Genetic bases of short stature

Bases genéticas de la talla baja

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Brazil

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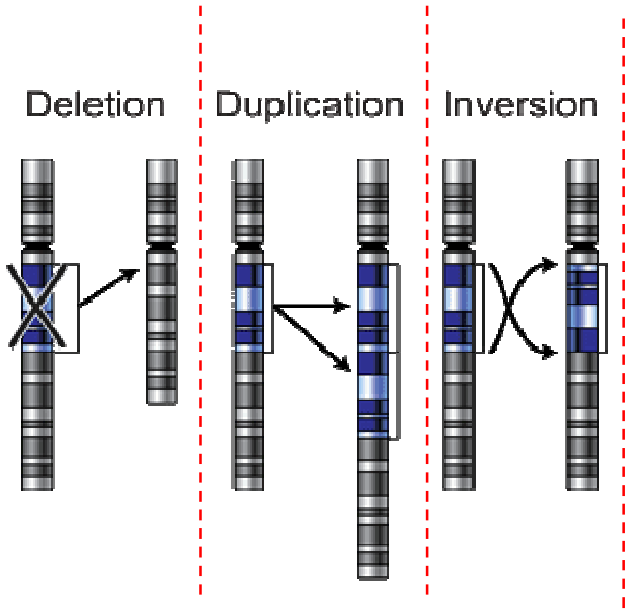
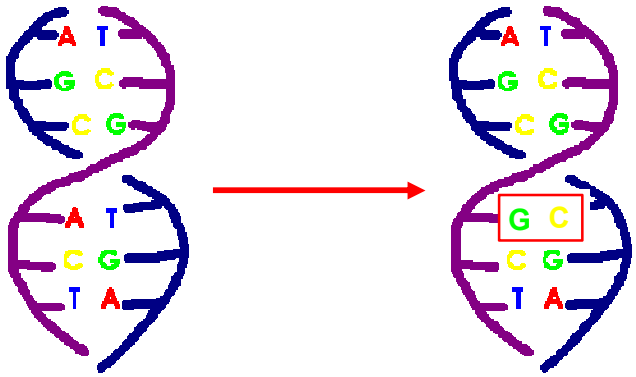
Nothing to disclose

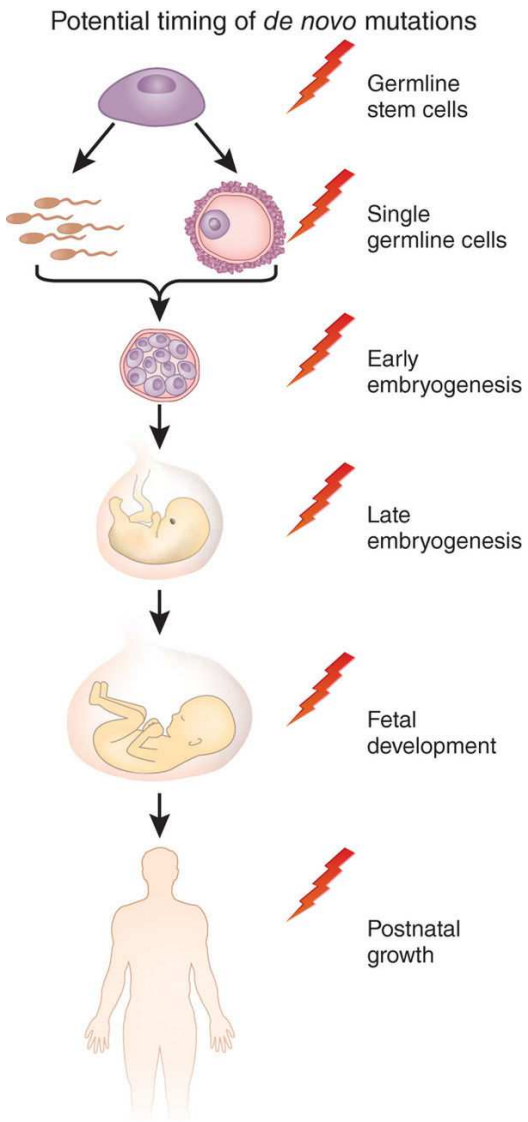
Genetic disorders

A genetic disorder is a disease caused in whole or in part by a change in the DNA sequence away from the normal sequence

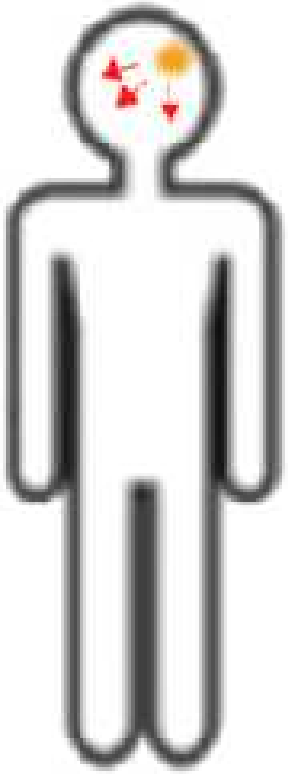




Genetic defects

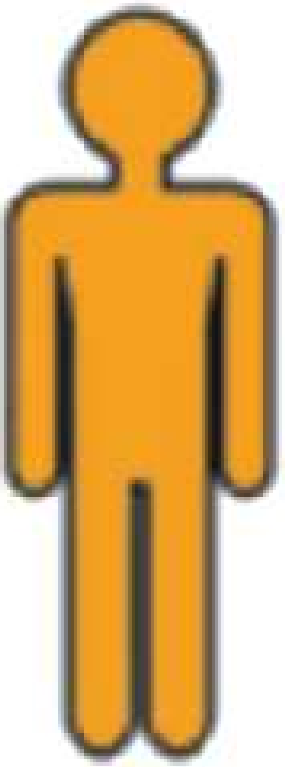


Somatic

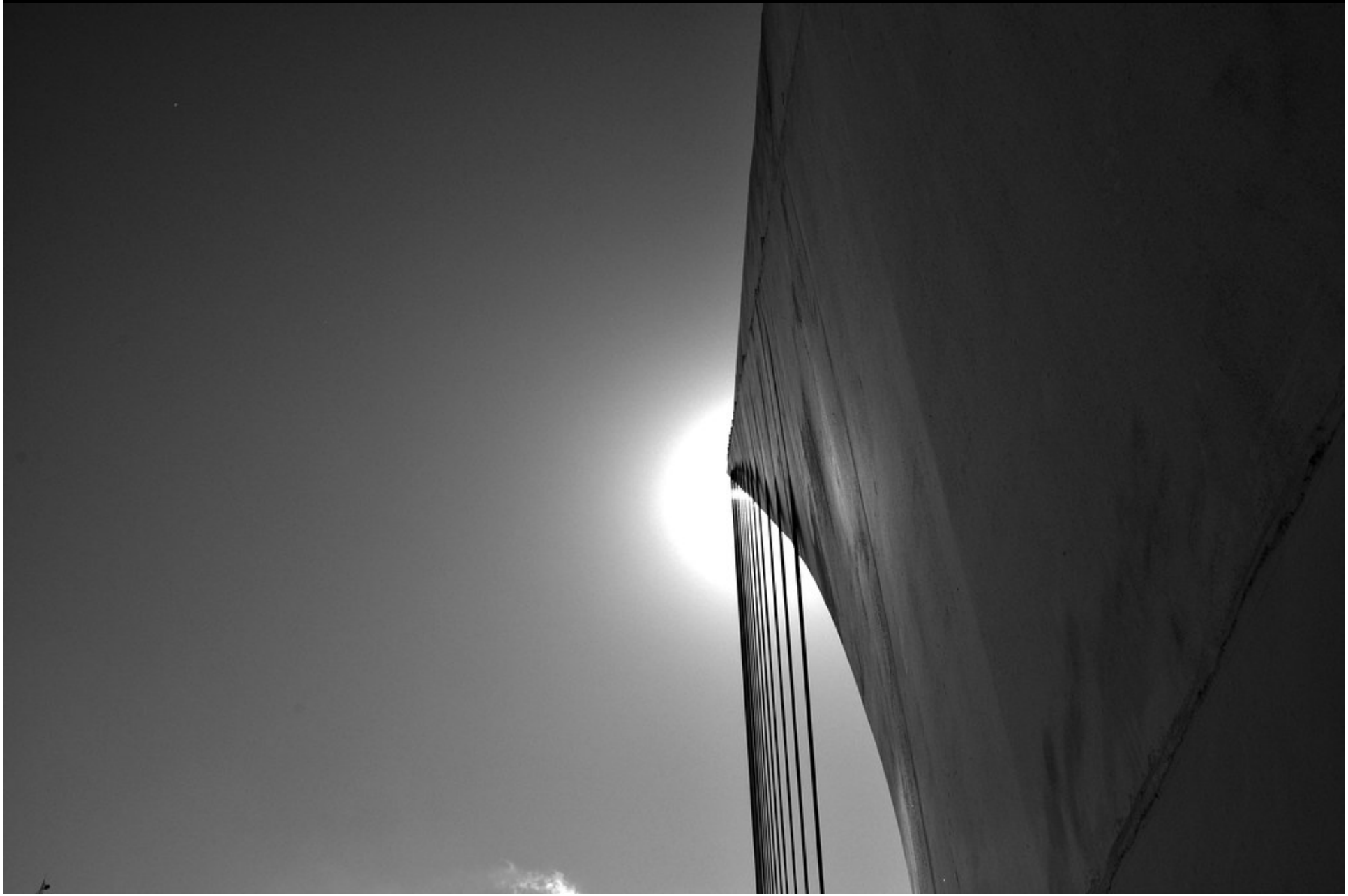


Mosaicism

Germline



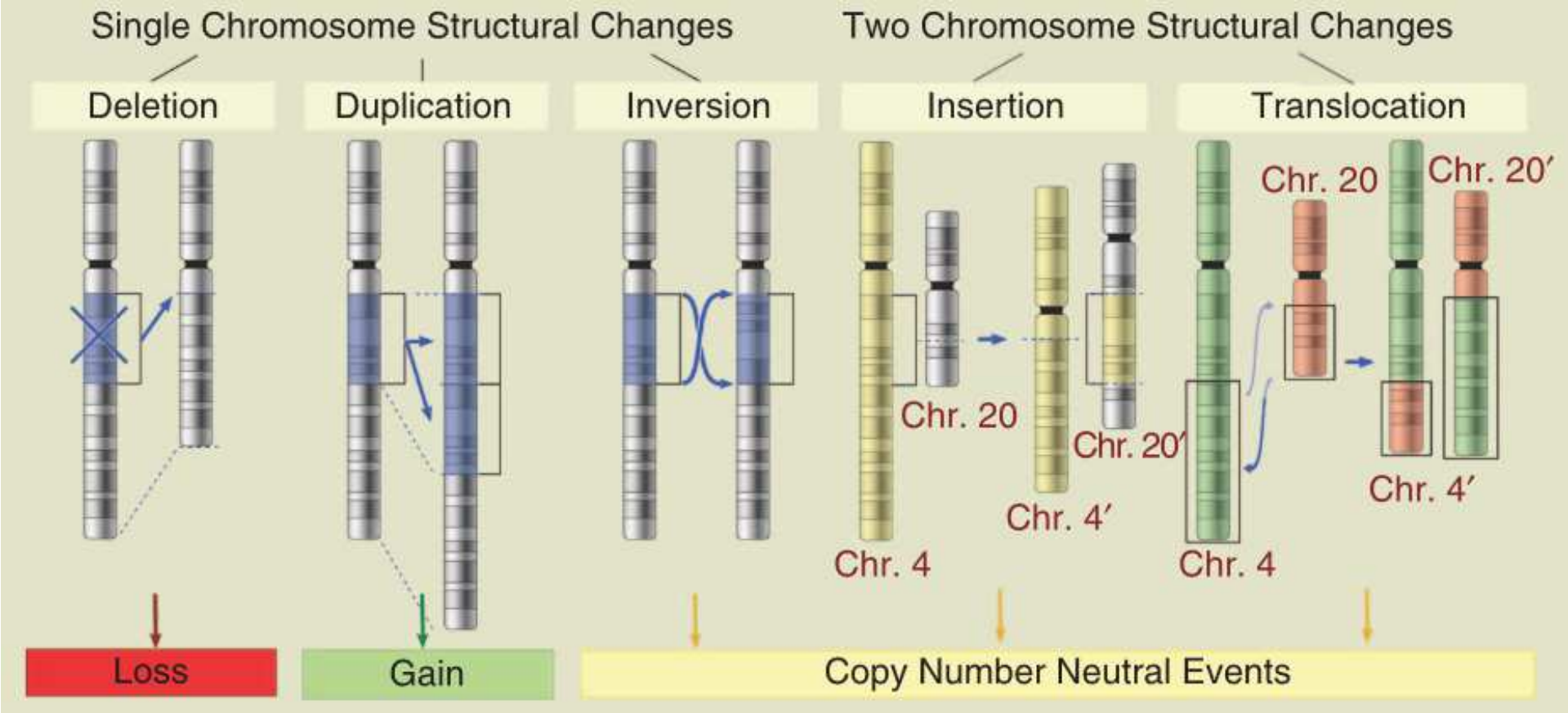
Types of genetic defects



Chromosome abnormality

- Numerical changes (aneuploidies)
- Structural changes (chromosomal rearrangements)

  Most microduplication/deletion syndromes are ≥ 500 kb



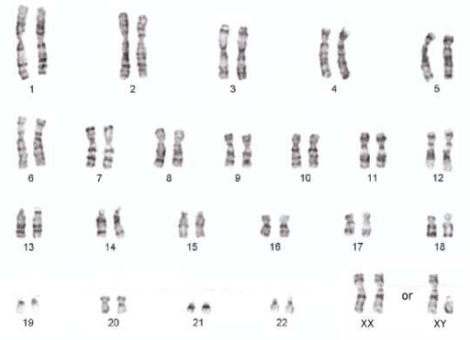
Miller *et al*. Am J Hum Genet **2010**;86(5):749-64

Techniques for analysis

Karyotype

Resolution >4Mb

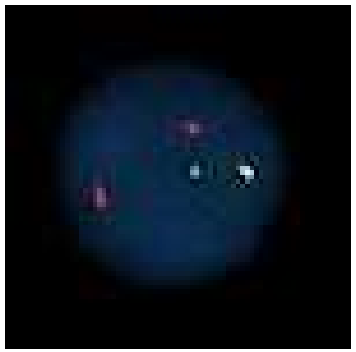
Genomic



FISH

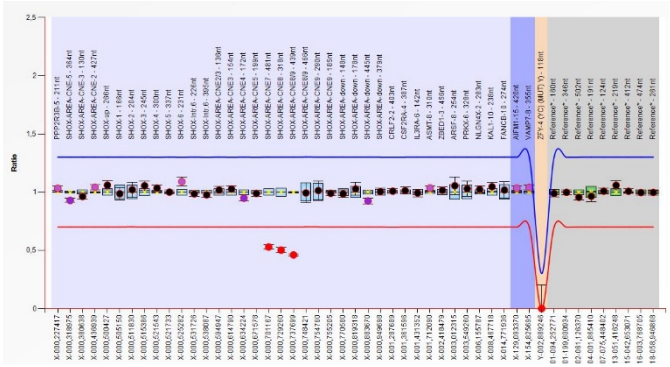
Candidate locus

Good for mosaicism



MLPA

Candidate locus/loci

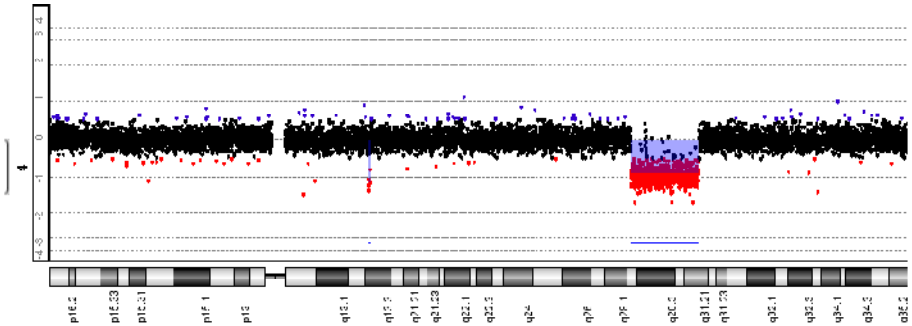


Molecular Karyotype

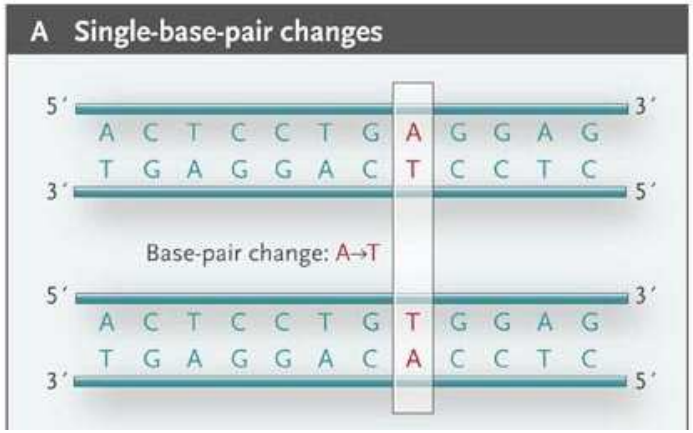
(SNP or CGH array)

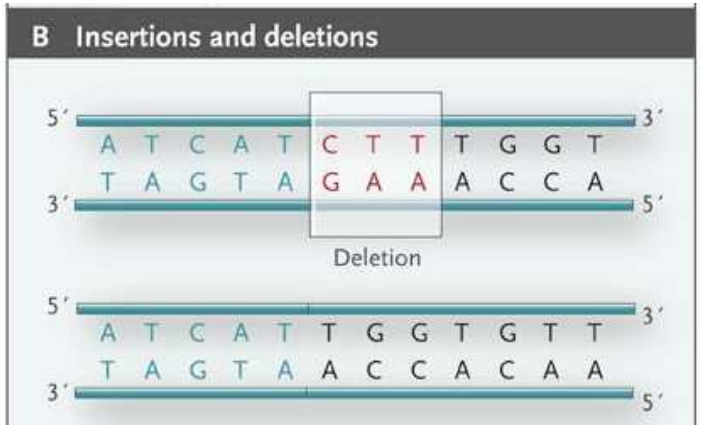
Resolution 20-50 kb

Genomic



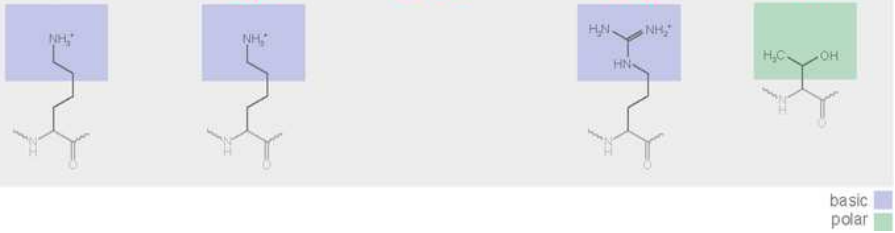
Abnormalities at the nucleotide level





if in the coding region…

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr



Several other mechanisms

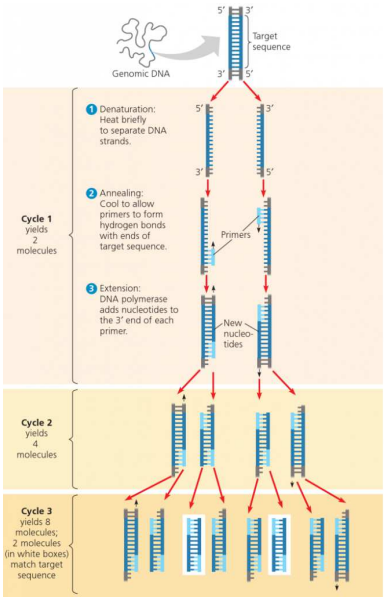
Frameshift
Inframe alteration
Splicing
Expression
mRNA
Stoploss
….

