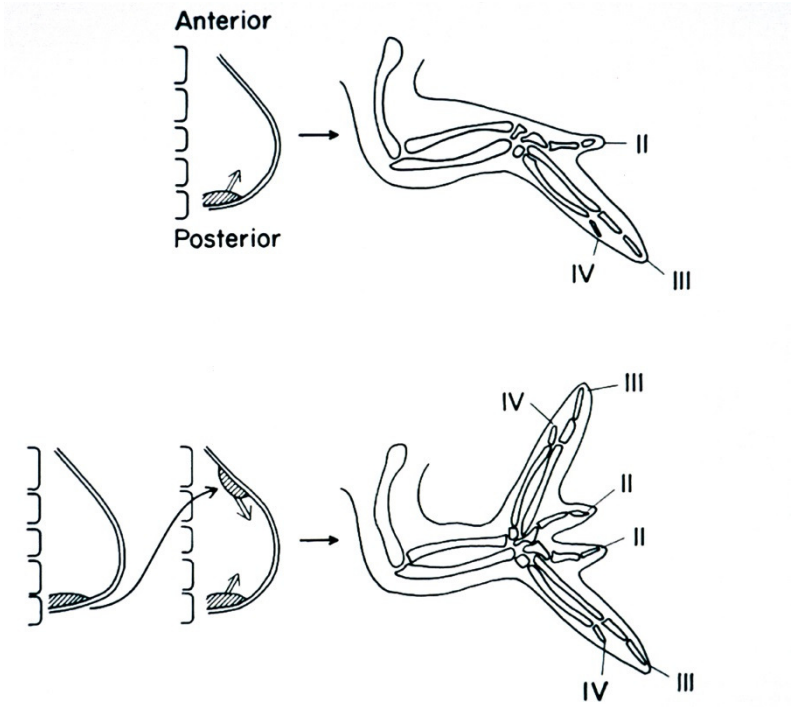
dorso-ventral

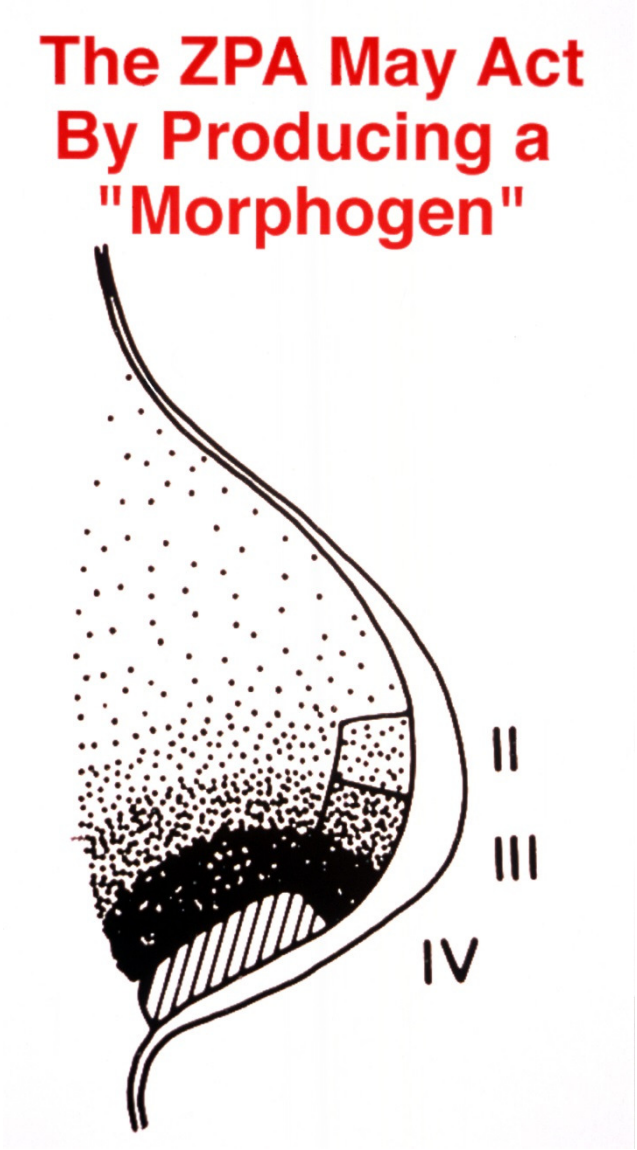
proximodistal

FGFs
AER

The Zone of Polarising Activity (ZPA)

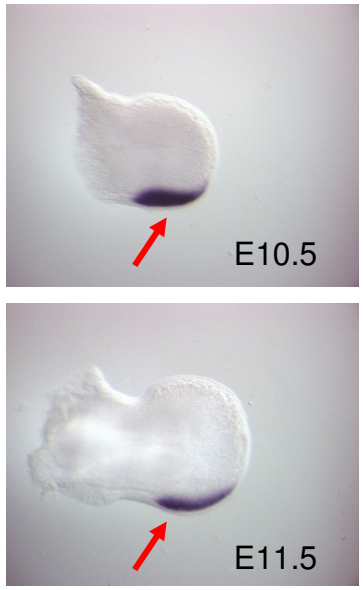
- control of anterior-posterior pattern -

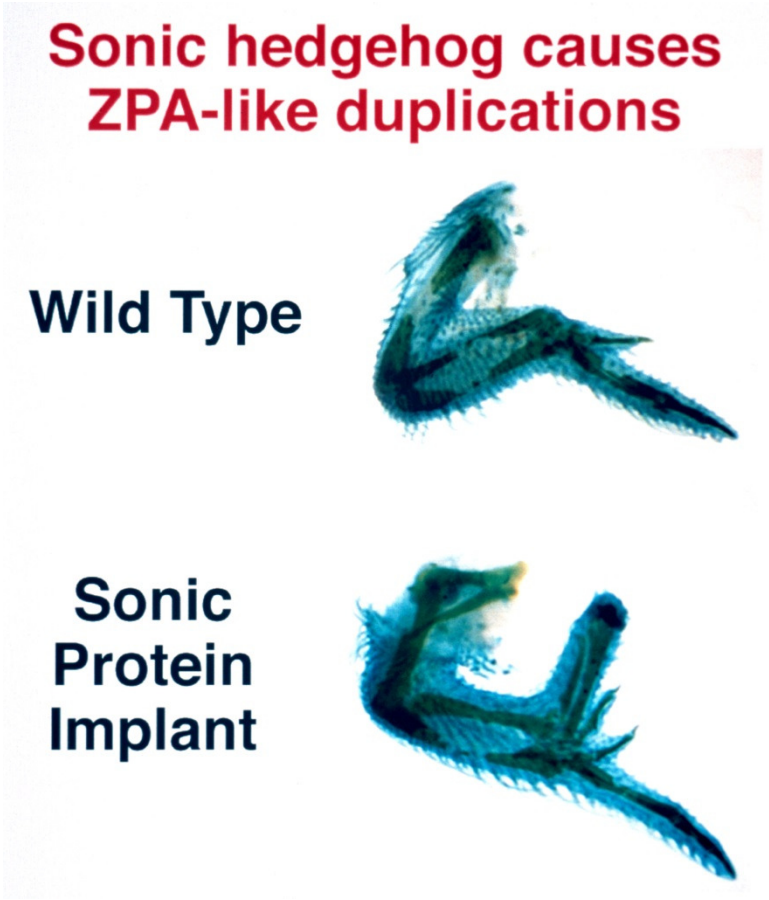




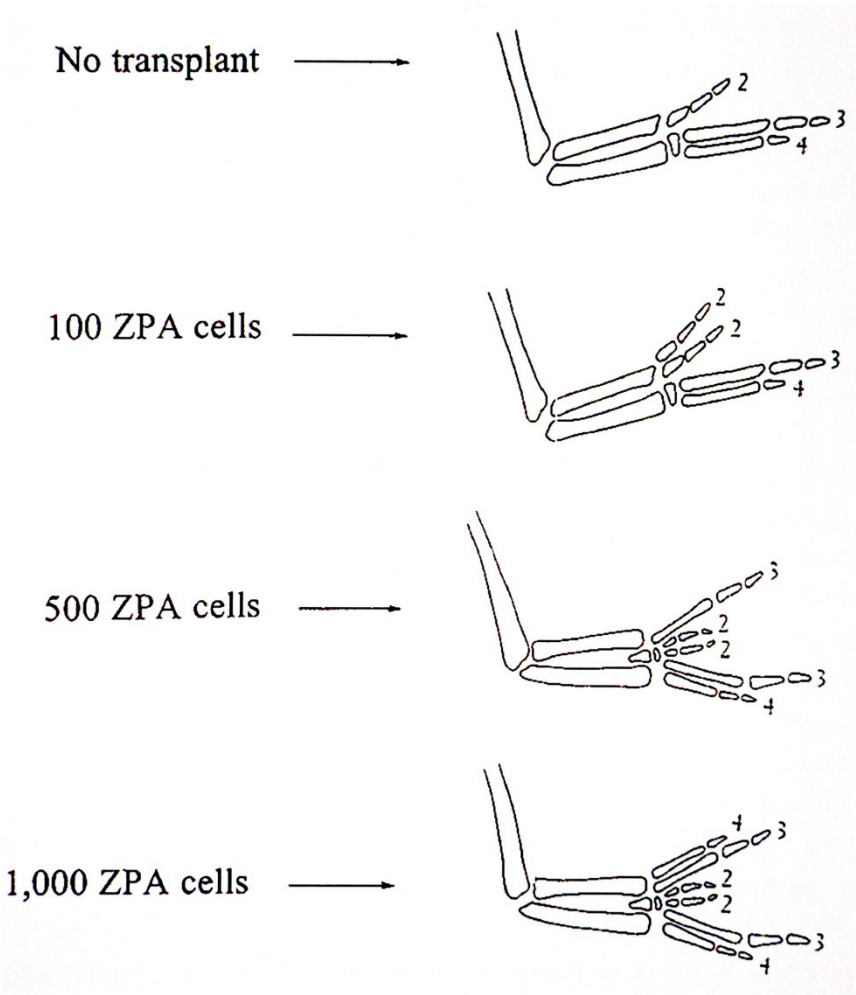
Sonic Hedgehog (Shh) mediates the ZPA signal

