# Aspectos genéticos y moleculares de la talla baja idiopática

# Genetic and molecular aspects of idiopathic short stature

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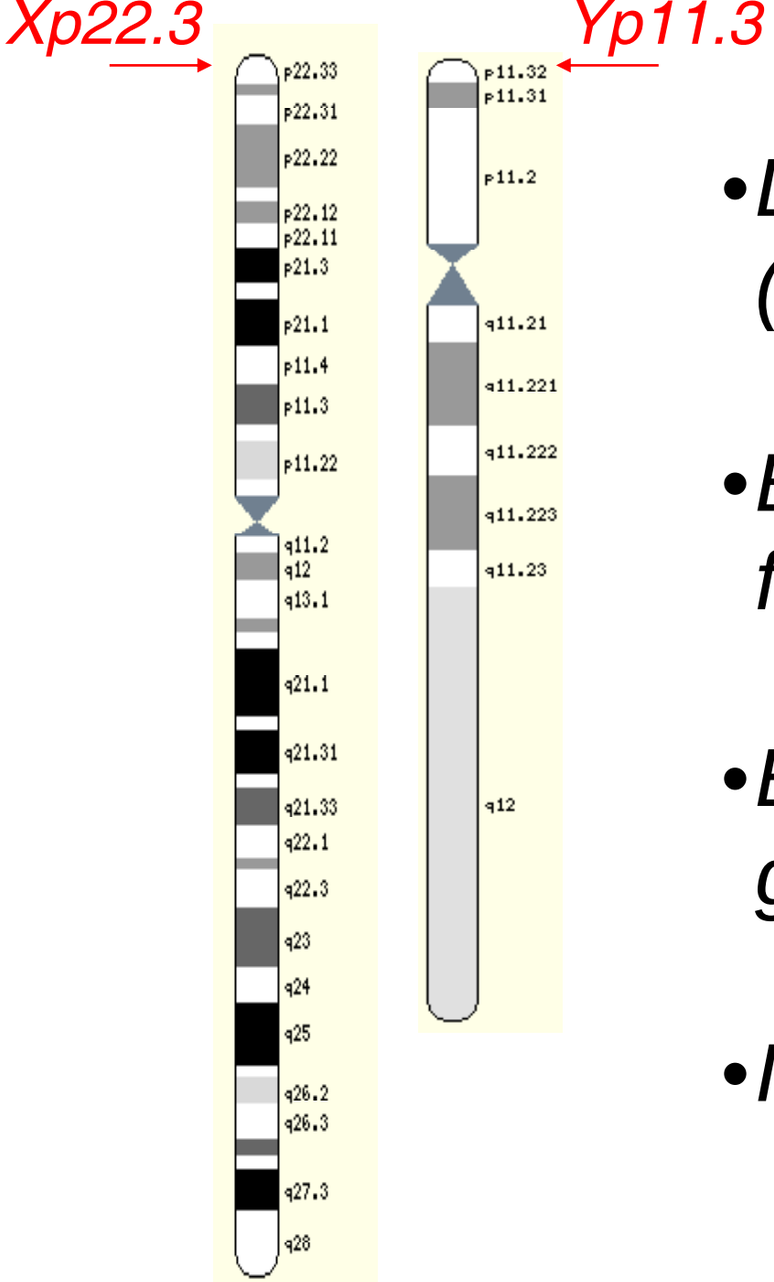
- Idiopathic short stature is a condition in which the height of the individual is more than 2 SDS below the mean height for a given age, sex and population, in whom no identifiable disorder is present.
- It can be subcategorized into familial and non-familial ISS, and according to pubertal delay.
- It should be differentiated from dysmorphic syndromes, skeletal dysplasias, small for gestational age, and systemic and endocrine diseases.
- ISS is the diagnostic group that remains after excluding known conditions in short children.

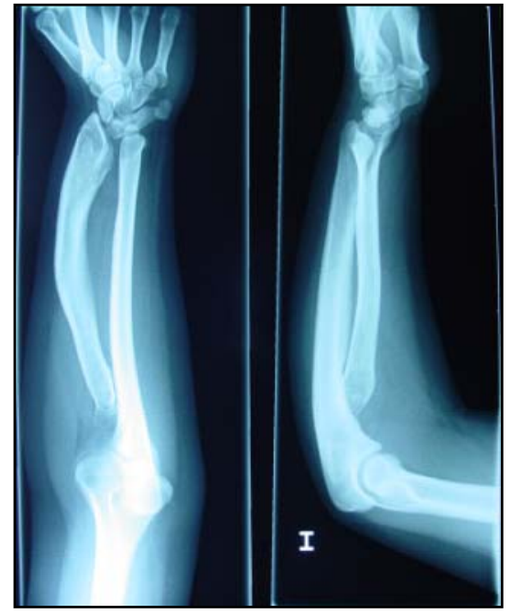
Wit JM, Clayton PE, Rogol AD, Savage MO, Saenger PH, Cohen P

- Growth hormone - insulin-like growth factor I (GH - IGF-I) axis - *GHR, IGF-ALS……*

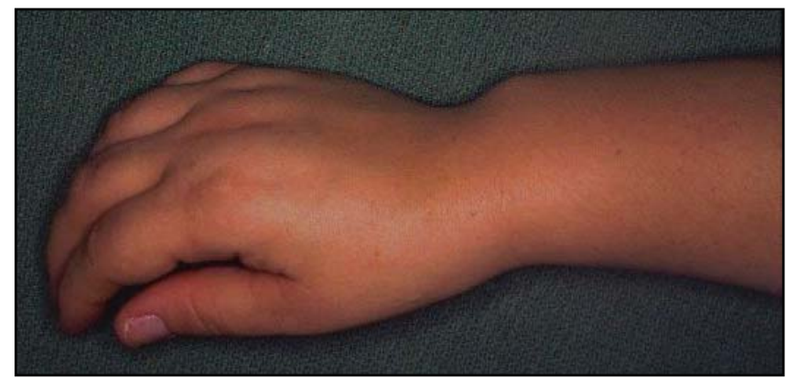
- *SHOX*
- *NPR2*
- *NPPC (CNP)*
- *ACAN*




- *Located on the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes*
- *Encodes a homeodomain transcription factor*
- *Expressed in chondrocytes of the human growth plate*
- *Involved in determining stature in humans*



Madelung deformity



*(-9 to 6.2 SD)*

***Langer mesomelic dysplasia (LMD)***

*(-4.6 to +0.6 SD)[1]*

***Léri-Weill dyschondrosteosis (LWD)***

*(< -2 SD)[2]*

***Idiopathic short stature (ISS)***

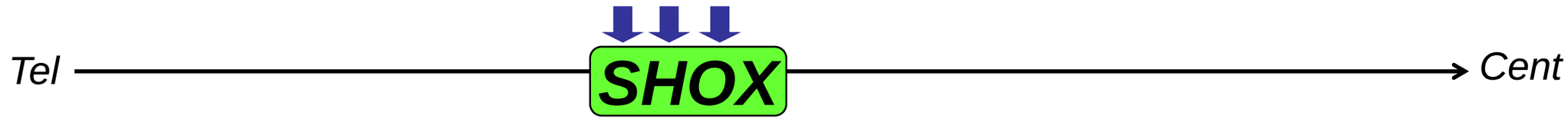
***Turner syndrome***

***0***

***1***

(Belin et al, 1998; Shears et al, 1998)

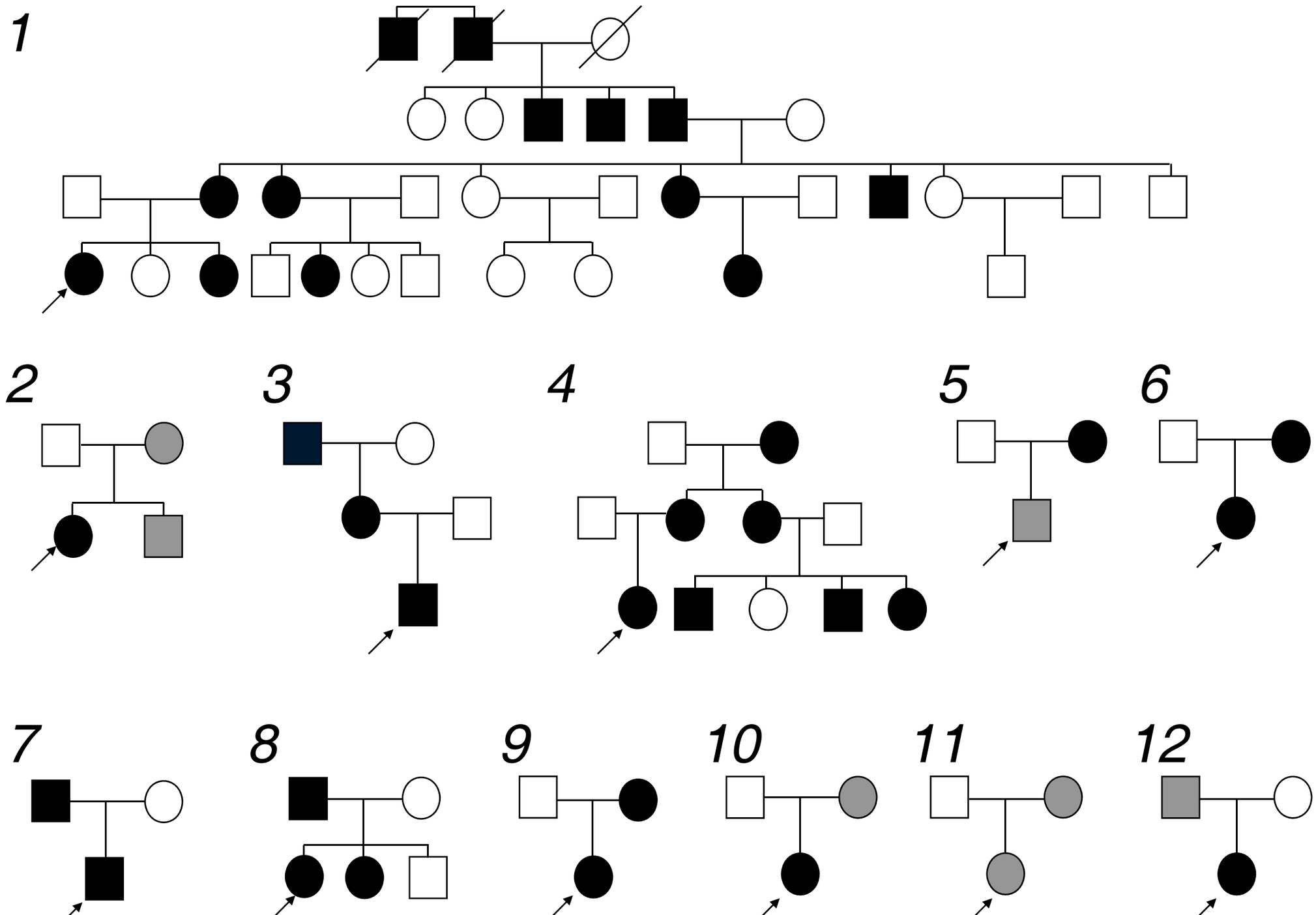
## *Mutations (1/3)*

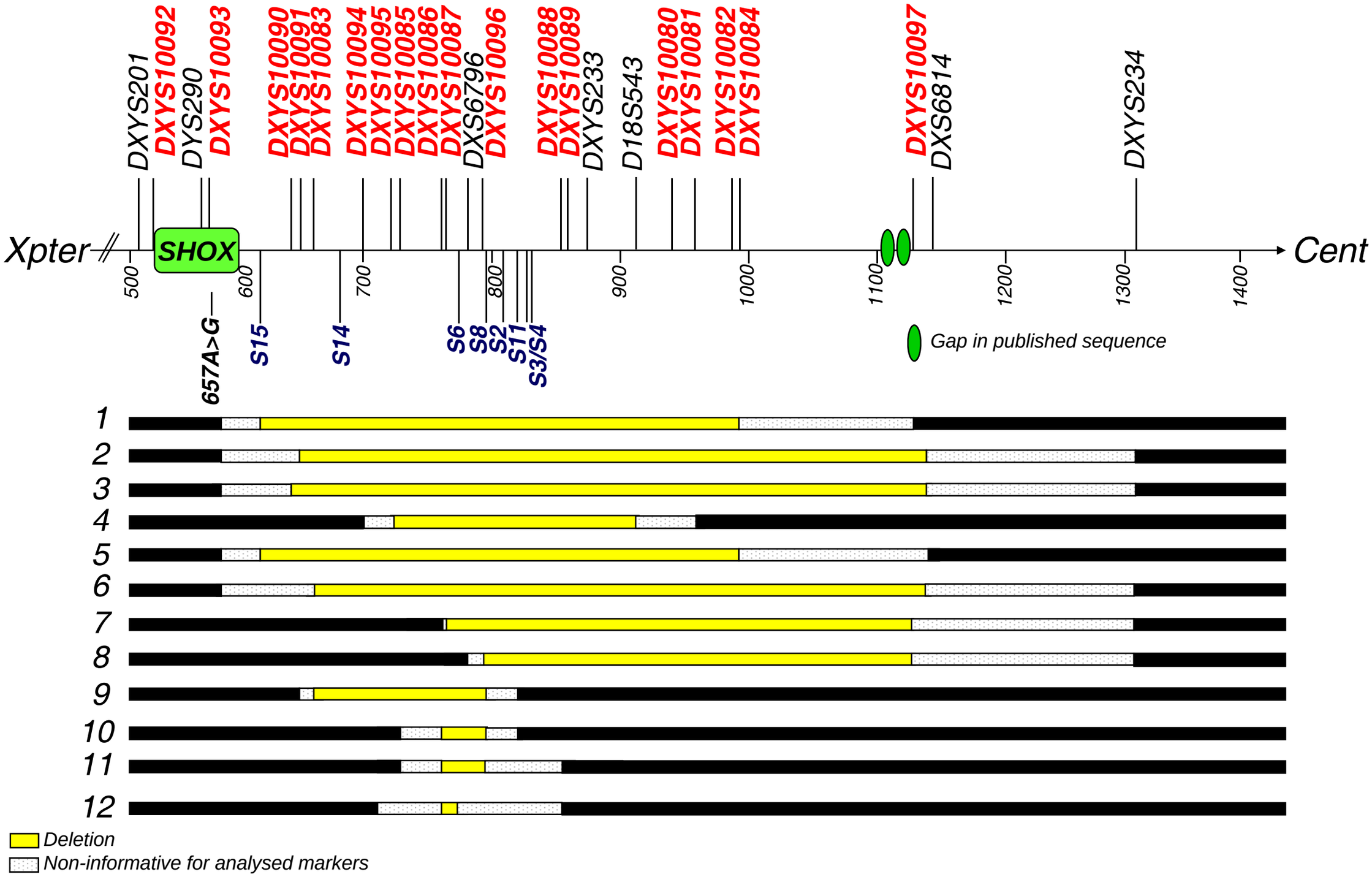


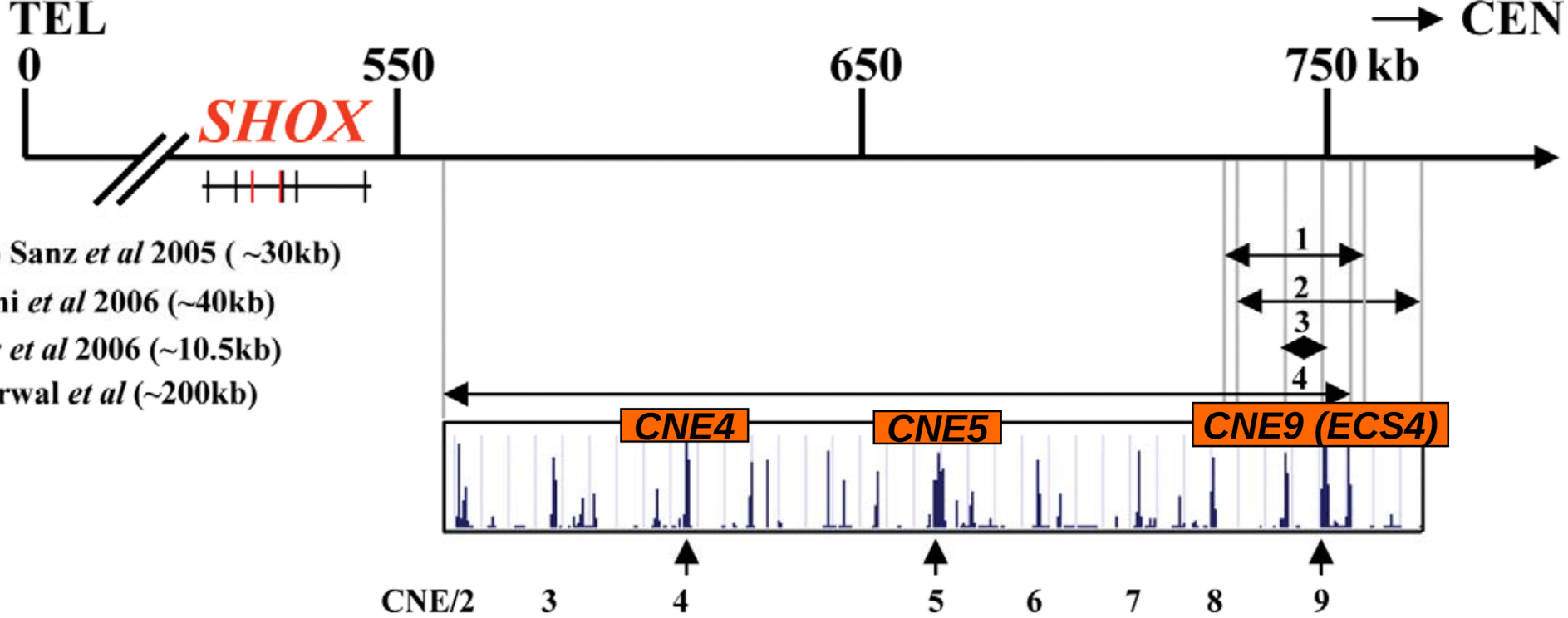

## *Complete SHOX deletions (2/3)*

(Rao et al, 1997; Ellison et al, 1997)

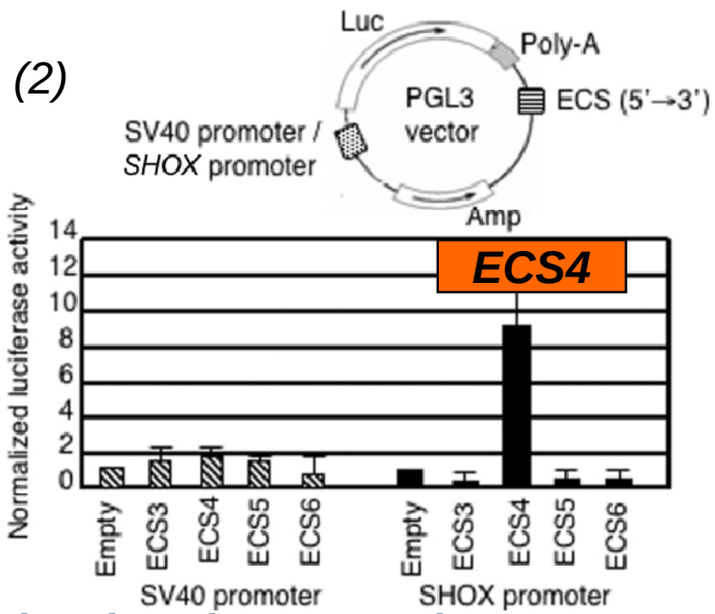
- *~ 50% of LWD cases*
- *~ 80% of LMD cases*
- ***~ 2.5% of ISS cases***


1
2
3
4
5
6
7
8
9
10
11
12


DXYS201
DXYS10092
DYS290
DXYS10093
DXYS10090
DXYS10091
DXYS10083
DXYS10094
DXYS10095
DXYS10085
DXYS10086
DXYS10087
DXS6796
DXYS10096
DXYS10088
DXYS10089
DXYS233
D18S543
DXYS10080
DXYS10081
DXYS10082
DXYS10084
DXYS10097
DXS6814
DXYS234
Xpter
SHOX
Cent
500
600
700
800
900
1000
1100
1200
1300
1400
657A>G
S15
S14
S6
S8
S2
S11
S3/S4
Gap in published sequence
1
2
3
4
5
6
7
8
9
10
11
12
Deletion
Non-informative for analysed markers

