NGS
in the perinatal setting

Pr.K.Devriendt and Pr.I.Maystadt

Belgische Vereniging voor Neonatologie
Groupement Belge de Néonatologie
14.11.2019

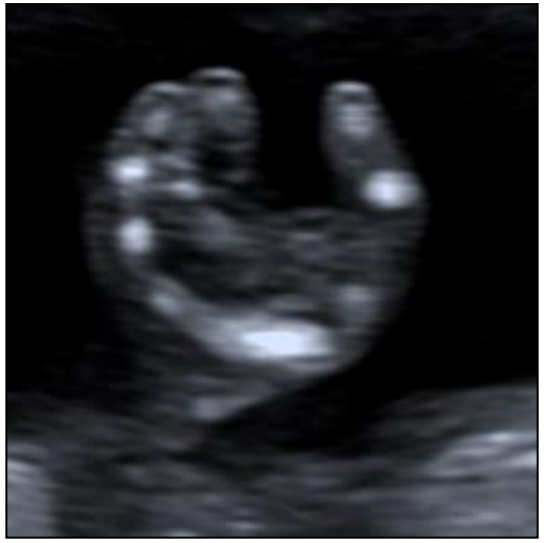
KU LEUVEN

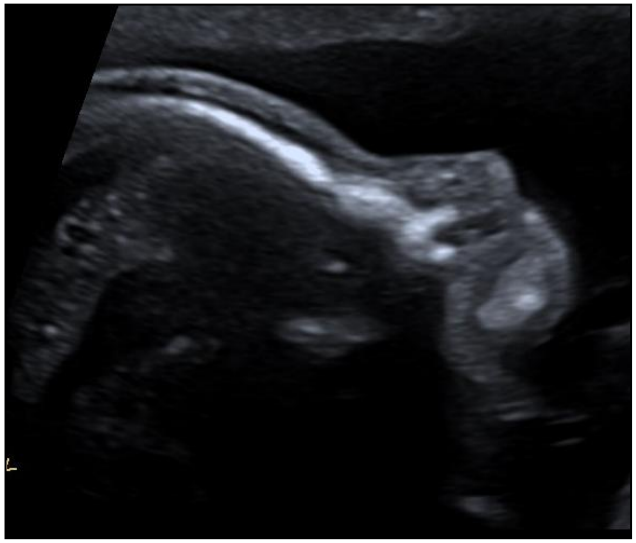
IPG

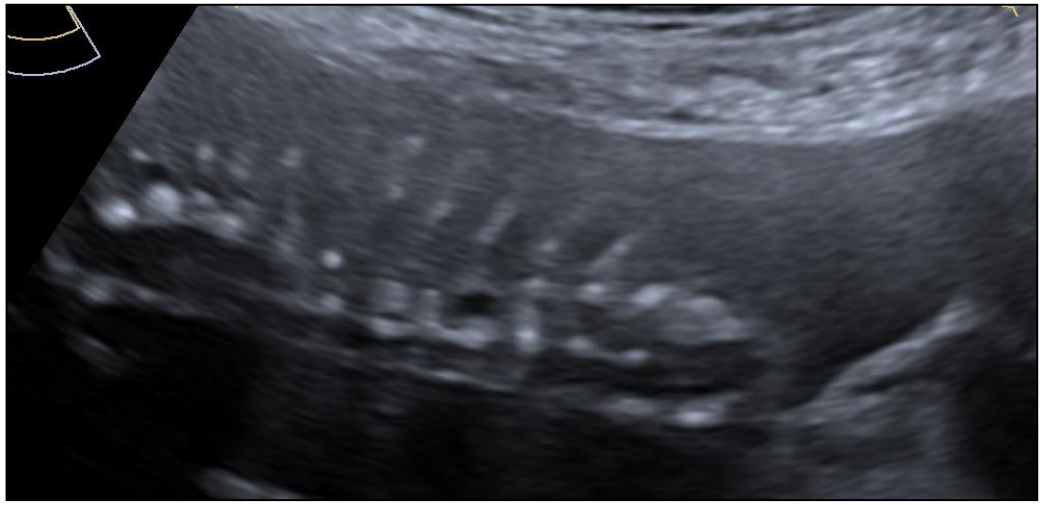
Prenatal

<u>17 weeks of gestation:</u>

- very flat midface
- short palate
- radial deviation of index fingers
- 1 rocker bottom foot aspect
- Vertebral abnormalities (lumb / thorac)
- Normal growth



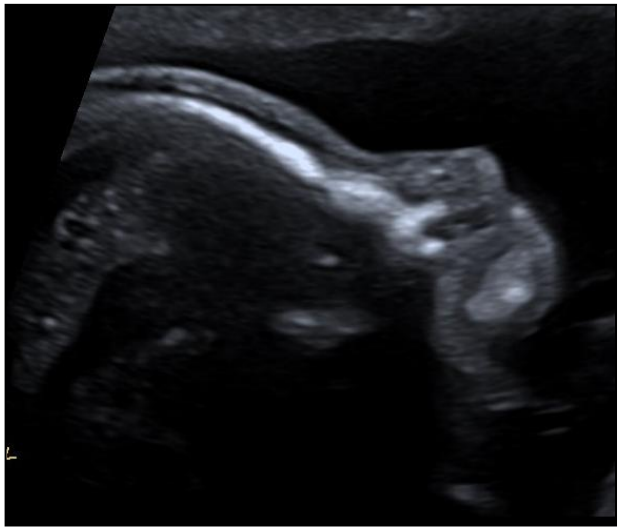




Postnatal

Early epilepsy
Progressive microcephaly
Severe developmental delay
Hypotonia

“epileptic encephalopathy”



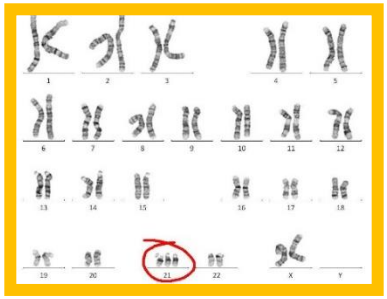
Diagnosis ?

Prognosis ?

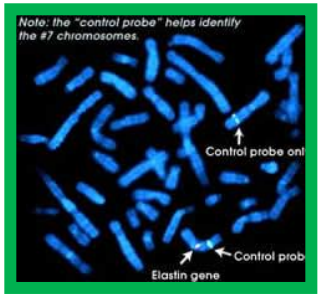
Recurrence risk ?