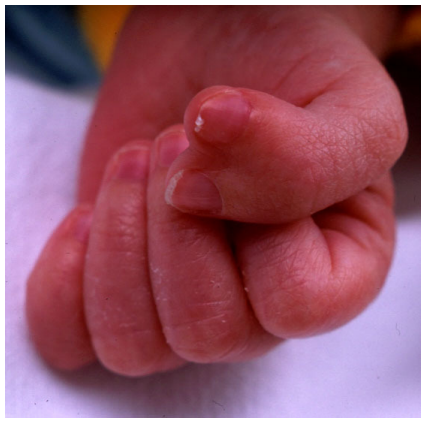
Shh co-localizes with the ZPA





The degree of digit duplication is dosage dependent



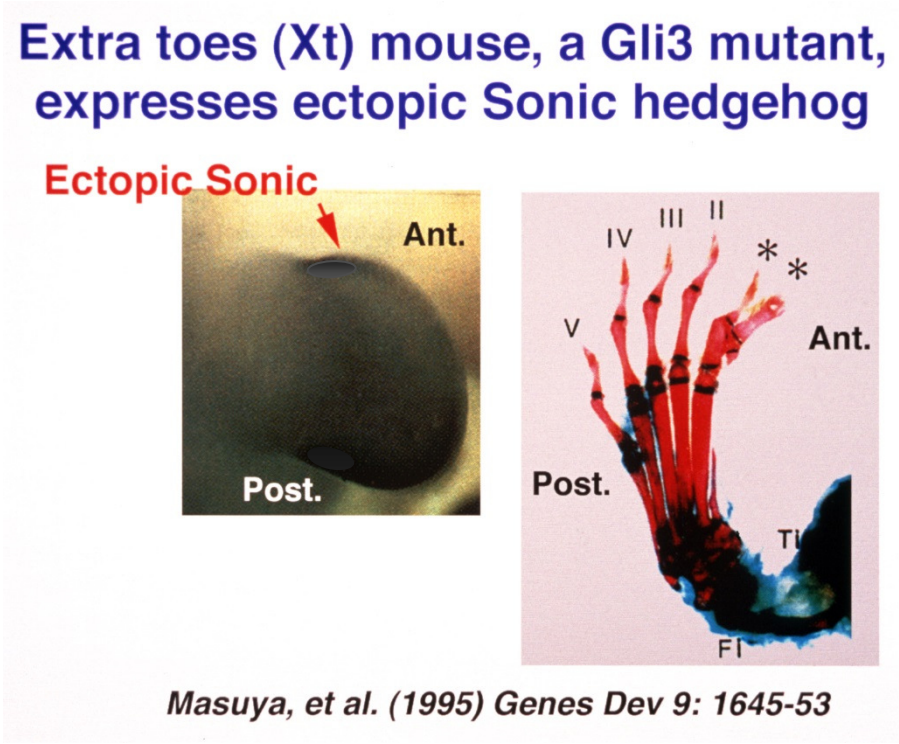


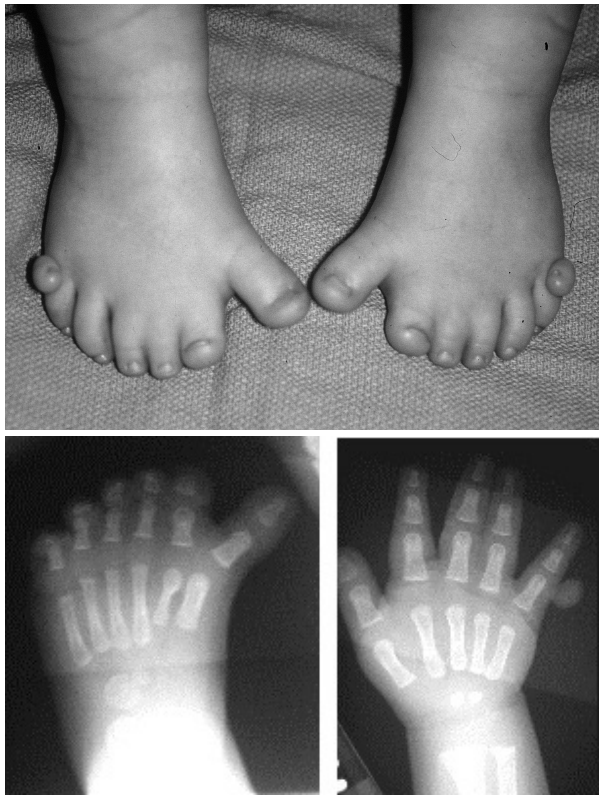
Pathogenesis of Polydactyly?

Mutations within the Hedgehog pathway

Polydactyly phenotype

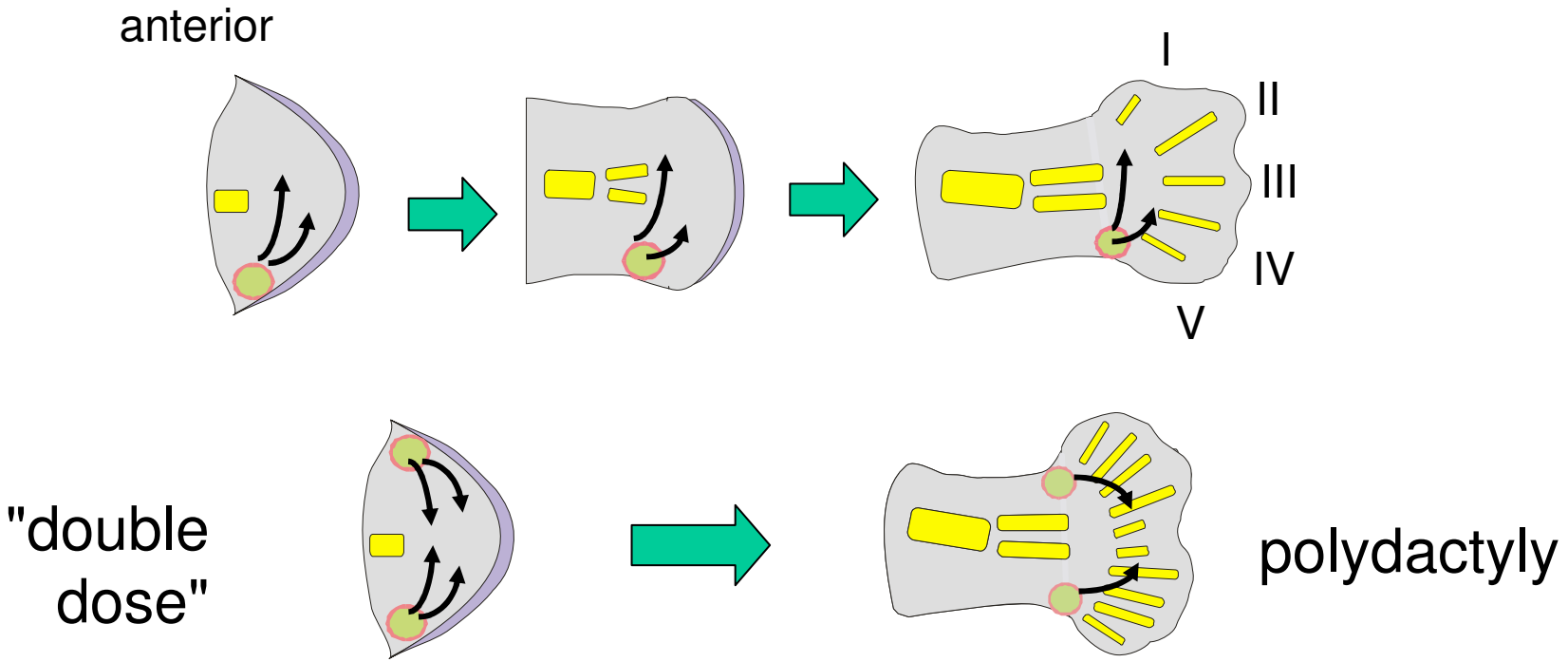
Cephalopolysyndactyly
(Greig syndrome)
Mutations in GLI3
Polydactyly





Mechanisms of Polydactyly

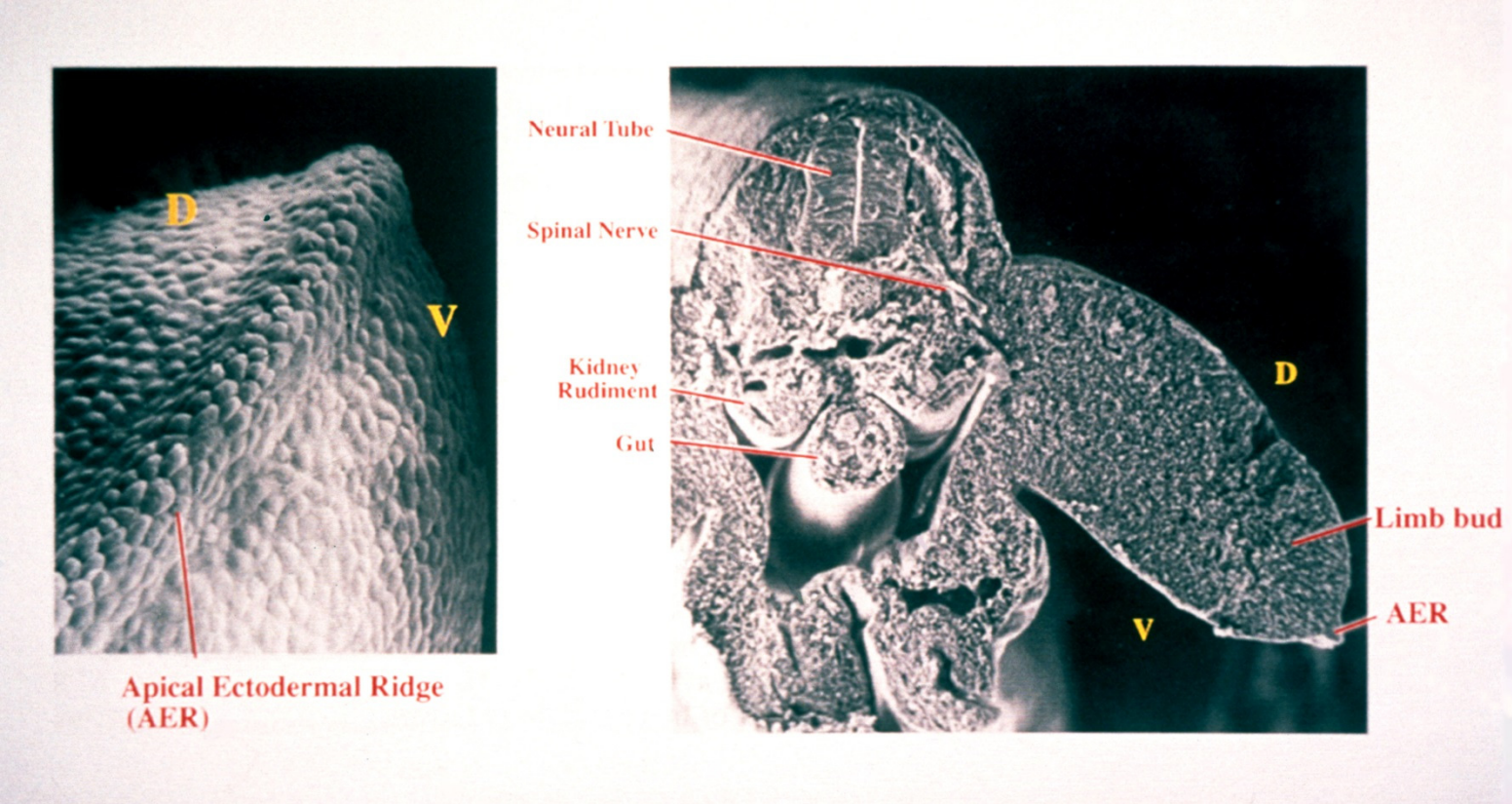
- ectopic expression of Shh -



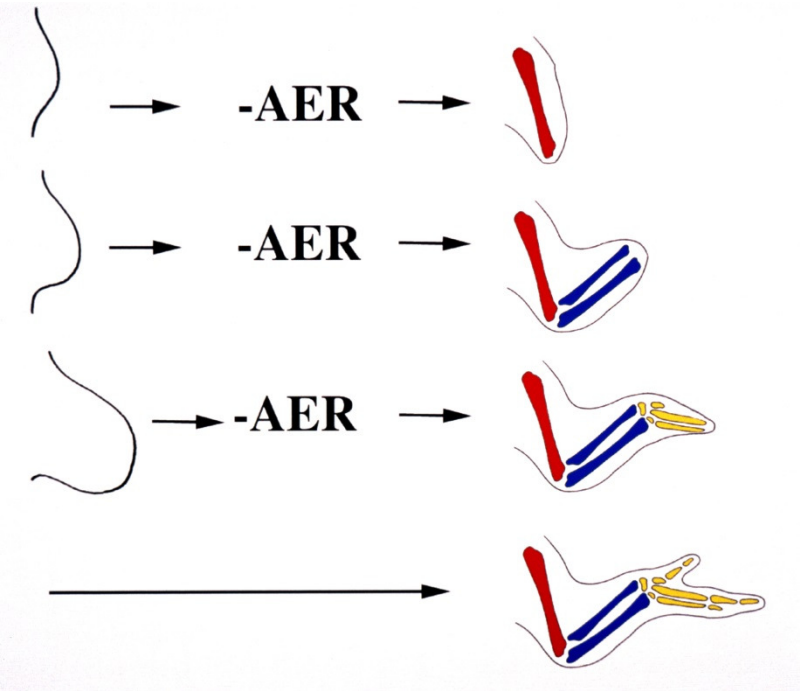
- Zone of polarising activity (ZPA)
  Expression of Shh