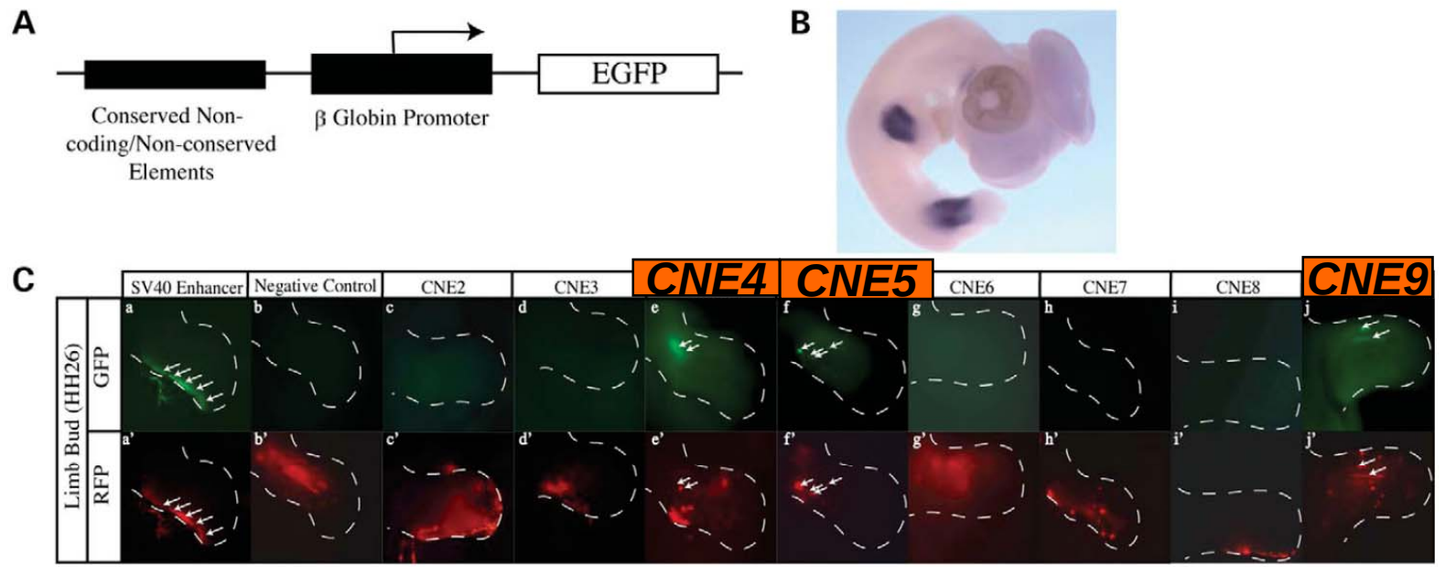



(2)



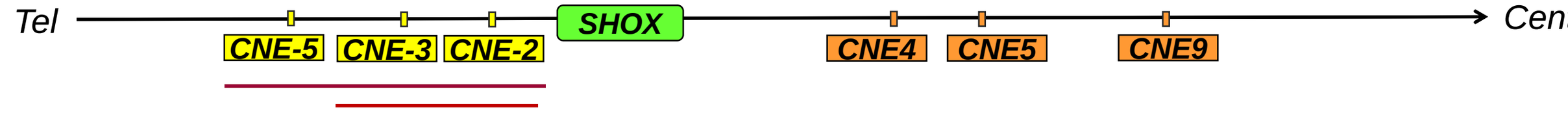

Fukami et al 2006, Am j Hum Genet 78:1768-8

(4)




Sabherwal et al, 2007; Hum Mol Genet 16(2):210-222

**Three enhancers identified downstream of *SHOX***




ISS

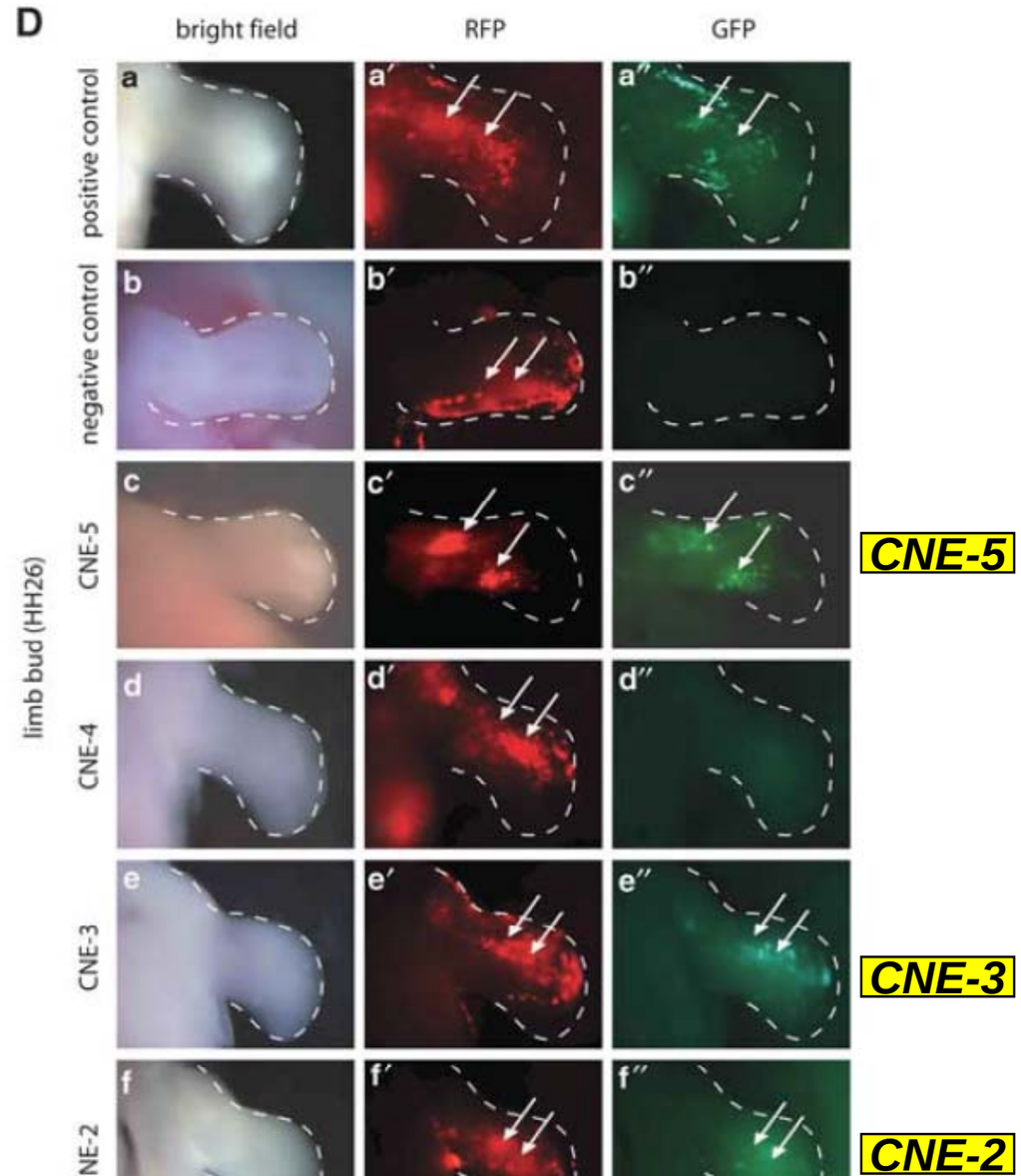
## *Upstream enhancer deletions*

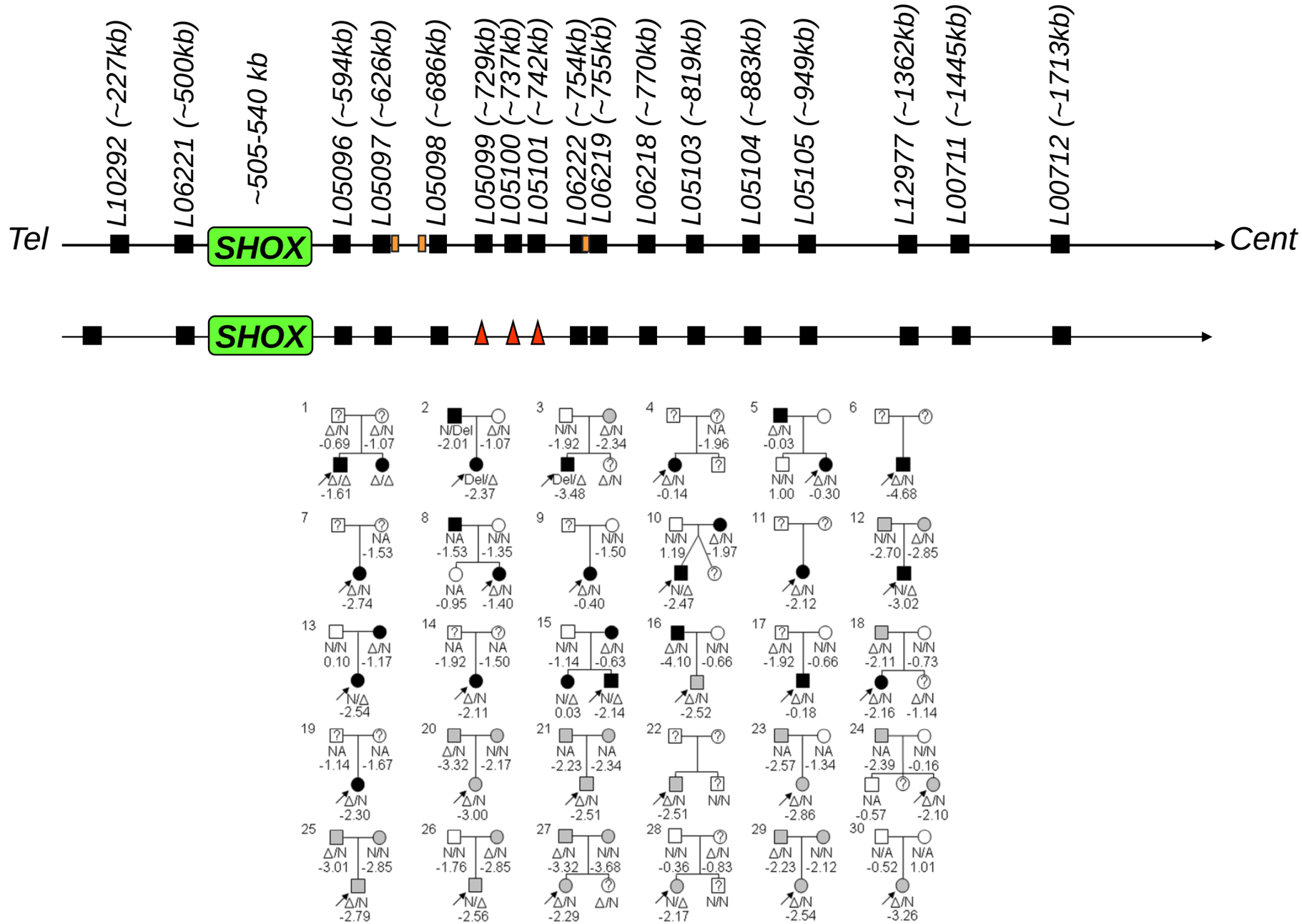
*Benito-Sanz et al, Eur J Hum Genet 2012*
*Verdin et al, Sci Rep 2015*

## *Upstream enhancer duplications*

*Benito-Sanz et al, J Clin Endocrinol Metab 2011*
*Verdin et al, Sci Rep 2015*

**Three enhancers located upstream of *SHOX***



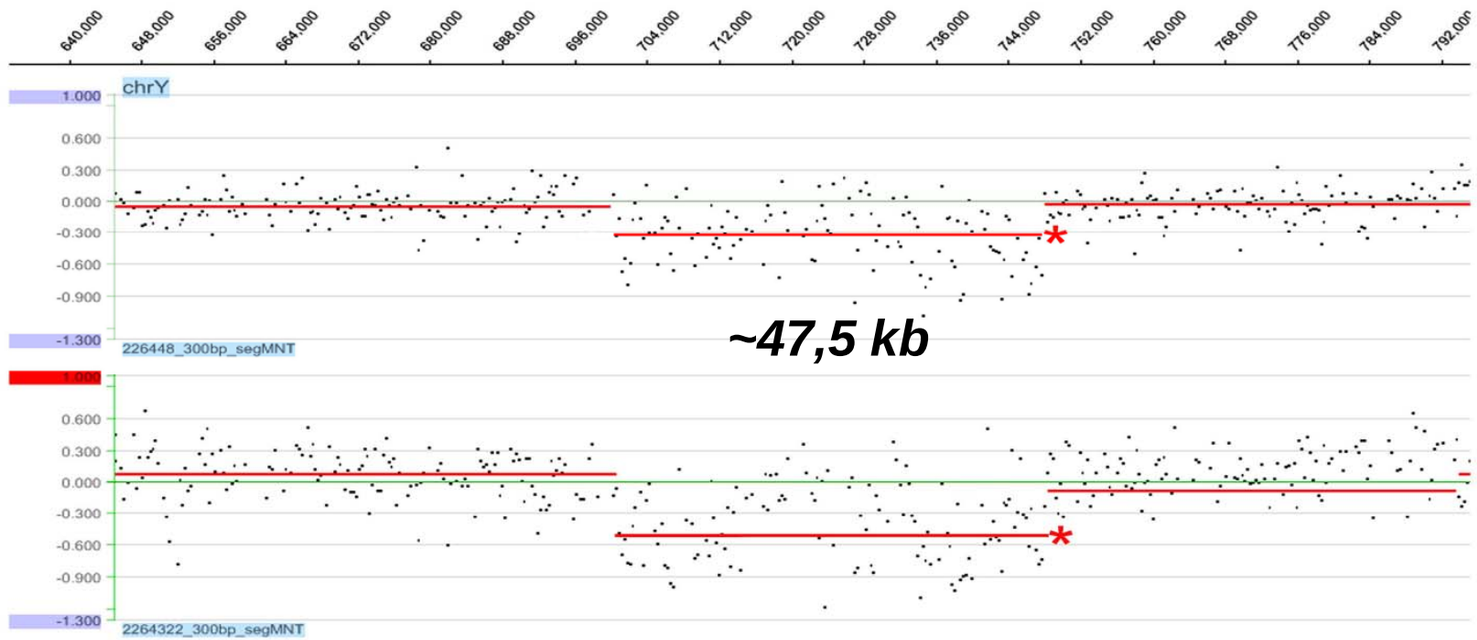



**Recurrent deletion in 30 patients (19 LWD & 11 ISS)**


*Array CGH*

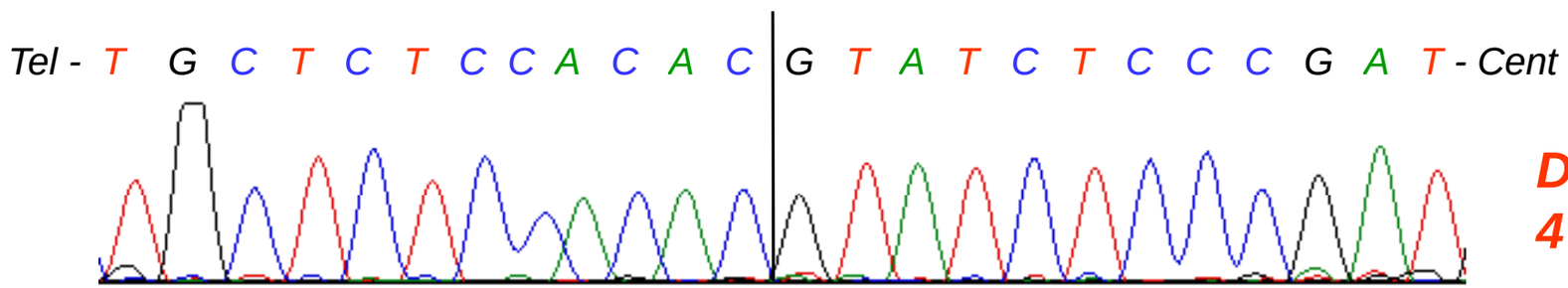
*Chr Y*

*log2 ratio*




***(NCBI36/hg18)***

***700549:748093***

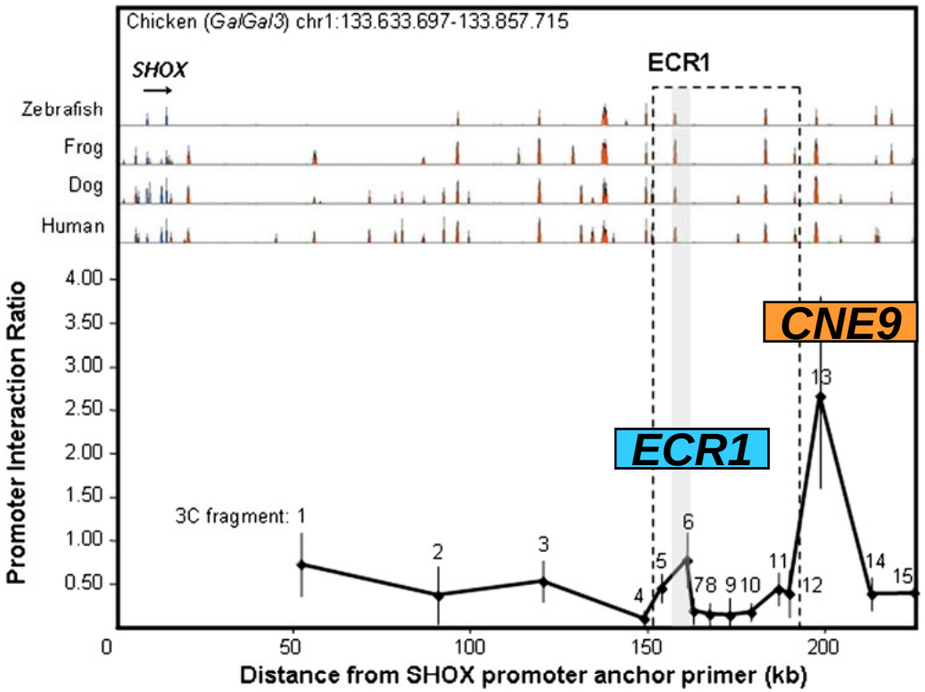



***Deletion 47543 bp***

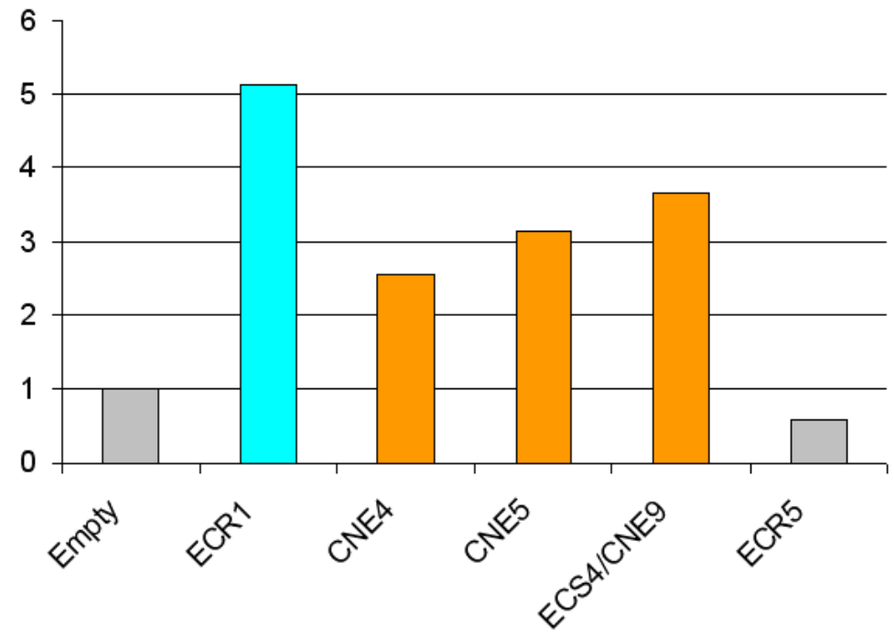
*LINE (L1P12)/no repetitive sequence*

**Recurrent deletion – NHEJ/unknown mechanism**

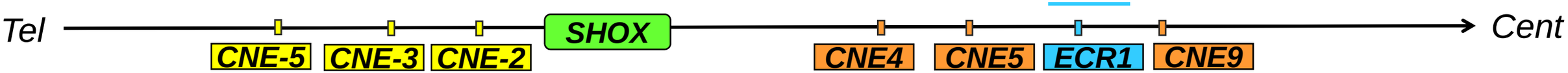
## Chromosome Conformation Capture (3C) analysis of ECRs in Chicken Limb Embryos