Pre/postnatal cytogenetic analyses: chromosomal abnormalities

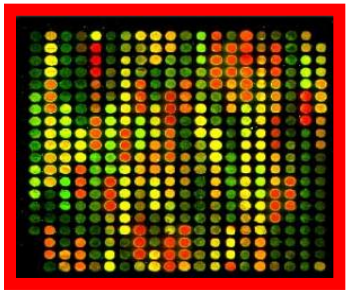
Karyotype



FISH



Array-CGH



1966 — 1994 — 2005

<u>Aneuploidies :</u>
Down, T18
T13, Turner.
<u>Large CNV's</u>
<u>(copy number variaties)</u> :
4p-, 5p- …

<u>Interphase FISH</u>
= quick diagnosis of aneuploidies
<u>Microdeletions</u> :
- *del22q11.2*
- *Prader-Willi*

multiple structural abnormalities without diagnosis
⇒ *genome-wide screening for smaller deletions / duplications*
⇒ *= CNVs*

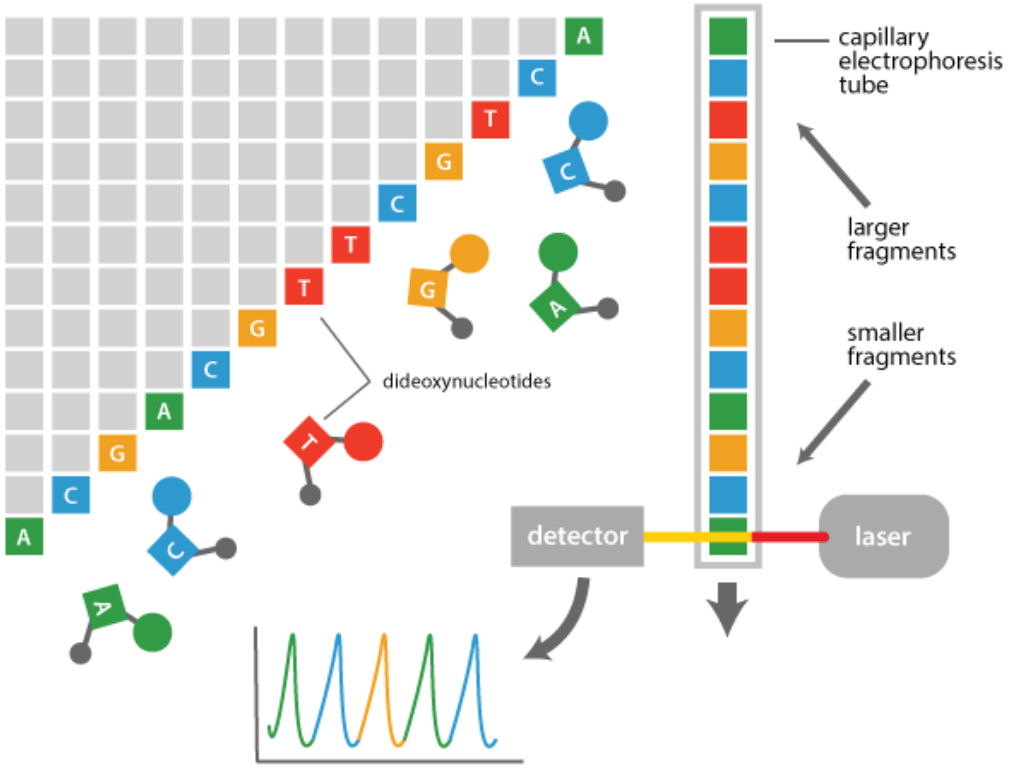
DNA

Prenatal DNA analyses: monogenic diseases

Sanger sequencing

1978	1986	1989	1999	2005
sikkel cell anemia	Cystic fibrosis		thanatoforic dysplasia	Noonan Syndrome
	↓ linkage analysis	↓ mutation analysis CFTR-gene	FGFR3-gene	PTPN11

Classical Sanger sequencing → 1 target gene



EEG
brain MRI
Metabolic tests
Ophthalmo

Array-CGH

MECP2
(Rett)

Metabolic

STXBP1

CDKL5

SCN1A

PCDH19

UBE3A

FOXG1

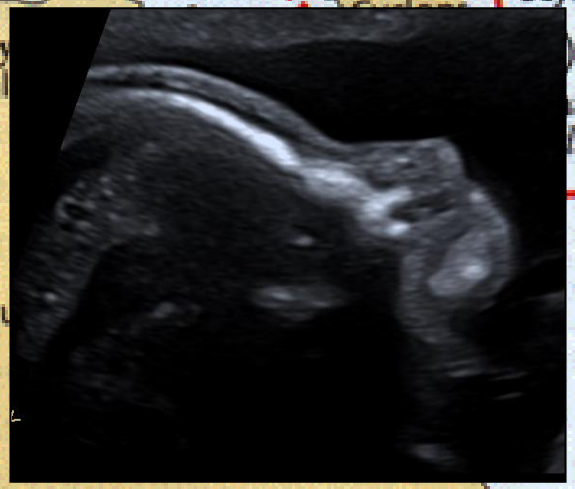
DYRK1A

"epileptic encephalopathy"

Etiology = FOXG1 mutation!

The diagnostic Odysseus: a journey of many years

Pregnancy: a few weeks to months …



Achondroplasia *Cystic fibrosis* *Kabuki* *Noonan* *deafness* *epilepsy* *Intellectual deficiency*







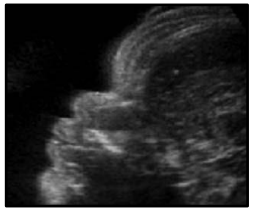
1 mutation | 1 gene | 2 genes | 7 genes | 65 genes | 85 genes | >500 genes

1 or a few genes
(1-5)

PCR
+ Sanger sequencing

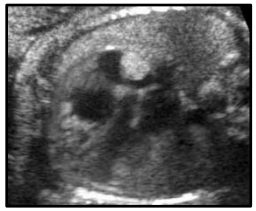
?

Achondroplasia

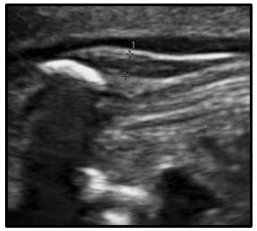


Cystic Fibrosis

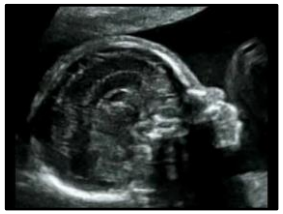
Tuberous Sclerosis



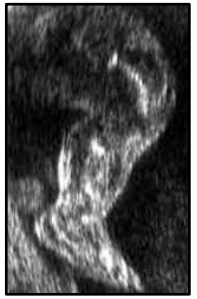
Noonan



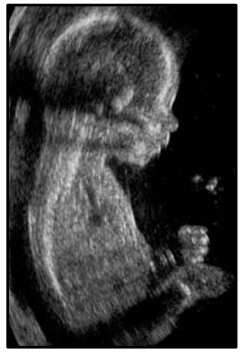
Brain Malformation



Skeletal dysplasia



Multiple malformations



1 mutation

1 gene

2 genes

14 genes

95 genes

111 genes

>1000 genes

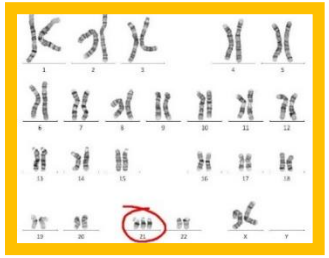
1 of a few genes
(1-5)

PCR
+ Sanger sequencing

?

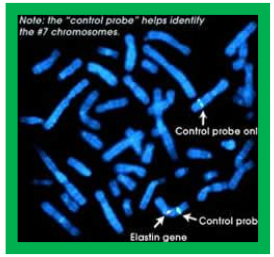
New technologies in genetic testing?

Karyotype

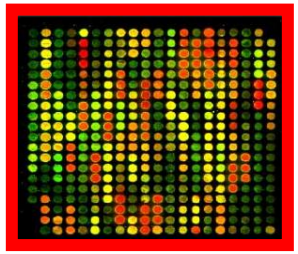


DNA

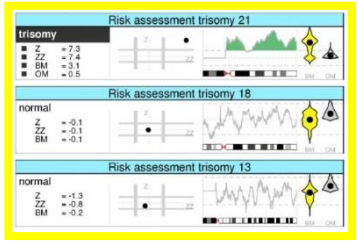
FISH



Array-CGH



NIPT



NGS°

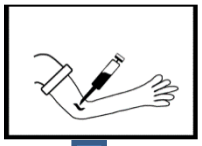
1966 1978 1994 2005 2011 2015

° Next generation sequencing


Le Séquenceur
Nouveau
est arrivé !