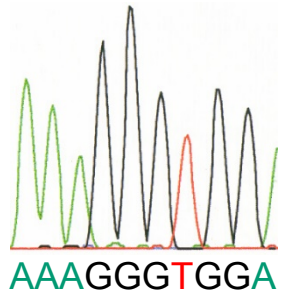
Feero et al N Engl J Med. **2010**;362(21):2001-11

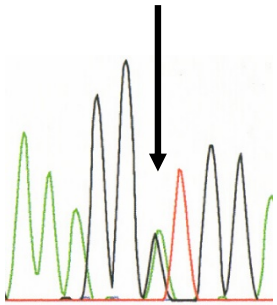
Abnormalities at the nucleotide level

Sanger sequencing

A candidate gene approach
Good for specific variant
Small genes
Few candidate genes

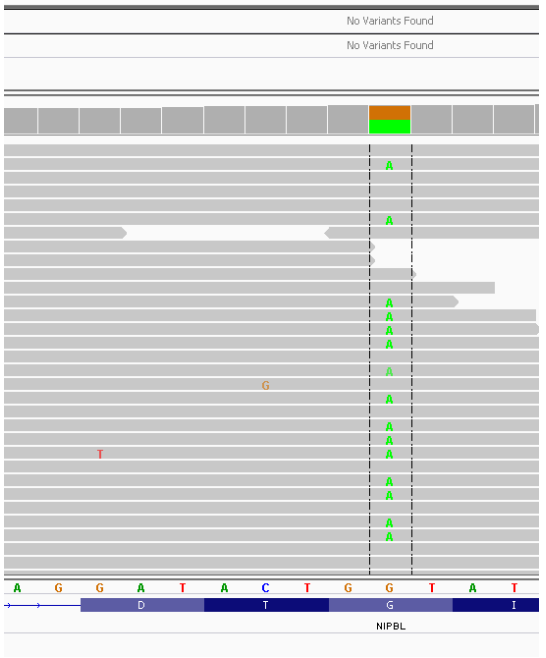






Massive parallel sequencing (NGS)

Whole exome or genome sequencing
Great for analyzing multiple regions simultaneously
More automated analysis

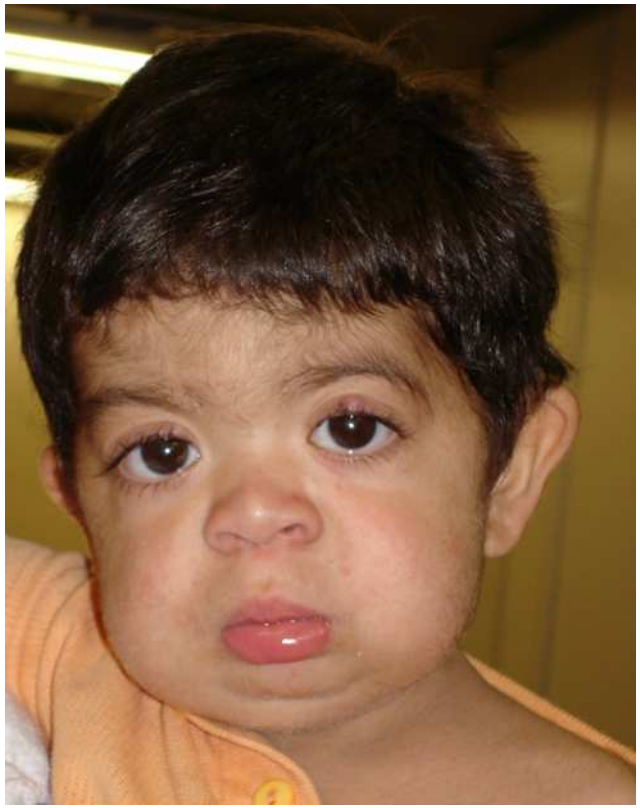


Katsanis & Katsanis. Nat Rev Genet 2013;14(6):415-26

Let's start with growth disorders

Differential diagnoses of short stature

Chronic systemic diseases



- Endocrine conditions (hypothyroidism)
- Celiac disease
- Psychosocial deprivation
- Primary and secondary undernutrition
- Chronic kidney disease,
- Gastrointestinal disease
- Rheumatologic disease,
- Hematological,
- Cardiac disease
- Pulmonary disease,
- Muscular and neurological disorders
- Medication (glucocorticoid)

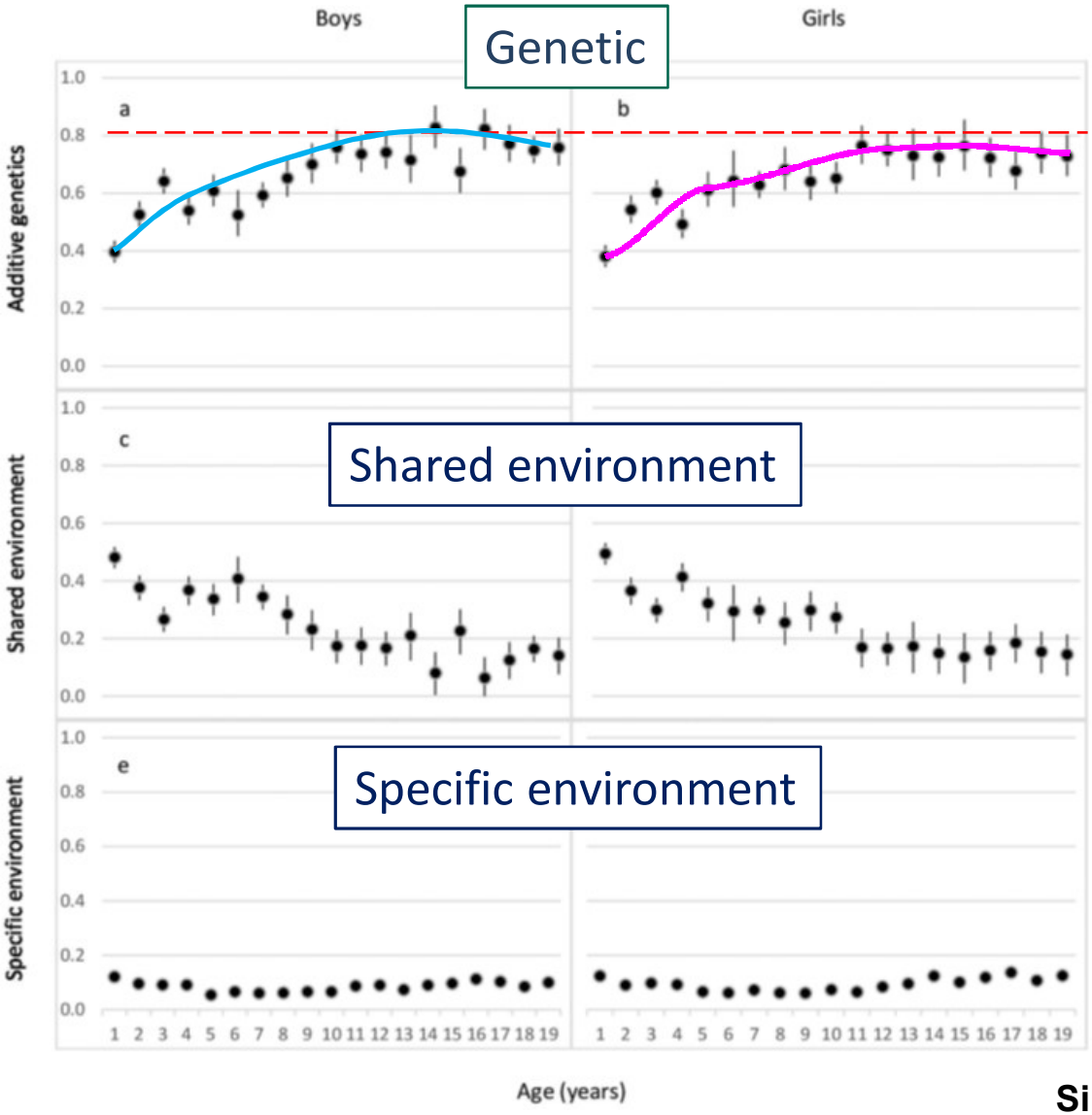
Differential diagnoses of short stature

Chronic systemic diseases	<1%
Disorders of the GH/IGF axis	~2%
Syndromic short stature	5-7%
Skeletal dysplasia	~2%
Isolated short stature ("normal variants of growth")	>60%

- Idiopathic short stature
- Familial short stature
- Constitutional delay of growth and puberty
- Children born small for gestational age

Collett-Solberg *et al*. Growth Horm IGF Res. **2019**; 44:20-32

Heritability of height



Large family studies consisting of thousands of families have also estimated the heritability of height is between 0.79-0.98

Study with 45 twin cohorts (180,520 pairs 1-19 years old)

This heritability is lower in early childhood, increasing with age, reaching a estimated heritability of 0.68-0.94

Jelenkovic *et al.* Sci Rep **2016**; 23;6:28496
Silventoinen *et al.* Twin Res **2003**; 6(5):399-408
Wu *et al.* European Journal of Human Genetics **2003**; 11(3), 271–274

Genetic regulation of growth

Severe phenotype

Monogenic conditions

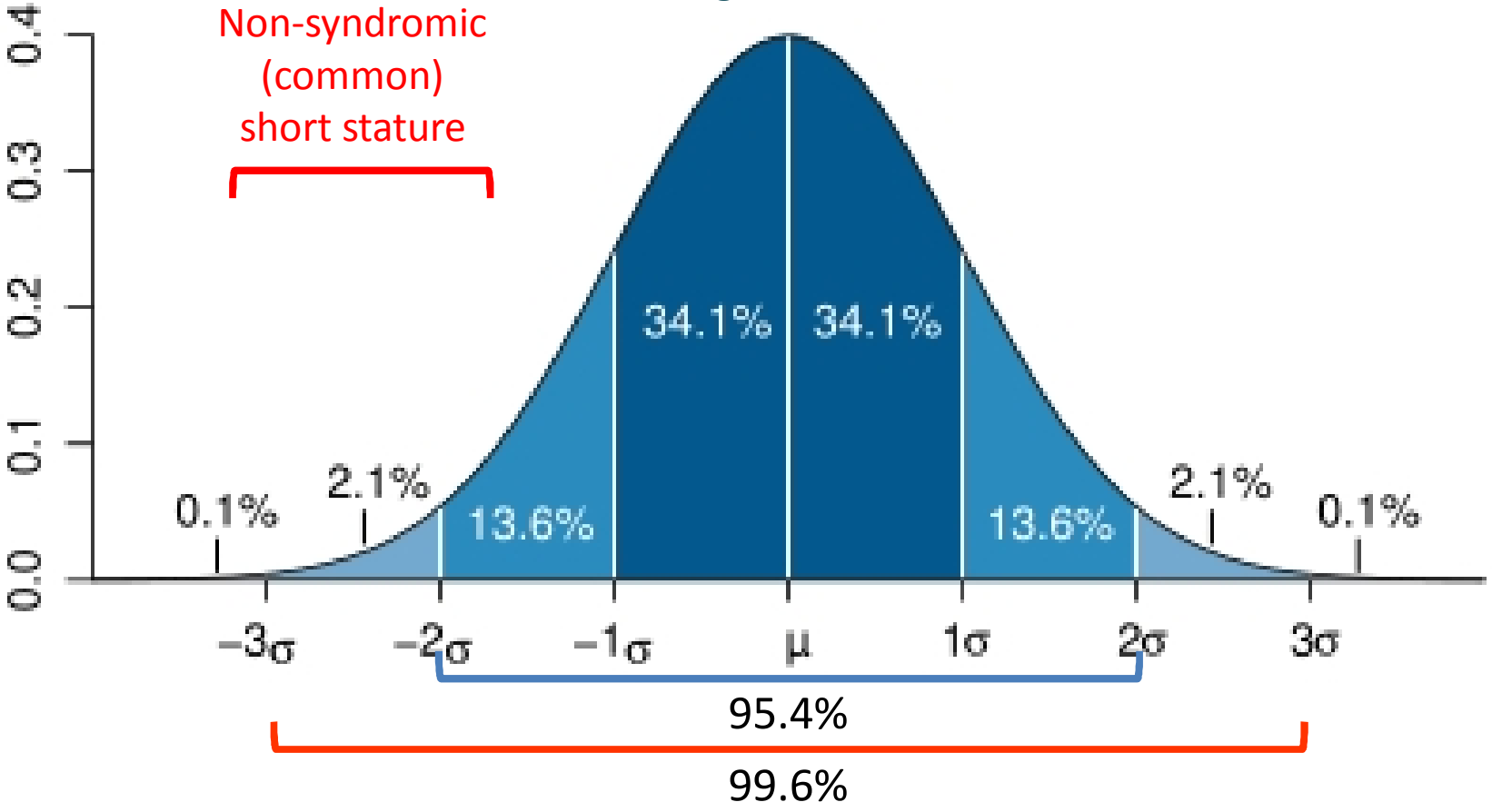
Height SDS < -3

Height variation

polygenic traits

Height SDS ± 2

Non-syndromic (common) short stature



OMIM
Online Mendelian Inheritance in Man

Johns Hopkins University

5,533 – phenotypes for which the molecular basis is known

3,318 – phenotype under investigation

8,851

Clinical synopses: "short stature" OR "Small for gestational age" OR dwarfism OR dwarf OR "intrauterine growth restriction" OR "Intrauterine growth retardation" OR "Postnatal growth retardation"

Results: 1,099 entries

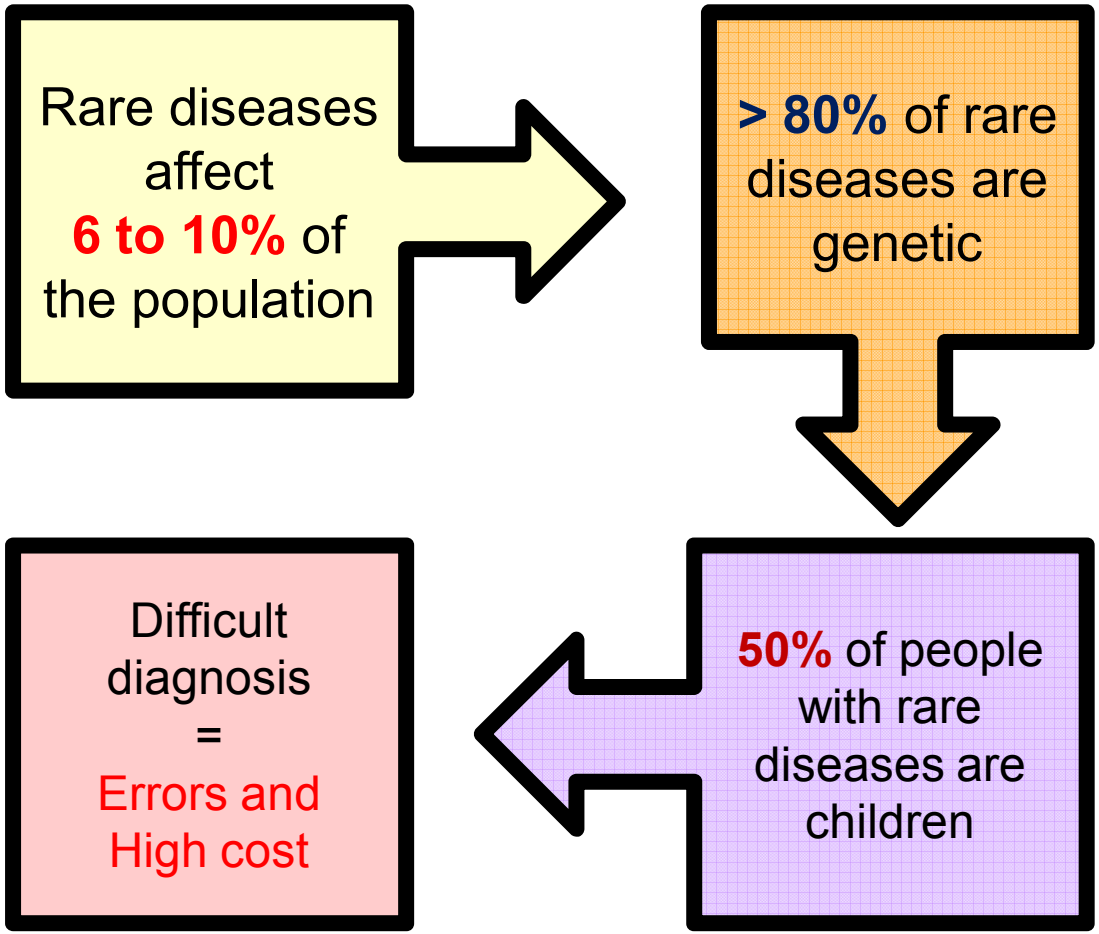
- 12% of inherited/genetic diseases have growth disorder as an important phenotype