## Luciferase assays in U2OS cells




***Recurrent ~47.5 kb enhancer deletion***

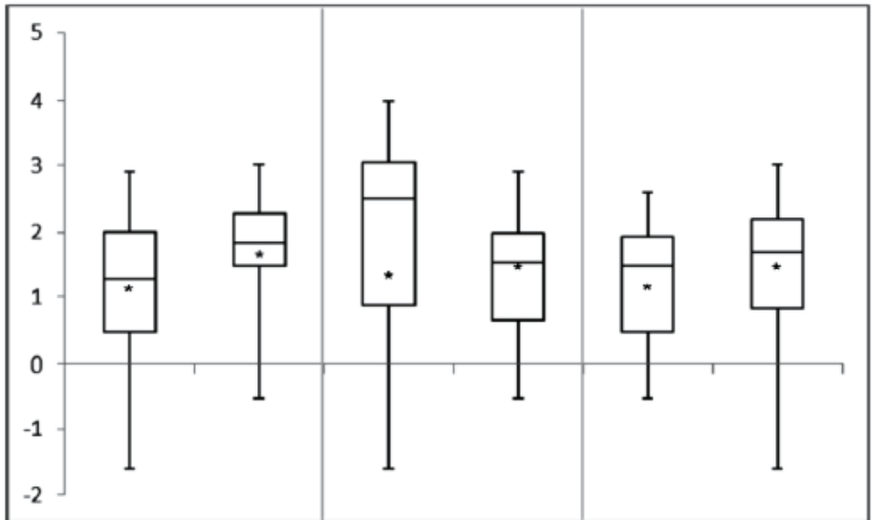



**Identification of 4th downstream *SHOX* enhancer – ECR1**

TABLE IV. The 17 Patients With a 47.5 kb Deletion 160 kb 3′ of the *SHOX* Gene, Showing the Genotype (Mut, mutation; PM, point mutation) and Phenotype (Phe, Phenotype; Hgt, Height in cm; SD, Standard Deviation of Height From the National Mean; Meso, Mesomelia; Mad, Madelung Deformity; Bilat, Bilateral) of the Proband and Both Parents Where Available, Plus the Country of Referral for All Probands

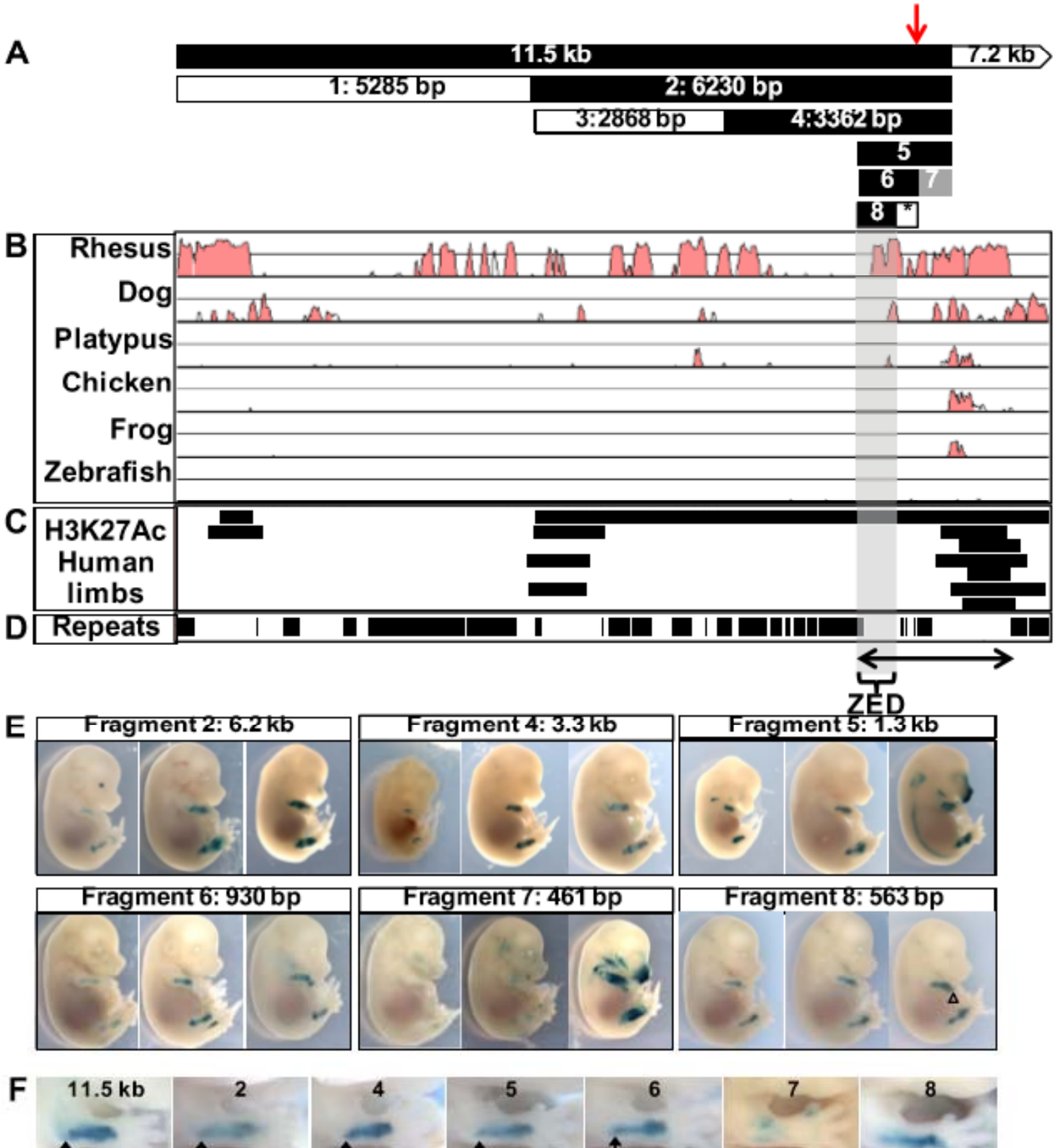
| | | | Proband | | | | | | | Mother | | | | | | Father | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fam | Origin | Mut2 | Age | Sex | Phe | Hgt | SD | Meso | Mad | Mut | Phe | Hgt | SD | Meso | Mad | Mut | Phe | Hgt | SD | Meso | Mad |
| 33 | England | — | 18+ | F | LWD | 150 | −2.3 | Mild | Bilat | None | N | 157 | −1 | No | No | Δ47.5 | N | 170 | −1 | No | Mild |
| 52 | England | — | 15 | F | LWD | | | Yes | Bilat | Δ47.5 | SS | | | No | No | None | N | | | No | No |
| 95 | England | — | 18+ | F | LWD | 162 | −0.5 | No | Bilat | None | N | 152 | −2 | No | No | Δ47.5 | N | 188 | +1.6 | No | No |
| 117 | England | — | 18+ | M | LWD | 160 | −2.3 | Yes | Bilat | None | N | 160 | −0.7 | No | No | Δ47.5 | N | 178 | +0.4 | No | No |
| 141 | England | — | 12 | M | SS | 115 | −3 | No | No | Δ47.5 | SS | 152 | −2 | No | No | None | N | 175 | −0.3 | No | No |
| 158 | England | — | 18+ | M | LWD | | | Yes | Bilat | | | | | | | | | | | | |
| 171 | Norway | — | 18+ | F | LWD | | | Yes | Bilat | Δ47.5 | LWD | | | Mild | Mild | None | N | | | No | No |
| 193 | England | — | 18+ | F | LWD | | | Yes | Bilat | | | | | | | | | | | | |
| 239 | England | — | 18+ | F | LWD | | | Yes | Bilat | | | | | | | | | | | | |
| 292 | England | — | 11 | F | LWD | | | Yes | Mild | Δ47.5 | LWD | 150 | −2.1 | Yes | Bilat | None | N | | | No | No |
| 301 | England | — | 12 | F | LWD | 124 | −3 | Yes | Mild | Δ47.5 | LWD | 148 | −2.3 | Yes | No | None | N | 176 | −0.3 | No | No |
| 312 | England | — | 13 | F | LWD | | | Yes | Bilat | None | N | | | No | No | Δ47.5 | LWD | 180 | +0.6 | Mild | Mild |
| 349 | England | — | 18+ | F | LWD | | | Yes | Mild | Δ47.5 | N | | | No | No | None | N | | | No | No |
| 311 | England | Dup gene | 14 | F | LWD | | | Yes | Bilat | **Dup** | **N** | | | **No** | **No** | Δ47.5 | N | | | No | No |
| 91 | Denmark | PM | 18+ | M | LWD | 165 | −2.3 | Yes | Bilat | Δ47.5 | N | 155 | −2.3 | No | No | **PM** | **LWD** | **185** | **+0.3** | **Mild** | **Bilat** |
| 181 | England | Del gene | 4 | F | LWD | | | Yes | Bilat | Δ47.5 | N | | | No | No | **Del** | **LWD** | | | **Yes** | **Bilat** |
| 222 | Scotland | 3′del | 18+ | F | LWD | 153 | −1.5 | Yes | Bilat | Δ47.5 | SS | 157 | −1 | No | No | **Del 3′** | **LWD** | **164** | **−1.8** | **Yes** | **Mild** |

Pare… s are shown in bold.




Bunyan et al, 2013; Am J Med Genet 161A(6):1329-38

# Sequence of the recurrent 47.5 kb deletion

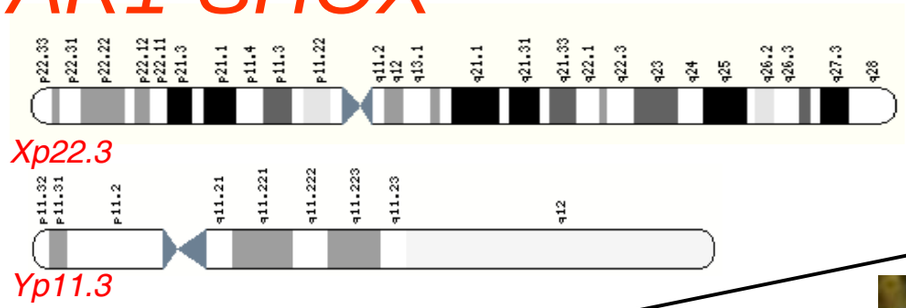


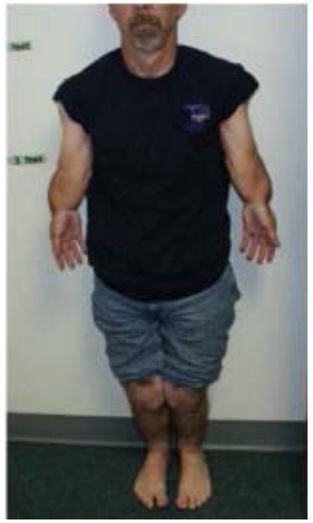

ZED – 563 bp enhancer

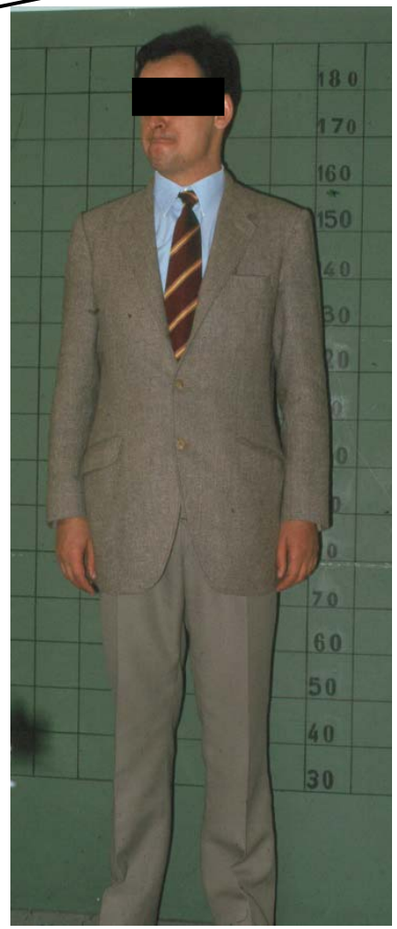
No mutation has been identified in this region in 126 possible LWD with no *SHOX* defect.

But virtually all enhancer deletions include the ZED enhancer, ie not only the recurrent 47.5 kb deletion.

# PAR1-SHOX





(-9 to 6.2 SD) (-4.6 to +0.6 SD) (< -2 SD) (-2 to +2 SD) (>+2 SD)