Newest genetic diagnostic testing: NGS

from sequential to parallel

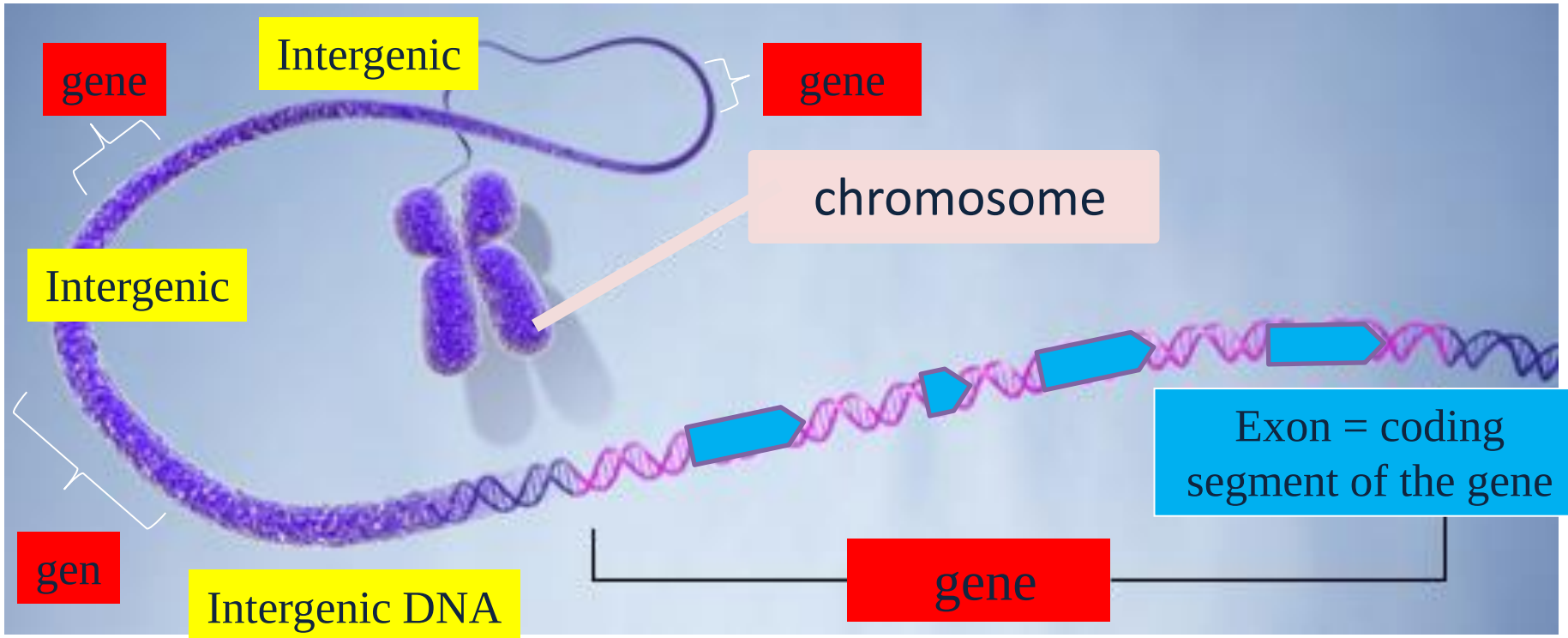


blood DNA

ATTGTACGTGATGACCAGTGGAAT
ACCGTAAGGTAAAGTACCGTGTAC
TTGGTTGGAACGTAGACTGAATGC
CAACCCTGGTATTGGTGTCCCGTG
TACAAGGTTAGTAATGTACCATTGA
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC
TGTGGTGCAGTACAGATACAGTAC
AGATTAGCAGAAATGCAGATTTAGT

Massive Parallel Sequencing = Next Generation Sequencing

DNA sequencing



Exome = 180,000 exons, comprising most of the mutations
= sequencing of all coding segments of all genes
= 1% of the whole genome