50 YEARS OMIM Human Genetics Knowledge for the World

Updated May 1st, 2019

The common problem of rare diseases

Rare diseases: those that affect <1: 1,500 to 1: 2,500



Knight & Senior Med J Aust. **2006**; 185(2):82-3

Main genetic conditions associated with short stature

Defects affecting:

- Chromosomal abnormalities and CNVs
- GH/IGFs axis
- Intracellular pathways
- Fundamental cellular processes

(syndromic short stature)

- Paracrine factors in the growth plate
- Cartilage extracellular matrix

(Skeletal dysplasias)

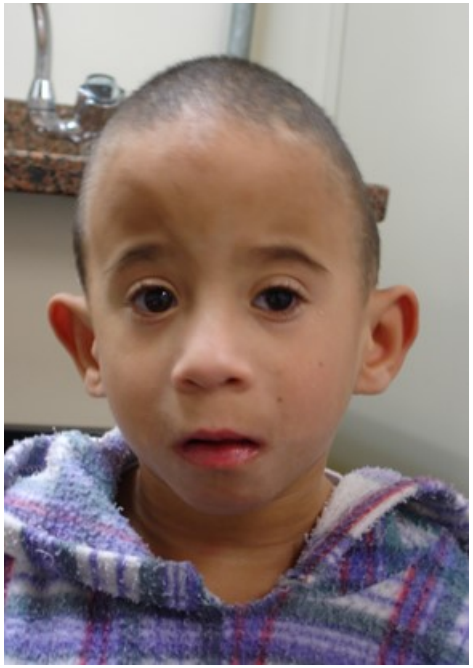
- Imprinting disorders

Wit *et al. EJE* **2016**; 174(4):145-173

to keep in mind that not always is genetic

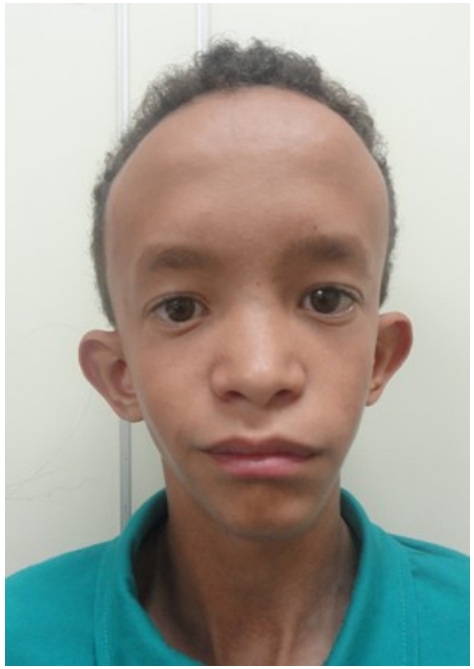
Syndromic short stature

Environment



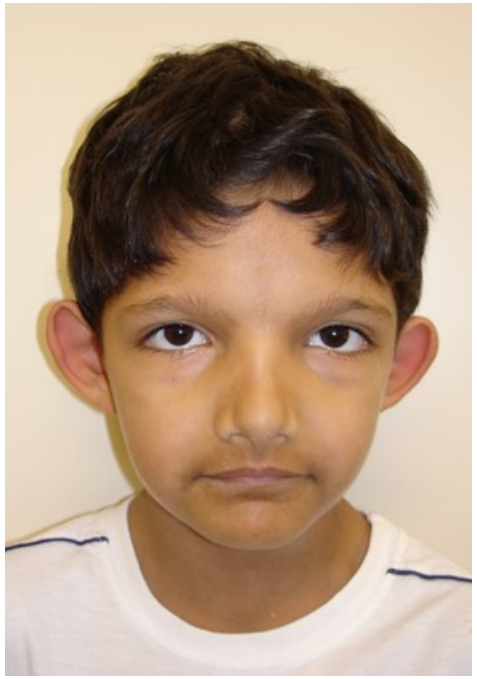
Fetal alcohol syndrome

Epigenetic (Epimutation)



Silver-Russell syndrome (11p.15 epimutation)

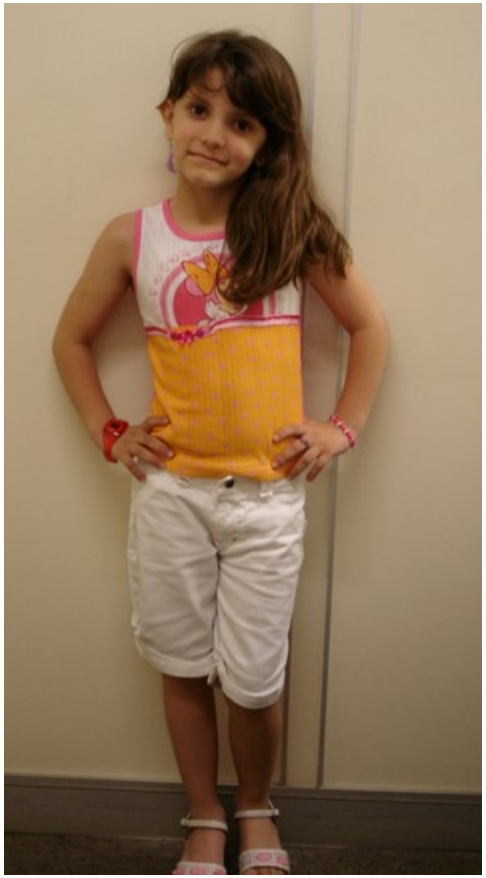
Genetic



9p trisomy syndrome

Main genetic conditions associated with short stature

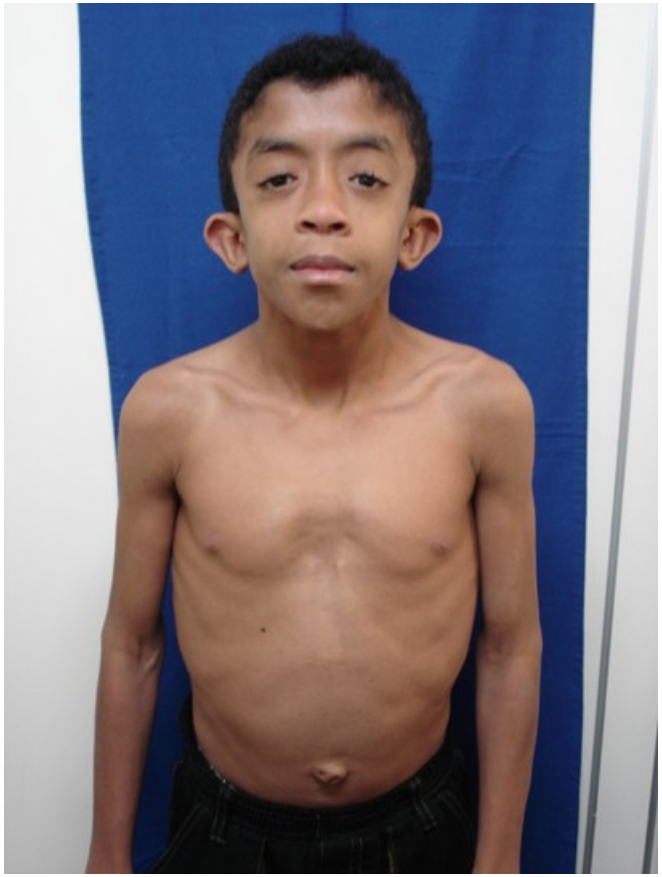
Turner Syndrome



45,X
and variants

1 in 2,000 live female births

Noonan Syndrome



PTPN11
HRAS
KRAS
NRAS
SOS1
SOS2
BRAF
RAF1
MEK1
MEK2
...

1 in 1,000 and 1 in 2,500 children

Main genetic conditions associated with short stature

Achondroplasia and hypochondroplasia

FGFR3

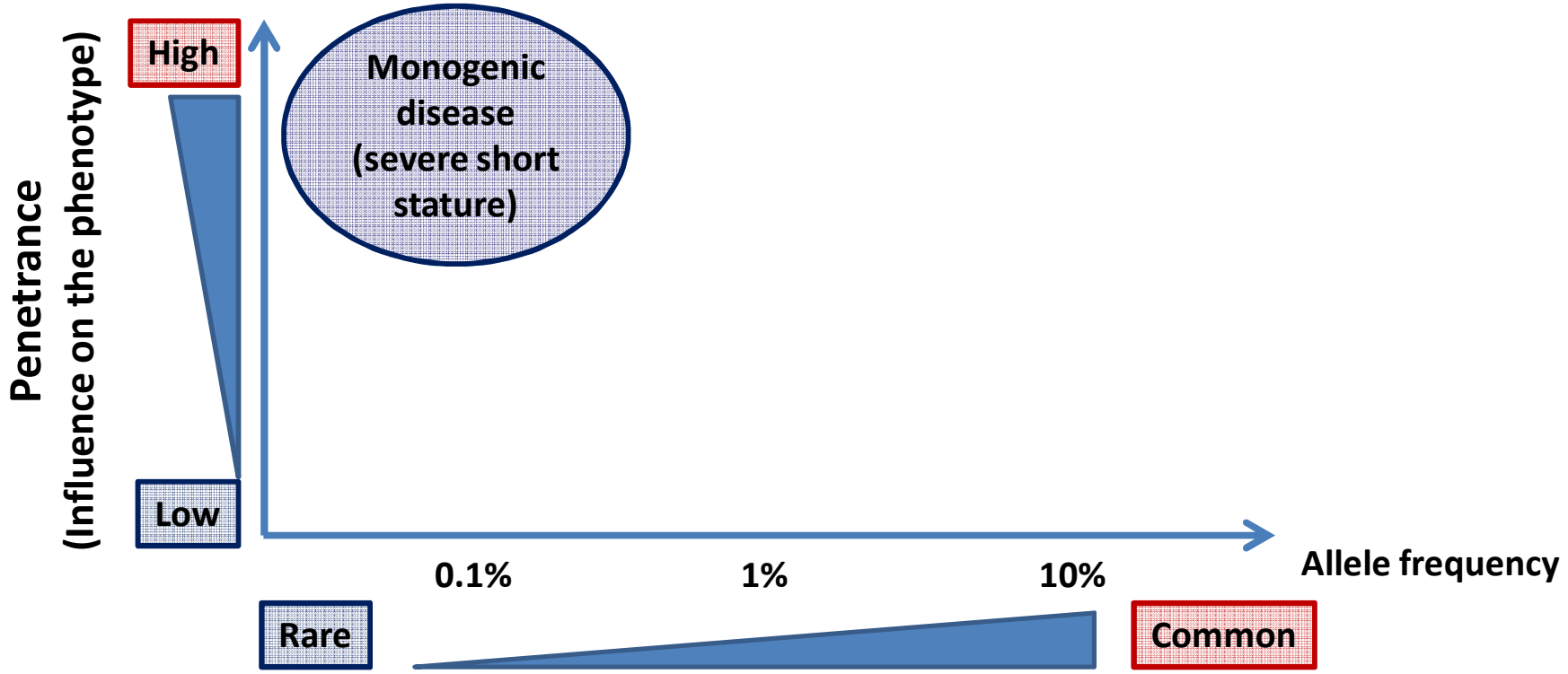
1 in 15,000 to 40,000 newborns

Léri-Weill dyschondrosteosis

SHOX

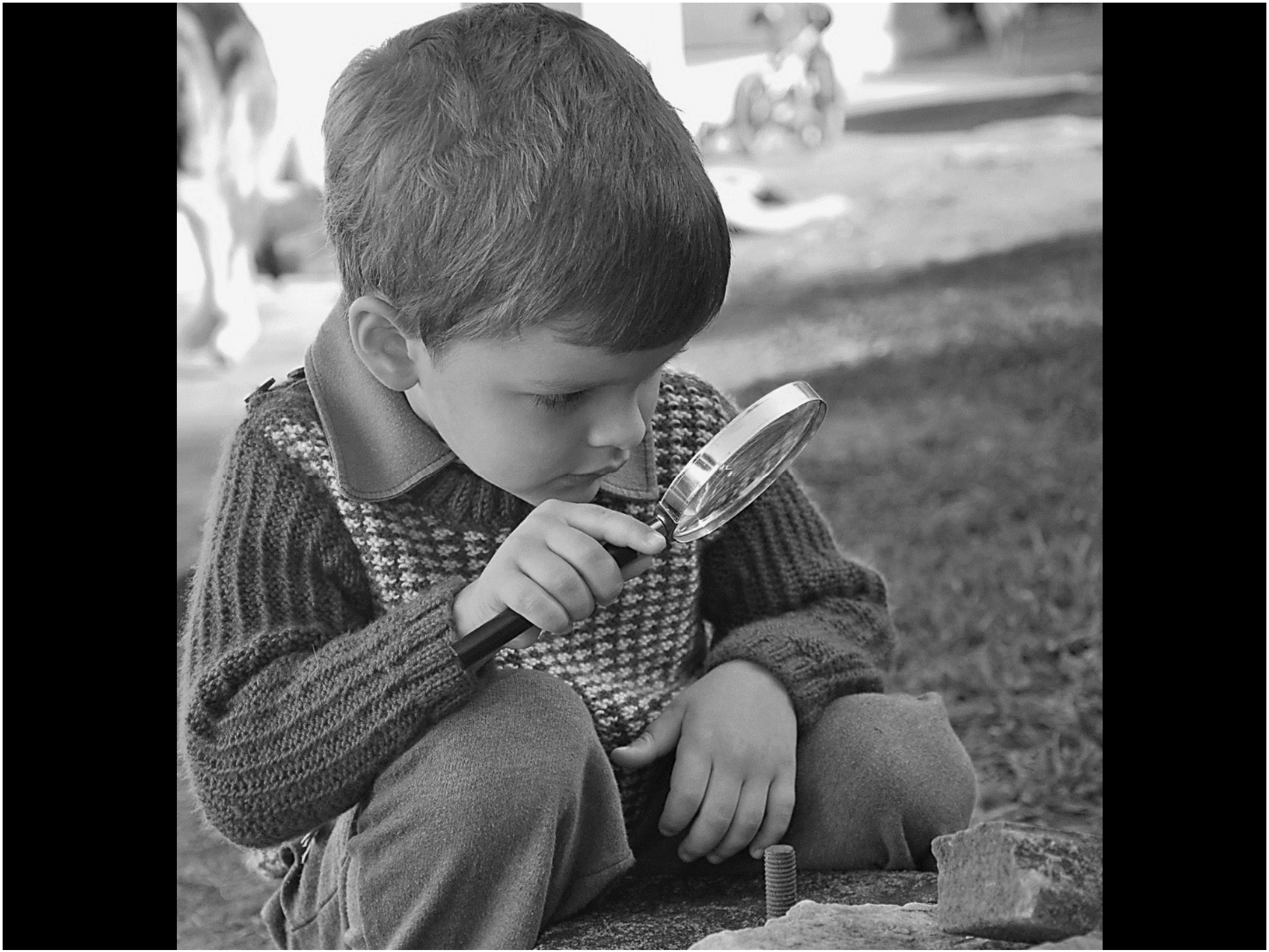
Estimate prevalence: 1 in 1,000-2,000

Genetic influences on height



Rare condition = Rare genetic variant with high penetrance

McCarthy *et al.* Nat Rev Genet. **2008**; 9(5):356-69
Manolio *et al. Nature.* ***2009****; 461(7265):747-53*

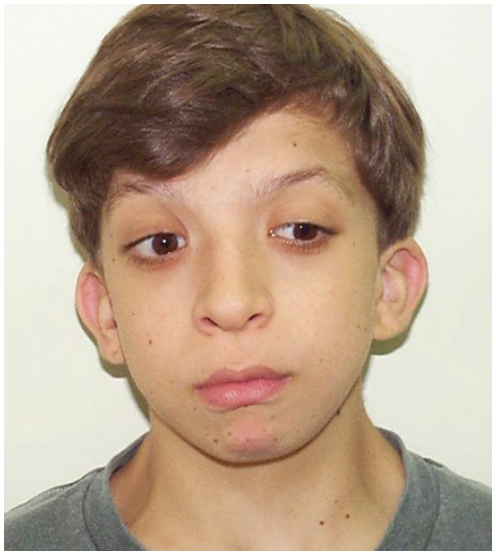


Diagnostic process and pattern recognition

Short-limb
Height SDS -4.7
SH:H SDS +13
Rhizomelic shortening
Exaggerated lumbar lordoses
Low nasal bridge
Trident hand

(Achondroplasia)

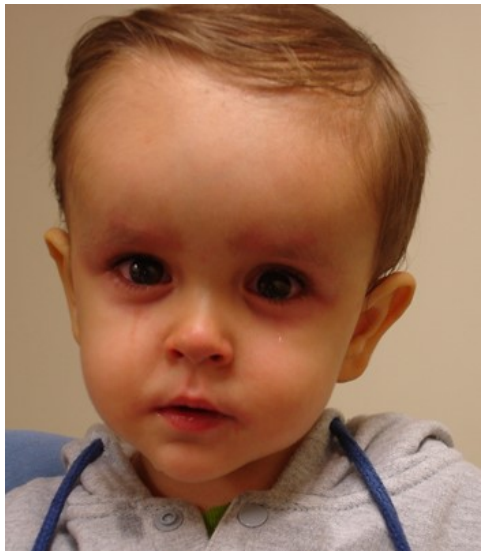
FGFR3



Typical phenotype
Height SDS -3.5
Hypertrophic cardiomyopathy
Father 184 cm
Mother 162 cm

(Noonan syndrome)

PTPN11, RAF1, ...