*Langer mesomelic dysplasia (LMD)*

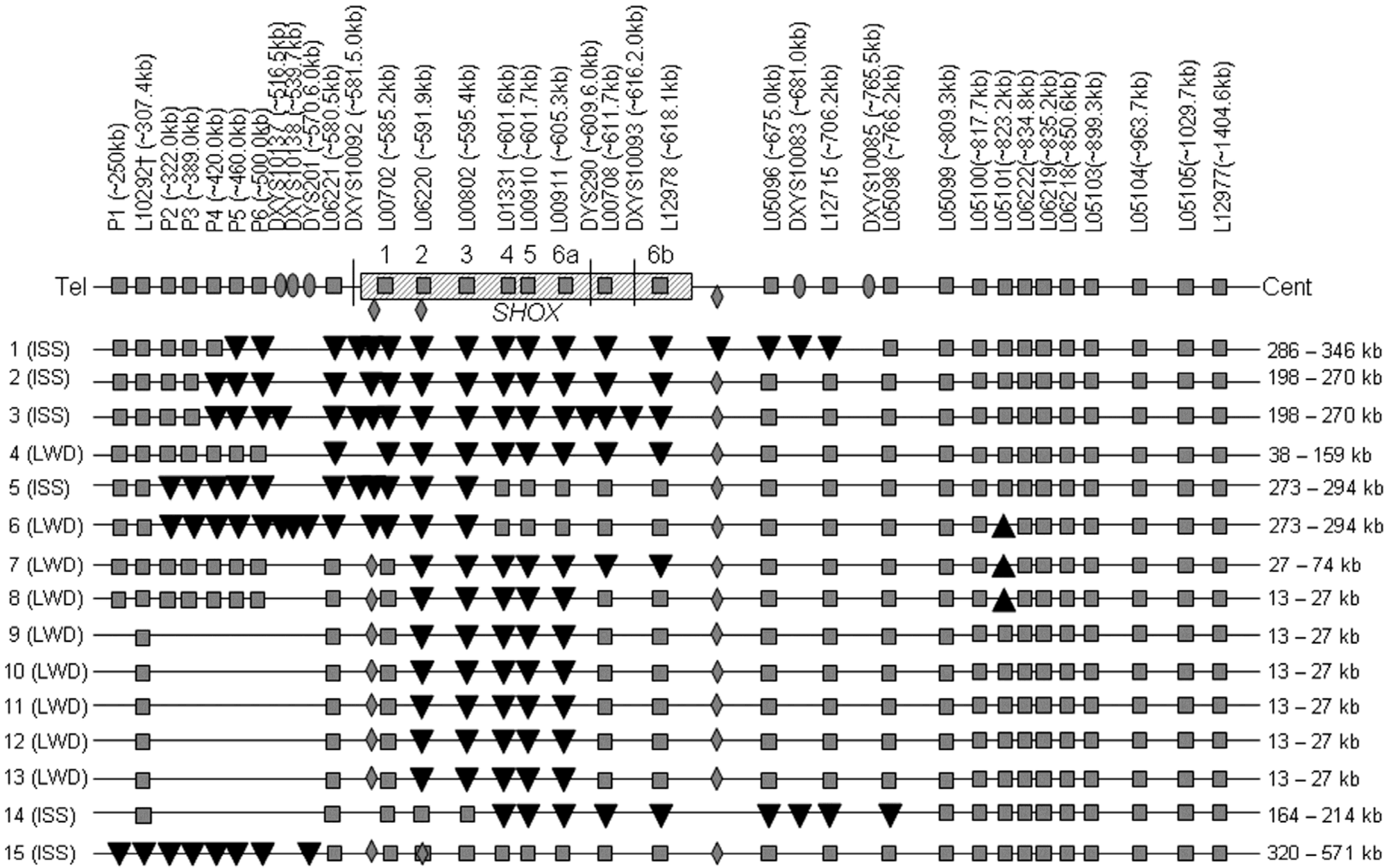
*Léri-Weill dyschondrosteosis (LWD)*

*Idiopathic short stature (ISS)*

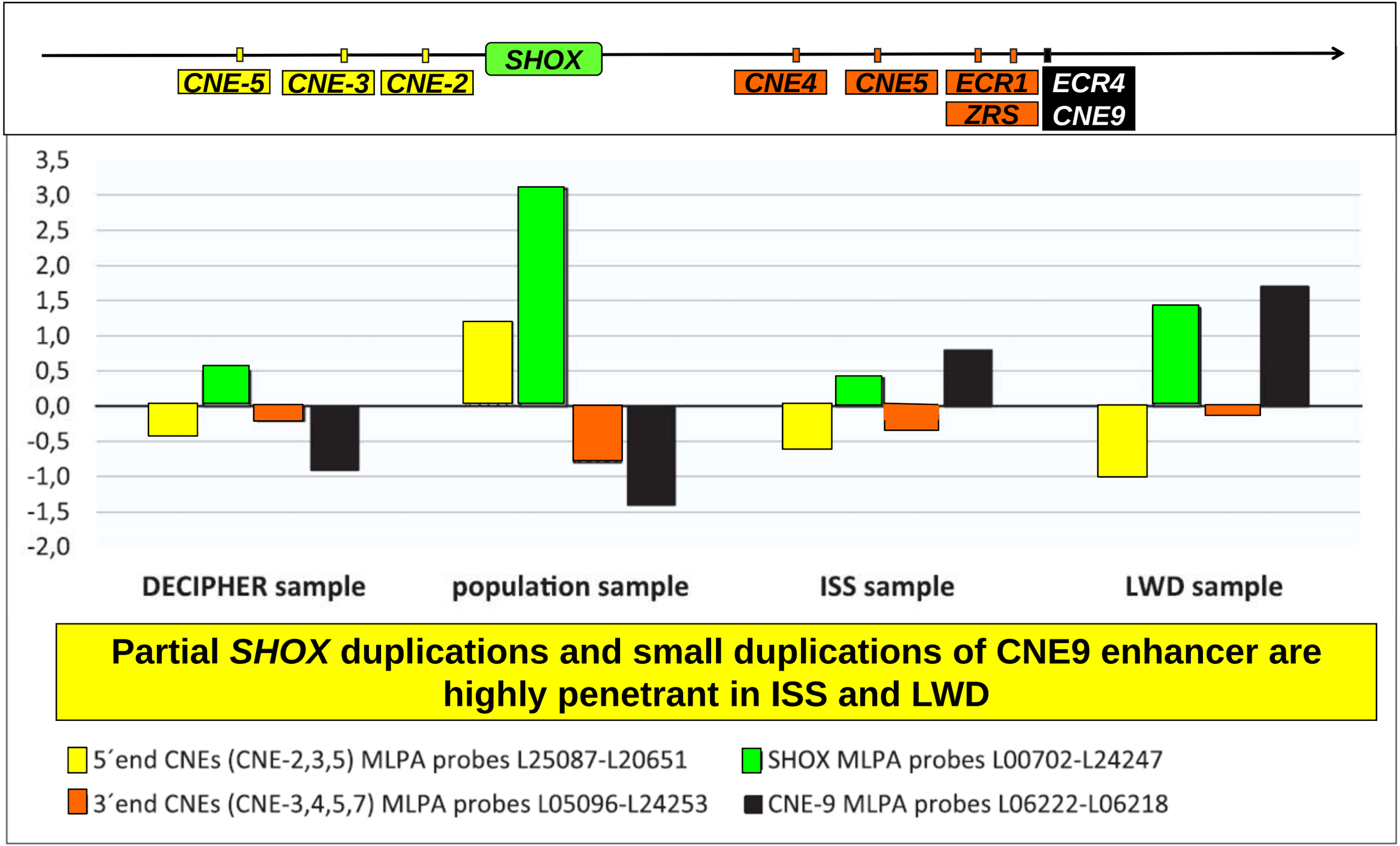
*Turner syndrome*

*Normal stature*

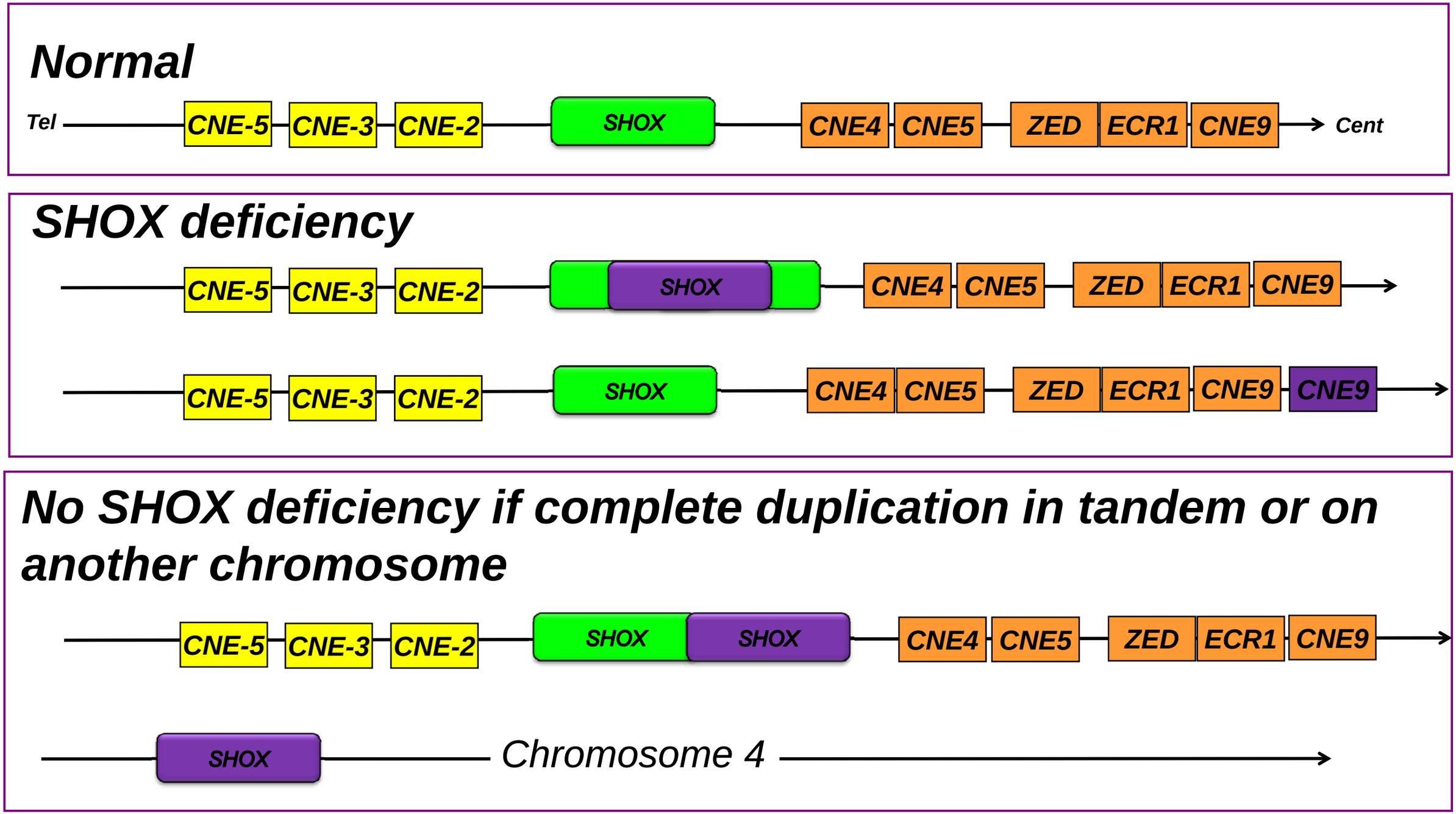
*Trisomy X*
*Klinefelter syndrome*
*XYY syndrome….*


P1 (~250kb)
L10292† (~307.4kb)
P2 (~322.0kb)
P3 (~389.0kb)
P4 (~420.0kb)
P5 (~460.0kb)
P6 (~500.0kb)
DXYS10137 (~516.5kb)
DXYS10138 (~539.7kb)
DYS201 (~570.0kb)
L06221 (~580.5kb)
DXYS10092 (~581.5.0kb)
L00702 (~585.2kb)
L06220 (~591.9kb)
L00802 (~595.4kb)
L01331 (~601.6kb)
L00910 (~601.7kb)
L00911 (~605.3kb)
DYS290 (~609.6.0kb)
L00708 (~611.7kb)
DXYS10093 (~616.2.0kb)
L12978 (~618.1kb)
L05096 (~675.0kb)
DXYS10083 (~681.0kb)
L12715 (~706.2kb)
DXYS10085 (~765.5kb)
L05098 (~766.2kb)
L05099 (~809.3kb)
L05100(~817.7kb)
L05101(~823.2kb)
L06222(~834.8kb)
L06219(~835.2kb)
L06218(~850.6kb)
L05103(~899.3kb)
L05104(~963.7kb)
L05105(~1029.7kb)
L12977(~1404.6kb)
1
2
3
4
5
6a
6b
Tel
Cent
SHOX
1 (ISS)
286 – 346 kb
2 (ISS)
198 – 270 kb
3 (ISS)
198 – 270 kb
4 (LWD)
38 – 159 kb
5 (ISS)
273 – 294 kb
6 (LWD)
273 – 294 kb
7 (LWD)
27 – 74 kb
8 (LWD)
13 – 27 kb
9 (LWD)
13 – 27 kb
10 (LWD)
13 – 27 kb
11 (LWD)
13 – 27 kb
12 (LWD)
13 – 27 kb
13 (LWD)
13 – 27 kb
14 (ISS)
164 – 214 kb
15 (ISS)
320 – 571 kb

***(NCBI37/hg19)***


SHOX
CNE-5
CNE-3
CNE-2
CNE4
CNE5
ECR1
ZRS
ECR4
CNE9
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
-1,5
-2,0
DECIPHER sample
population sample
ISS sample
LWD sample
Partial SHOX duplications and small duplications of CNE9 enhancer are highly penetrant in ISS and LWD
5´end CNEs (CNE-2,3,5) MLPA probes L25087-L20651
SHOX MLPA probes L00702-L24247
3´end CNEs (CNE-3,4,5,7) MLPA probes L05096-L24253
CNE-9 MLPA probes L06222-L06218

# and enhancer duplications



# Pseudoautosomal region 1 (PAR1)

## Upstream enhancer duplication

Benito-Sanz et al, J Clin Endocrinol Metab 2011
Verdin et al, Sci Rep 2015

## Downstream enhancer deletions (Region 2)

Benito-Sanz et al, Am J Hum Genet 2005

## Recurrent ~47.5 kb enhancer deletion

Benito-Sanz et al, J Med Genet: 2012

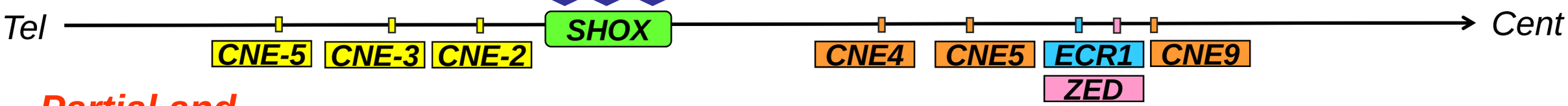
## Upstream enhancer deletions (Region 3)

Benito-Sanz et al, Eur J Hum Genet 2012
Verdin et al, Sci Rep 2015

## Mutations

## Non-pathogenic CNVs

Benito-Sanz et al, Am J Med Genet 2011




## Partial and complete SHOX deletions

Benito-Sanz et al, Am J Hum Genet 2006
Benito-Sanz et al, J Hum Genet 2016

## Downstream enhancer duplication

Hirschfeldova et al, 2011

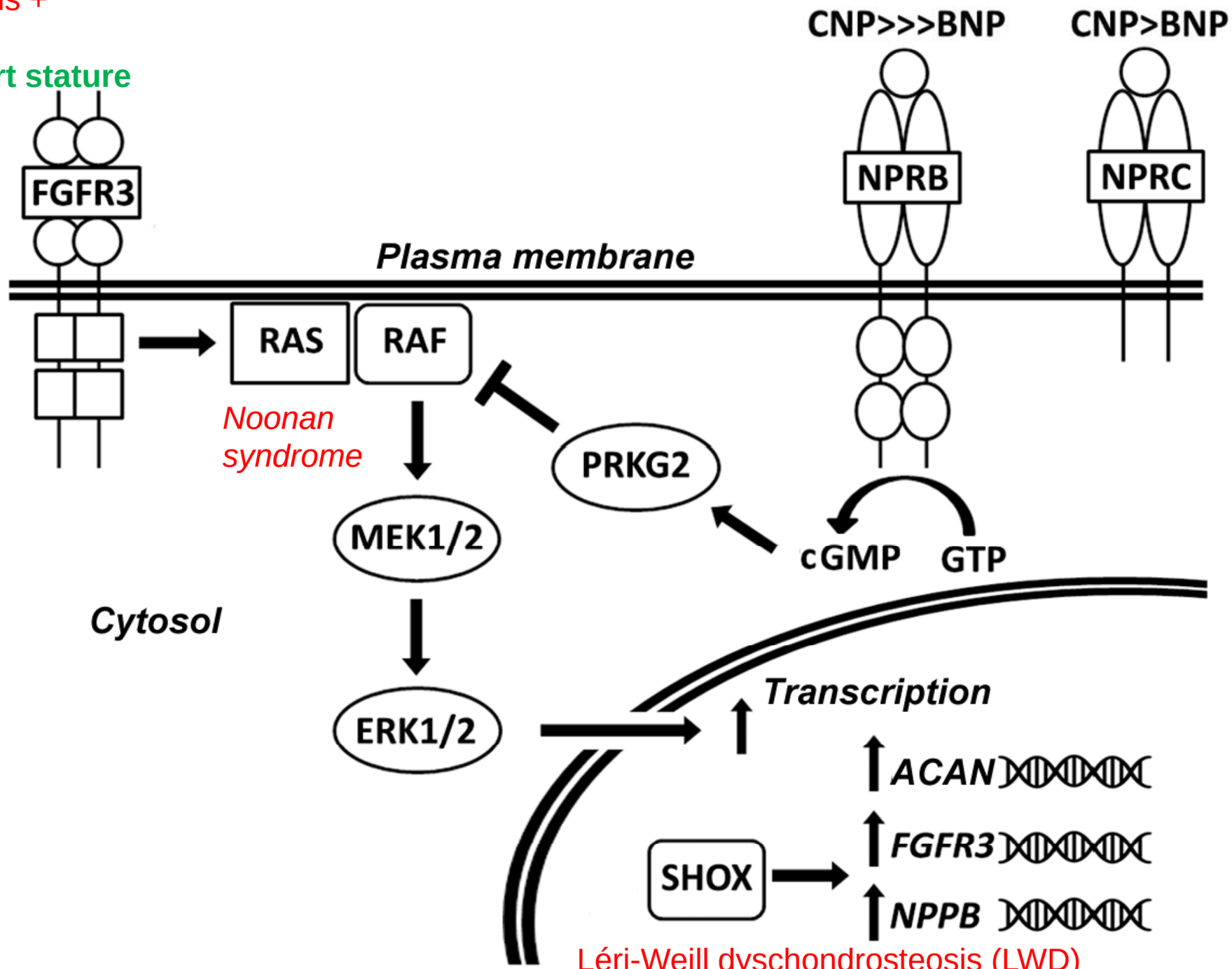
## Partial and complete SHOX duplications

Benito-Sanz et al, J Clin Endocrinol Metab 2011

## Region 4 Del/Dup

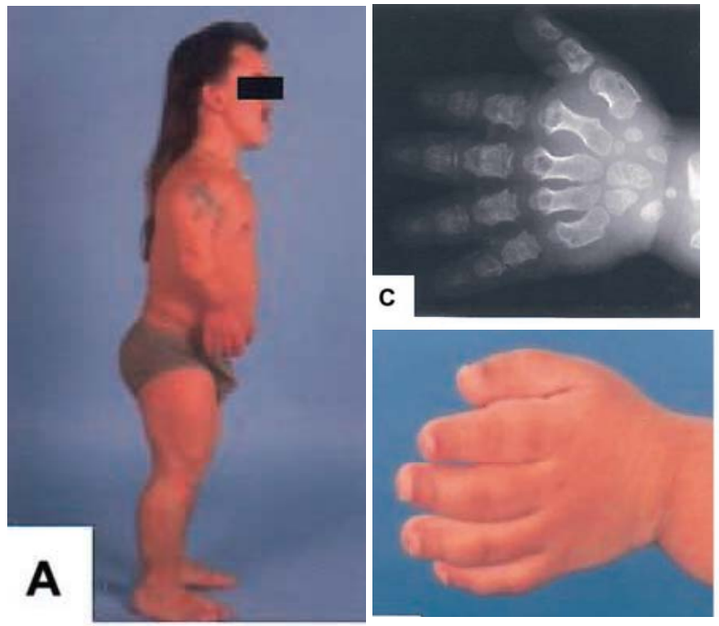
Tsuchiya et al, 2012
Bunyan et al, 2014
Benito-Sanz et al, unpub

Craniosynostosis +
others
**Idiopathic short stature**



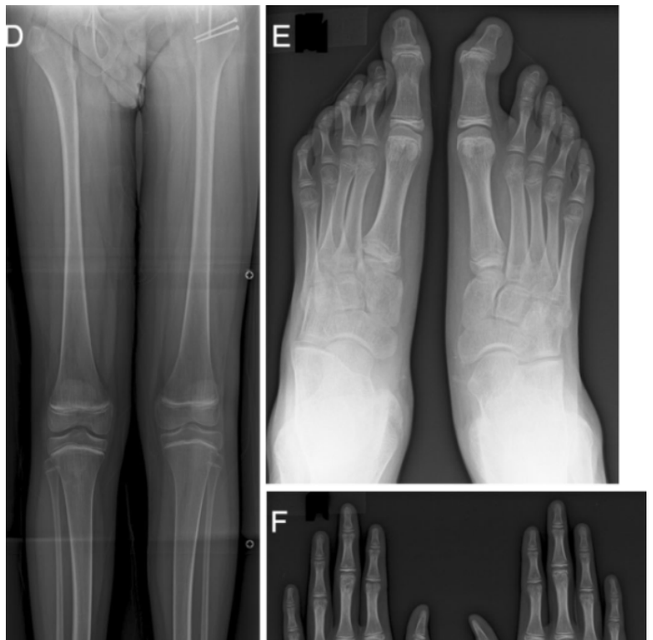

Léri-Weill dyschondrosteosis (LWD)
Langer mesomelic dysplasia (LMD)