HOW DOES IT WORK

1. SEQUENCING : massive and parallel

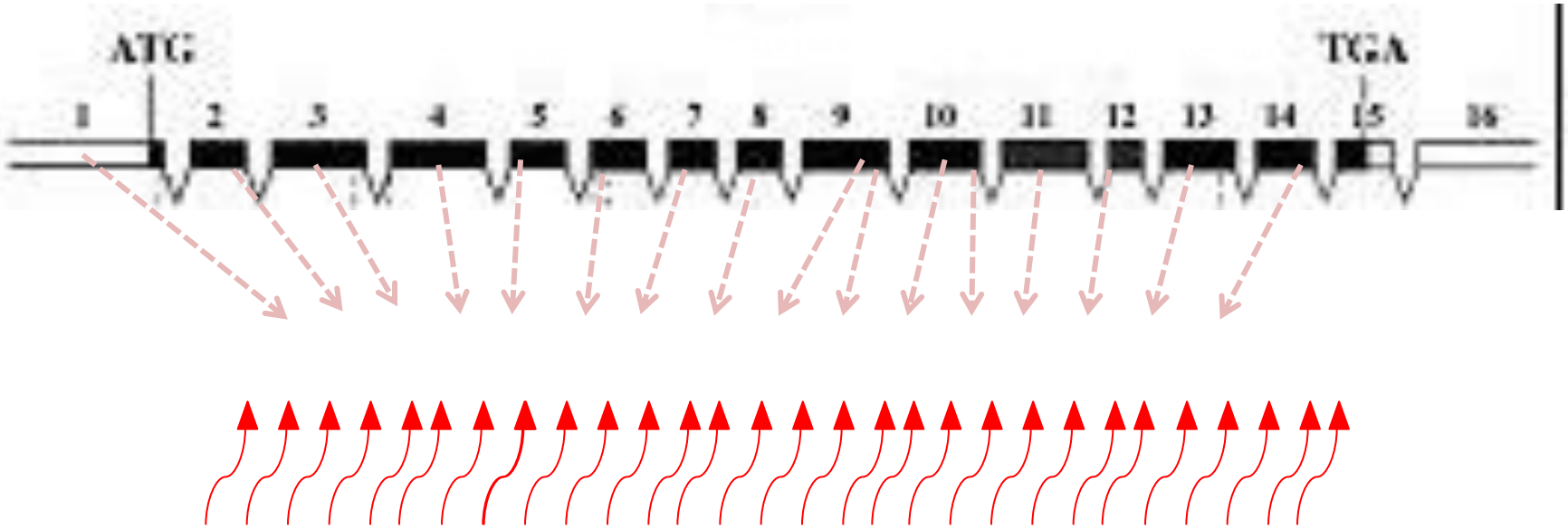
$\Rightarrow$ huge amount of data

2. IDENTIFICATION OF THE VARIANTS
   - What do we detect?
   - What do we miss?

3. INTERPRETATION OF THE VARIANTS

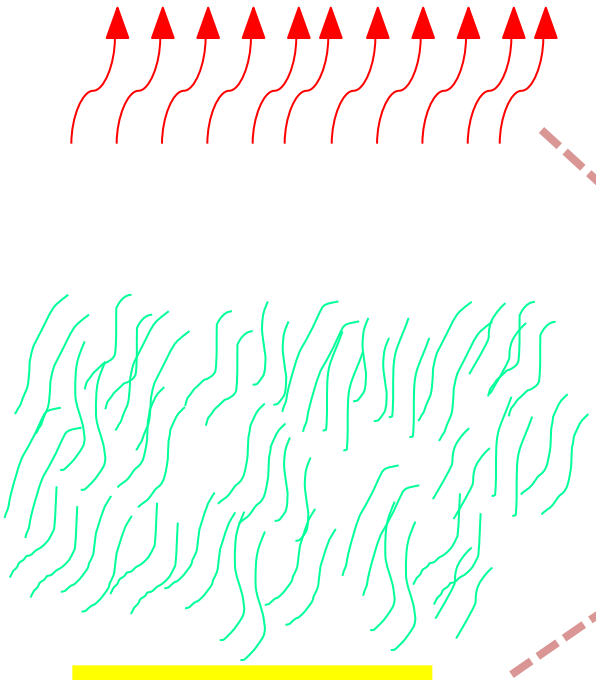
KU LEUVEN

IPG

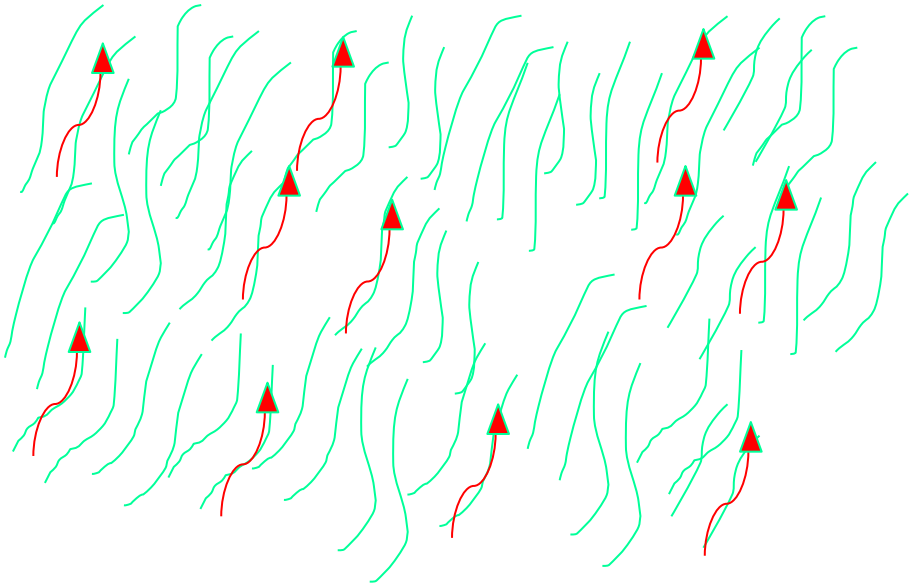


DNA fragments *(synthetic oligonucleotides)*
= complementary to targeted gene fragments that we want to investigate