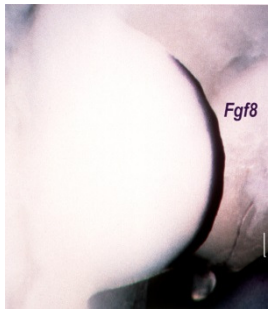
The Apical Ectodermal Ridge (AER)

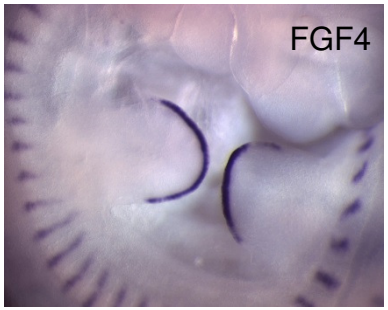
- control of proximo-distal pattern/outgrowth -



Removal of AER Results in Truncation







FGFs are the AER signal

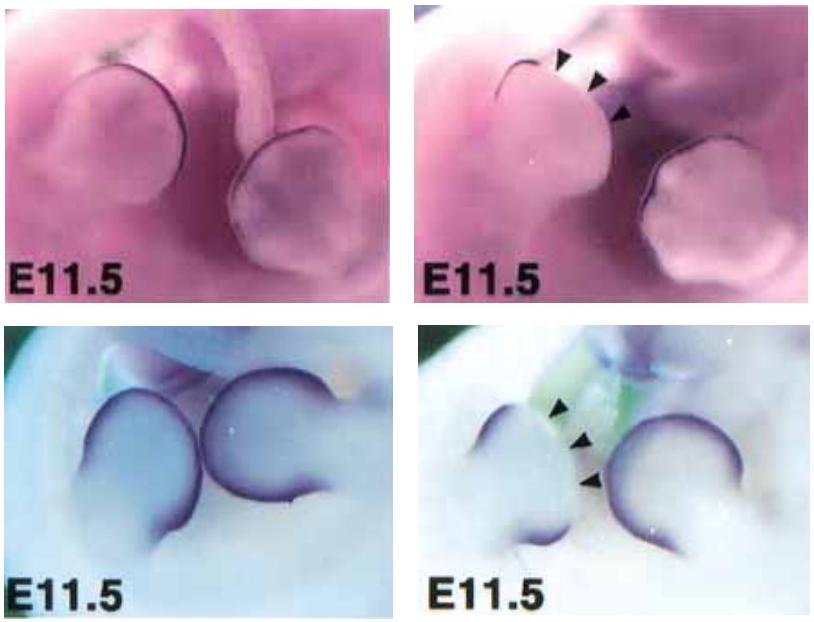
Dlx5/6$^{-/-}$ Mouse as a Model for Split-Hand-Foot Malformations

- **Distal-less Homeoboxgenes Dlx5/6**

Robledo et al. Genes Dev. 2002

Dlx5/6$^{-/-}$ Mouse Shows Loss of AER-Markers

- Loss of medial AER Structures
- Dlx5/6 important for maintenance of AER



Fgf8

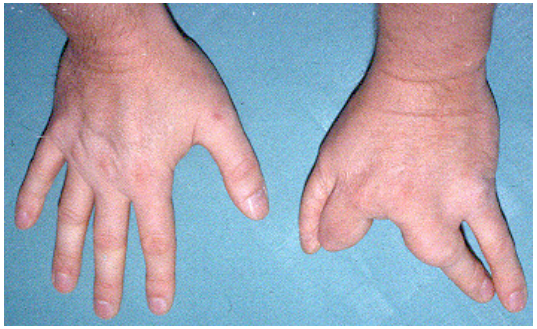
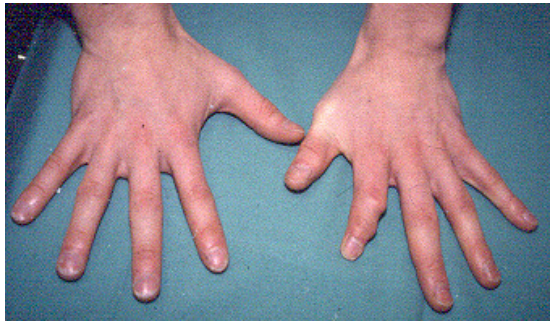
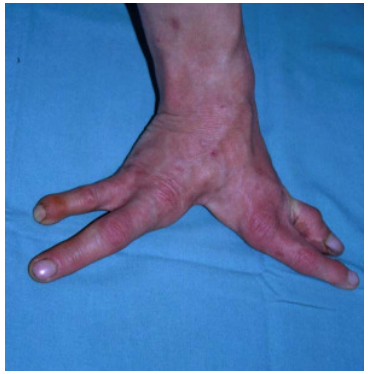
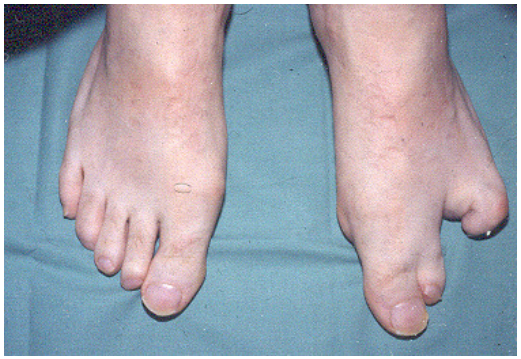
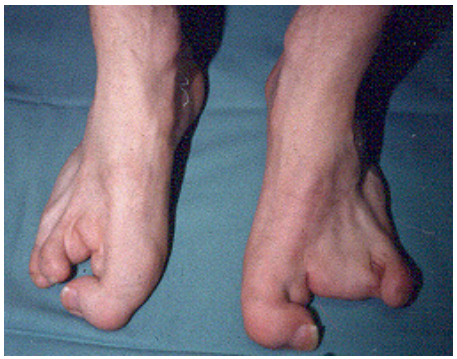
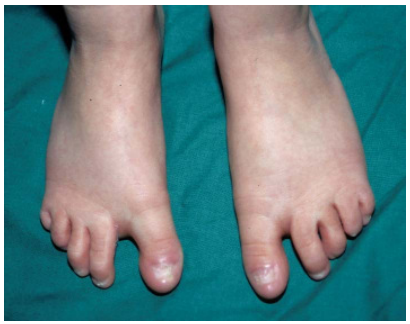
Msx2