Postnatal short stature
CA 2.2 yeas
Height SDS -4.8
Hypoglycemia
Typical face
- prominent forehead
- midface hypoplasia

(GHD)

> 20 genes

Diagnostics not so simple

SGA (39 w; 2260 g)
CA: 9y
Height SDS -2.7 (proportional)
BMI SDS -0.3
Delayed speech
Language development disorder

Short father (height SDS -2.5)

Elevated IGF-1 (above > 3 SDS)

A candidate gene approach

SGA

Height SDS -2.7

Language development disorder

Short father (height SDS -2.5)

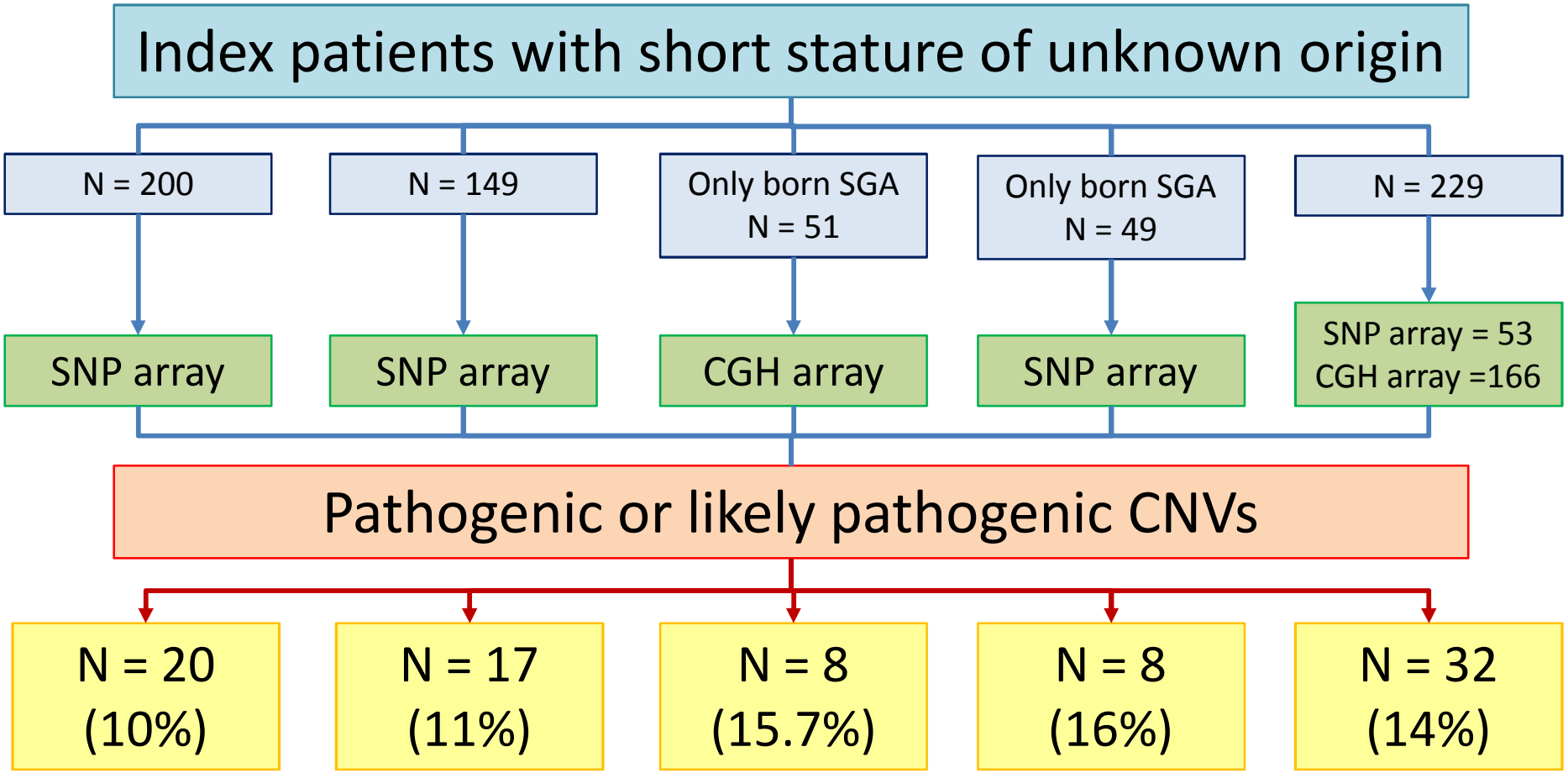
Elevated IGF-1 (above > 3 SDS)

Could be a defect in the *IGF1R* leading a partial IGFs insensitivity?

Normal *IGF1R* sequencing (21 exons)

Normal *IGF1* sequencing (6 exons)

Molecular karyotype in growth disorders



Zahnleiter *et al.*
PLoS Genet **2013**

van Duyvenvoorde *et al.*
Eur J Hum Genet **2014**

Canton *et al.*
Eur J Endocrinol **2014**

Wit *et al.*
Horm Res Paediatr **2014**

Homma *et al.*
Horm Res Paediatr **2018**

Molecular karyotype in growth disorders

Syndromic short stature

↓

671 patients from 5 different cohorts

↓

Molecular karyotype (SNPa or aCGH)

↓

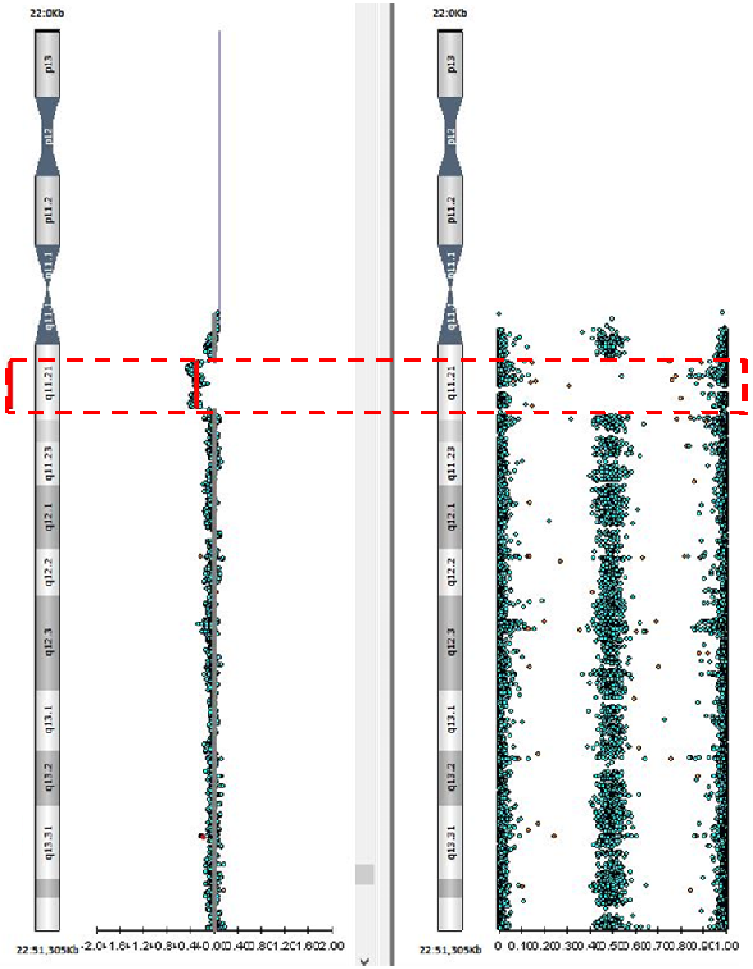
13% pathogenic CNVs
(95%CI: 10.4% - 15.5%)

↓

7 recurrent CNVs (only deletions)

22q11.21	15q26 (*IGF1R*)	Xp22.33 (*SHOX*);	1q21.1;
10.3%	9.6%	1p36.33	2q24.2;
		3.4%	17p13.3
			2.3%

Chromosome 22q11.21 deletion on SNParray

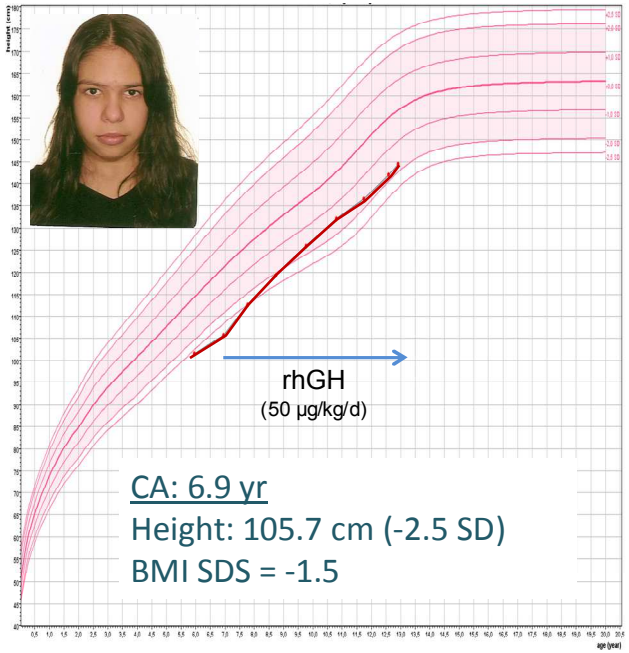


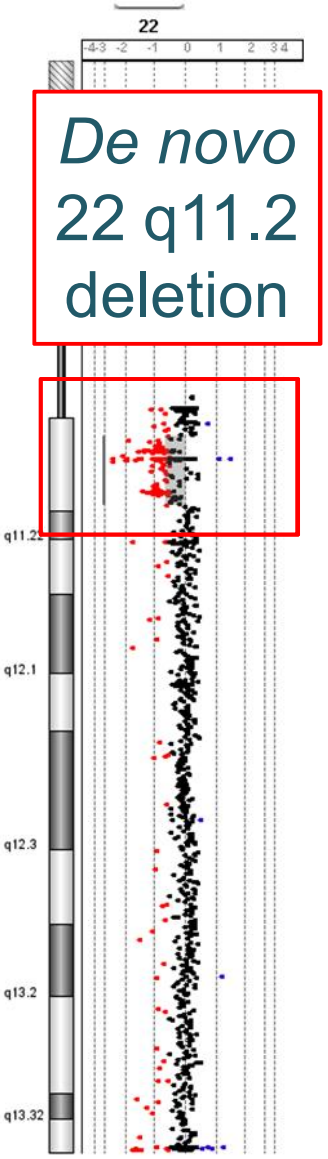
Homma *et al.* Horm Res Paediatr 2018; 89(1): 13-21

An example of the use of the molecular karyotype

A girl born SGA
Non-syndromic short stature
At the age of 11, she started experiencing seizures that were difficult control and without apparent cause

Normal laboratory evaluation
Karyotype 46,XX





De novo
22 q11.2
deletion

Compatible with 22q11.2 deletion syndrome
(DiGeorge or velocardiofacial syndrome)

Normal echocardiogram
Absence of palatal alterations
Normal immunology function
Normal behavior
Normal calcium, phosphorus and PTH

Canton *et al*. Eur J Endocrinol 2014;171(2):253-62

Genomic approach – CGH-array

SGA

Height SDS -2.7

Language development disorder

Short father (height SDS -2.5)

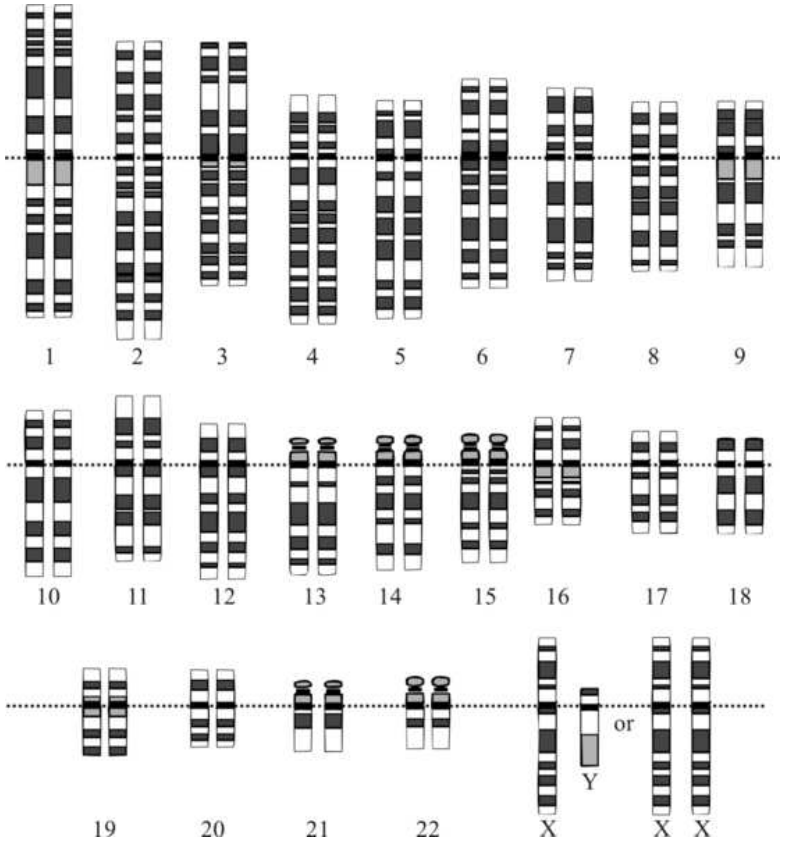
Elevated IGF-1 (above > 3 SDS)

Could be pathogenic CNV?

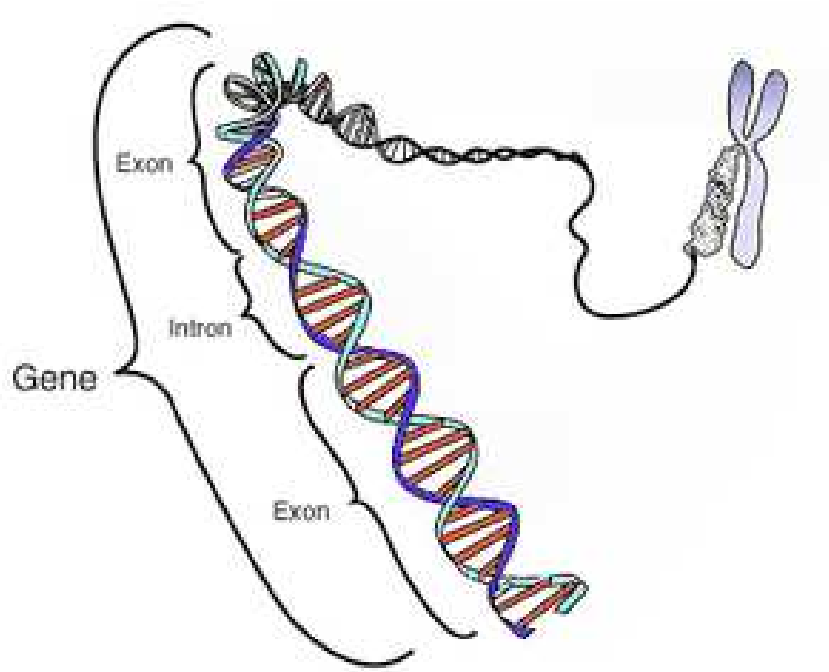
Normal molecular karyotype
(SNP-array)

Exome sequencing

Human genome = 100%
(~ 3.235 Mb)



Exons – 1-2% of the genome
(20,576 protein-coding genes)
(~ 64 Mb)



de Bruin & Dauber Nat Rev Endocrinol 2015; 11(8):455-64

Using whole exome sequencing for the diagnosis of Mendelian disorders

2.000 patients
(88% children and neurologic phenotype)

↓

Exome sequencing

↓

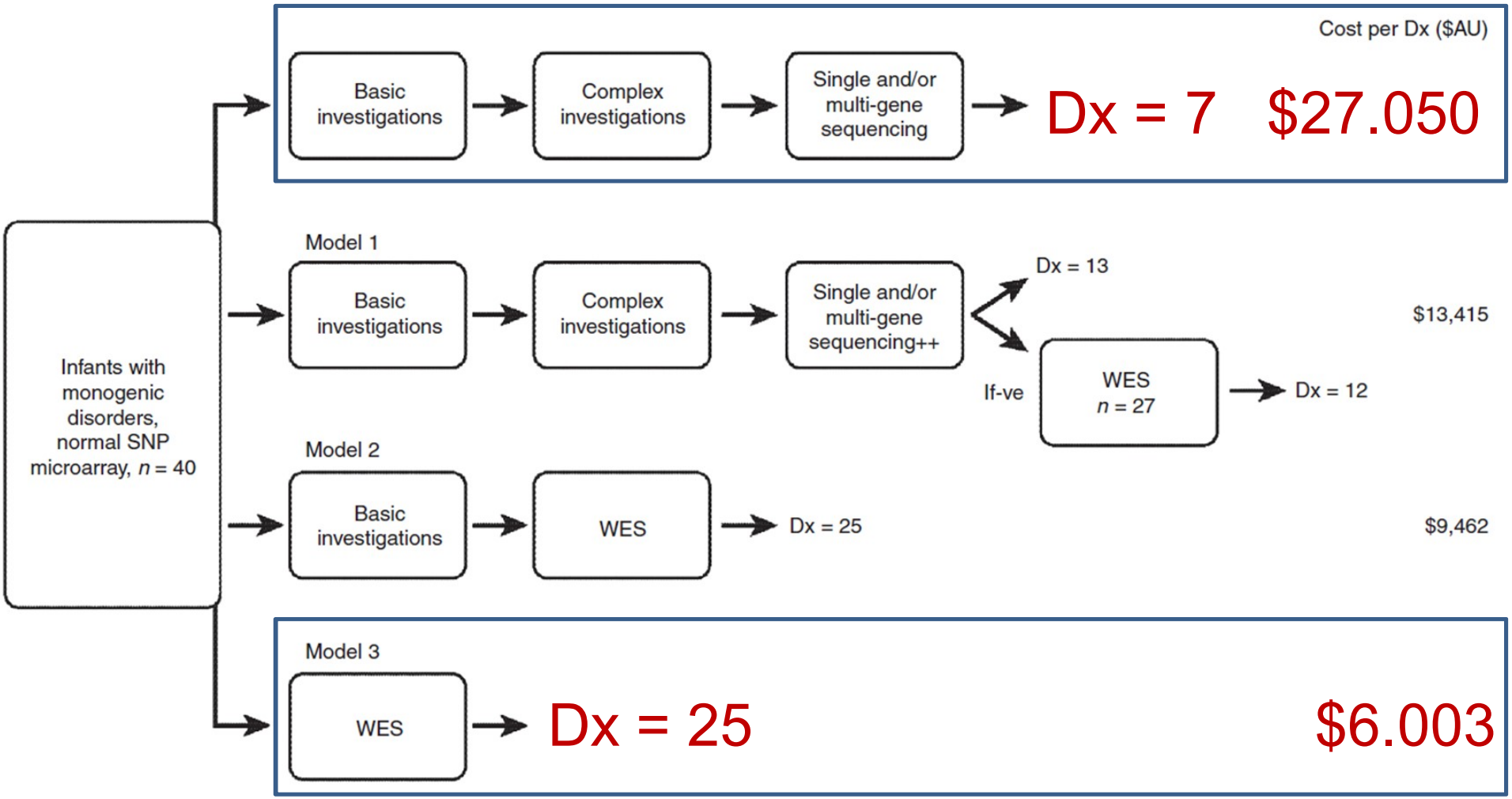
708 mutated alleles that were highly likely to be causative in 504 patients (25%, 95% CI 23-27%)

The success rate was higher when the family was evaluated (31%)

4.6% received two non-overlapping molecular diagnoses

Yang *et al*. JAMA **2014**;312(18):1870-1879

WES vs. traditional approach



Stark et al. Genet Med. **2017**; 19(8):867-874.