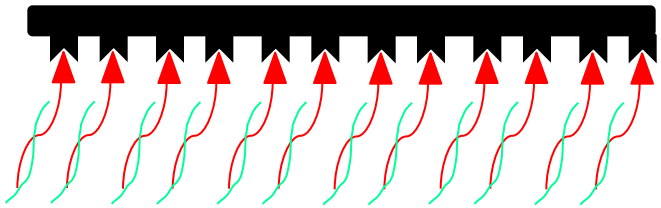
Capture probes

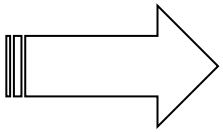


Patient DNA



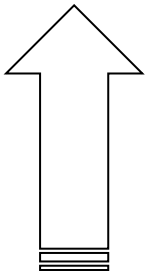
hybridisation



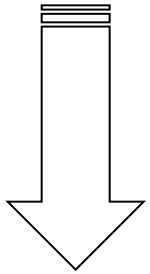


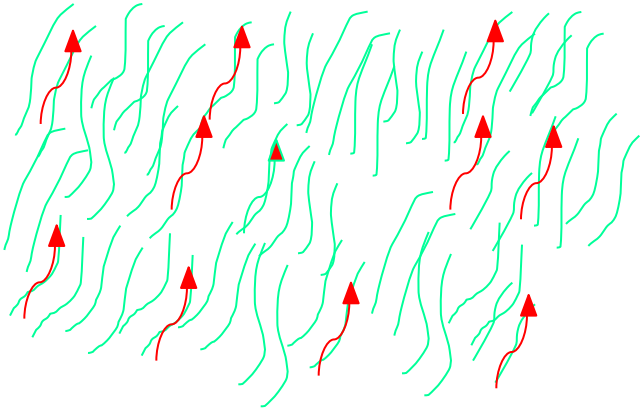
Enriched library

capture

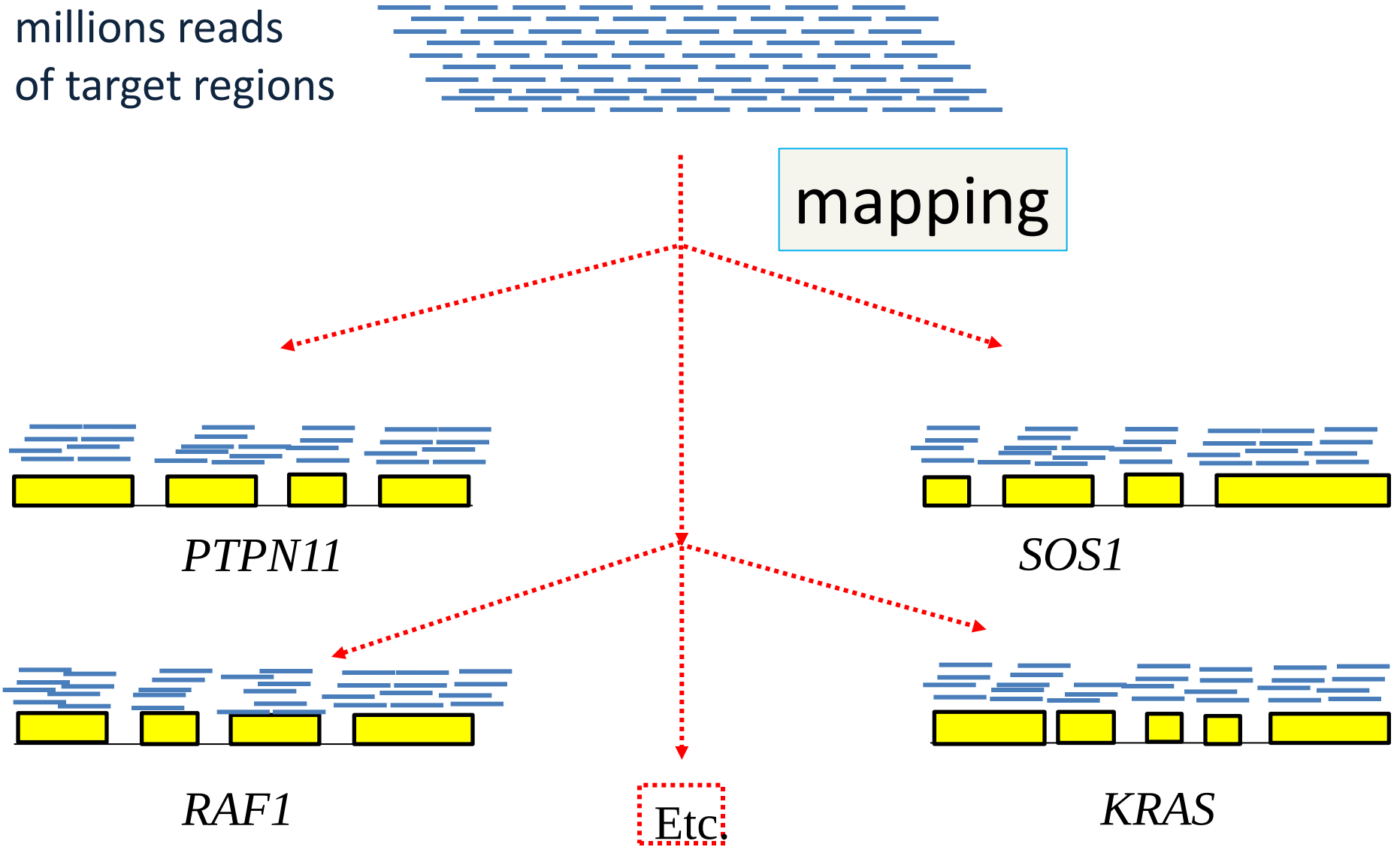


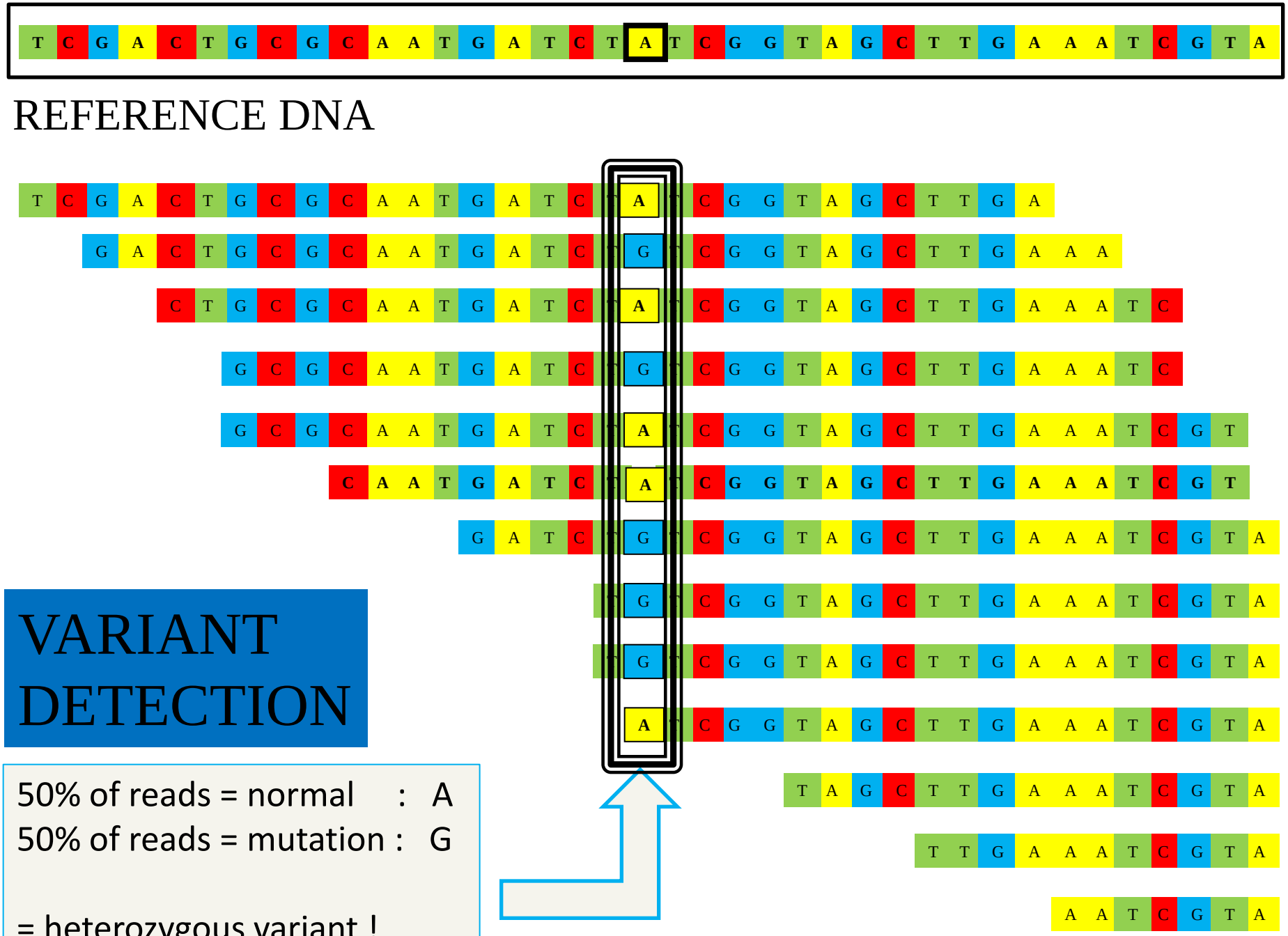
massive
parallel
sequencing





Millions of reads
= short sequences
of target regions


millions reads
of target regions
mapping
PTPN11
SOS1
RAF1
Etc.
KRAS


TCGACTGCGCAATGATCTATCGGTAGCTTGAAATCGTA
REFERENCE DNA
VARIANT DETECTION
50% of reads = normal : A
50% of reads = mutation : G
= heterozygous variant !

Gene panels :

target = specific clinical conditions with genetic heterogeneity

cardiomyopathies, nephropathies, cardiopathies, rasopathies, Neuromuscular diseases, aorta dilatation, immune disorders, epilepsy, brain malformations, …

Mendeliome / Clinical exome :

target = all exons of the genes already linked to genetic disorders

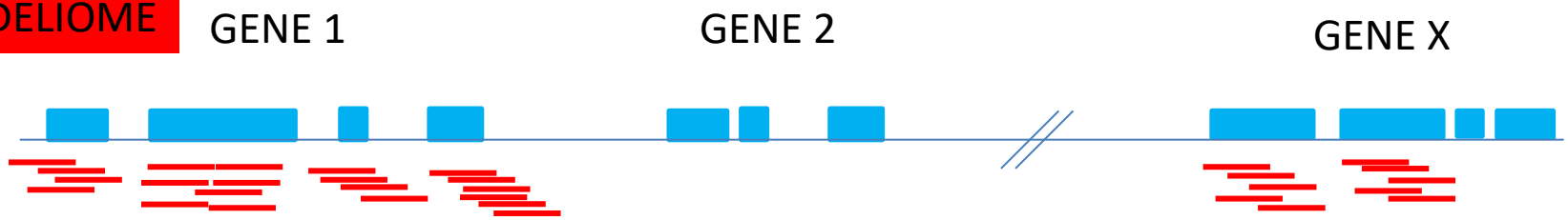
Whole exome (WES) :

target = all exons (coding fragments) of all genes

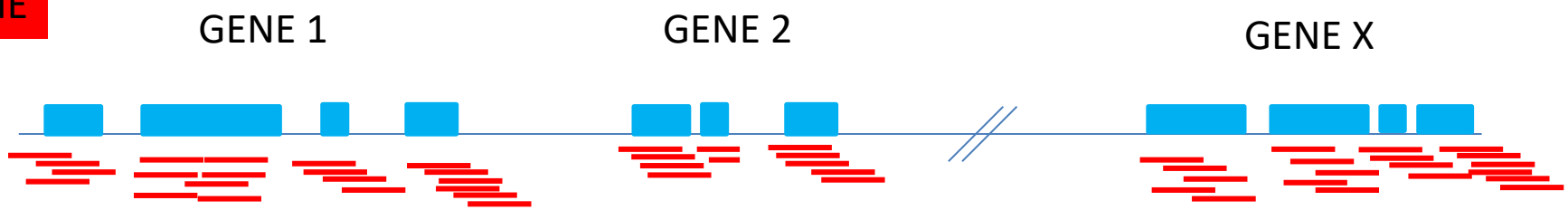
Whole genome (WGS) :