wt Dlx5/6 $^{-/-}$

Robledo et al. Genes Dev. 2002

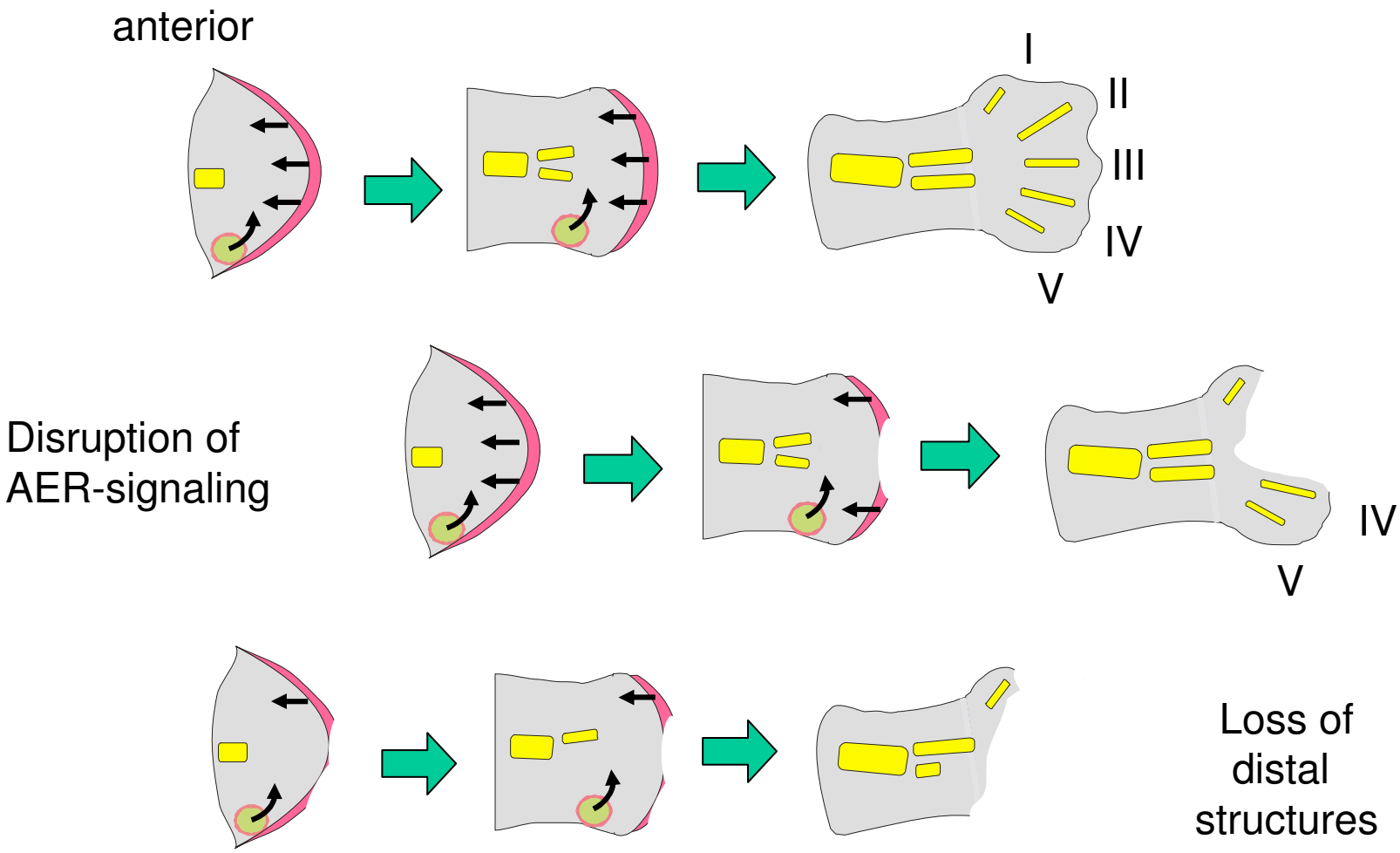
Split-Hand-Foot Phenotypes

- mutations in p63 -

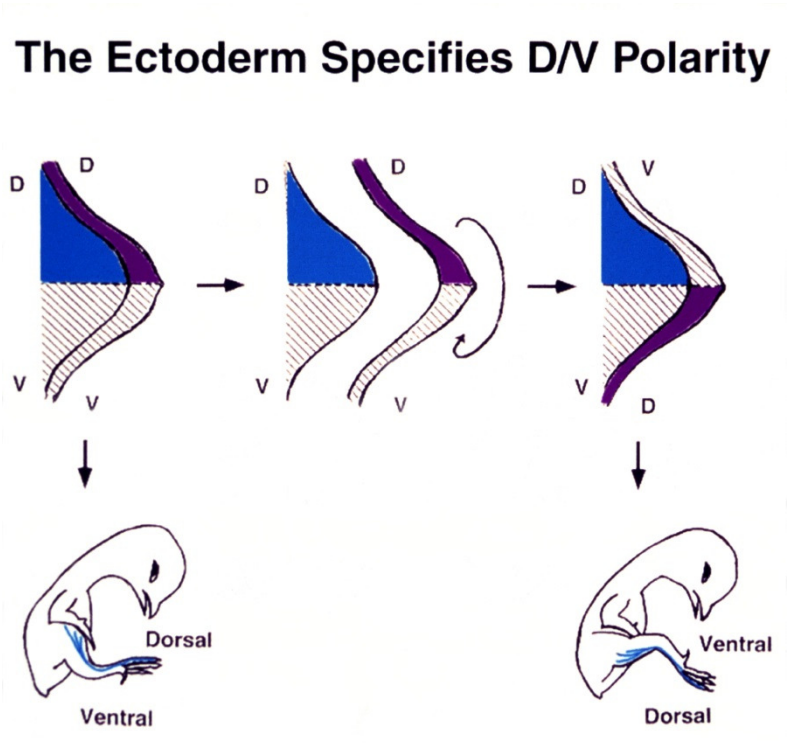
P63 controls devlopment of ectodermal structures

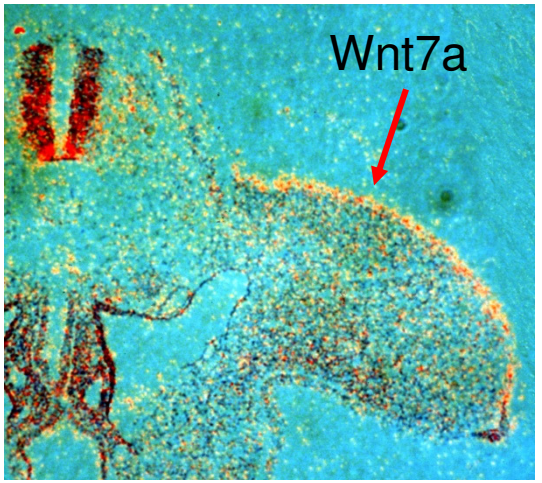
Mechanisms of Ectrodactyly

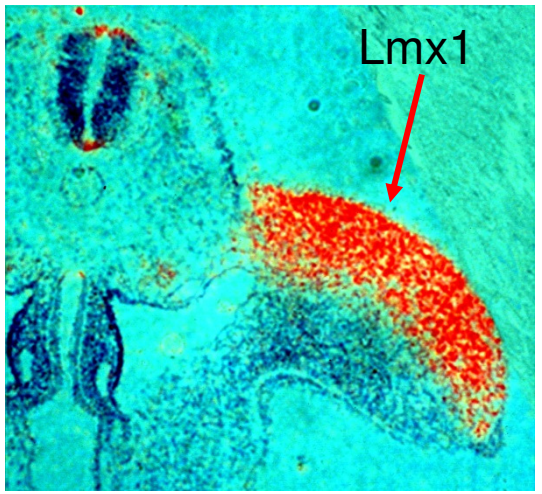


Dorso-ventral Polarity

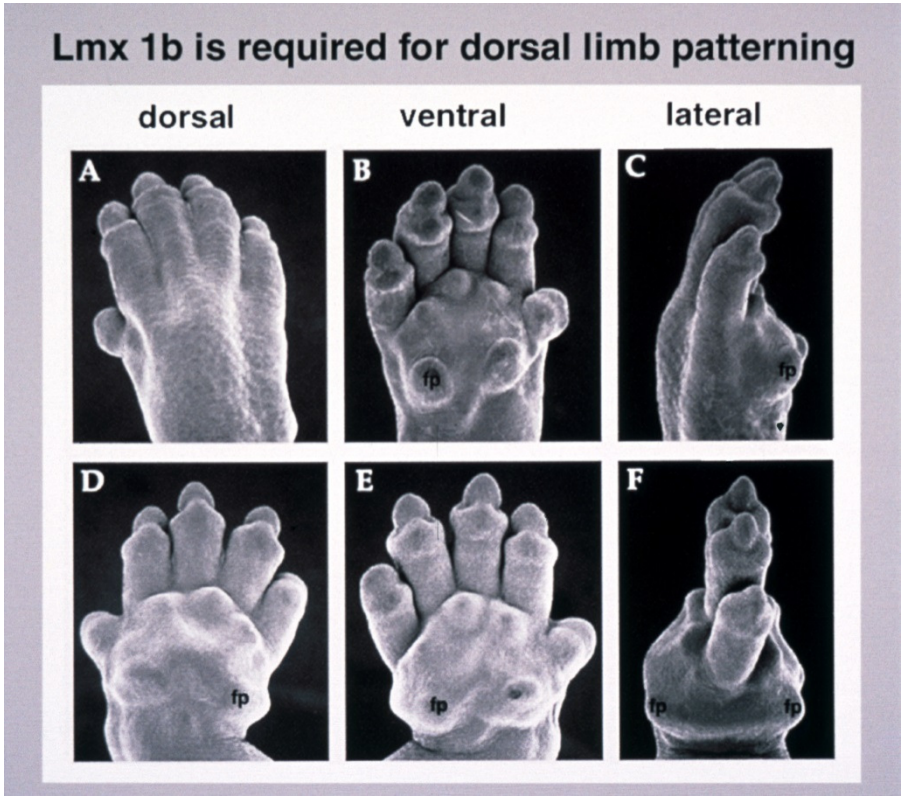
- specified by signal from the ectoderm (Wnt7a)