~~NPR2~~
~~NPR2~~ → *Acromesomelic dysplasia, type Maroteaux (<5 SDS)* →

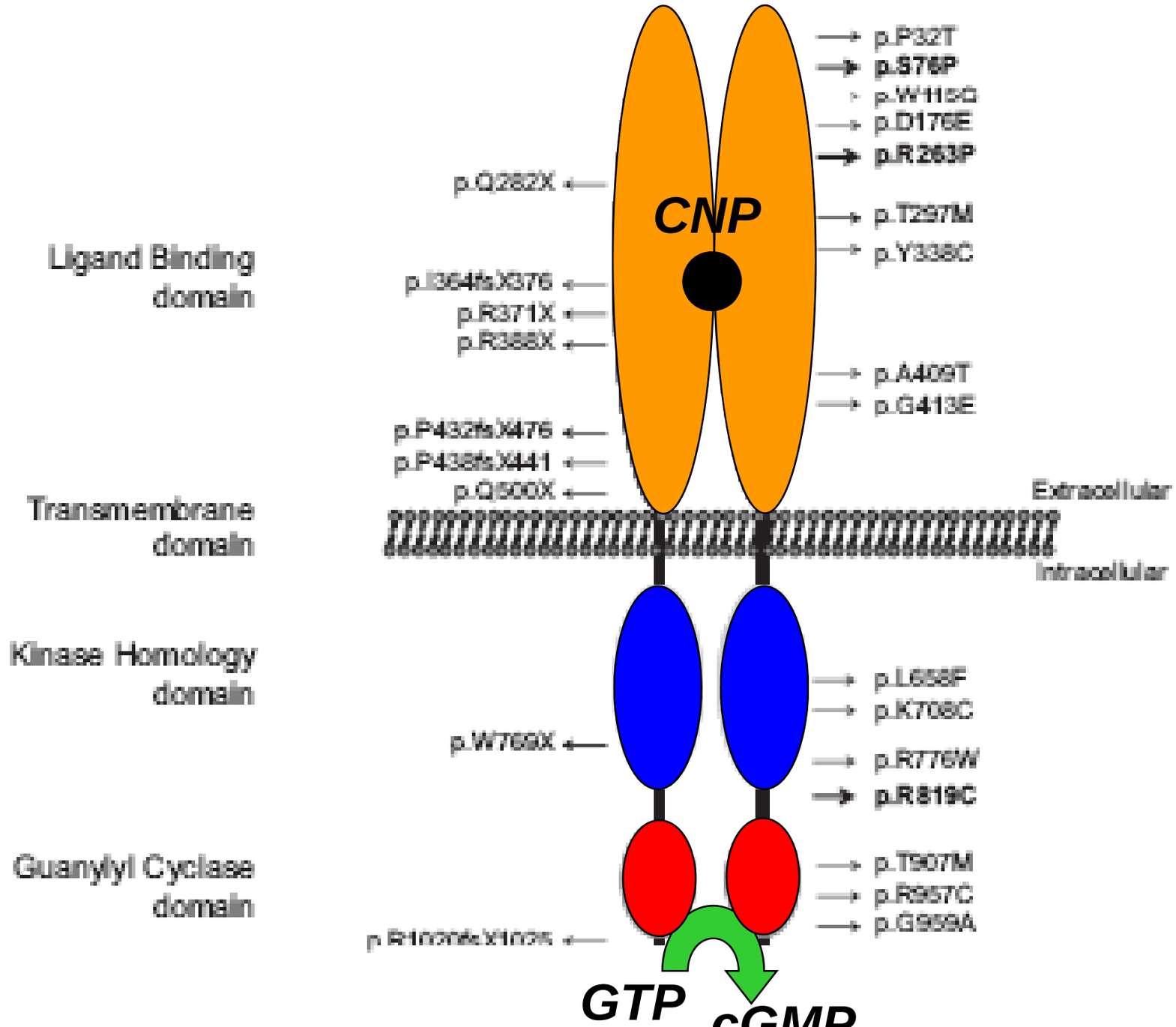



~~NPR2~~
NPR2 → ***3% Idiopathic short stature (ISS)***

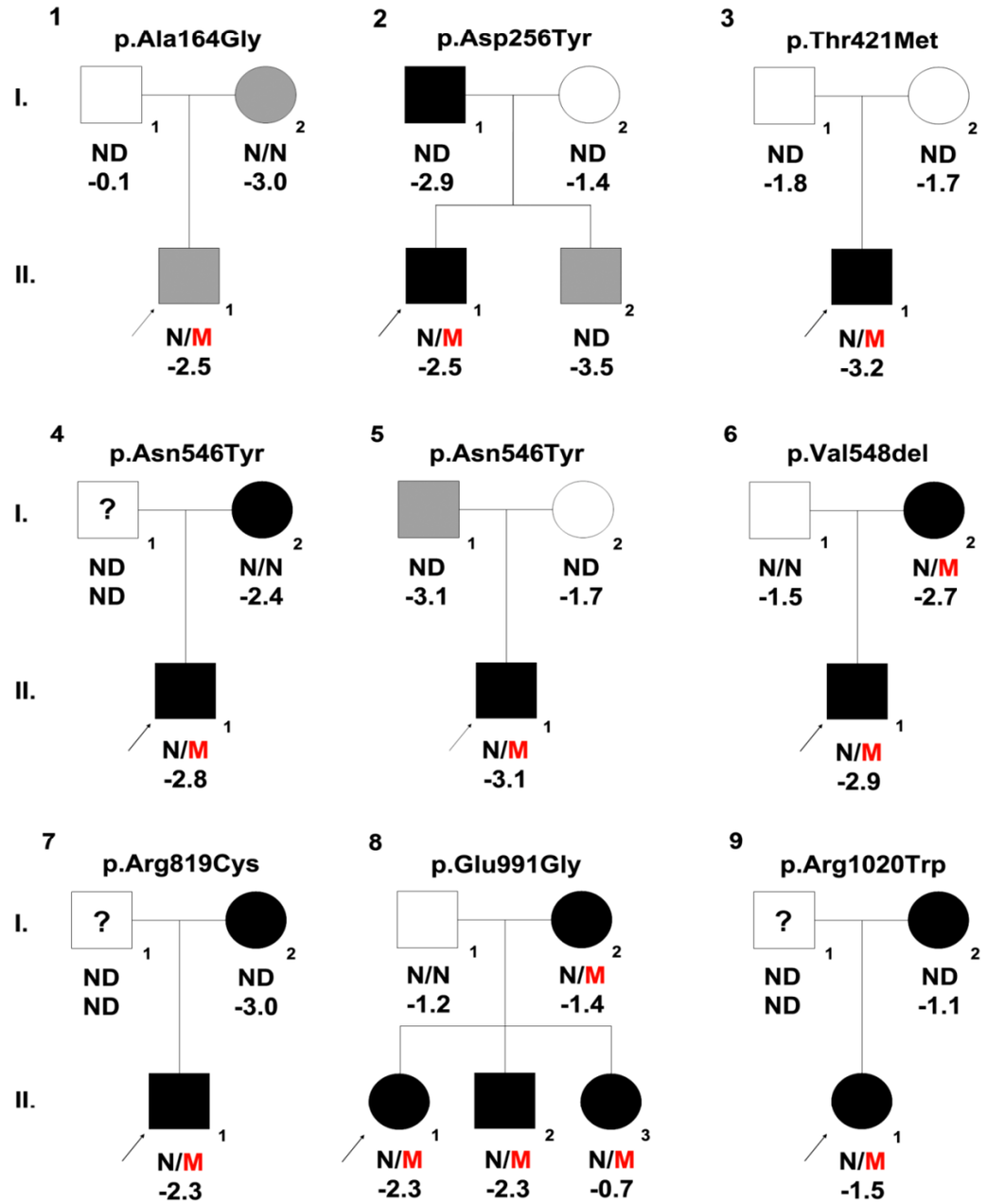
NPR2*
NPR2 → *Extremely tall stature with* →

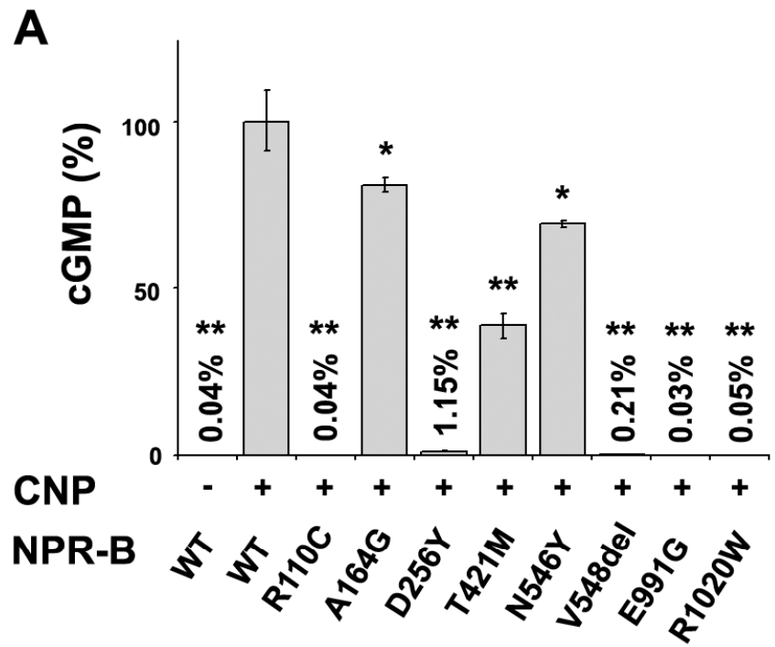


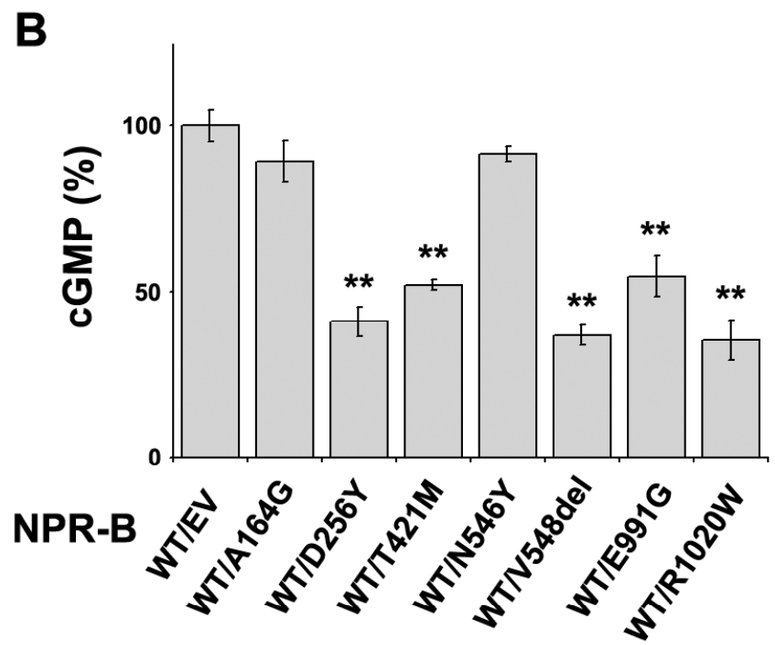
- Homodimer receptor.
- Union with its ligand, CNP causes a post-receptor signalling cascade.
- Expressed in cartilage, growth plate, brain, hypothalamus.



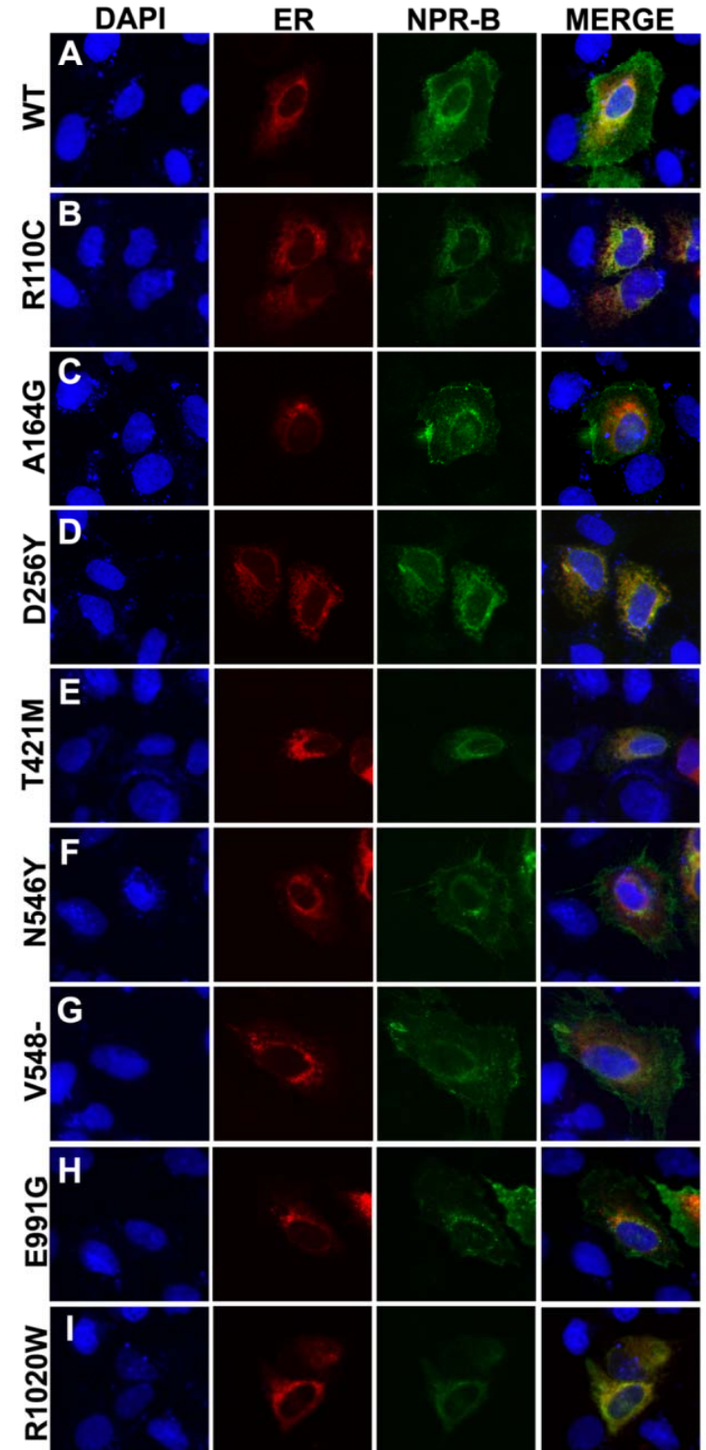
# Detected 8 variants in *NPR2* en 9 patients; 7 LWD and 2 ISS.






*Celular localization – ability to transport to ER*




*ER*

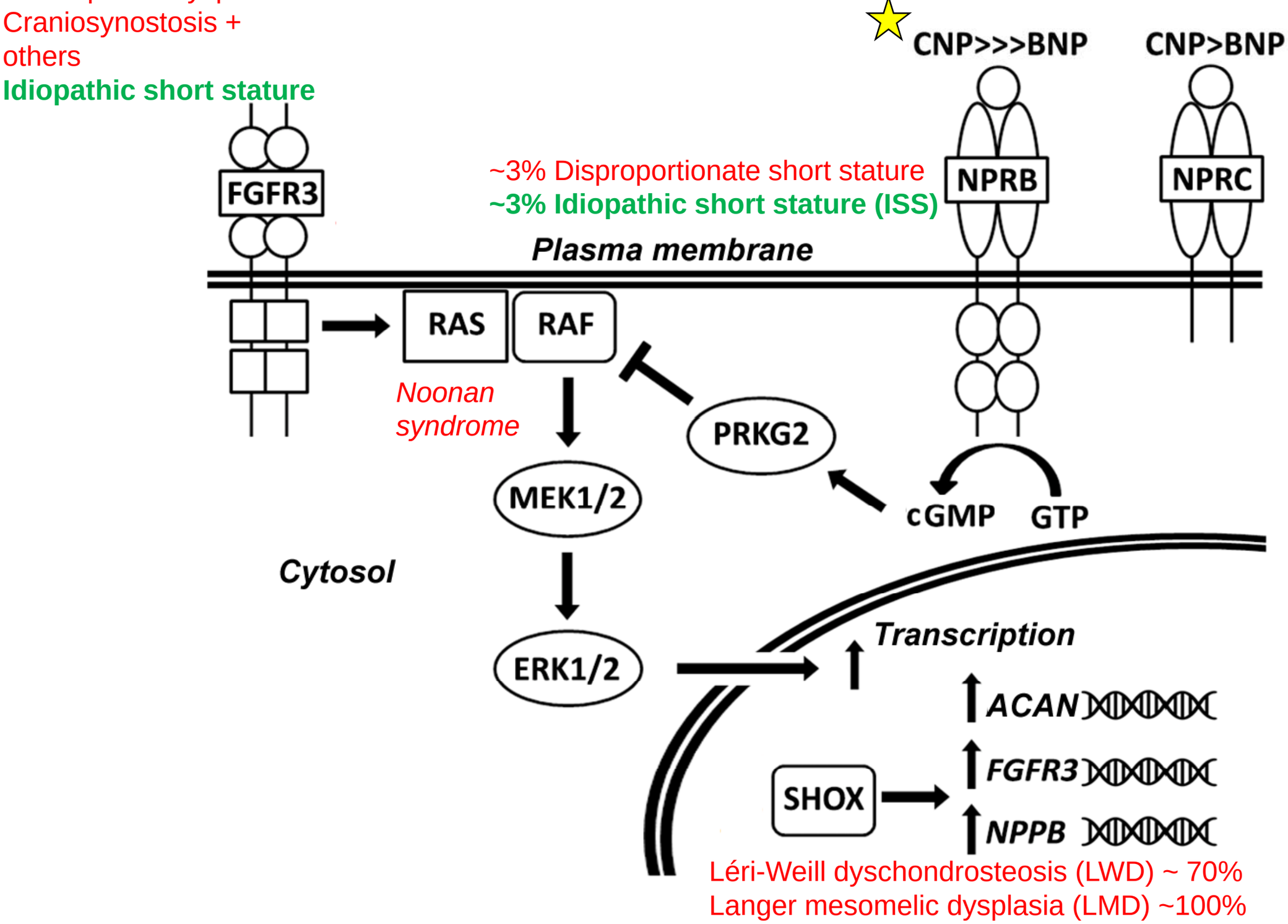
*ER*

*ER*

*ER*

# Heterozygous *NPR2* mutations

- Seven mutations were confirmed to be pathogenic.
- Approx 3% of suspected LWD cases had a pathogenic *NPR2* mutation. No ISS patient had a mutation.
- Similar phenotype to those referred for *SHOX* screening but without the Madelung deformity:
  - Short stature with limb shortening
  - Height:armspan < 0.965
  - Brachydactyly
  - Frontal bossing
  - Muscular hypertrophy
  - High-arched palate


Craniosynostosis +
others
Idiopathic short stature
CNP>>>BNP
CNP>BNP
FGFR3
NPRB
NPRC
~3% Disproportionate short stature
~3% Idiopathic short stature (ISS)
Plasma membrane
RAS
RAF
Noonan
syndrome
PRKG2
MEK1/2
cGMP
GTP
Cytosol
ERK1/2
Transcription
ACAN
FGFR3
SHOX
NPPB
Léri-Weill dyschondrosteosis (LWD) ~ 70%
Langer mesomelic dysplasia (LMD) ~100%

***Cohort:***

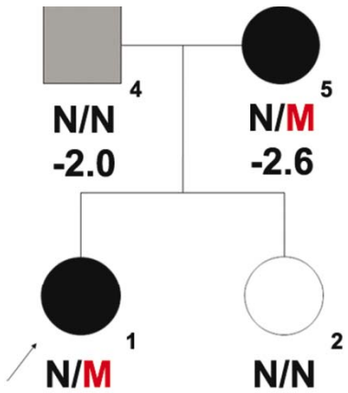
*697 patients:*

- *357 with disproportionate short stature and mild skeletal defects*
- *340 with ISS*

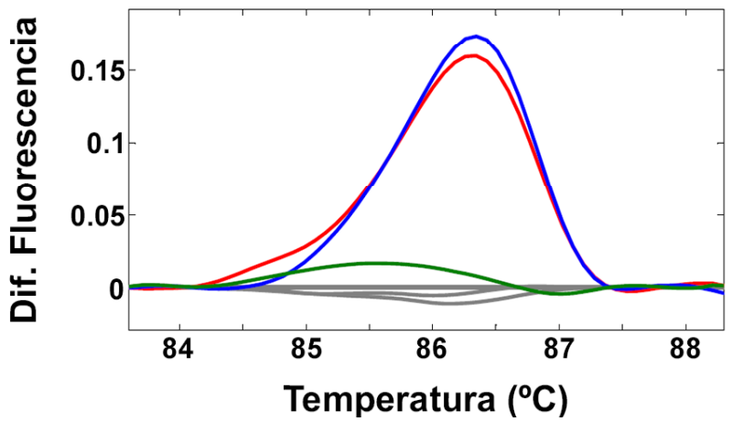
***Methods:***

- *Whole exome sequencing*
- *HRM & Sanger sequencing of candidate genes*

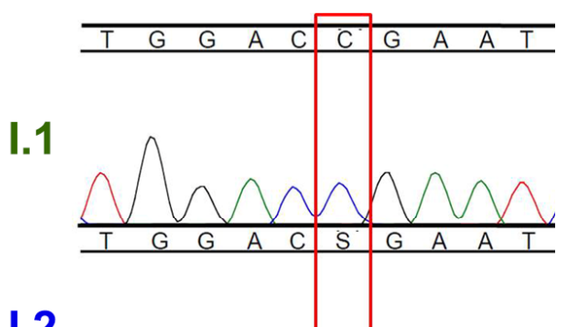
## Family 1: c.349C>G (p.Arg117Gly)

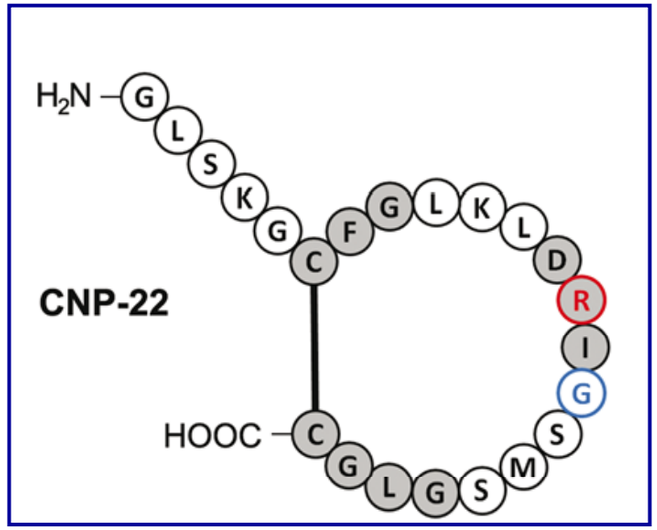



### HRM

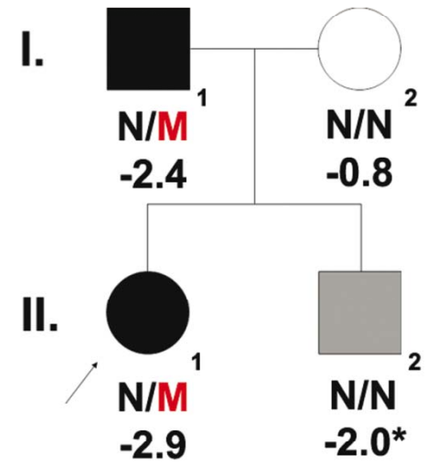



### Sanger




## Family 2: c.55G>T (p.Gly119Cys)




Variants in patient II.1 (SNVs and In/dels)
(n = 55,081)

↓

Located in exons or splice site
(n = 7,207)

↓

Low frequency in public database
MAF <0.001 (n = 963)