Complete genome (without prior capture)

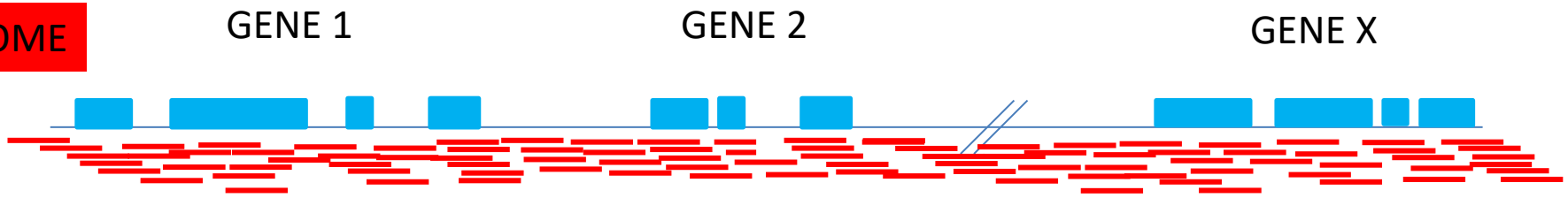
PANEL / MENDELIOME



EXOME



GENOME



1. SEQUENCING : massive and parallel

$\Rightarrow$ huge amount of data

2. IDENTIFICATION OF THE VARIANTS

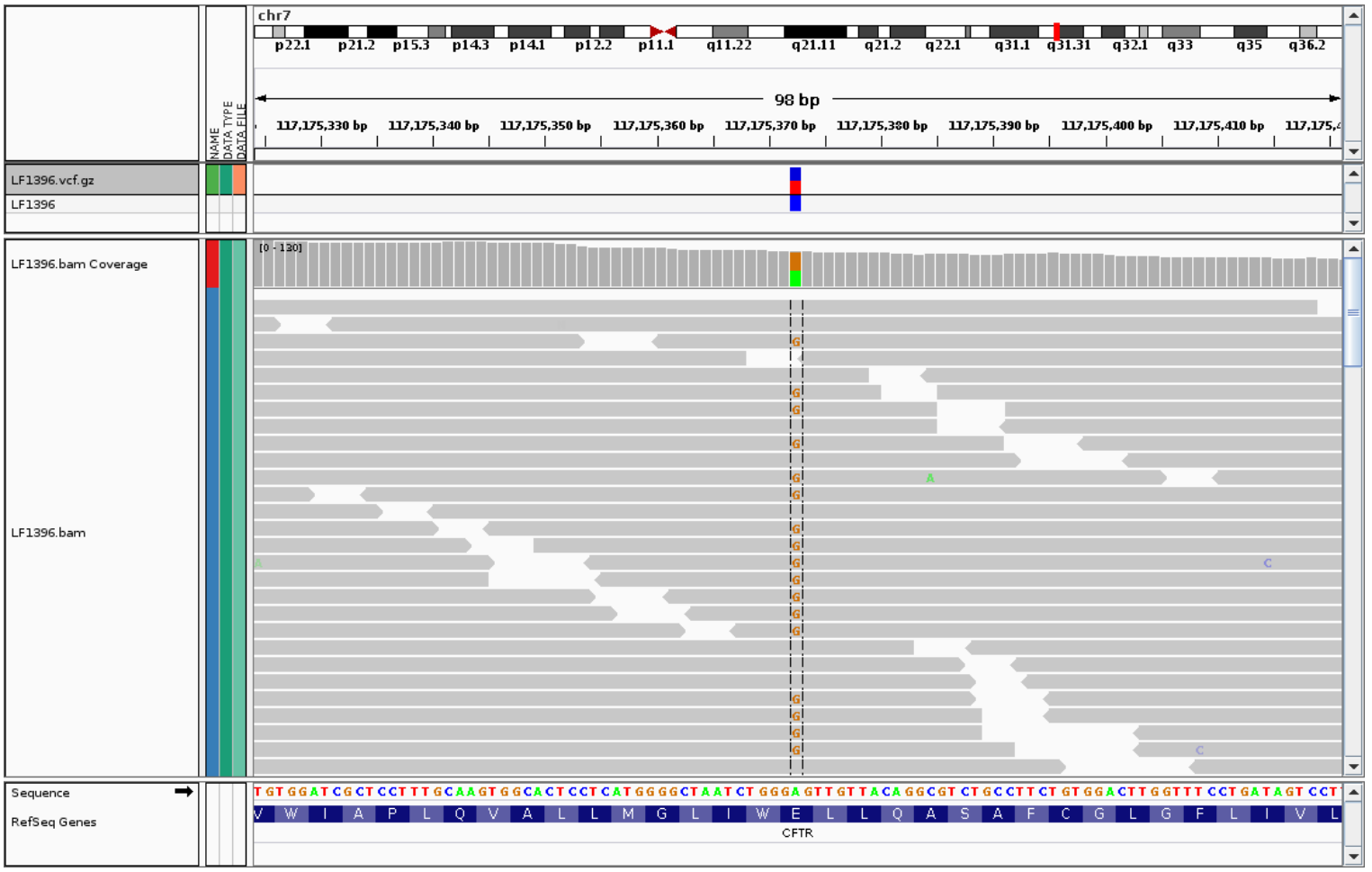
- What do we detect?
- What do we miss?

3. INTERPRETATION OF THE VARIANTS

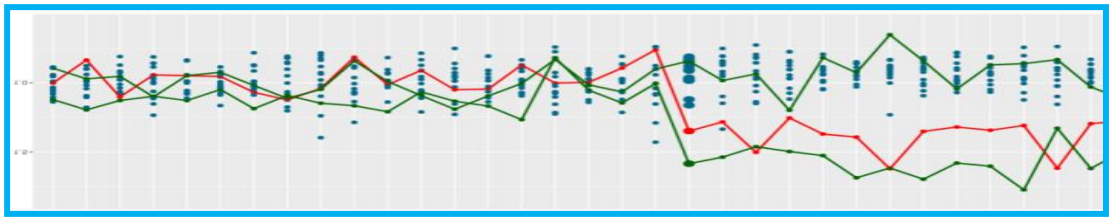
KU LEUVEN

IPG

1. Nucleotides substitutions/del/ins



2. CNVs (Copy Number Variations)



But CNVs detection is still challenging …

Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study

Rauch et al. Lancet, 2012

45%

Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability

De Light NEJM 2012

16%

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

Yang, NEJM 2013

25%

Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data

Wright Lancet 2014

27%

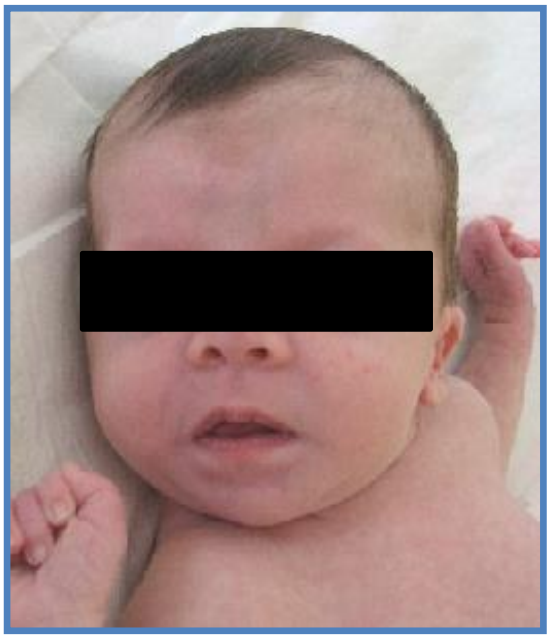
Examples of NGS testing in perinatal setting