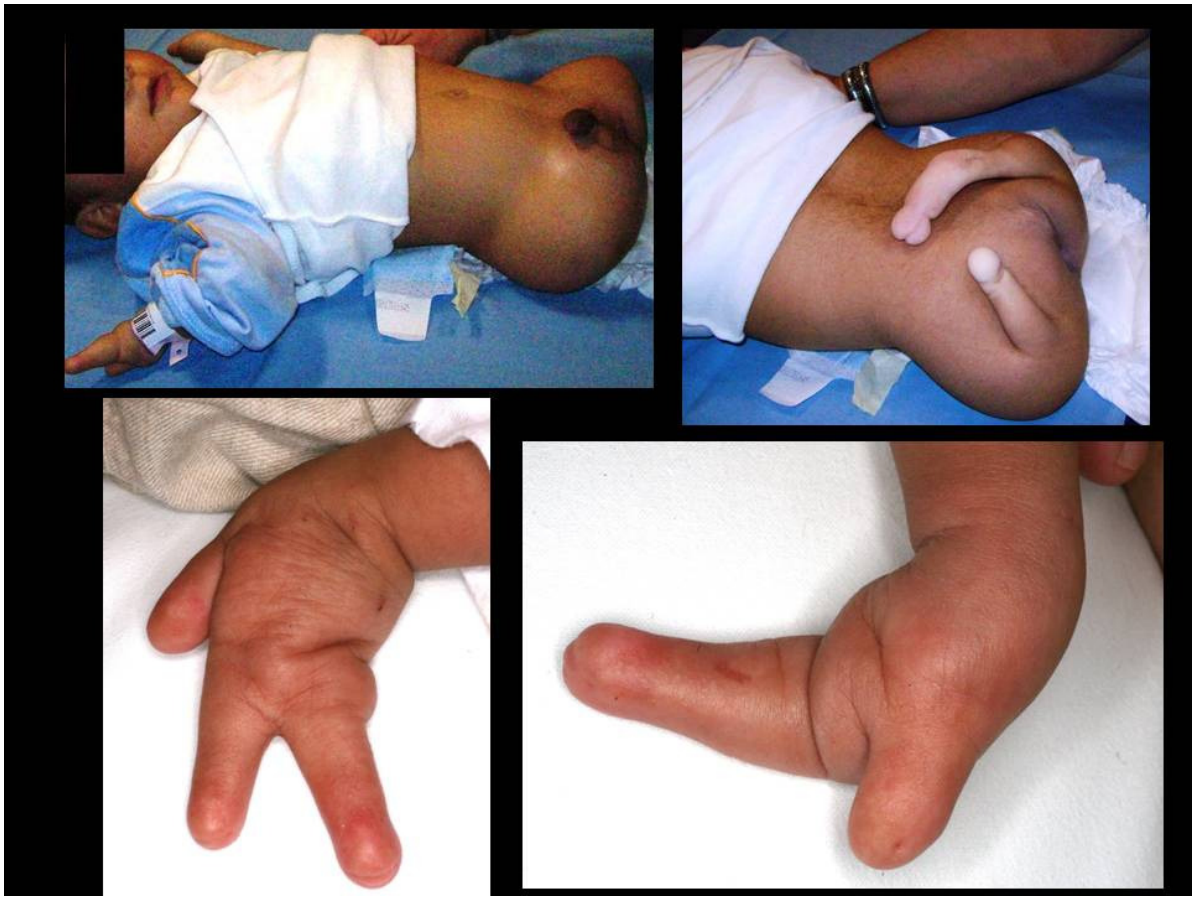
Lmx1 is Essential for Dorsal Pattern



Lack of dorsal structures in Lmx1 knock out mice

Mutations in LMX1 cause Nail-Patella syndrome

Breakdown of Signaling Centers Results in Truncations

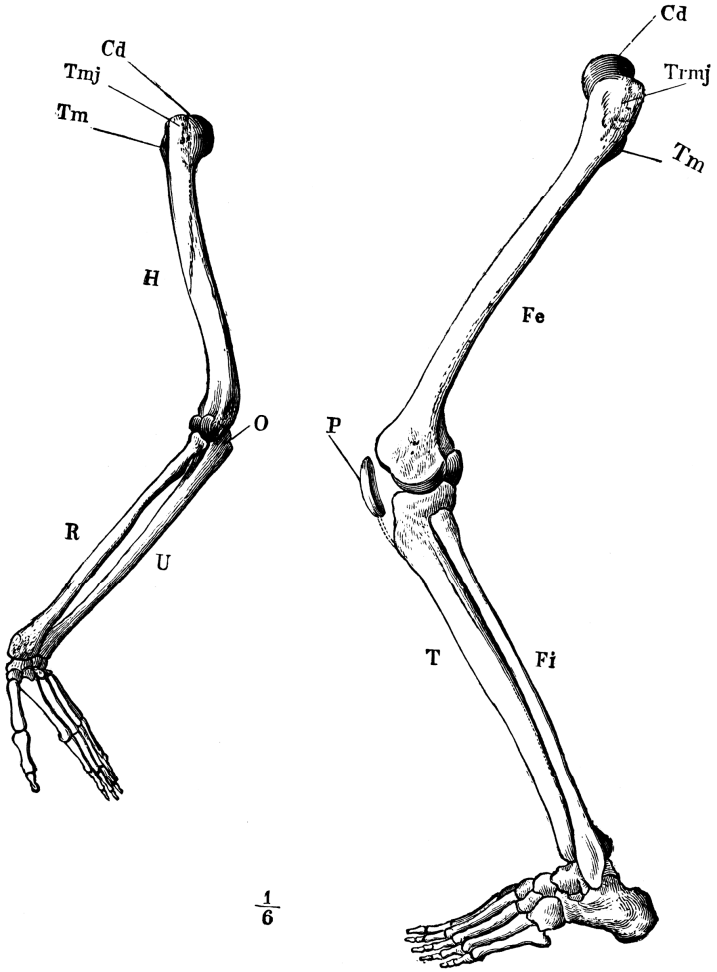


Mutation in WNT7

Loss of:
- distal structures
- asymmetry
- nails
- dorso-ventral pattern

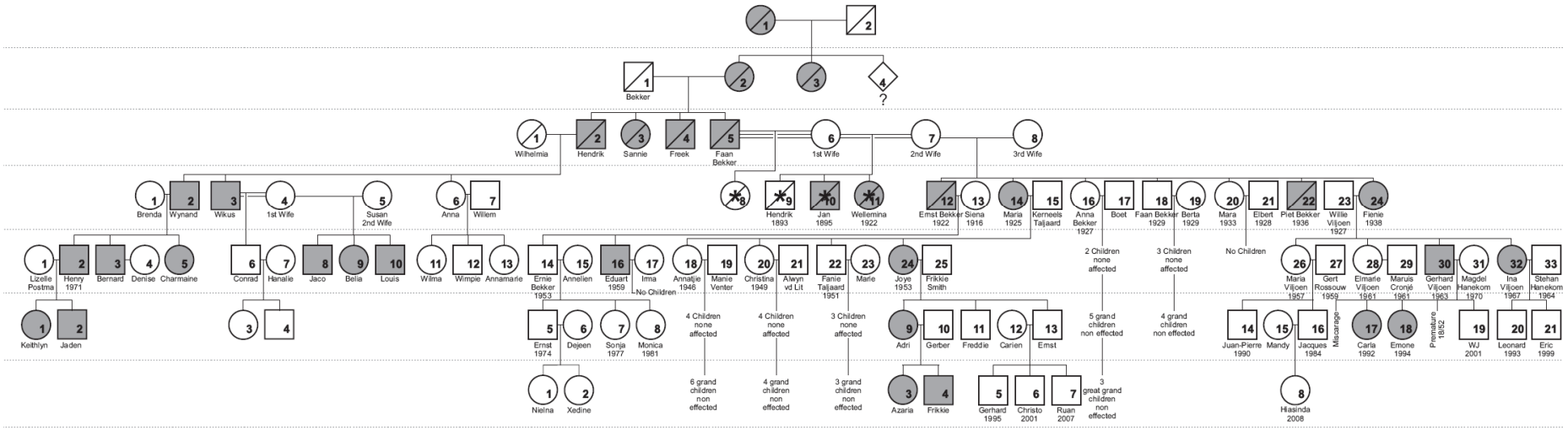
Limb Identity

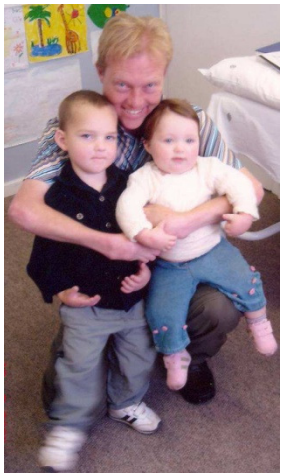
Arm vs. Leg

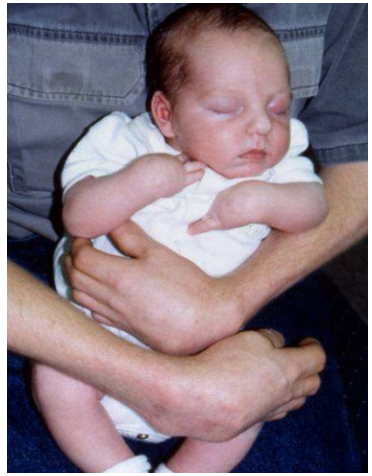


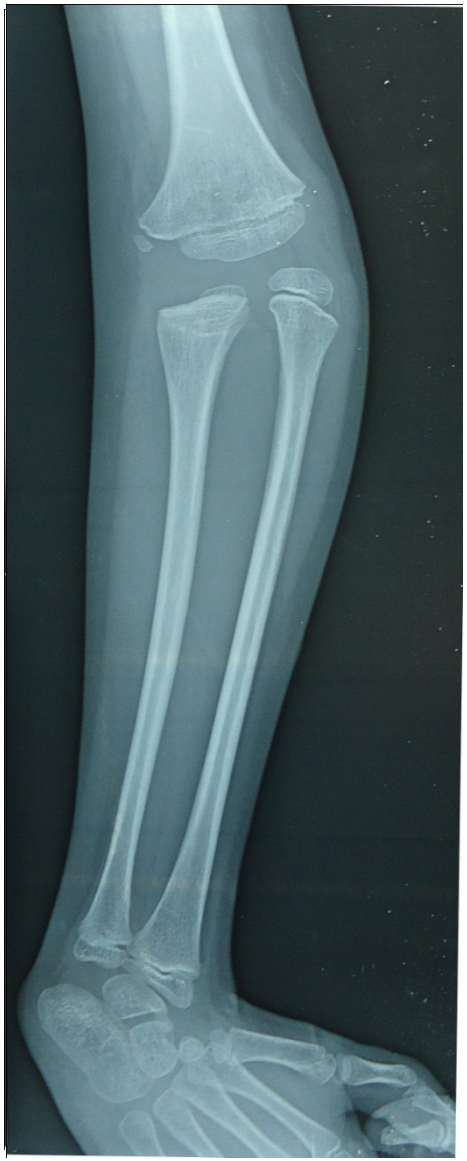
Pedigree with Abnormal Wrists and Elbows

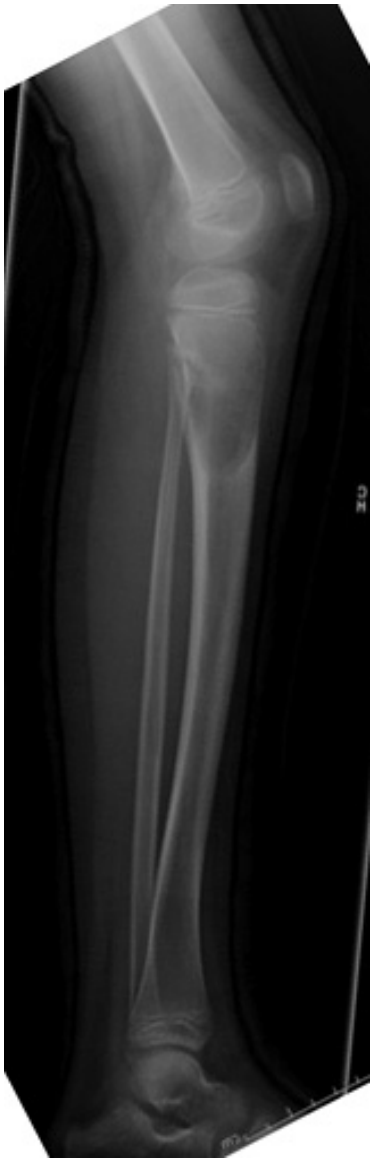
Liebenberg Syndrome





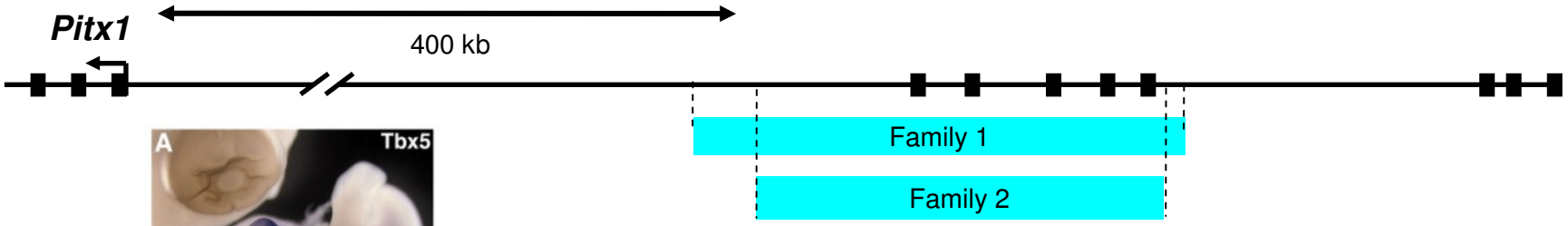




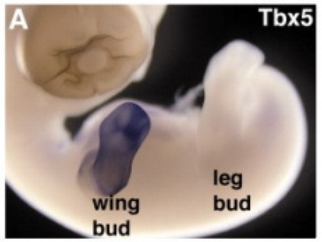


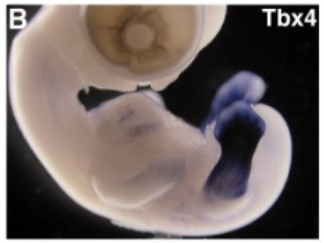
Arm to Leg Transformation

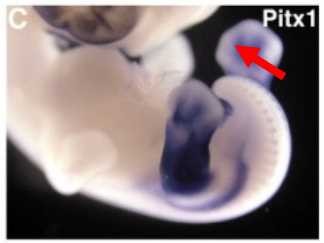
associated with rearrangements at the PITX1 locus