↓

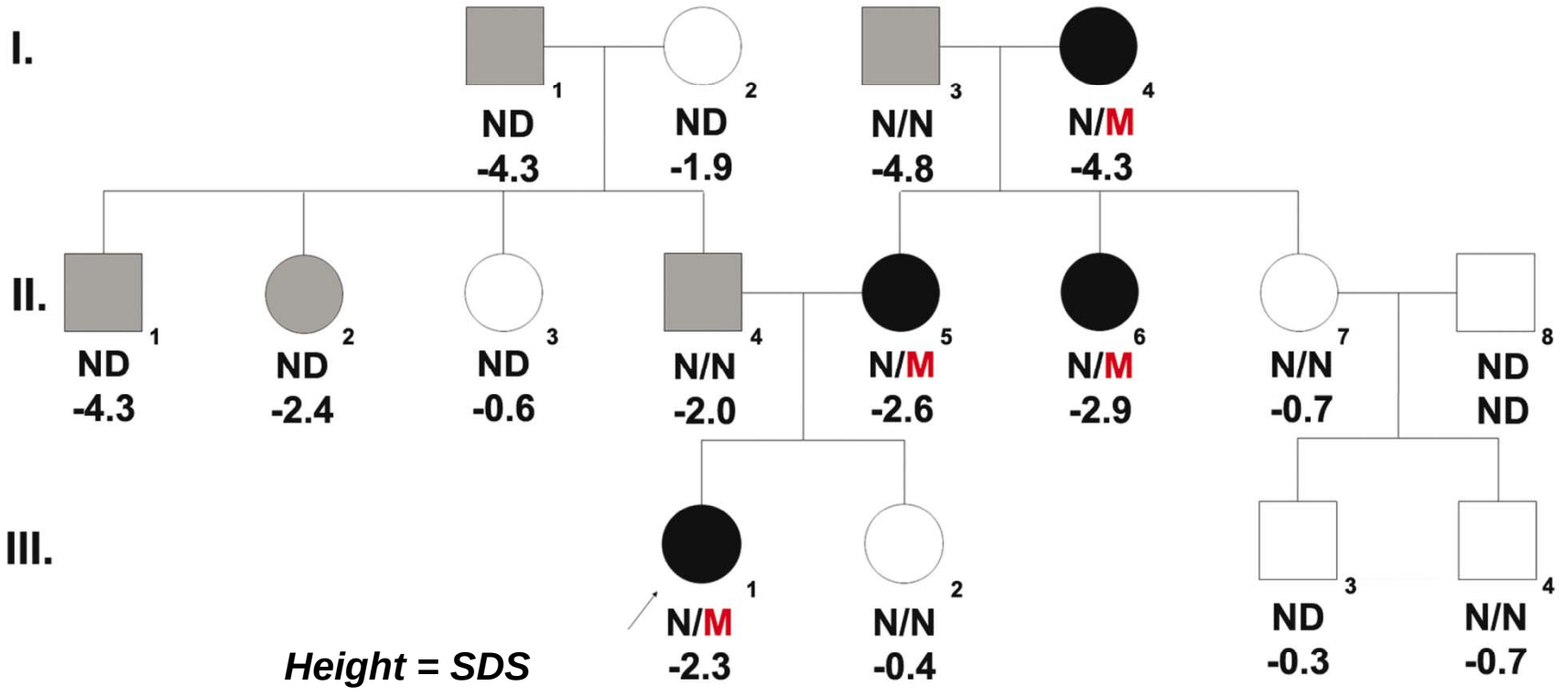
Absent in our internal database
(n = 350)

↓

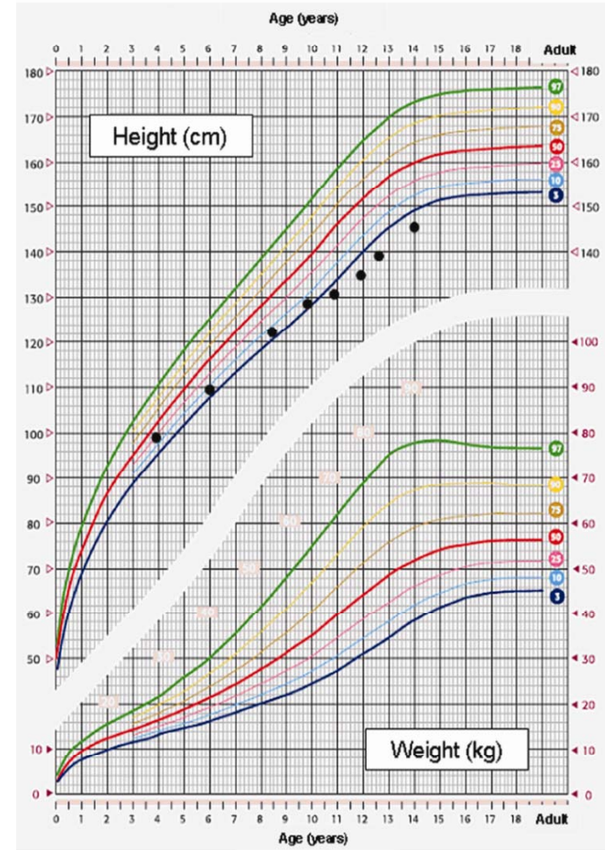
Absent in her brother (II.2) and
present in her father (I.1)
(n = 71)

↓

Predicted to be deleterious (nonsynonymous)
or to be loss of function
(n = 15)

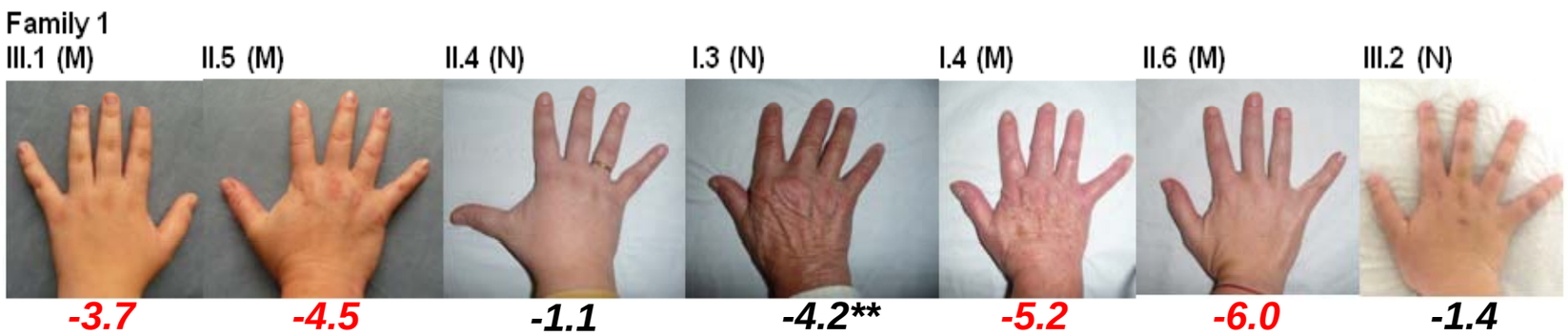


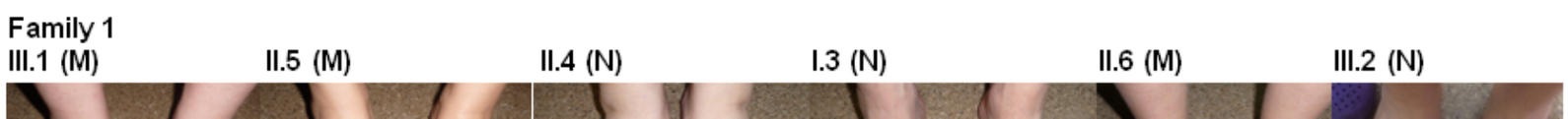

III.1



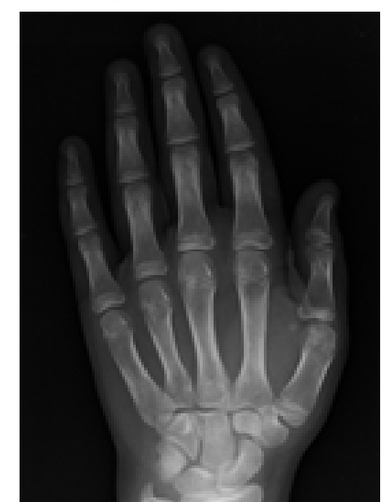

## Proportionate short stature and small hands

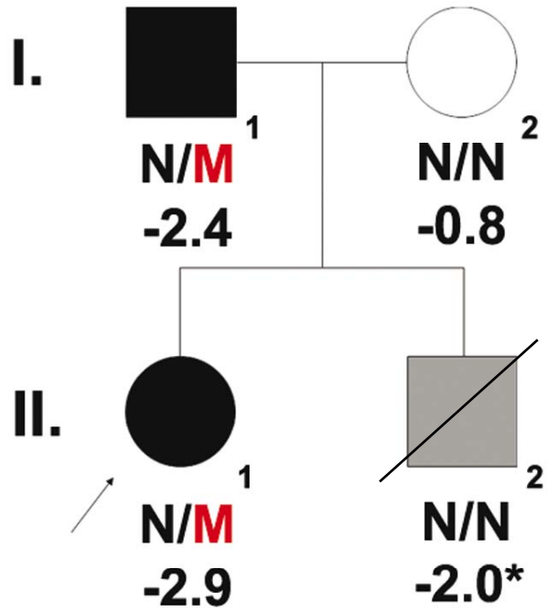
### Hand length (SDS)

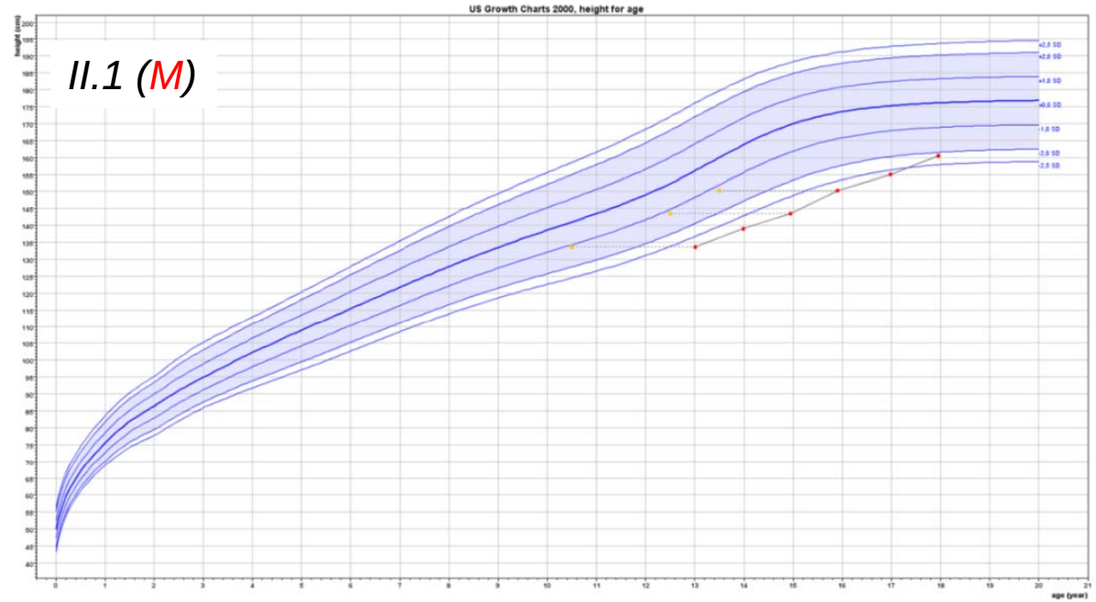


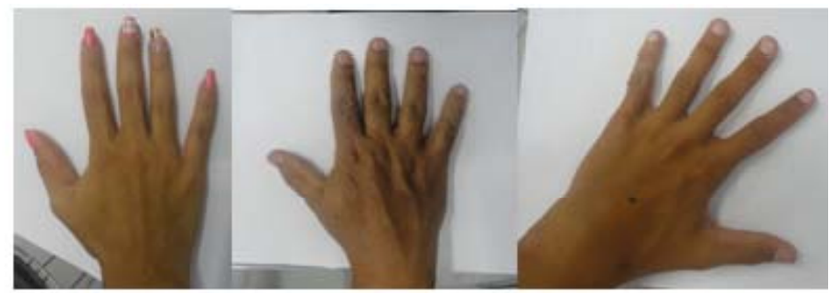

III.1 (M)





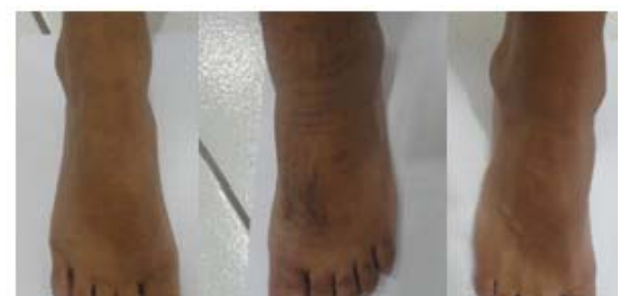




II.1 (M) I.1 (M) II.1 (N)



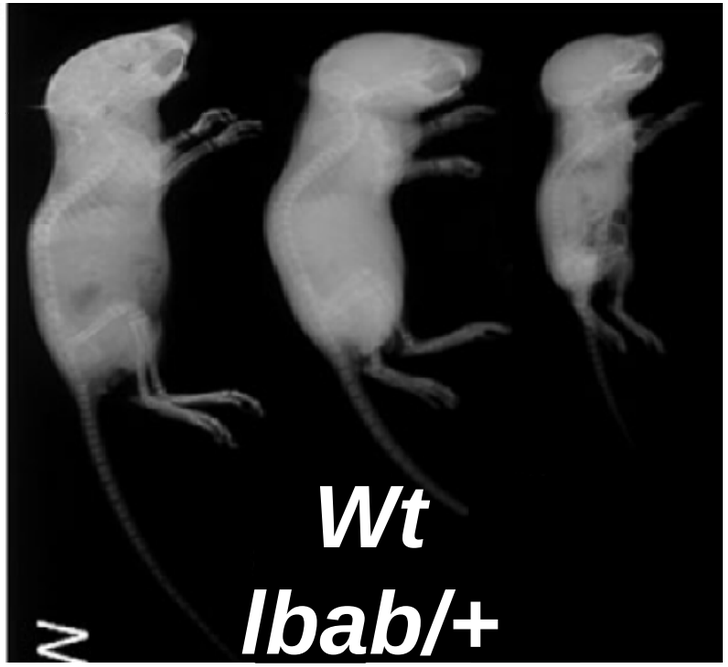
-2.1 -2.1 -1.3

II.1 (M) I.1 (M) II.1 (N)

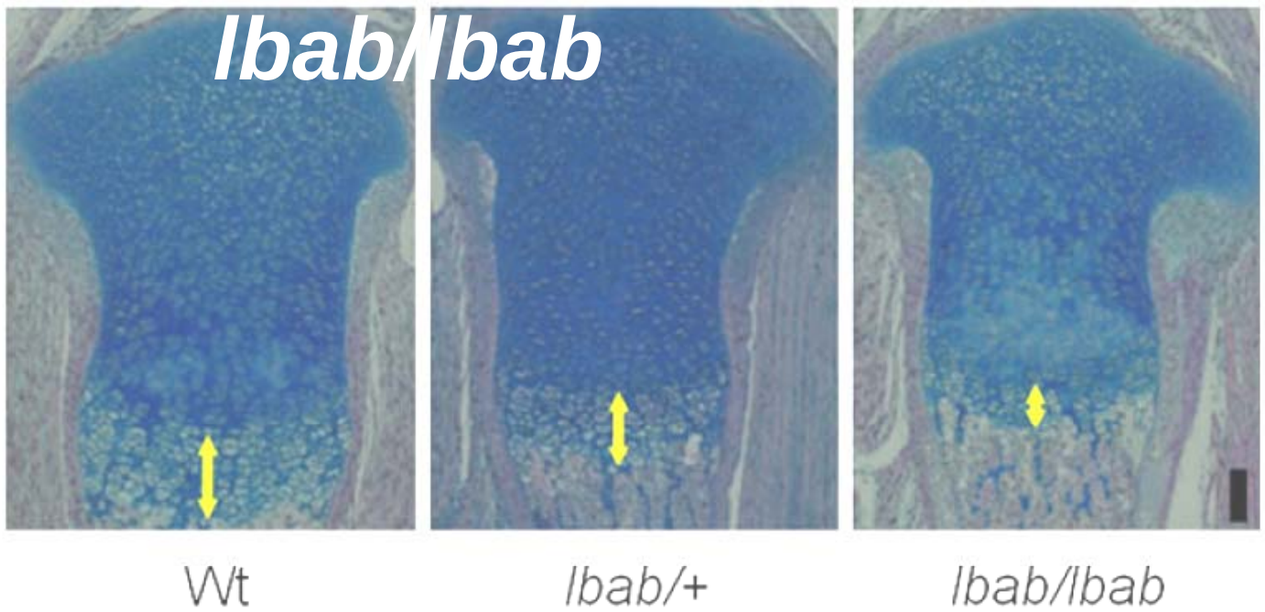


**Short stature and short hands**

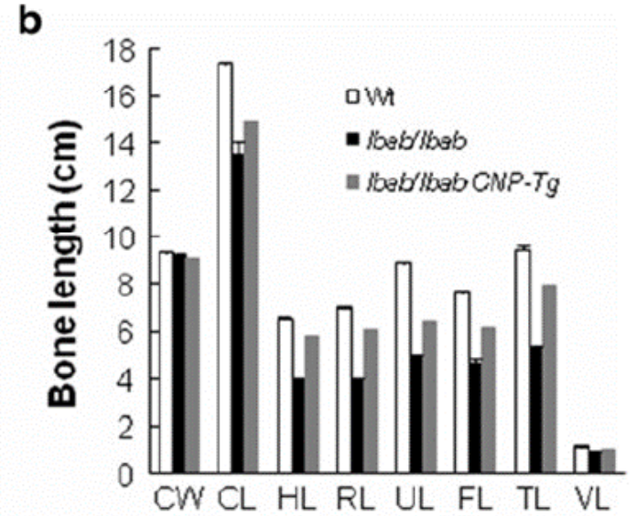
# abnormality" (lbab/lbab) with CNP p.Arg117Gly mutation