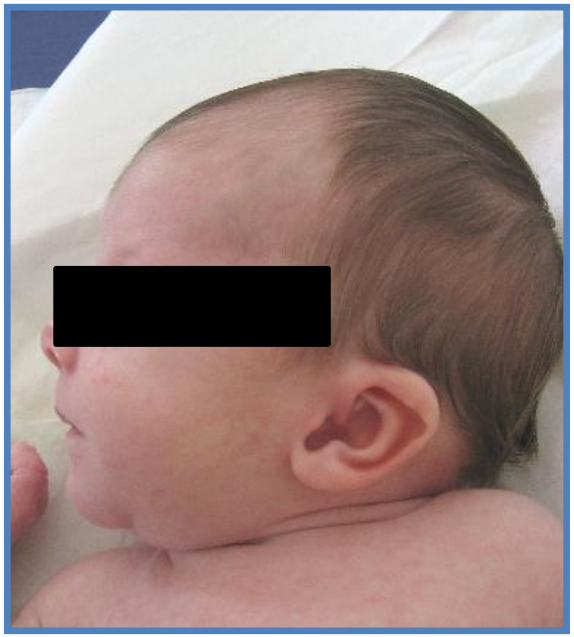
Succes stories

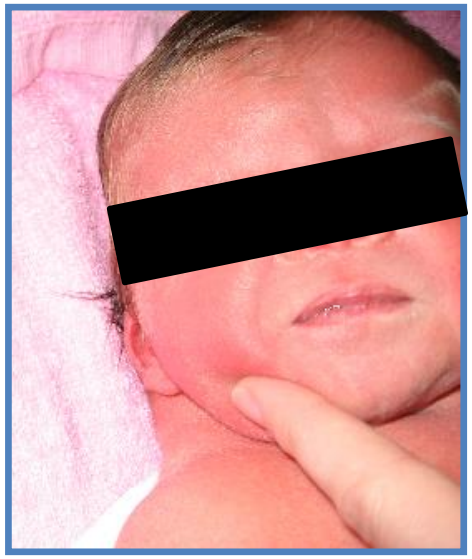
KU LEUVEN

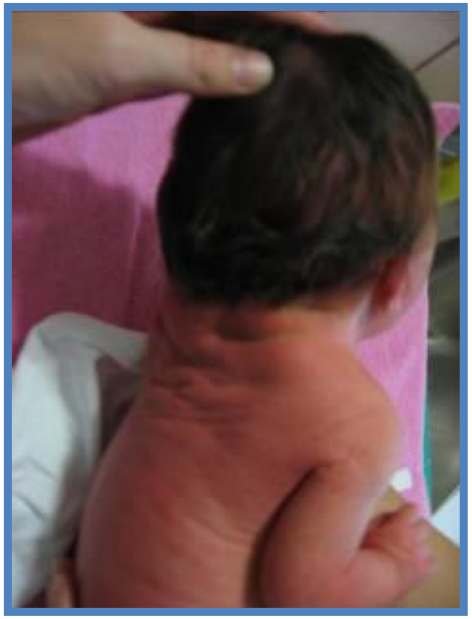
IPG

Case 1





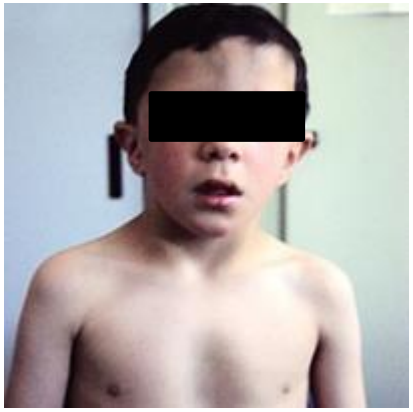


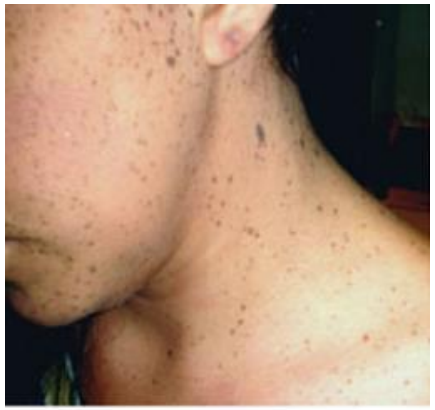


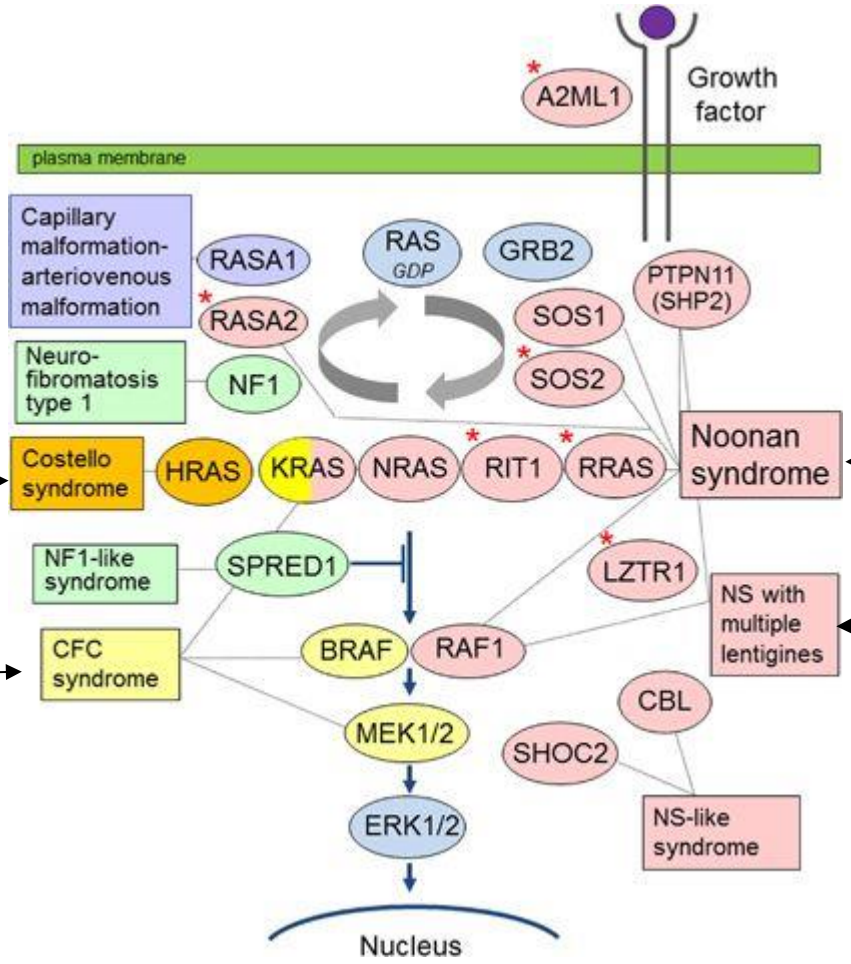
- Facial dysmorphism
- Pterygium colli
- Growth delay
- Supravalvular pulmonary stenosis
- Unilateral hydronephrosis