Deletion

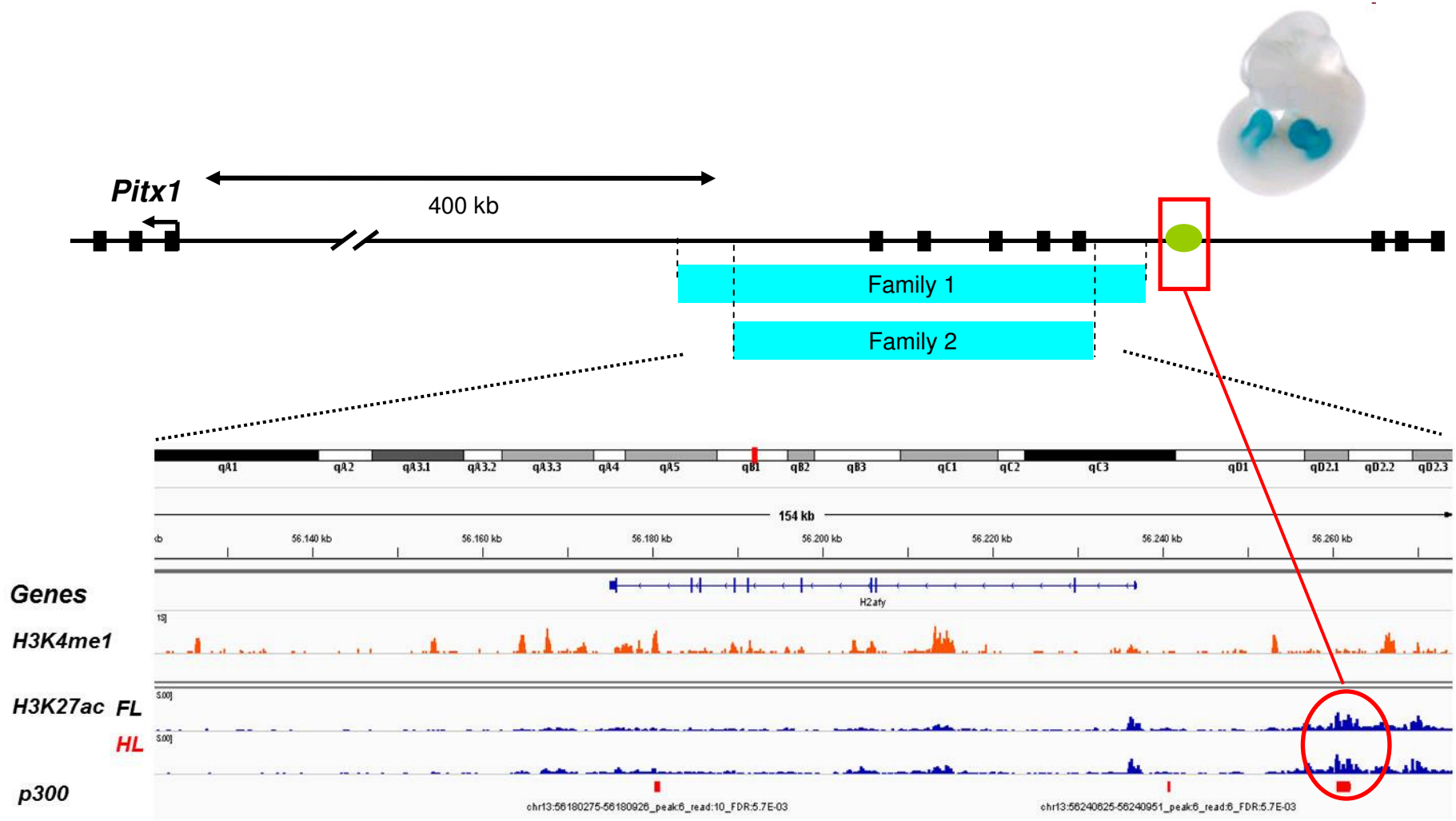




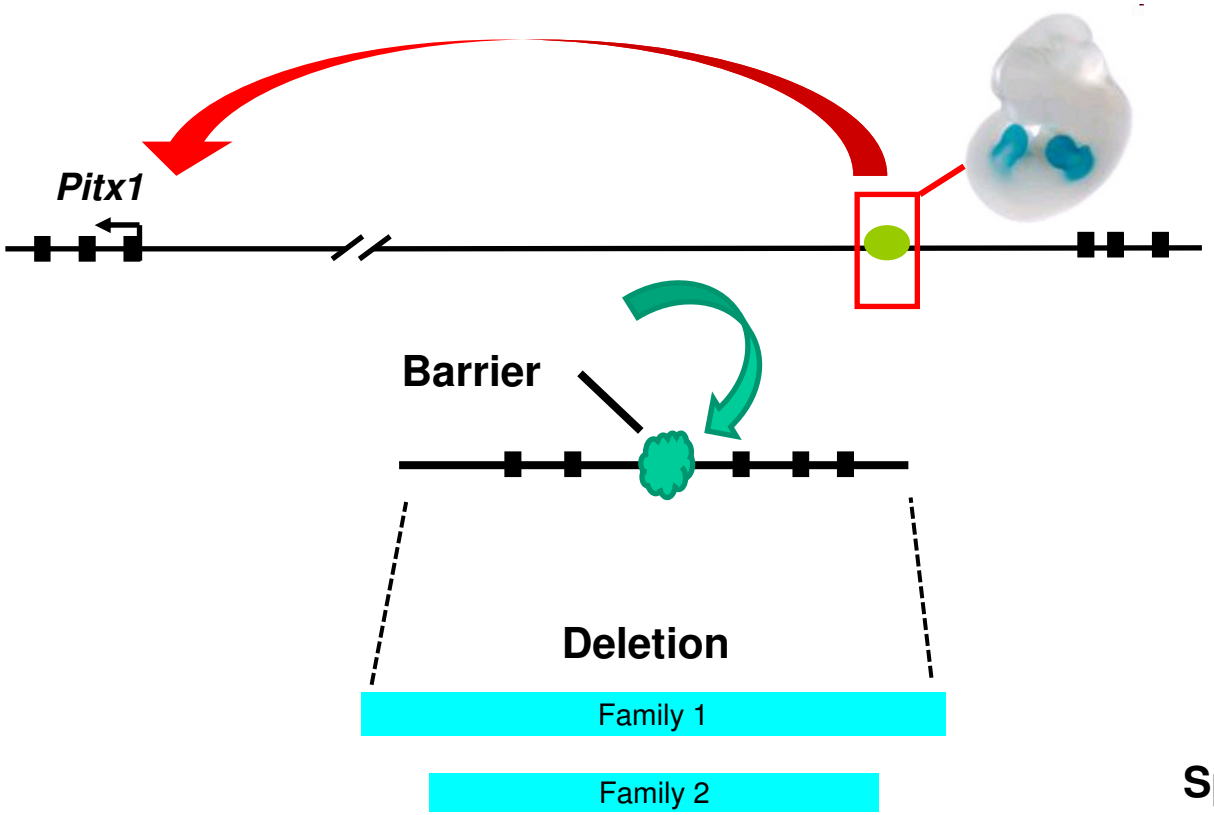


Pitx1 is expressed in hind limb only

Regulatory Elements at the Deletion

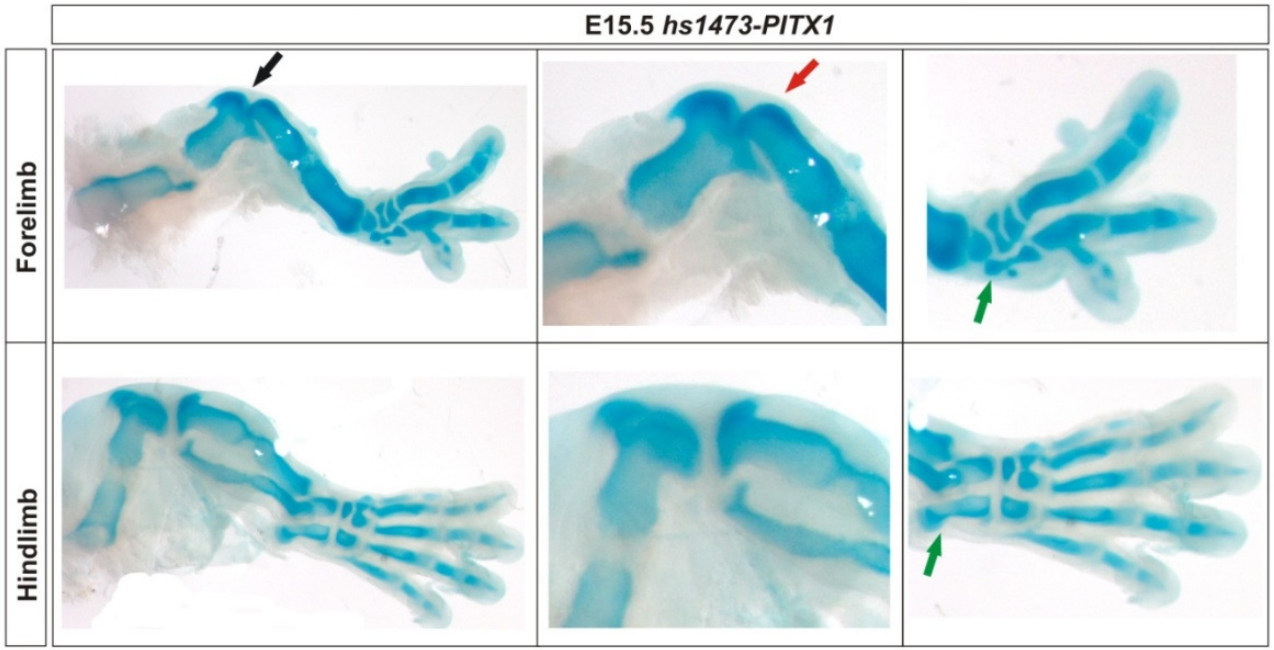


Enhancer Adoption at the PITX1 locus

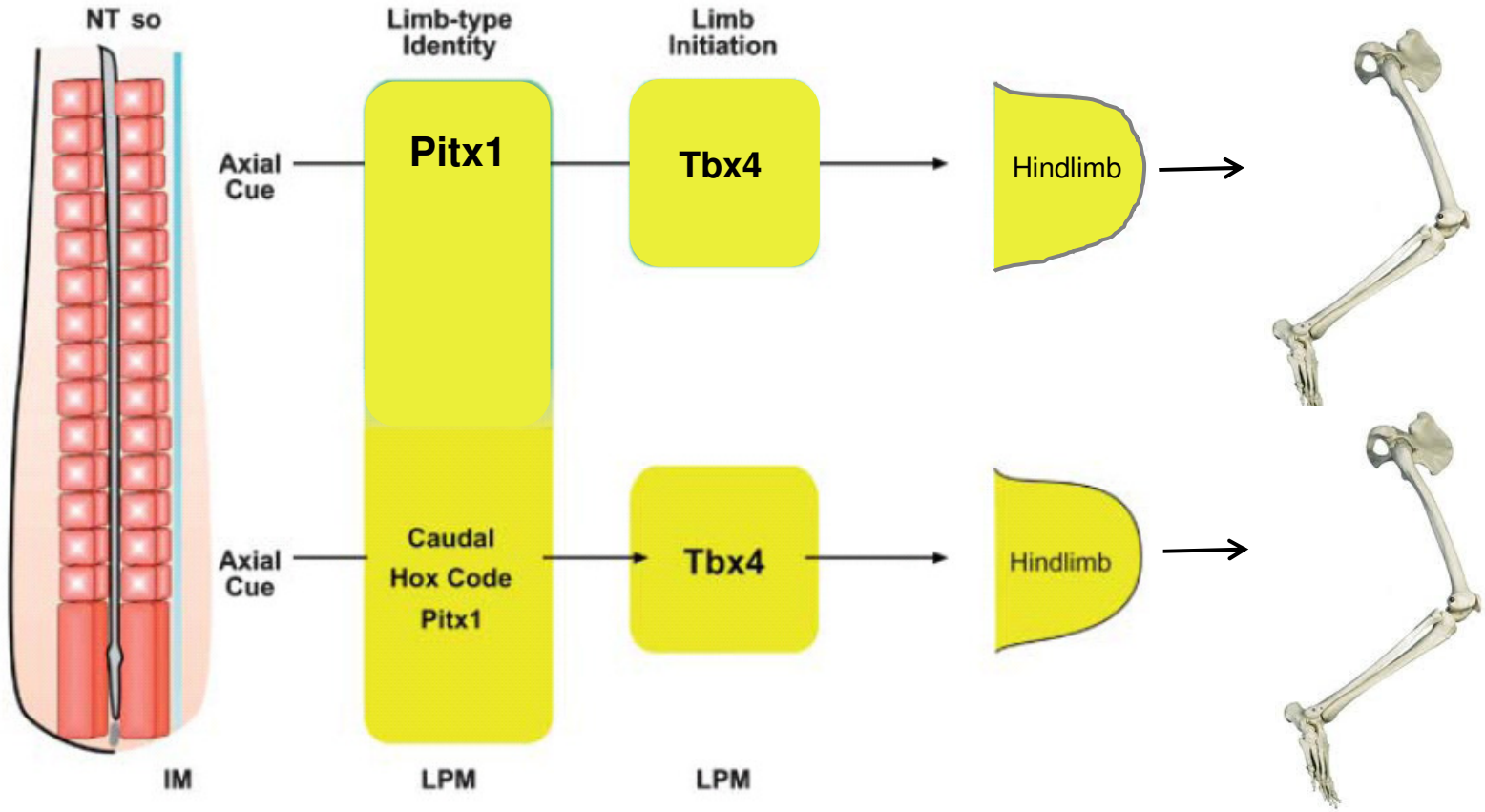


Malte Spielmann

Transgenic expression of Pitx1 under Hs1473 enhancer results in forelimb to hindlimb transformation



Molecular Determinants of Hindlimb/Forelimb Identity

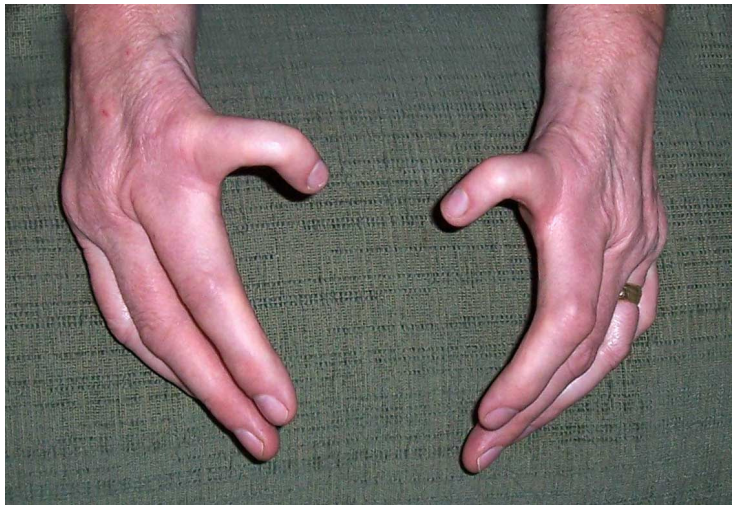


Minguillon and Logan 2005

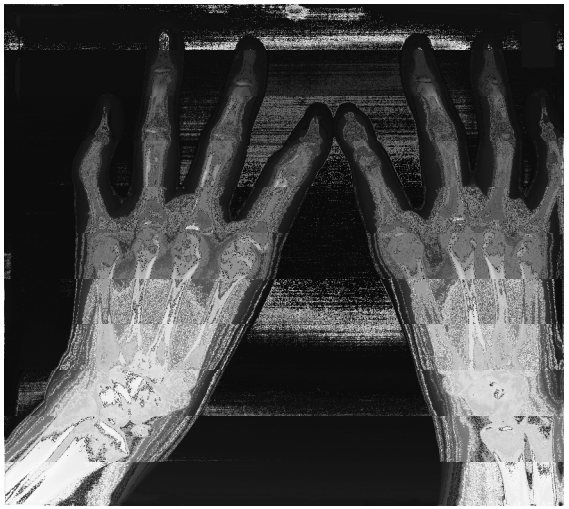
Genetic Testing

in congenital disease

Genetic disease or Thalidomide?

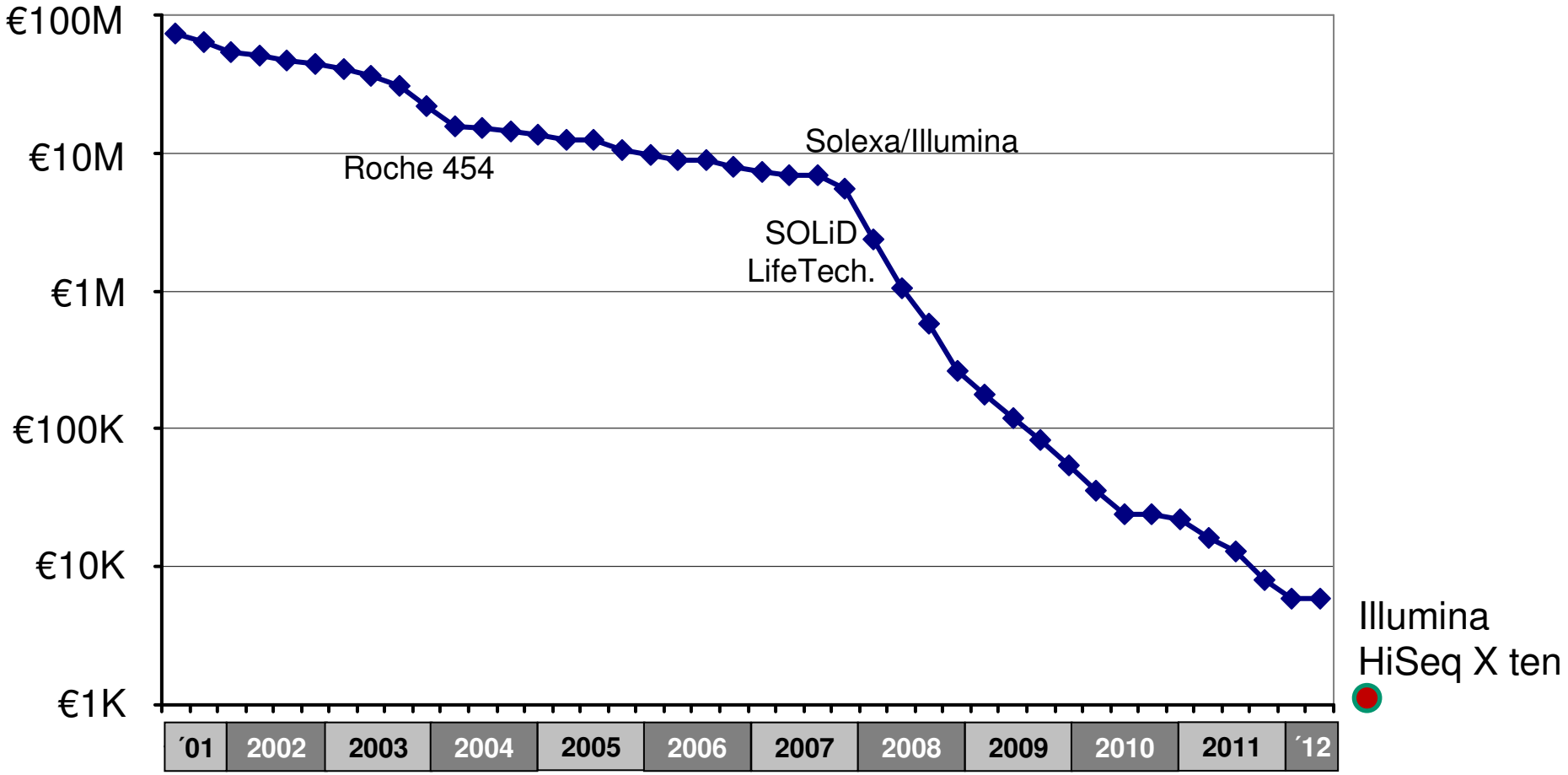


hypoplastic thumbs