Exome sequencing

44 patients born SGA with unknown syndromic short stature analyzed by WES

16:44 (36%) positive findings

- ***BRCA1*** *– 3rd description*
- ***BCL11B*** *– 1st description*
- *PCNA – 2nd case*
- *SRCAP (2x)*
- *ACTG1; AFF4, ANKRD11, CDKN1C, COL2A1, GINS1, INPP5K, KIF11, KMT2A, POP1A*

New genes under investigation

Homma, Freire et al. Submitted (under review) 2019

Davor *et al.* Brain **2018**; 141(8):2299-2311

Homma *et al.* Horm Res Paediatr **2018**; 89(1): 13-21

Freire & Homma *et al.* Eur J Med Genet. **2018**; 61(3):130-133

Genomic approach – WES

SGA

Height SDS -2.7

Language development disorder

Short father (height SDS -2.5)

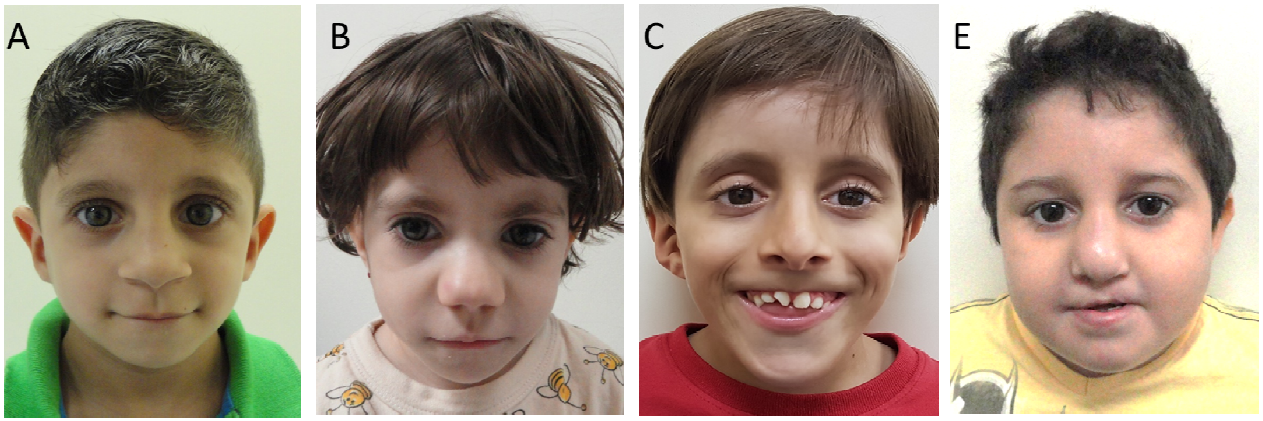
Elevated IGF-1 (above > 3 SDS)

Exome sequencing:

SRCAP (exon34) c. 7330C>T (p.R2444*)

Heterozygous de novo

= Floating-Harbor syndrome



Homma, Freire et al. in preparation to be submitted 2019

Isolated short stature

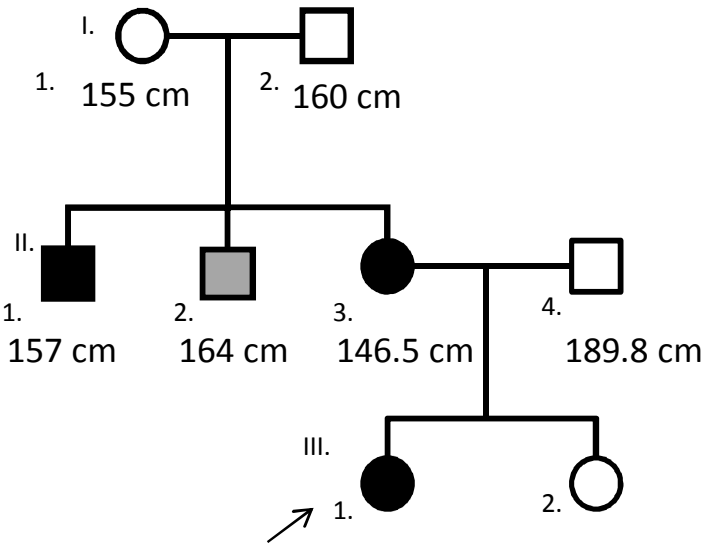
SGA for length

Healthy

Short stature

Absence of other findings

Mother with short stature



	III.1	III.2
Gestational age (ws)	38	
Birth weight (kg)	2785	
Birth length (cm)	44	
Age (y)	7.9	2.8
Height (cm)	112.2	96
Height SDS	-2.2	+1.2
SH:H SDS	+3.44	-1.2

Isolated short stature

- **Apparently, isolated growth disorder**
  - Minimal laboratory evaluation
    Including IGF-1 (IGFBP-3), TSH/LT4, Celiac disease screening (+ karyotype for girls)
  - Wrist x-ray for bone age
  - Abnormal body proportions with normal or with unspecific findings in skeletal survey
  - Familial short stature with dominant autosomal inheritance pattern

Genetic regulation of growth

Severe phenotype

Monogenic conditions

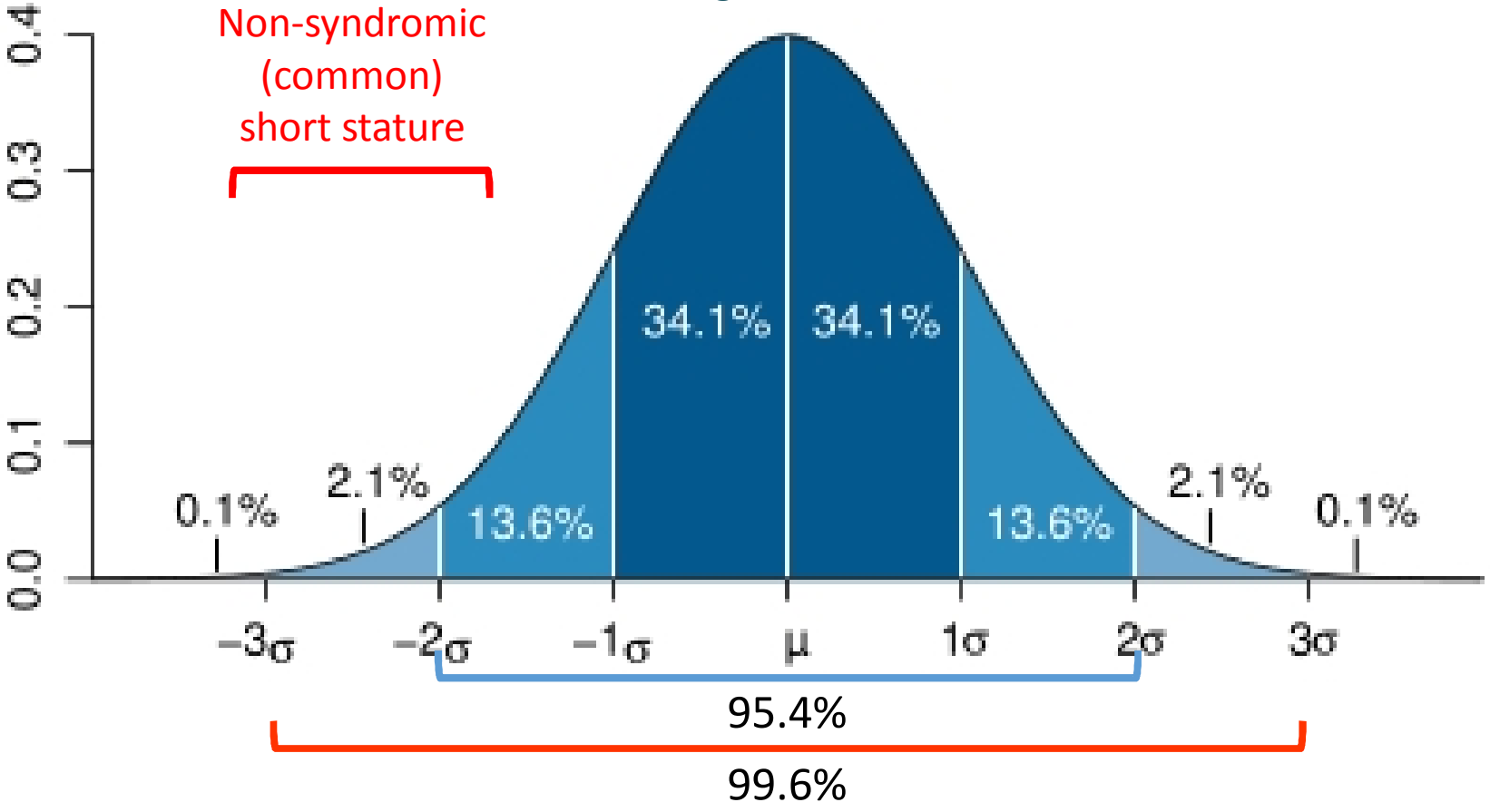
Height SDS < -3

Height variation

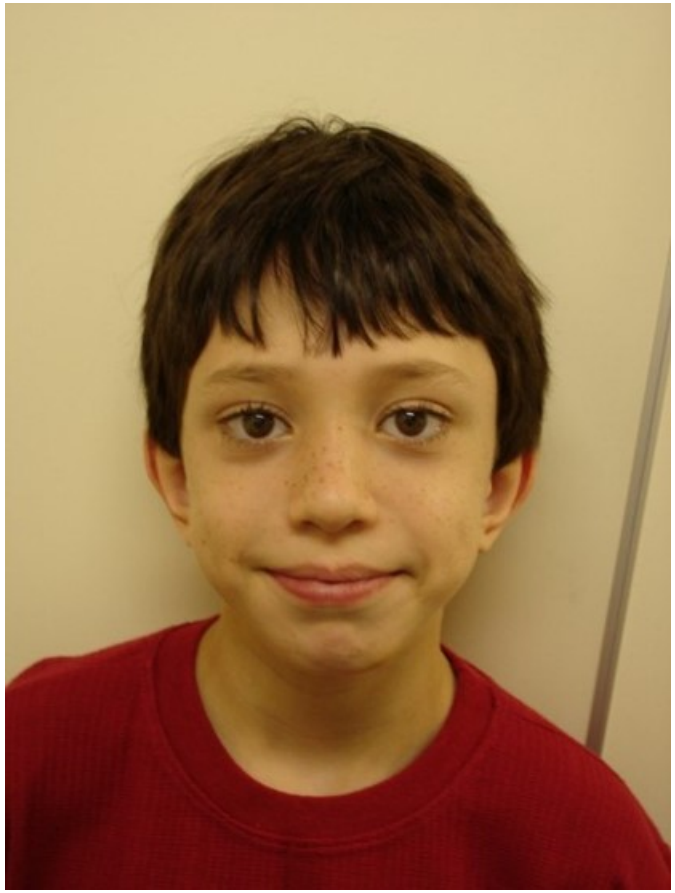
polygenic traits

Height SDS ± 2

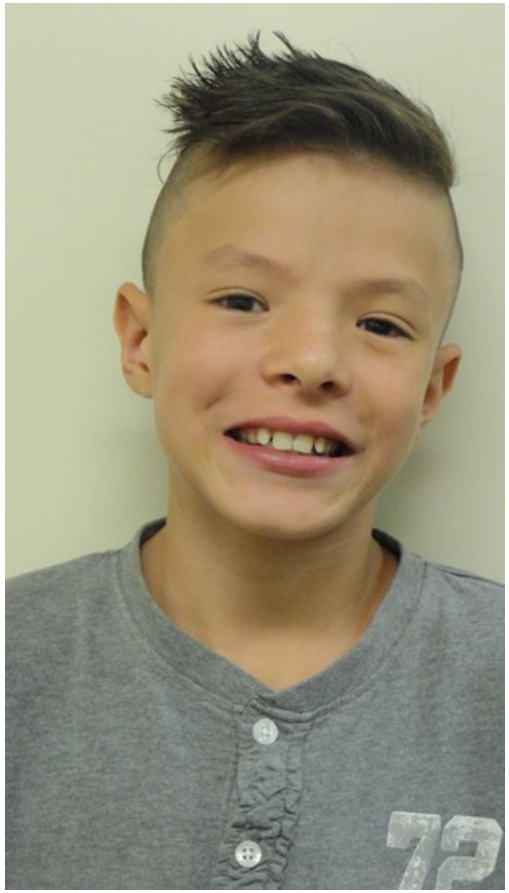
Non-syndromic (common) short stature



Mild phenotype of monogenic conditions?



c.922A>G (p.Asn308Asp)



c.417G>C (p.Glu139Asp)

c.922A>G (p.Asn308Asp)

PTPN11 mutations associated with Noonan syndrome

Mild clinical suspicion

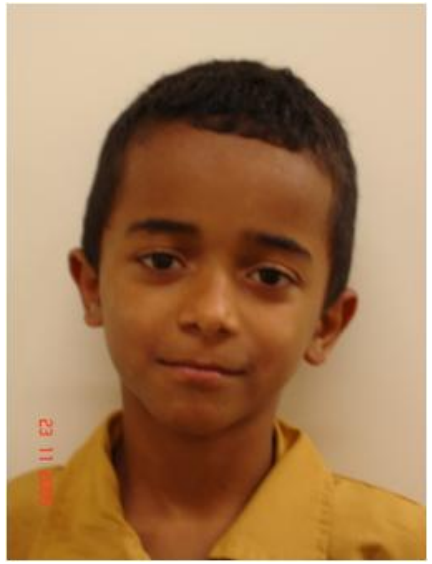
Children born SGA

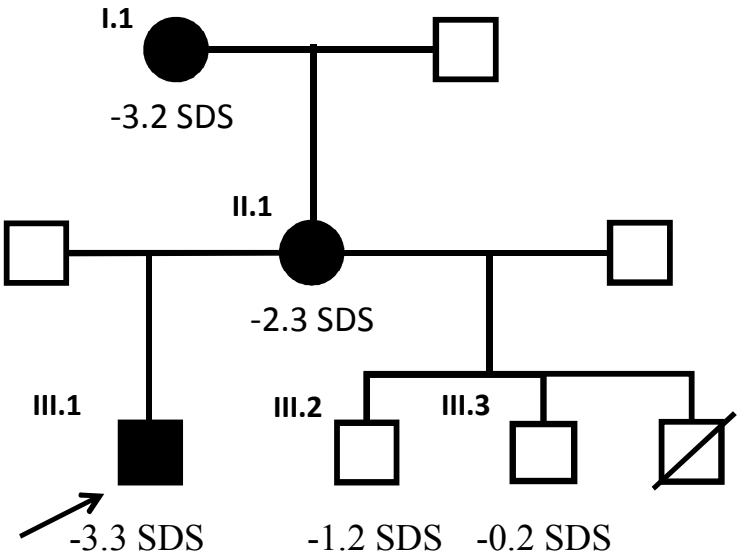
- 38 weeks
- 2480 g (-2.1 SDS)
- 47 cm (-2.0 SDS)

Normal head circumference
Normal neurodevelopment
Difficulty in school

CA 8.9 years
2 Cafe-au-lait spots
Height SDS -3.1

> 25 laboratory tests were done, including the GH release test and ophthalmologic evaluation





Mild clinical suspicion

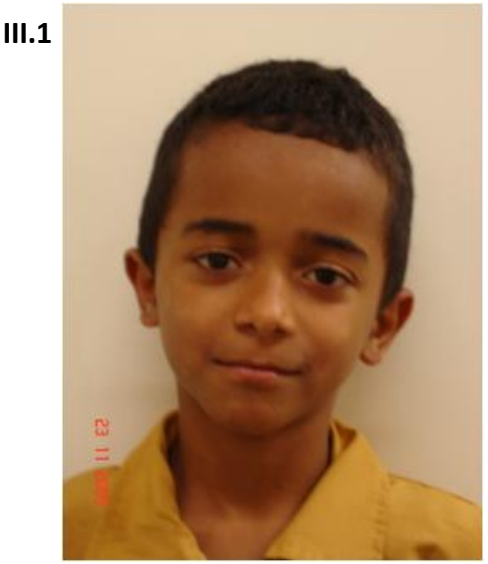
SGA without catch-up growth
Familial short stature
Difficulty in school
2 Cafe-au-lait spots