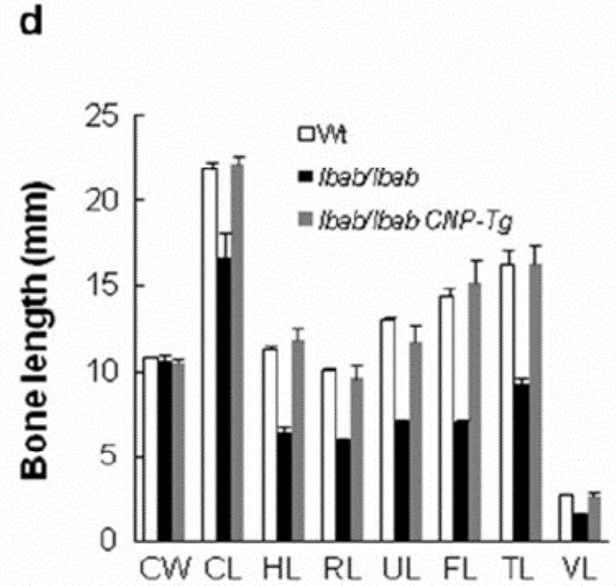

**lbab/lbab mice – 60% smaller than WT**




**Hmz and htz lbab mice have a reduction in the**

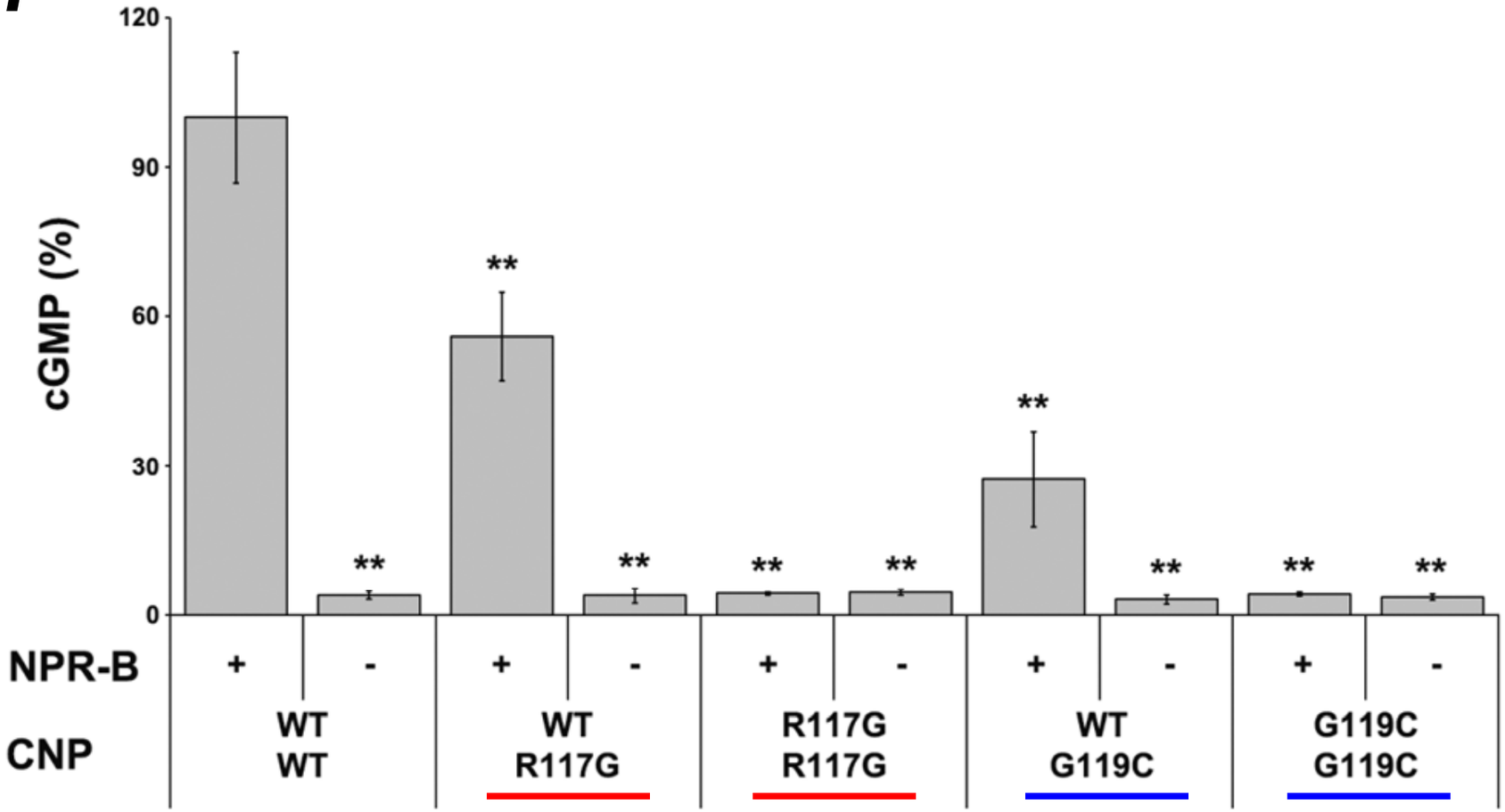


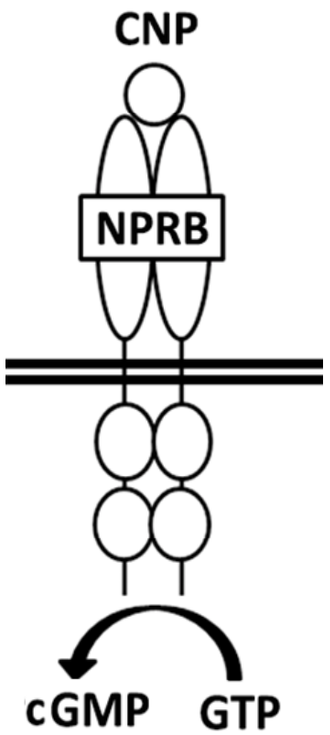

**CNP-22 rescues phenotype**



# *cGMP ELISA in COS7*

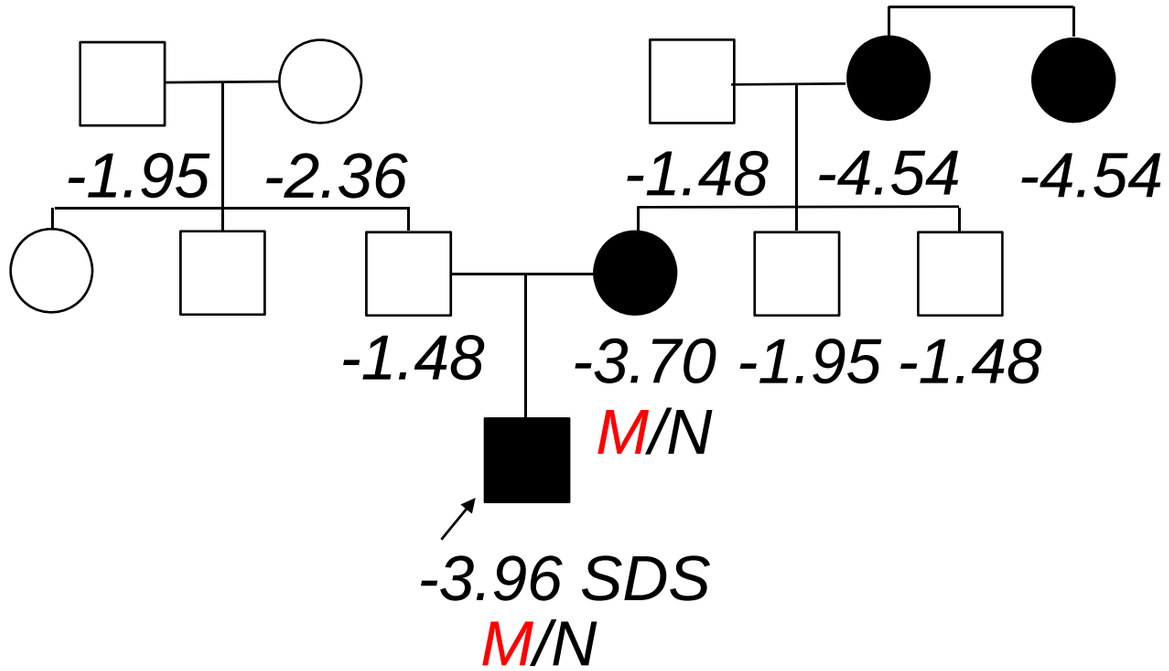
## *** = p<0.01*

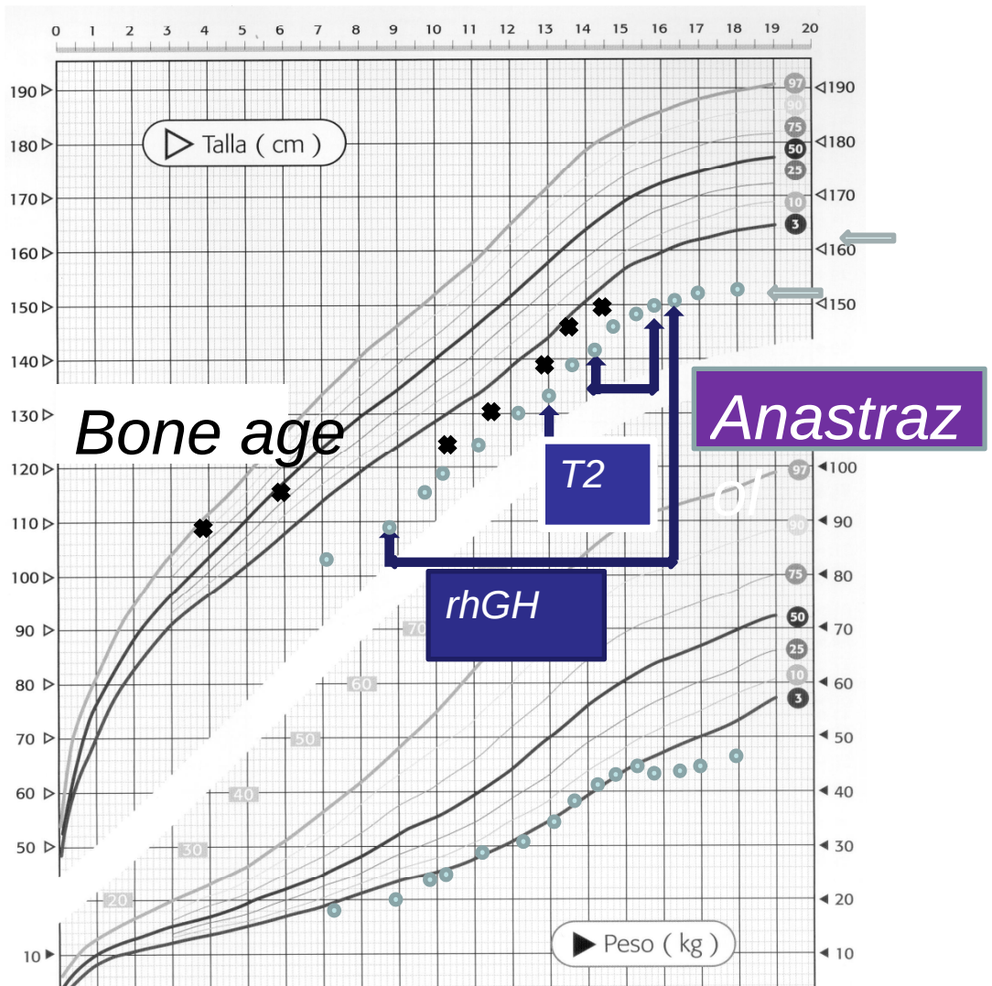


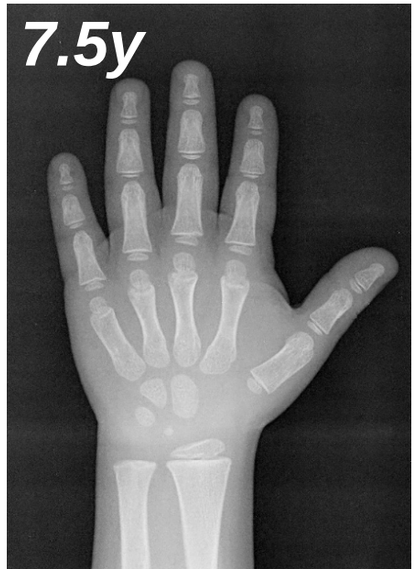



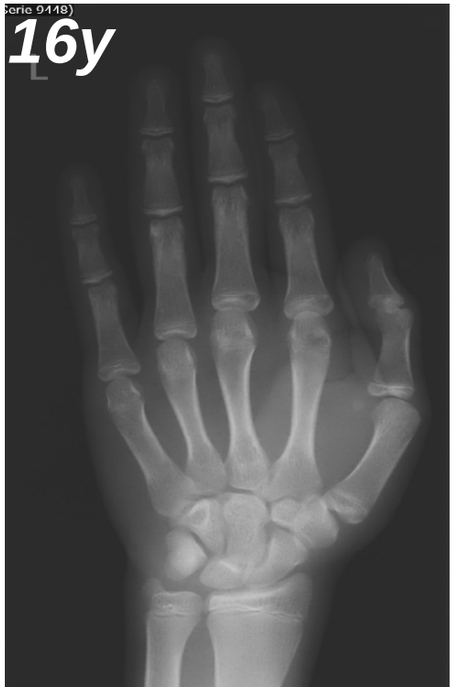

***Both CNP variants lose their capacity to synthesize cGMP in the homozygous state, and a reduction in the heterozygous state, therefore confirming their pathogenicity.***

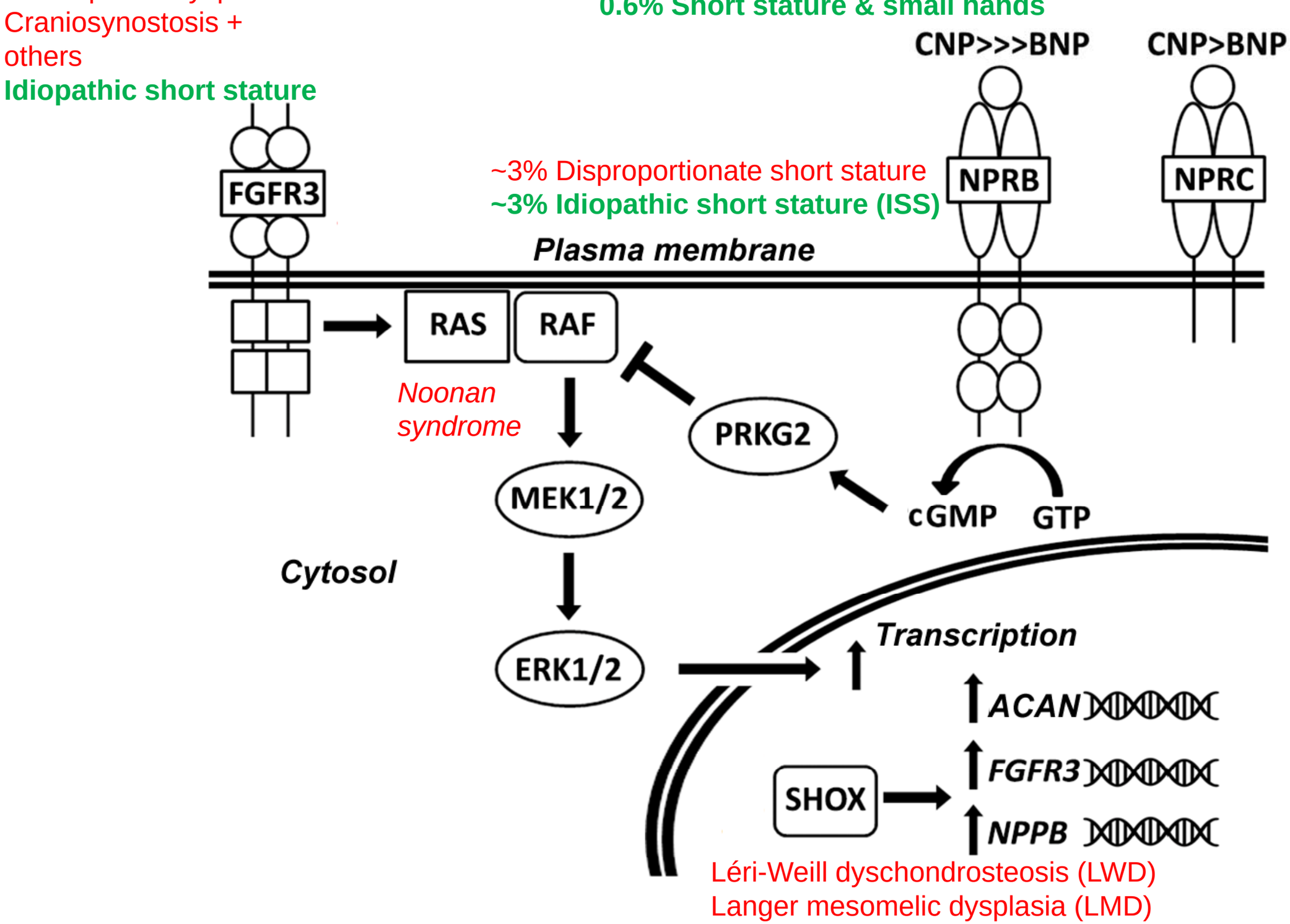
## Proportionate short stature and small hands



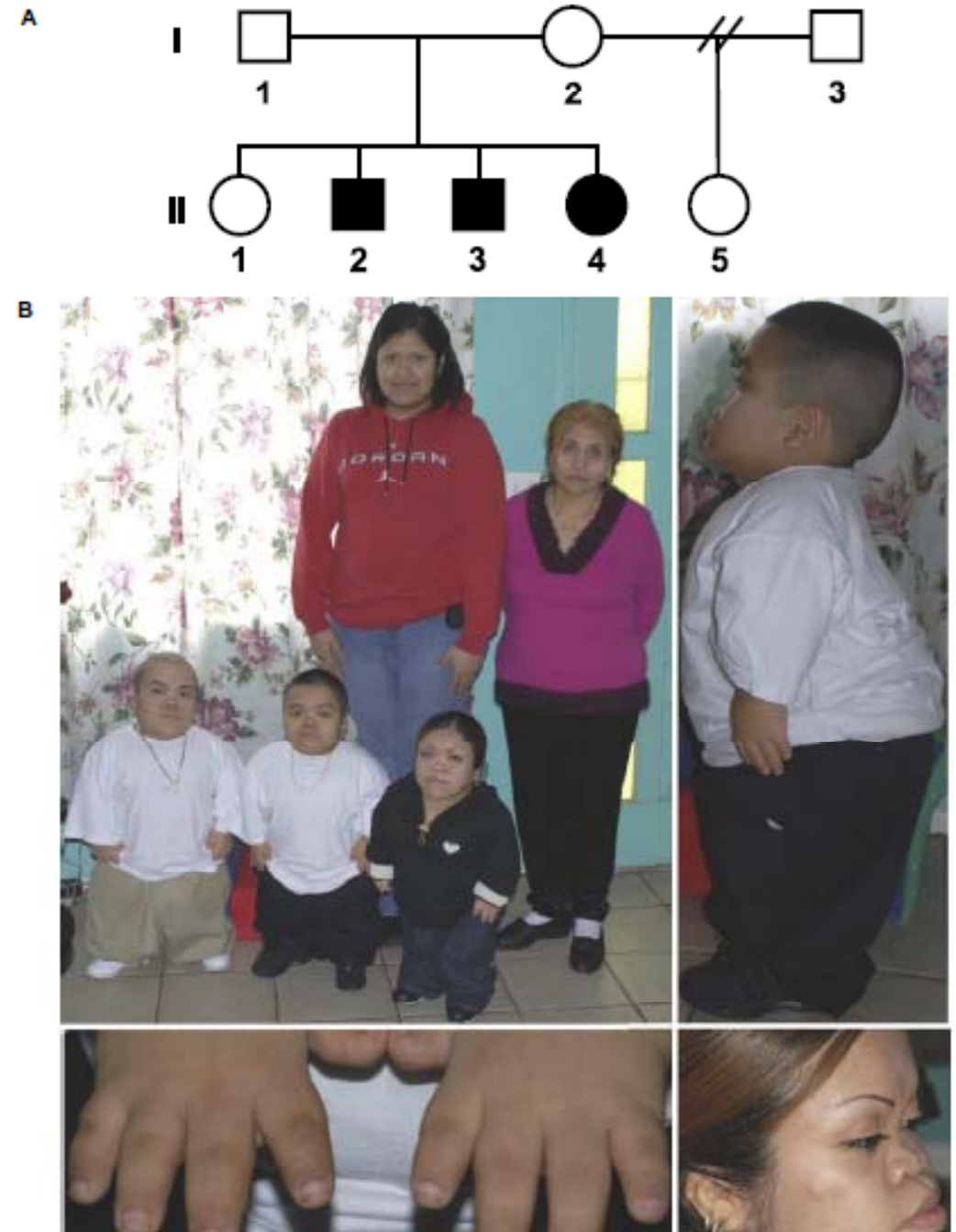



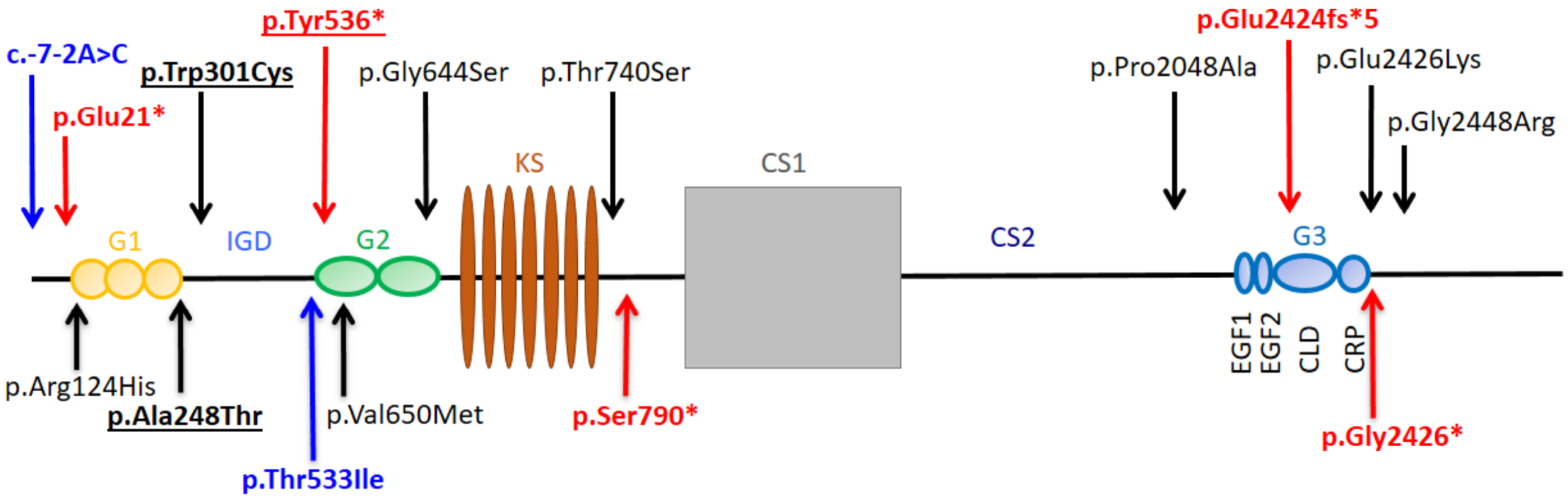

Craniosynostosis +
others
Idiopathic short stature
0.6% Short stature & small hands
CNP>>>BNP
CNP>BNP
FGFR3
~3% Disproportionate short stature
~3% Idiopathic short stature (ISS)
NPRB
NPRC
Plasma membrane
RAS
RAF
Noonan
syndrome
PRKG2
MEK1/2
cGMP
GTP
Cytosol
ERK1/2
Transcription
ACAN
FGFR3
SHOX
NPPB
Léri-Weill dyschondrosteosis (LWD)
Langer mesomelic dysplasia (LMD)