absent thumbs

Sequencing Cost / Genome



www.genome.gov/sequencingcosts

Applications of NGS

in genetic diagnostics

Gene Panel Diagnostics

all genes that are associated with a disease / phenotype

currently established

Immunologye	**Lipid metabolism**	**Cardiology**	**Deafness**
188 Genes	27 Genes	96 Genes	90 Genes
CDG	**Osteoporosis**	**Skelettal dysplasia**	**HPO**
76 Genes	90 Genes	167 Genes	2776 Genes

Skeletal Dysplasia Panel – Design

167 genes

Target region of 658 kbp

Criteria: **Causative for monogenic skeletal dysplasias + dysostoses**

no **predominant connective tissue involvement or abnormal bone mass**

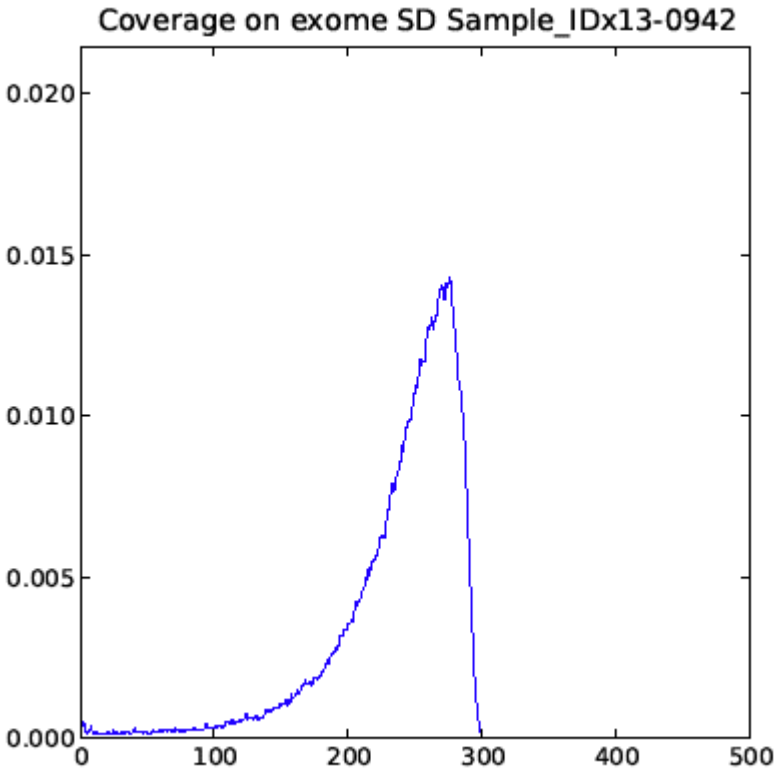
no **lysosomal storage disorder (group 27 of nosology)**