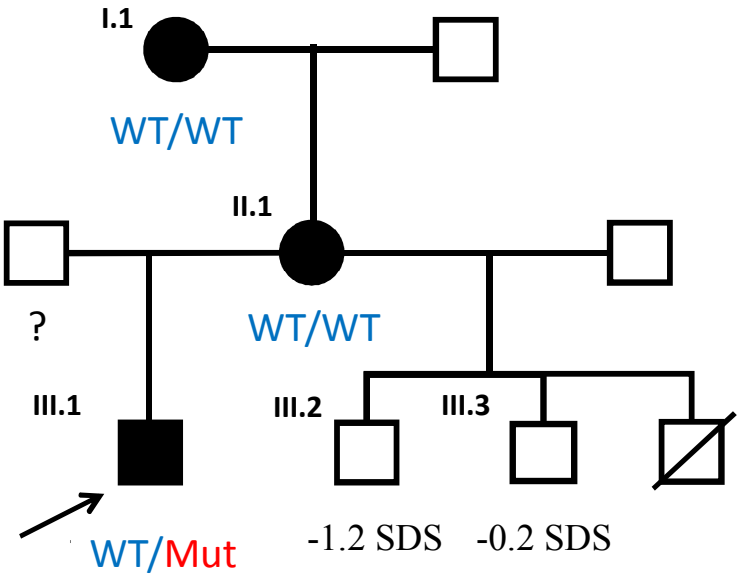
Treatment with rhGH was started (9y)

Treatment was discontinued

NF1
Heterozygous c.1261-1G>C (intron 11)

During the follow-up a Plexiform neurofibroma was diagnosed (age 18 y)

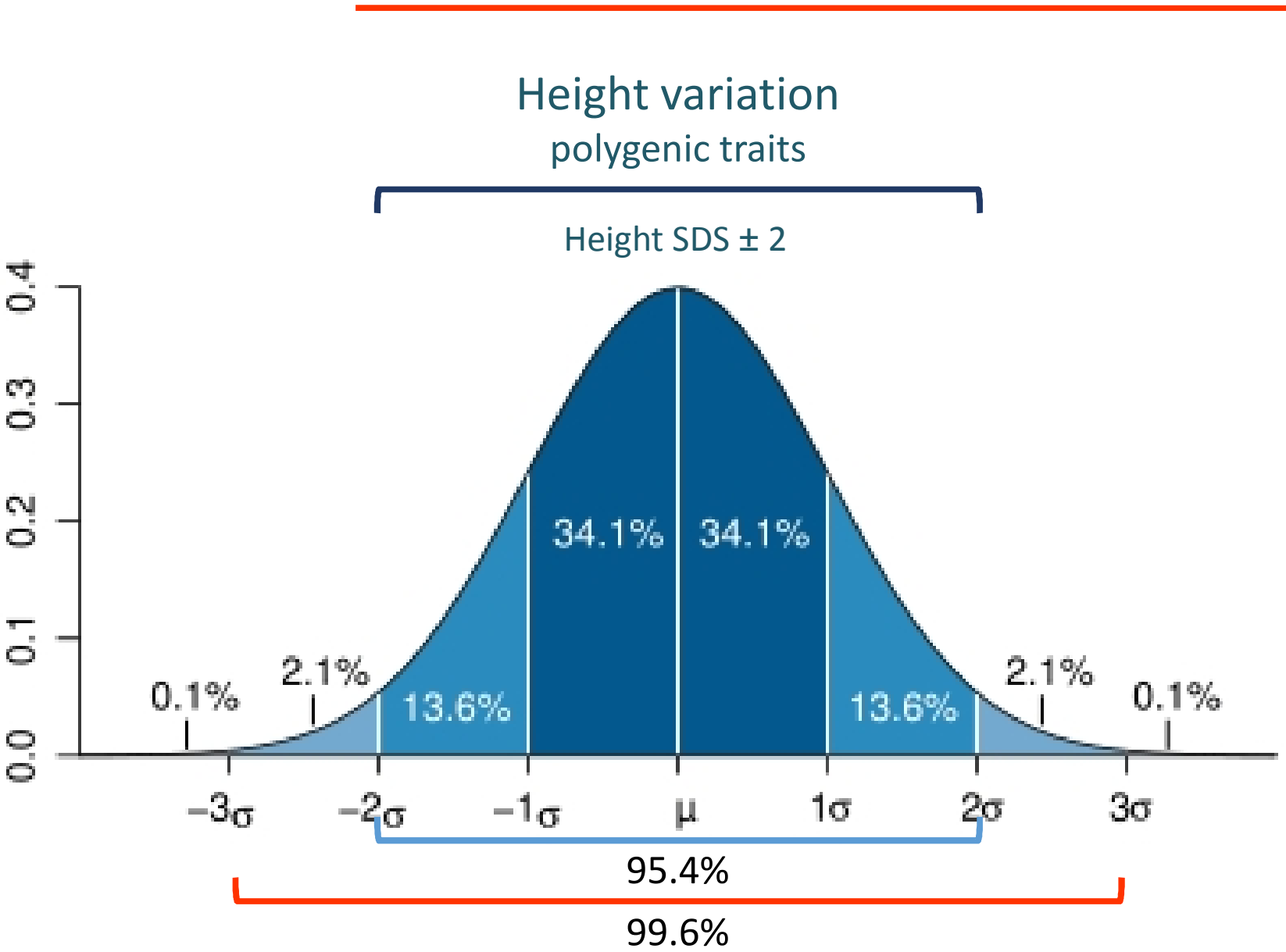




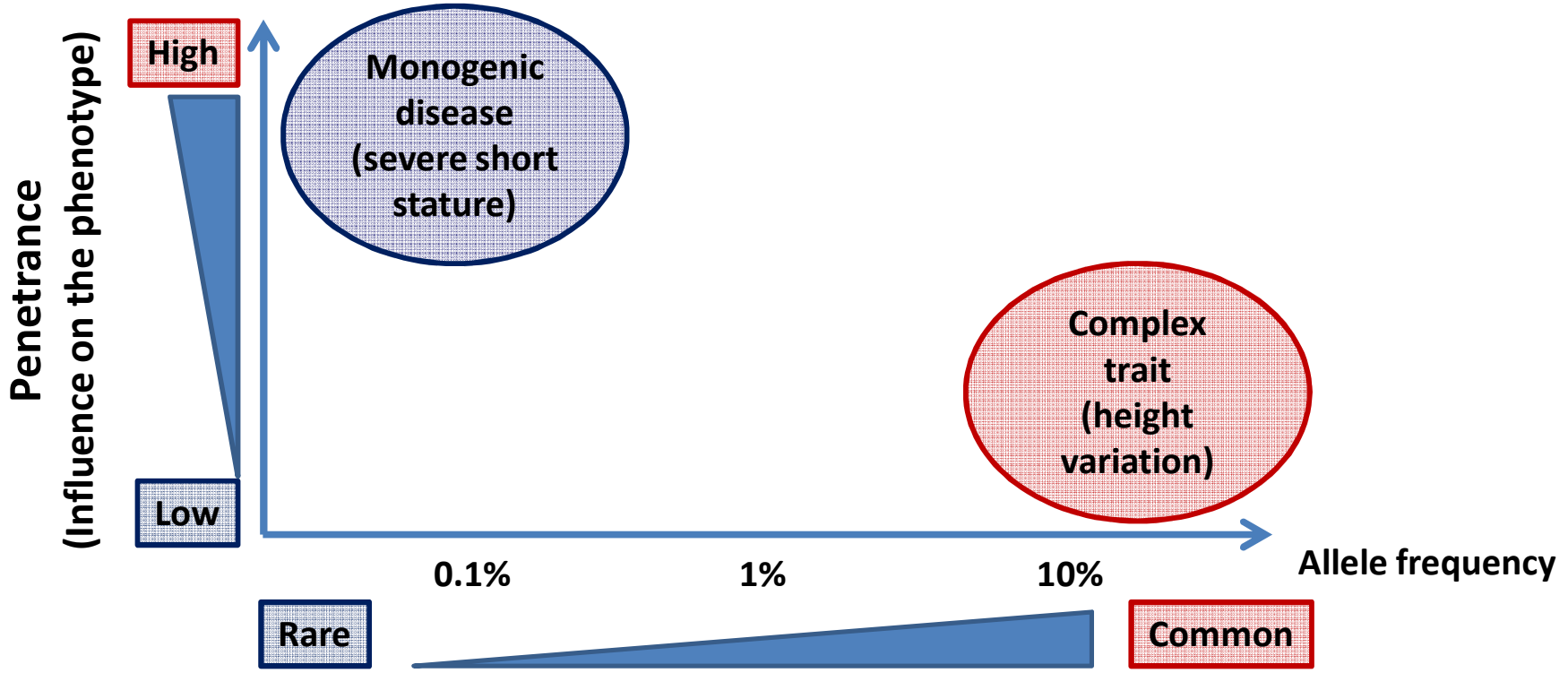
Genetic regulation of growth

Height variation

polygenic traits



Genetic influences on height

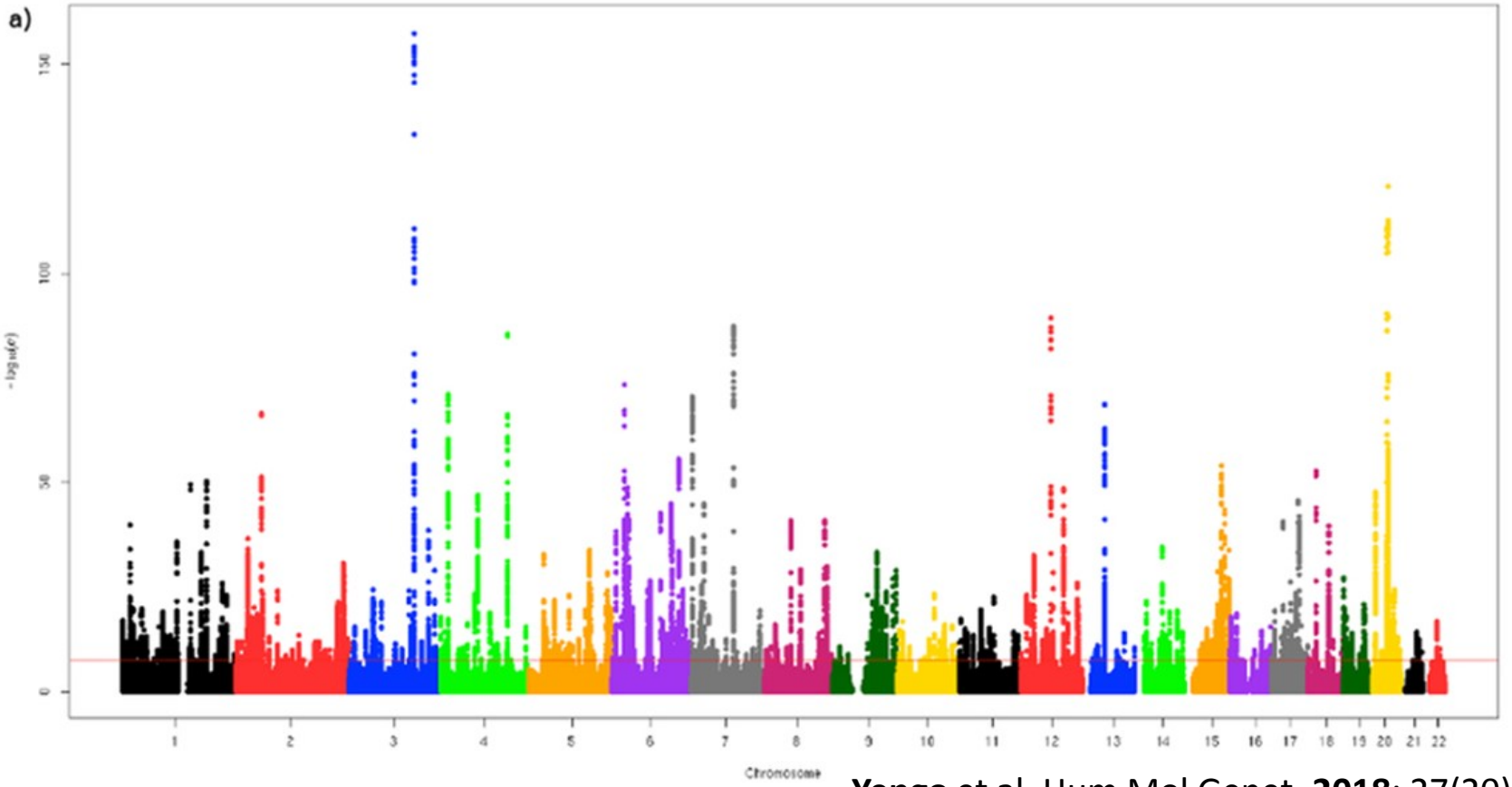


Common short stature = common variant with small effect size (polygenic)

Weedon *et al*. Nat Genet. 2007 Oct;39(10):1245-50
Wood et al. Nat Genet. 2014 Nov;46(11):1173-86

Meta-analysis of GWAS for height

Data from ~700,000 individuals
It was identified 3,290 variants (in 712 loci) that explained ~24.6% of the heritability for adult height (MAF > 5%)
Nearly all of the identified variants alter height by less than 1 mm



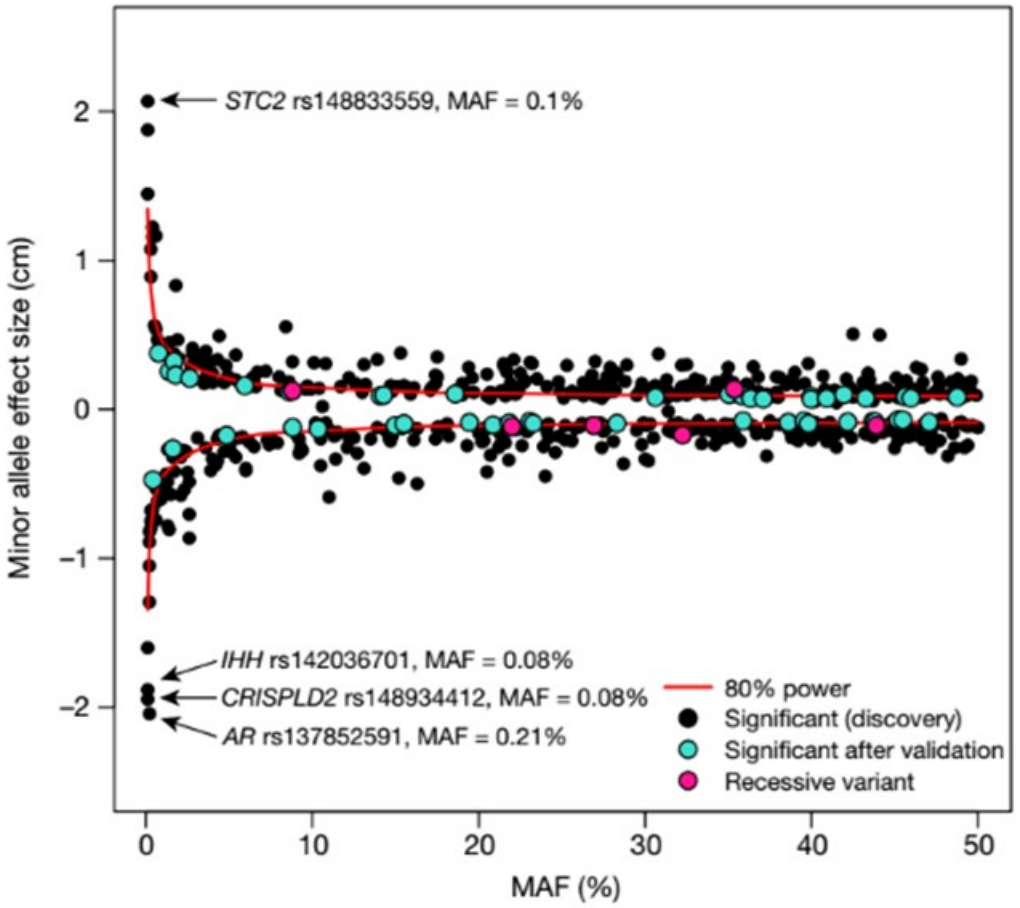
Yengo et al. Hum Mol Genet. **2018**; 27(20):3641-3649
Wood et al. Nat Genet. **2014**; 46(11):1173-86

Rare variants and adult height

A recently GWAS involving 711,428 adults and 241,453 variants (coding variants with a MAF≤5%).