- Spondyloepiphyseal dysplasia, Kimberley type (SEDK) – AD
- Familial osteochondritis dissecans – AD
- Spondyloepimetaphyseal dysplasia, aggrecan type (SEMD) - AR




Tompson et al, 2004;

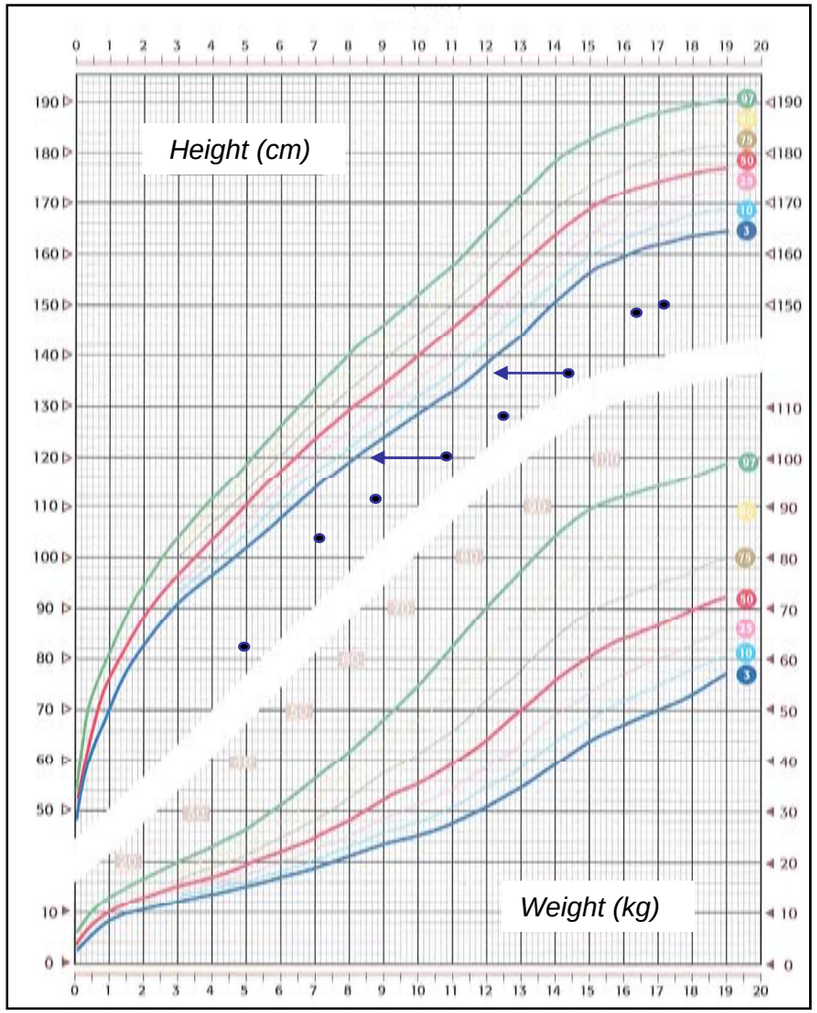
# heterozygous ACAN mutations



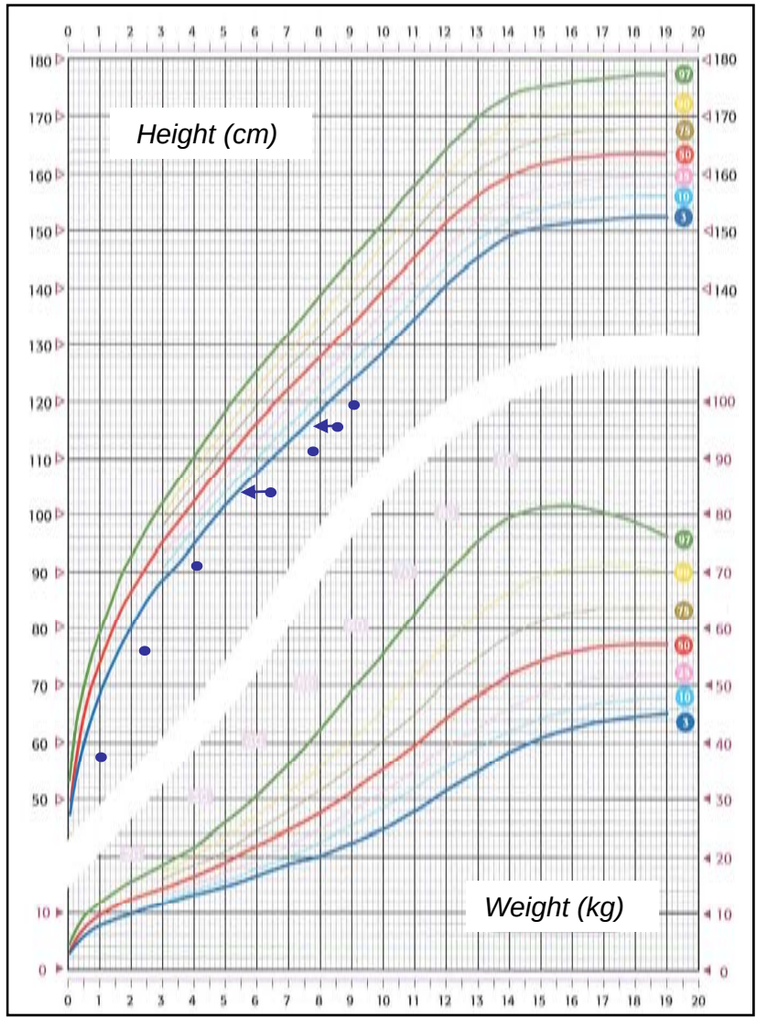

14 mild to moderate short stature and brachydactyly (missense, nonsense and frameshift). Those with osteoarthritis and/or discopathy.

2 Spondyloepiphyseal dysplasia, Kimberley type (SEDK)

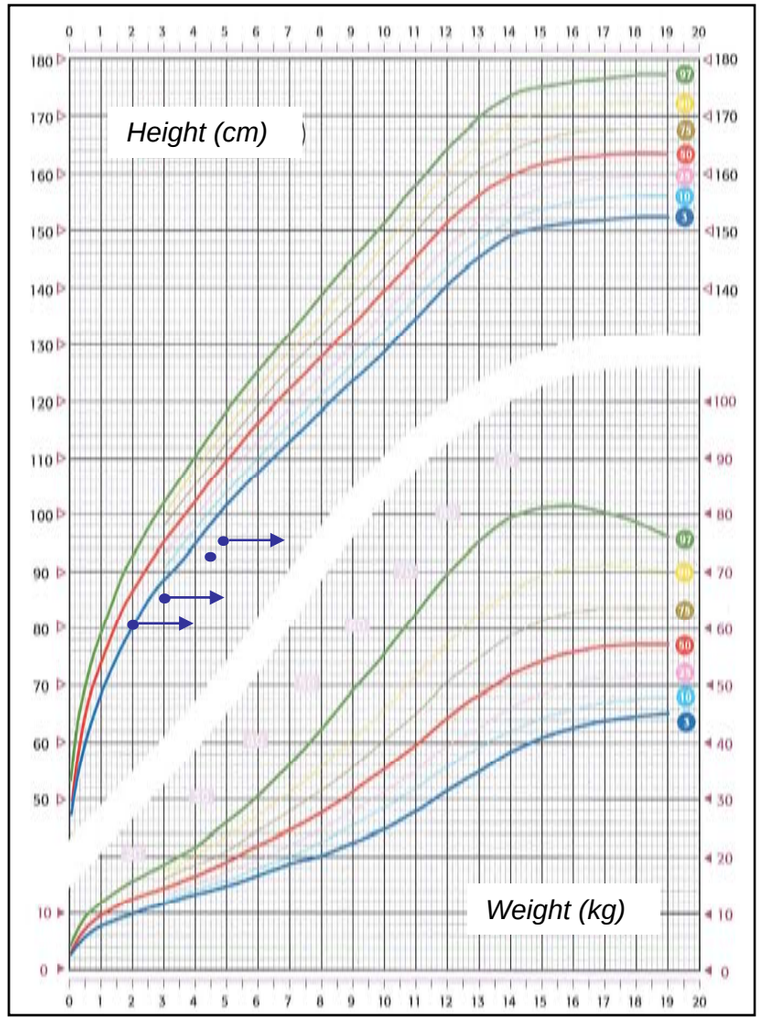
**Delayed bone age (n=4)**




**BA=CA (n=5)**




**Advanced BA (n=5)**



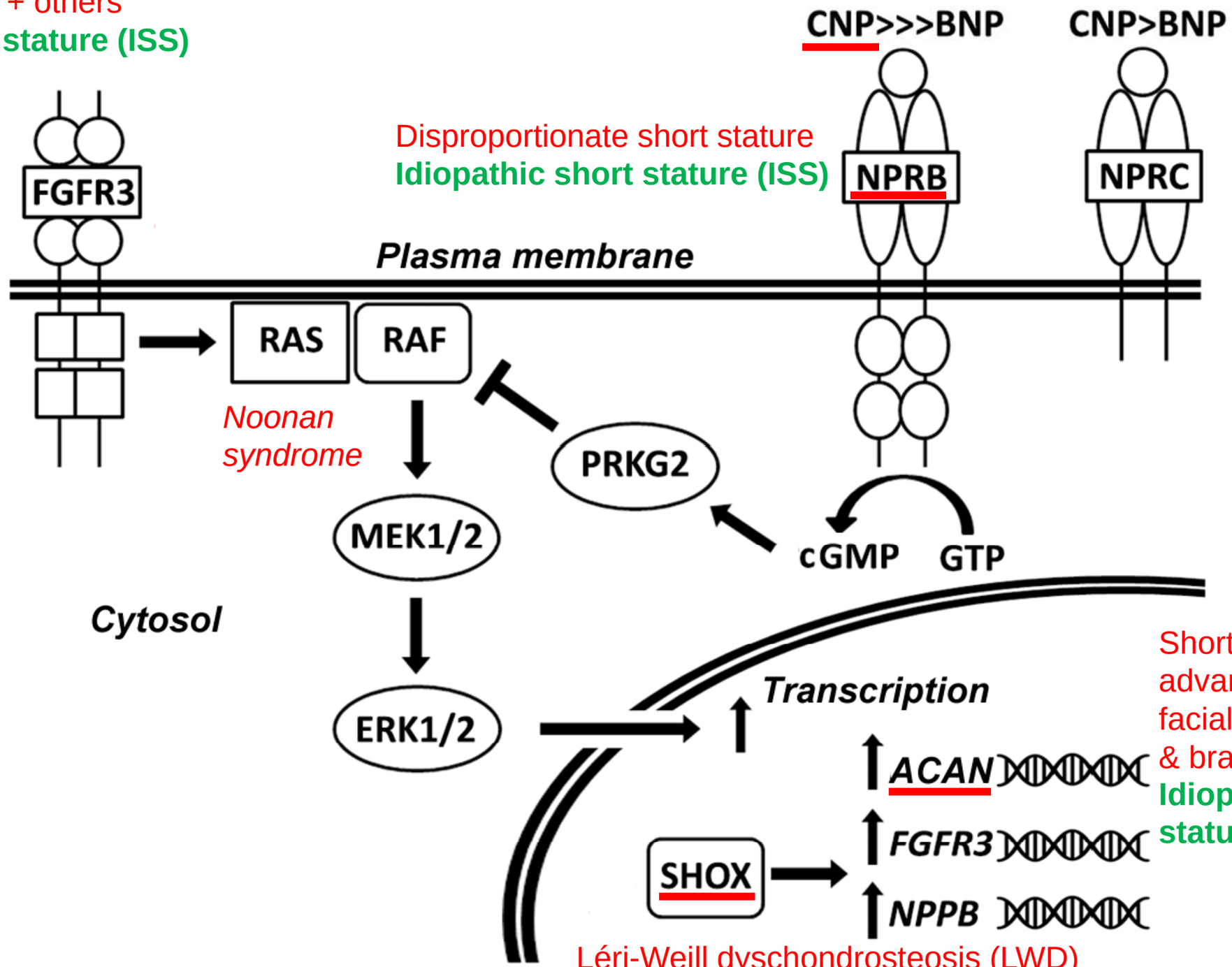

Median height SDS in children was -2.91 (range -4.30/-1.86 SDS)

Median height SDS in adults was -3.77 (range -5.40 SDS/-1.79 SDS)

| Study reference | Number of patients (Children/adults) from X families | SGA | Range of height (SD) Children Adults | Advanced BA in children | Frontal bossing | Flat nasal bridge | Mid facial hypoplasia | Brachydactyly | Short thumbs, and/or short first metacarpal | Broad great toe | Hyperlordosis | Hip anomalies | Mild osteochondrosis knee defects | Early growth cessation (adults) | Early-onset arthrosis /OD (families) | Intervertebral disc disease (families) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nilsson et al, 2014 | 14 (5/9)*<br>3 fams | 2/4 | -4/-1.2<br>-3.8/-2.3 | 3/5 | NR | NR | 6/9 | 6/9 | 3/9 | NR | NR | NR | NR | 5/5 | 1/3 | 0/3 |
| Quintos et al, 2015 | 3 (1/2)**<br>1 fam | 0/1 | -2.7<br>-4.7/-2.6 | 1/1 | NR | NR | 1/3 | NR | NR | NR | NR | NR | NR | 2/2 | 0/1 | 0/1 |
| Manouk van der Steen et al, 2016 | 10<br>(4/6)<br>3 fams | 3/3 | -3.7/-2.4<br>-5.4/-3.7 | 3/4 | NR | NR | 9/10 | NR | 3/10 | 6/10 | 3/10 | NR | NR | NR | 3/3 | 0/3 |
| Gkourogianni et al, 2017 | 102<br>(32/70)<br>20 fams | NR | -4.2/-0.6<br>-5.9/-0.9 | 19/23 | 2/32 | 8/20 | 8/20 | 5/20 | 3/20 | NR | NR | NR | NR | 23/70 | 13/20 | 8/20 |
| Dateki et al, 2017 | 4 (2/2)<br>1 fam | 0/2 | -2.7/-2.5<br>-3.1/-3 | 2/2 | NR | NR | 3/4 | NR | NR | NR | 1/4 | NR | NR | NR | 0/1 | 1/1 |
| Hu et al, 2017 | 9(3/6)<br>3 fams | 1/1 | -4.3/-2.9<br>-5.4/-2.9 | 0/3 | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | 0/3 | 0/3 |
| Hauer et al, 2017 | 11(6/5)<br>6 fams | 1/6 | -3.9/-2 -<br>3.8/-1.8 | 2/5 | 3/6 | NR | NR | 3/6 | 2/6 | 2/6 | NR | 1/6 | NR | NR | 1/6 | NR |
| Current study | 32<br>(14/18)<br>14 fams | 4/13 | -4.3/-1.86<br>-5.4/-1.79 | 5/13 | 5/13 | 4/13 | 3/13 | 12/13 | 11/13 | NR | 3/13 | 5/13 | 3/13 | 6/6 | 3/14 | 1/14 |
| SUMMARY | 164<br>(59/105)<br>45 | 11/40 | -4.7/-0.6<br>-5.9/-0.9 | 30/45 | 9/49 | 4/13 | 22/39 | 19/28 | 19/38 | 8/16 | 7/27 | 5/13 | 3/13 | 27/74 | 20/45 | 10/39 |

Craniosynostosis + others
**Idiopathic short stature (ISS)**

Short stature & brachydactyly




Disproportionate short stature
**Idiopathic short stature (ISS)**

*Noonan syndrome*

Short stature, +/- advanced BA, facial features, & brachydactyly
**Idiopathic short stature (ISS)**

Léri-Weill dyschondrosteosis (LWD)
Langer mesomelic dysplasia (LMD)

- Idiopathic short stature is a condition in which the height of the individual is more than 2 SDS below the mean height for a given age, sex and population, in whom no identifiable disorder is prese

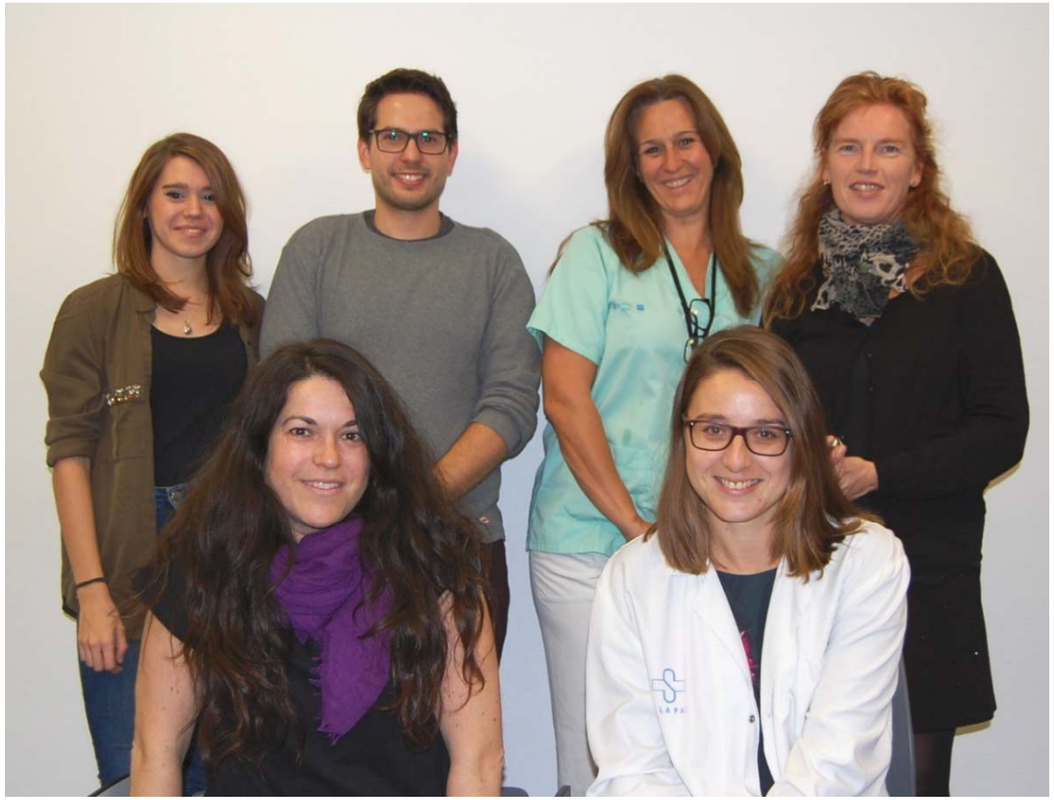
- It can be subca familial ISS, and according to pub

Many patients with ISS are actually often the mild forms of various skeletal dysplasias

- It s skeletal dysplasias ry to a small birth size (small for stemic and endocrine dise

- ISS is the diagnost group that remain after excluding known conditions in short children.

Wit JM, Clayton PE, Rogol AD, Savage MO, Saenger PH, Cohen P



**Group members**

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Lucia Sentchordi-Montané
Jimena Barraza-García
Carlos Rivera-Pedroza
Alba Ruzafa-Martín
María Rodríguez-Zabala
Beatriz Paumard-Hernández
David Medino
Carolina de la Torre