ACAN	DDR2	GUSB	DYNC2H1	DYNC2H1	RECQL4	TWIST1
ACP5	EFNB1	HDAC4	EBP	EBP	RMRP	WDR35
ACVR1	EIF2AK3	HOXA11	MNX1	MNX1	ROR2	WISP3
ADAMTSL2	EP300	HOXA13	MSX2	MSX2	RPGRIP1L	WNT3
AGA	ESCO2	HOXD13	MYCN	MYCN	RUNX2	WNT7A
AGPS	EVC	HSPG2	NAGLU	NAGLU	SALL1	
ALX1	EVC2	ICK	NEK1	NEK1	SALL4	
ALX4	EXT1	IDS	NEU1	NEU1	SBDS	
ANO5	EXT2	IDUA	NFIX	NFIX	SGSH	
ANTXR2	FAM58A	IFT122	NIPBL	NIPBL	SH3BP2	
ARSB	FBXW4	IFT43	NKX2-3	NKX2-3	SH3PXD2B	
ARSE	FGD1	IFT80	NLRP3	NLRP3	SHH	
BMP2	FGF10	IHH	NOG	NOG	SHOX	
BMPER	FGF9	IL1RN	NPR2	NPR2	SLC17A5	
BMPR1B	FGFR1	INPPL1	NSD1	NSD1	SLC26A2	
CANT1	FGFR2	KAT6B	NSDHL	NSDHL	SLC35D1	
CC2D2A	FGFR3	KIF22	OBSL1	OBSL1	SMARCAL1	
CDH3	FLNA	LBR	OFD1	OFD1	SOX9	
CEP290	FLNB	LFNG	PAPSS2	PAPSS2	SUMF1	
CHST14	FMN1	LIFR	PCNT	PCNT	TBCE	
CHST3	FUCA1	LMBR1	PDE4D	PDE4D	TBX15	
COG1	GALNS	LMX1B	PEX7	PEX7	TBX3	
COL10A1	GDF5	LPIN2	PITX1	PITX1	TBX4	
COL11A1	GDF6	LRP4	POLR1C	POLR1C	TBX5	
COL11A2	GLB1	MATN3	POLR1D	POLR1D	TCOF1	
COL2A1	GLI3	MESP2	POR	POR	THPO	
COL9A1	GNAS	MGP	PRKAR1A	PRKAR1A	TMEM67	
COL9A2	GNPAT	MKS1	PTH1R	PTH1R	TP63	
COL9A3	GNPTAB	MMP13	PTHLH	PTHLH	TRAPPC2	
COMP	GNPTG	MMP9	PTPN11	PTPN11	TRIP11	
CREBBP	GNS	DLL3	RAB23	RAB23	TRPS1	
CTSA	GPC6	DYM	RAB33B	RAB33B	TRPV4	
CUL7	GREM1					

Skeletal Dysplasias and Dysostoses Panel

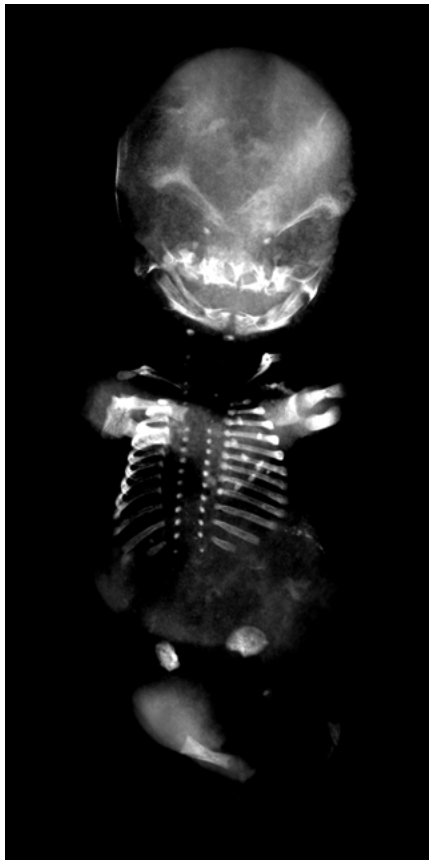
Paired-end 150 bp sequencing on MiSeq

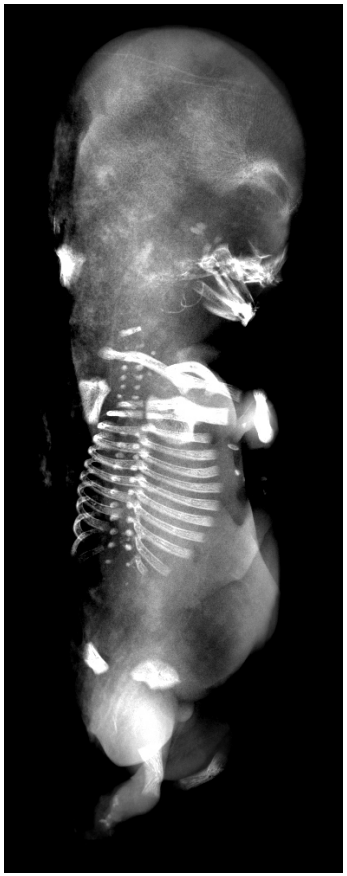
Excellent coverage:
Mapped reads: 2153679
Unmapped reads: 84020
Fraction with cov. >= 10 : 0.9854506
Fraction with cov. >= 20 : 0.9839304

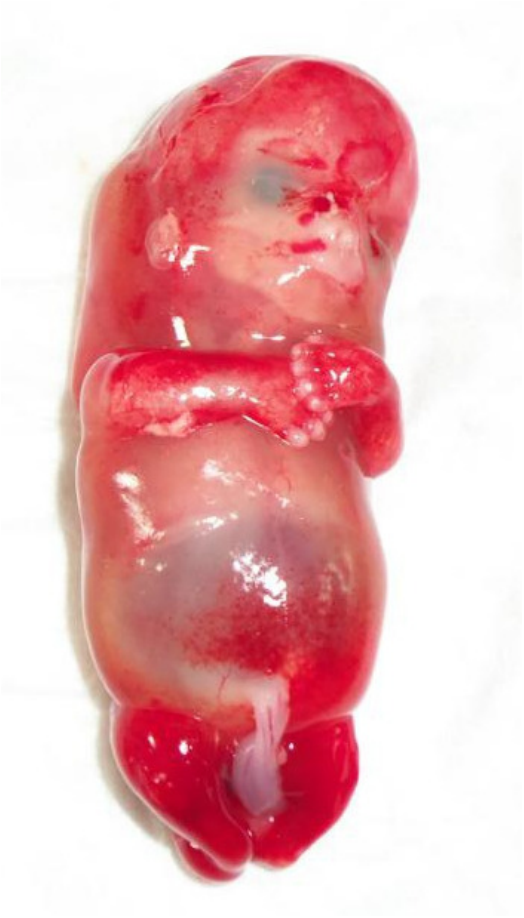


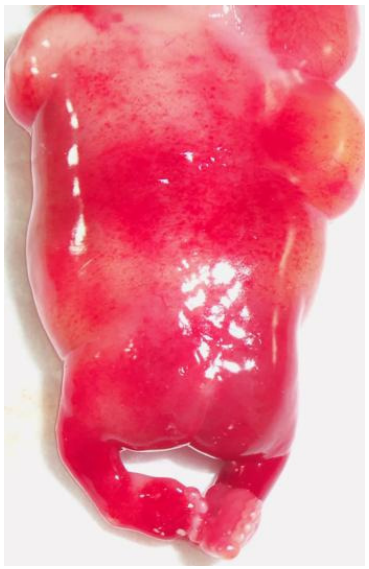
Skeletal Dysplasia Panel – Case 13-0812

Clinic: lethal chondrodysplasia

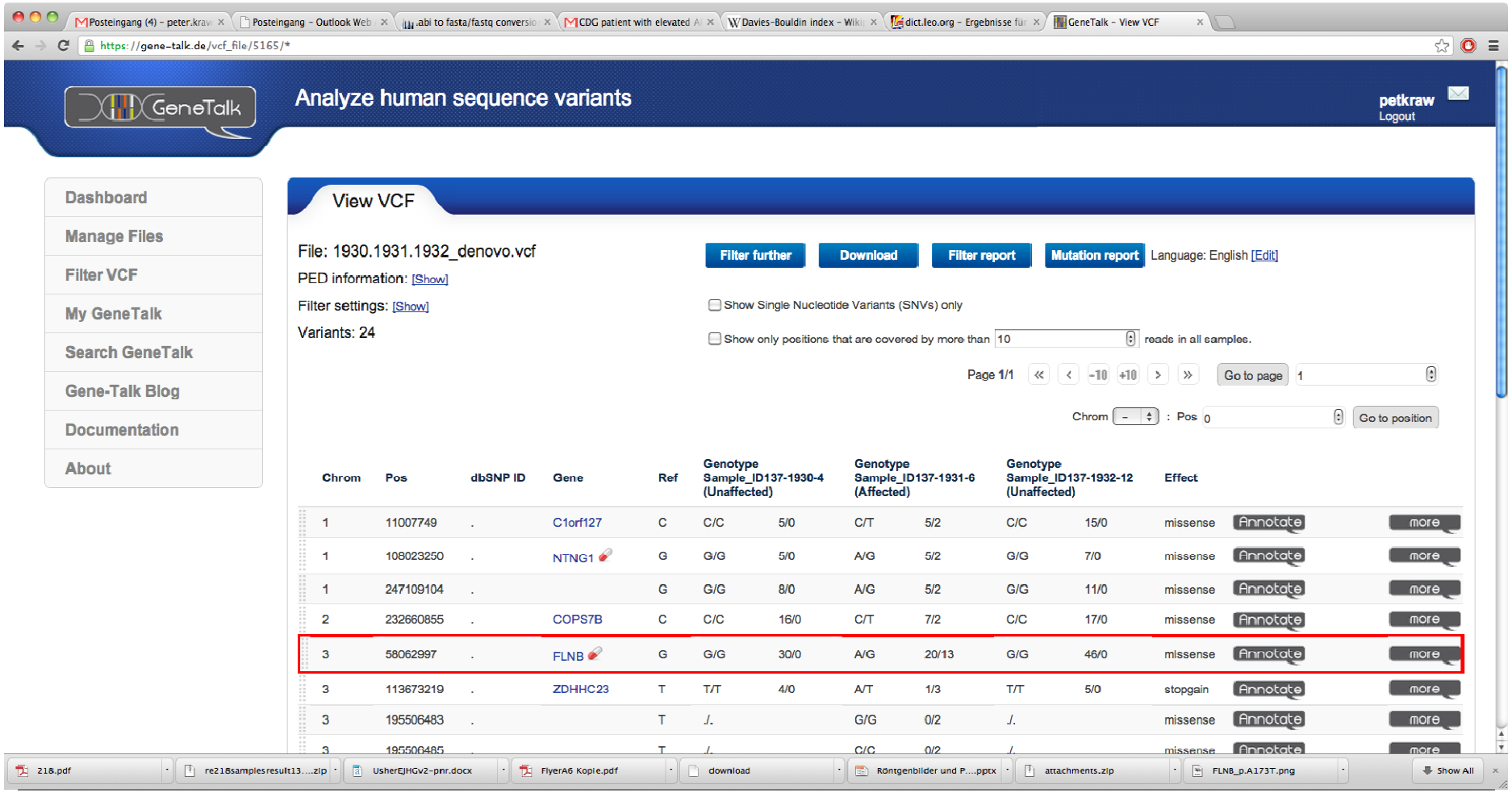








Analysis of Results



Fetus with Skeletal Dysplasia

Analysis of Data

FLNB:NM_001164317:exon2:c.517G>A:p.A173T

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