Identification of 83 rare coding variants that alter height by 2 cm per allele

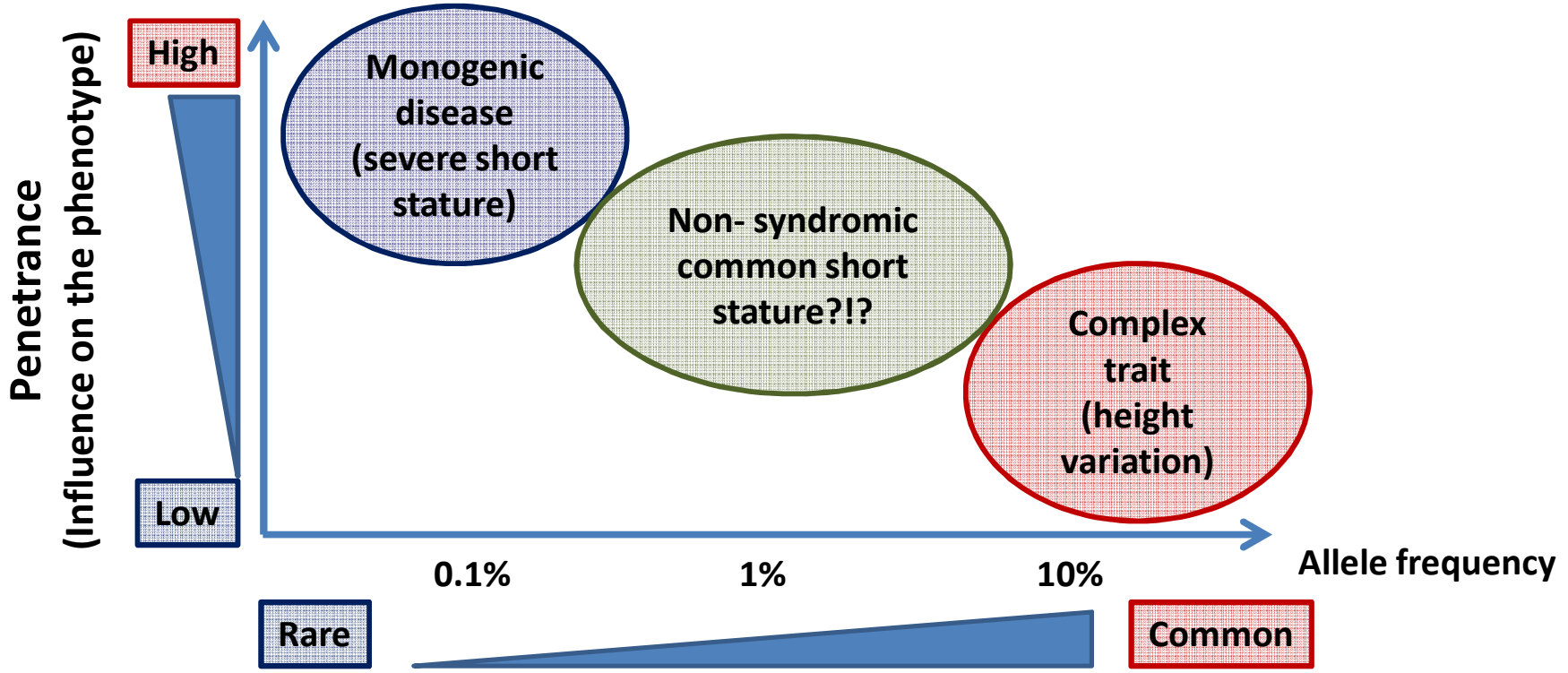
This effect is 10x greater than the average effect of common variants.



Although rare, these variants are important for predicting the risk of disease development for the individuals that carry them.

Marouli *et al.* Nature **2017**; 542(7640):186-190

Genetic influences on height



It is likely that among patients with less severe short stature, allelic variants with intermediate frequency and penetrance are responsible for the growth disorder

Genes and non-syndromic short stature

- *SHOX* – **Rao** *et al.* Nat Genet. **1997**; 16(1):54-63
- *NPR2* – **Vasques** *et al.* J Clin Endocrinol Metab ***2013***; *98(10):E1636-E1644*
- *ACAN* – **Nilsson** *et al.* J Clin Endocrinol Metab **2014**; 99(8):E1510-8
- *IHH* – **Vasques** *et al.* J Clin Endocrinol Metab ***2018***; *103(2):604-614*
- *IGF1R* – **Abuzzahab** *et al*. N Engl J Med **2003**; 349(23):2211-22
- *IGFALS* – **Domené** *et al*. N Engl J Med **2004**; 350(6):570-7
- *NPPC* – **Hisado-Oliva** *et al*. Genet Med. **2018**;20(1):91-97
- *GHSR* – **Pantel** *et al*. J Clin Invest **2006**; 116(3):760-8
- *GHR* – **Goddard** *et al. N Engl J Med.* ***1995***; *333(17):1093-8*
- *FGFR3* – **Kant** *et al*. Eur J Endocrinol **2015**; 172(6):763-70
- *Other…*

Each of these genes explain a small proportion of patients classified as ISS or SGA (≤ 2 %)

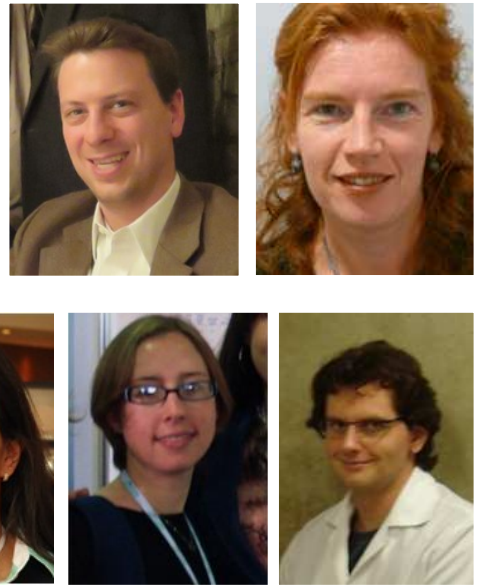
Exome sequencing in isolated short stature

30 families with autosomal dominant short stature analyzed by WES (2 to 5 individuals per family)

11:30 (37%) positive findings

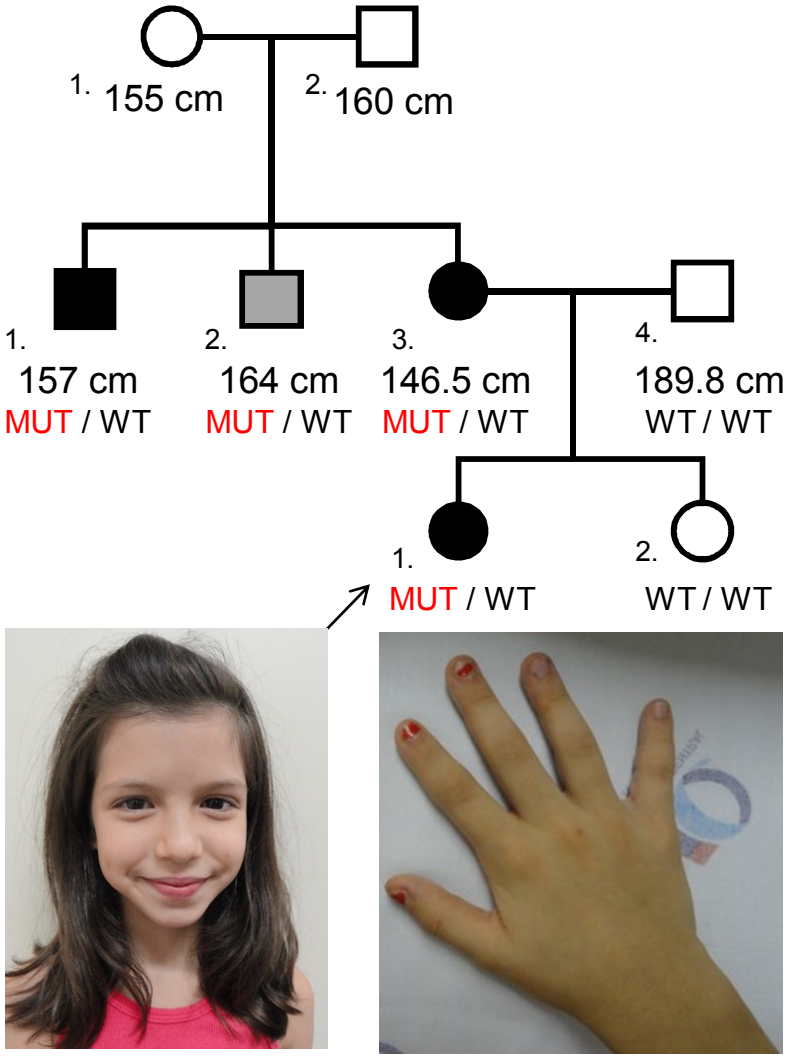
- ***NPPC*** *– 1st description*
- ***FBN1*** *– atypical or mild phenotype*
- *ACAN*
- *NPR2*
- *NF1 (2x)* – atypical or mild phenotype
- *SHOX*
- *LTB3*

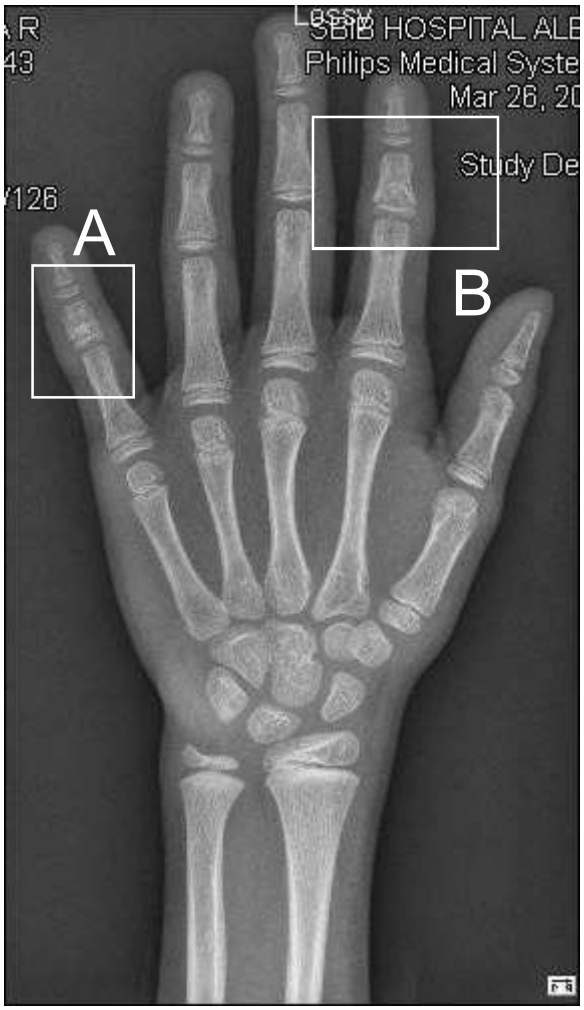
3 different heterozygous ***IHH*** variants identified in 3 of these families

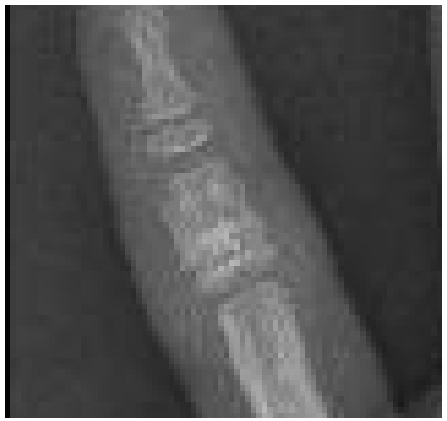


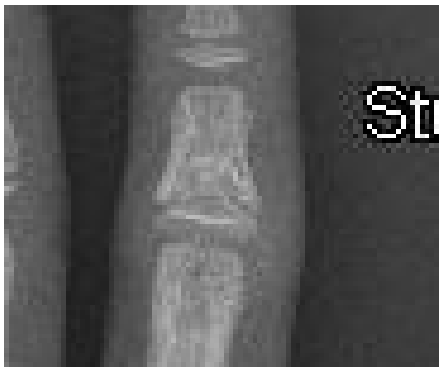
Vasques *et al*. JCEM **2018;** 103(2):604-614
Hisado-Oliva *et al*. Genet Med. **2018**; 20(1):91-97
Vasques *et al*. JPEM **2017**; 30(1):111-116
Gkourogianni *et al*. JCEM **2017**; 102(2):460-469
de Bruim *et al*. Horm Res Paediatr **2016**;86(5):342-348

Phenotype of ISS caused by *IHH* variants









Vasques et al. J Clin Endocrinol Metab. **2018**; 103(2):604-614

Multigene sequencing analysis of children born SGA with isolated short stature

55 Children born SGA with isolated short stature of unknown cause → Evaluation by exome (n = 16) or targeted gene panel (n = 39) sequencing

8 of 55 (15%) with pathogenic or probably pathogenic variants

Gene	Variant	Functional annotation	Inheritance pattern	ACMG/AMP
IHH	c.446G>A:p.Arg149His[1]	Missense	Inherited from affected mother	Likely Pathogenic
IHH	c.172G>A:p.Glu58Lys[1]	Missense	Inherited from affected father	Likely Pathogenic
NPR2	c.1249C>G:p.Gln417Glu[2]	Missense	Unavailable[4]	Pathogenic
NPR2	c.94C>A:p.Pro32Thr[2]	Missense	Inherited from affected mother	Likely Pathogenic
PTPN11	c.794G>A:p.Arg265Gln[2]	Missense	Unavailable[4]	Pathogenic
SHOX	c.503G>A:p.Arg168Gln[2]	Missense	Inherited from affected mother[5]	Likely Pathogenic
ACAN	c.532A>T:p.Asn178Tyr[1]	Missense	Inherited from affected mother	Likely Pathogenic
NF1	c.1261-1G>C[1]	Splice site acceptor	Unavailable[2]	Pathogenic

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Freire et al J Clin Endocrinol Metab. **2019**; 104(6):2023-2030

Take home messages

- In the coming years we will see several genetic causes of short stature revealed, explaining the phenotype of what we currently classify as short stature of unknown cause

- This knowledge will have a profound impact on the follow-up and treatment of these children

Muchas gracias

AlexJ
@endogenetica

AlexJ
@endogenetica

Emblematic patient

♀ SGA

- GA 40 1/7 weeks
- Weight 1630 g
- Length 39,5 cm
- Head circumference 25 cm
- (congenital syphilis)

Microcephalia
Developmental delays

Normal height parents,
- 3rd degree cousins

Age 2.5 y
Height SDS -5.8

BRCA1 (exon10)
c.2709T>A:p.C903* (homozygous)

Freire, Homma *et al.* Eur J Med Genet. **2018**; 61(3):130-133

Fanconi anemia and *BRCA1*

Autosomal recessive

SGA
Short stature
Microcephaly

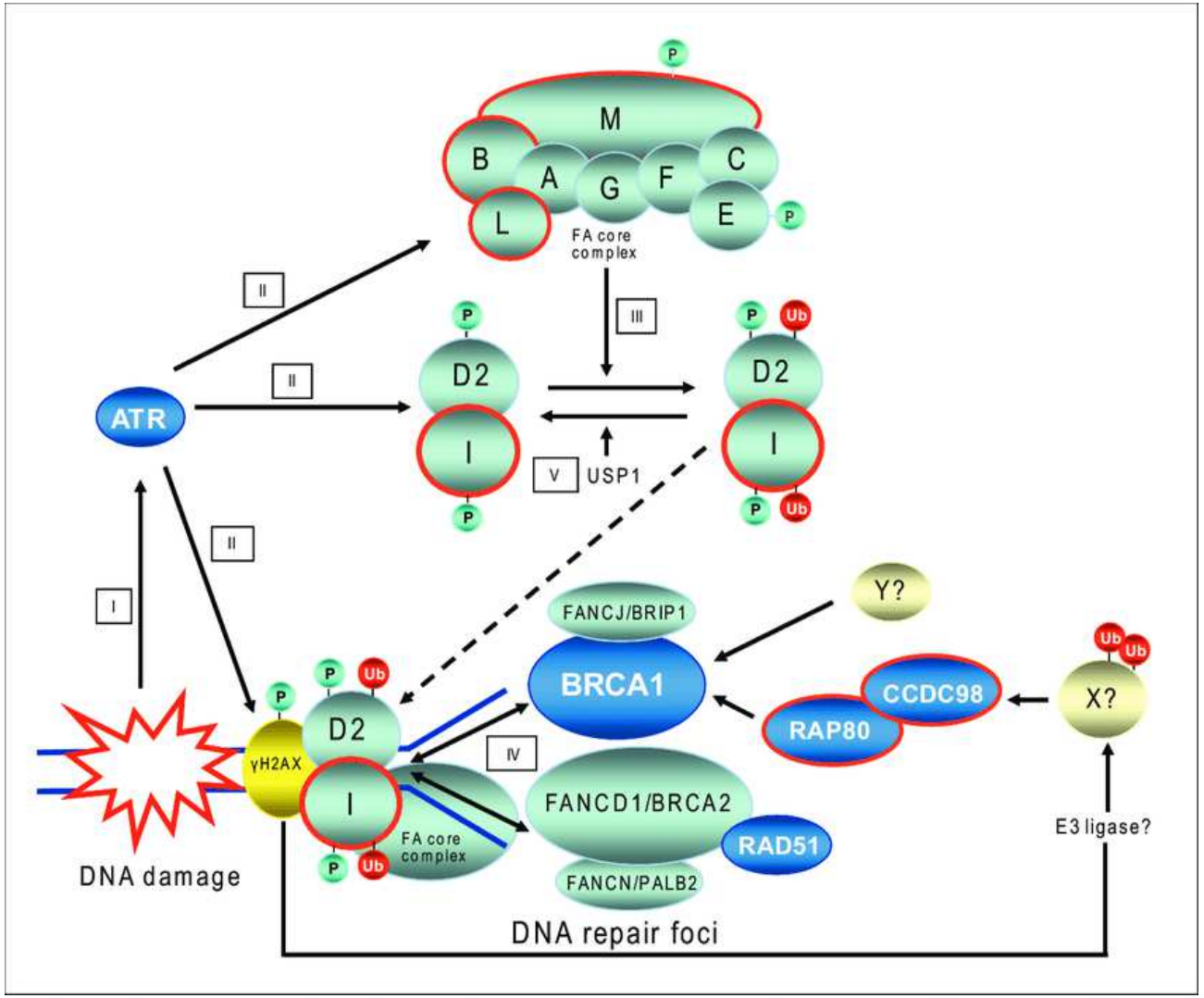
Congenital heart defect.

Thumb deformity
Thumb aplasia

Intellectual disability

Anemia
Neutropenia
Thrombocytopenia
Leukemia

Chromosomal breaks



Garcia & Benitez Clin Transl Oncol **2008**; 10:78-84

Emblematic patient

♀ SGA

- GA 40 1/7 weeks
- Weight 1630 g
- Length 39,5 cm
- Head circumference 25 cm
- (congenital syphilis)

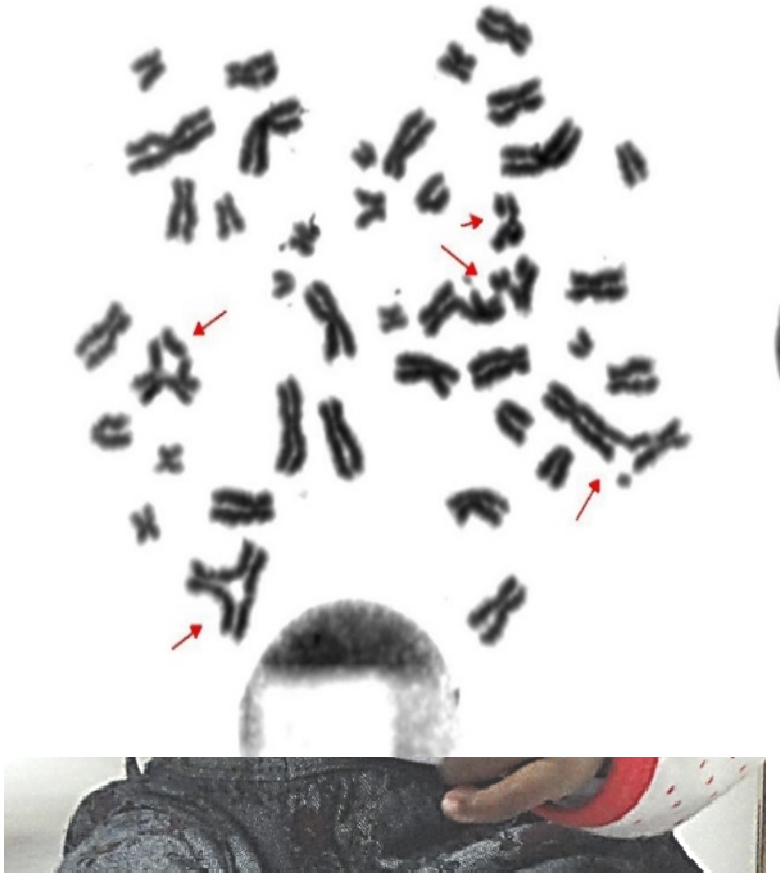
Microcephalia
Developmental delays

Normal height parents,
- 3rd degree cousins

Age 2.5 y
Height SDS -5.8

BRCA1 (exon10)
c.2709T>A:p.C903* (homozygous)

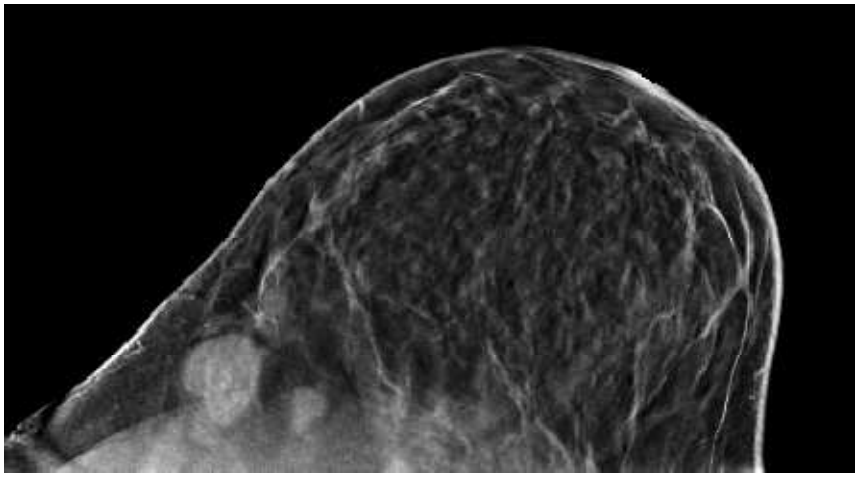
Chromosomal breakage induced by diepoxybutane (DEB)

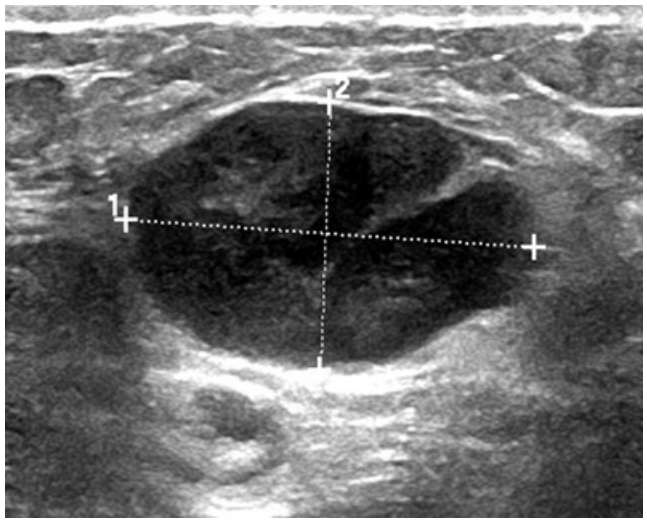


Freire, Homma *et al.* Eur J Med Genet. **2018**; 61(3):130-133

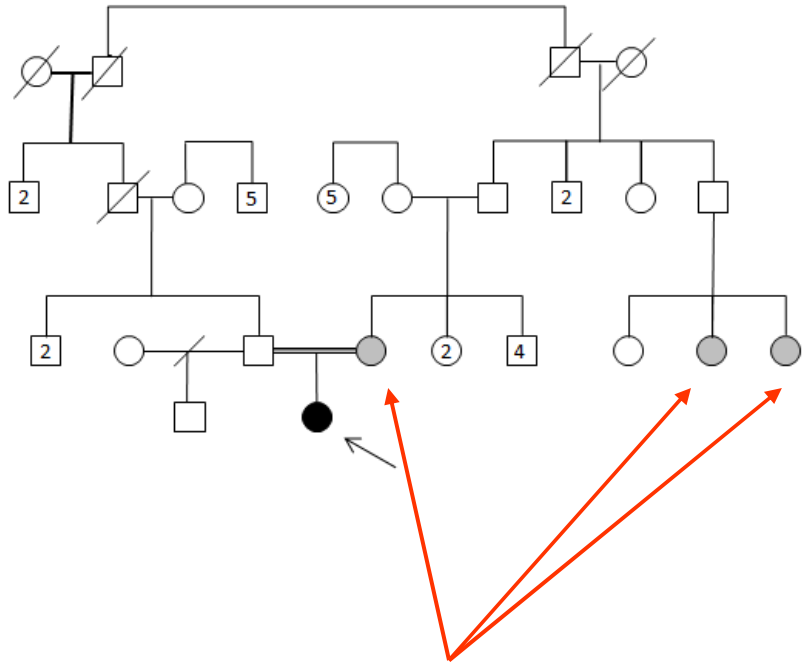
Emblematic patient

Mother (32 y)





Family



Brest cancer before the age of 40Y

Freire, Homma *et al.* Eur J Med Genet. **2018**; 61(3):130-133

Acknowledgments







SELA
Laboratório
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da FMUSP

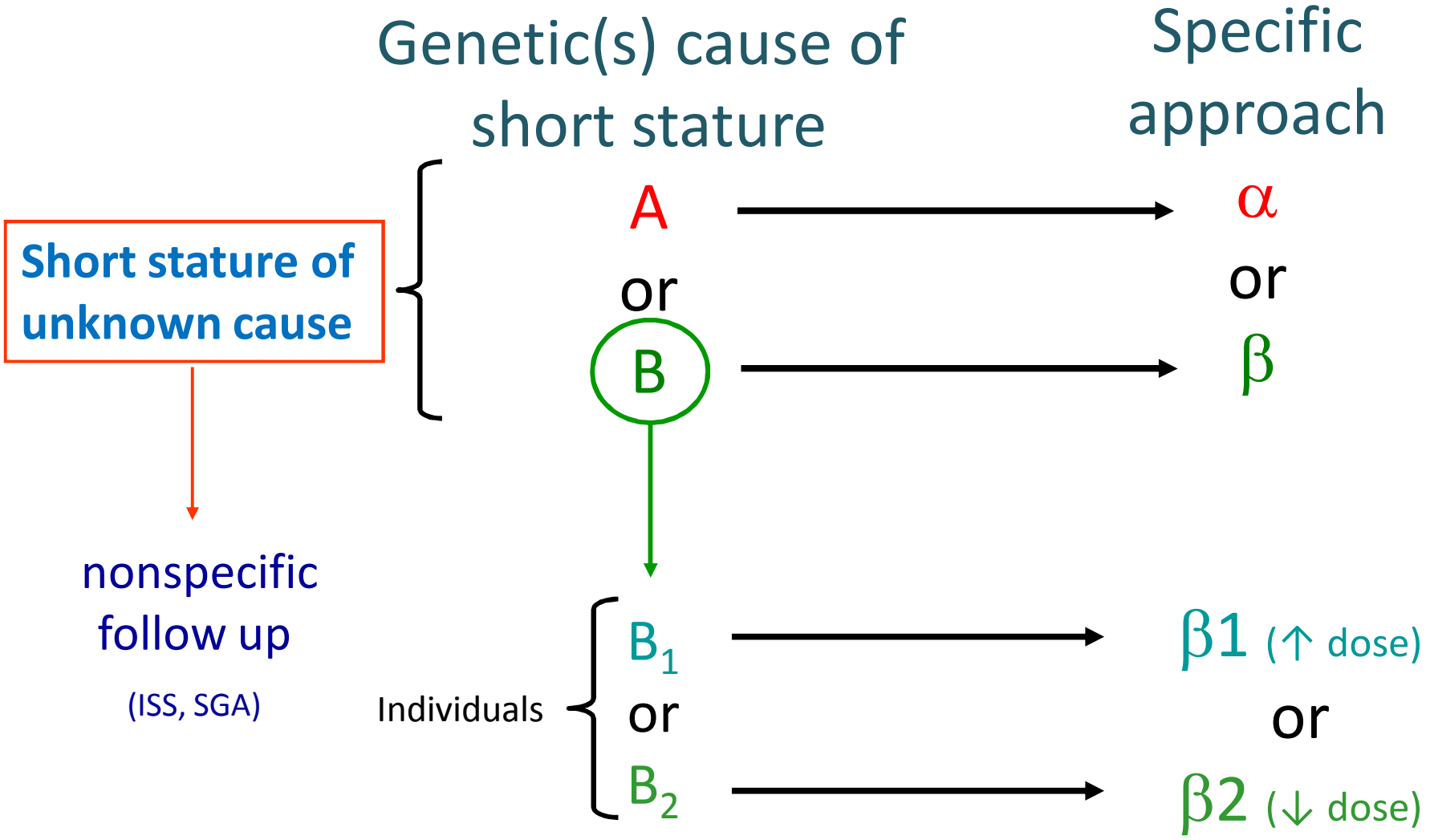
MEDICINA
USP

HC

FAPESP

CNPq

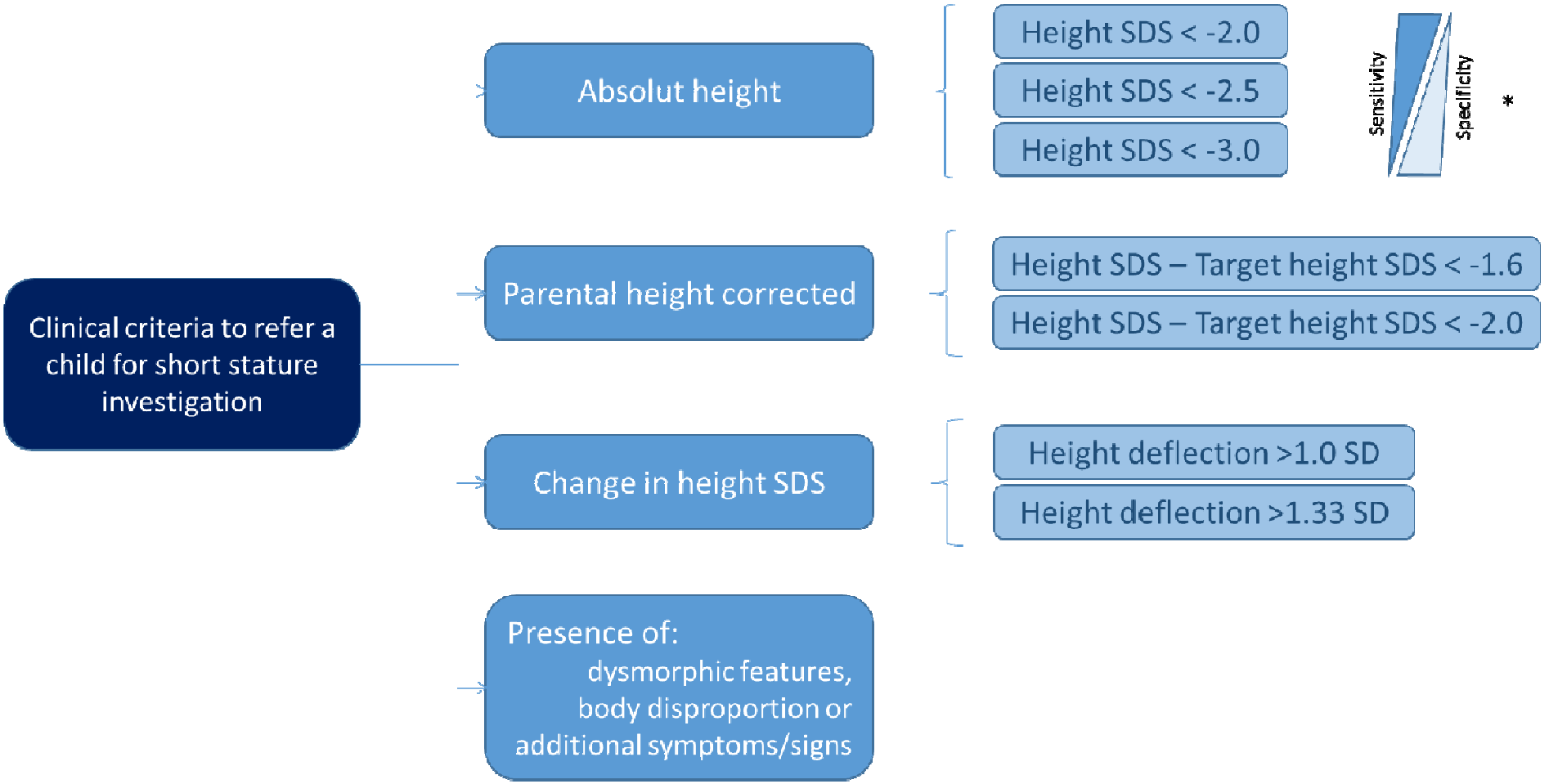
Moving toward precision medicine



Euan A Ashley. Nat Rev Genet **2016**;17(9):507-20

New paths to explore

Criteria for Investigation



Collett-Solberg *et al*. Growth Horm IGF Res. **2019**; 44:20-32

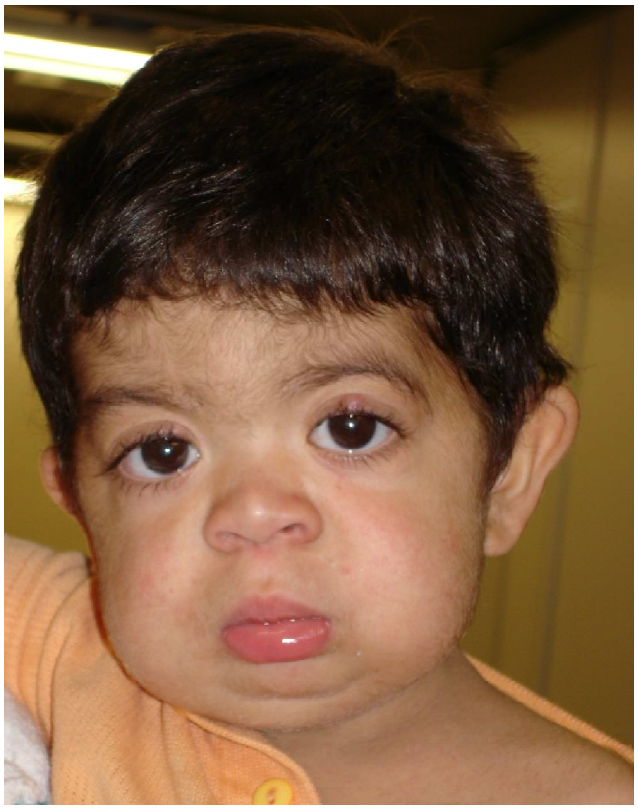
Final take home message

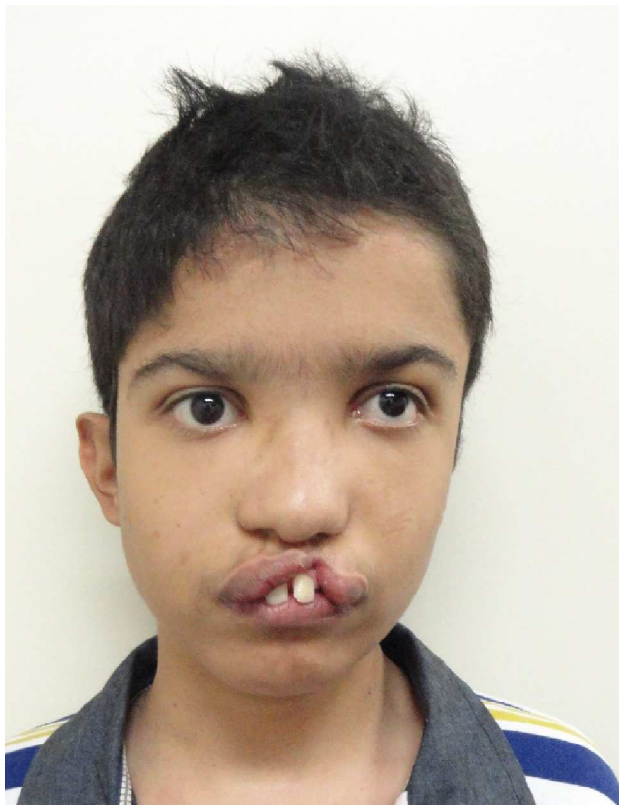
So when should we request an exome sequencing or molecular karyotype?

- After a detailed evaluation you are unable to obtain a clinical diagnosis and determine a candidate gene
- When the possible result has the potential to be useful for the patient and/or their family
- These exams need to be available and affordable
- We have to be aware that there is more possibility that the result will be negative

Differential diagnoses of short stature

Chronic systemic diseases





Syndrome

A syndrome is a set of medical signs and symptoms that are correlated with each other and, often, with a particular disease or disorder.