Suspicion of … Noonan syndrome

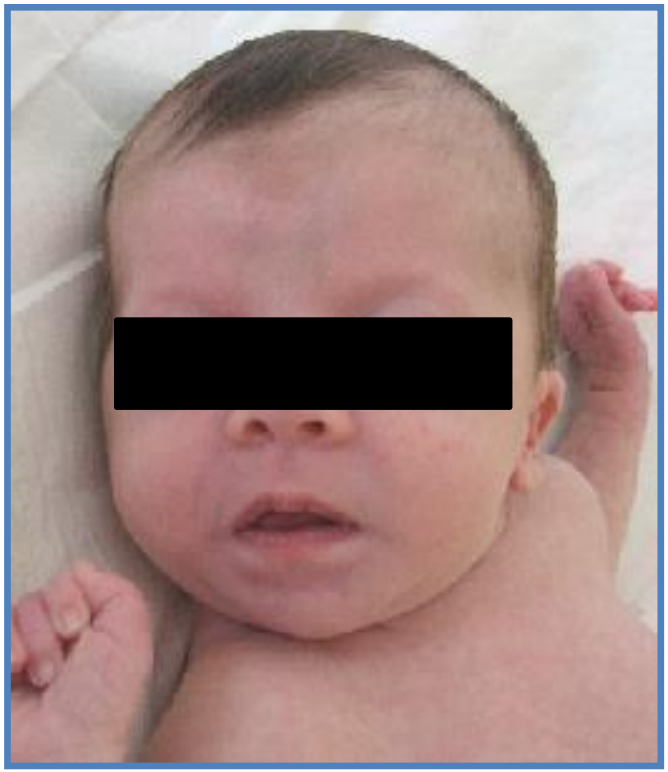
Ras/Raf/MEK/ERK Signal Transduction Pathway







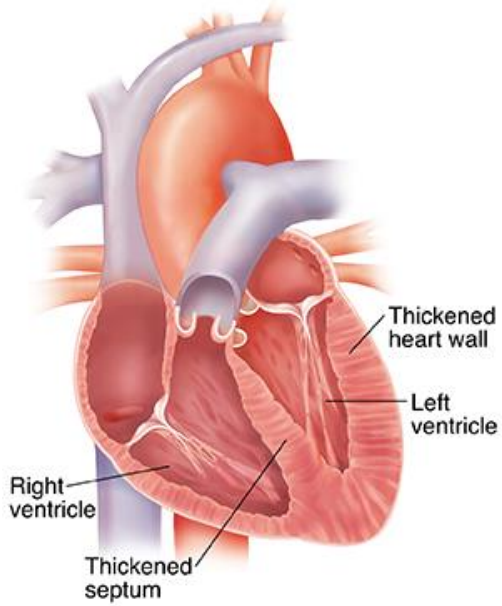
⇒ RASopathies gene panel



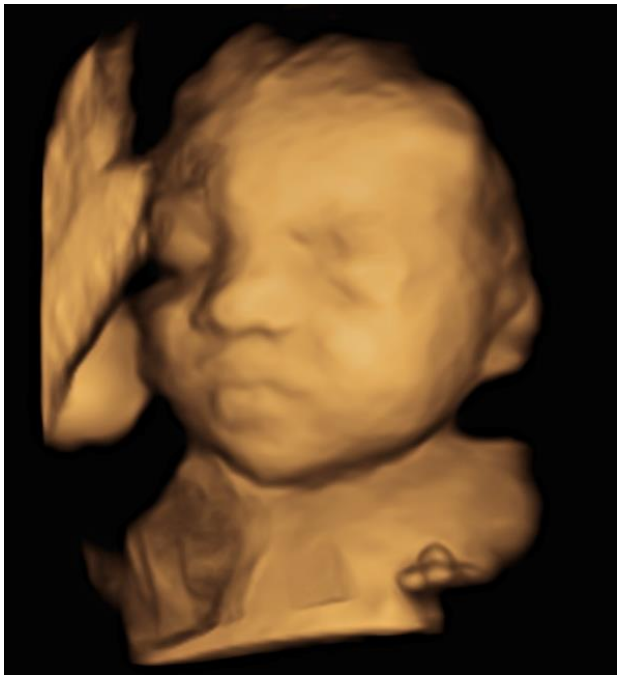
RAF1: Hz c.770C>T (p.Ser257Leu)

- de novo
- already described

RAF1: 95% hypertrophic cardiomyopathy (vs 18% in Noonan cohorts)



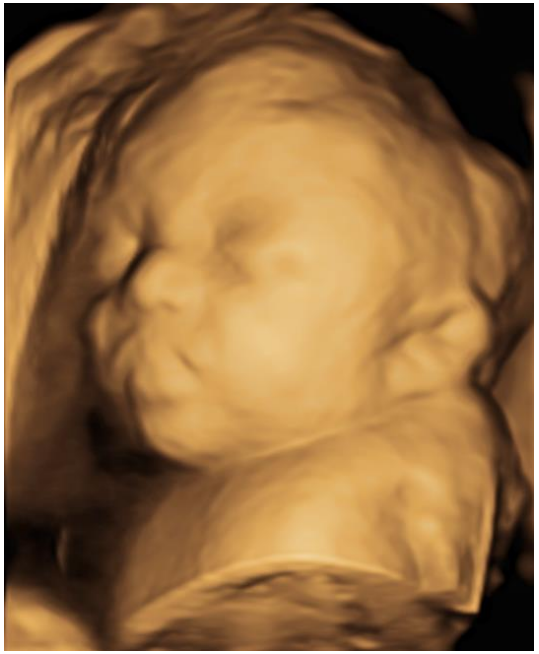
Diagnosis
Prognosis
Genetic counselling

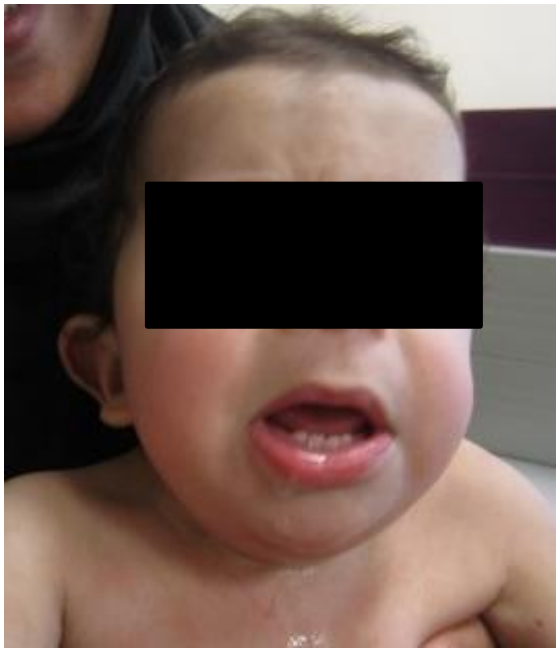


SOS1: Hz c.1294T>C (p.Trp432Arg)

- de novo
- already described

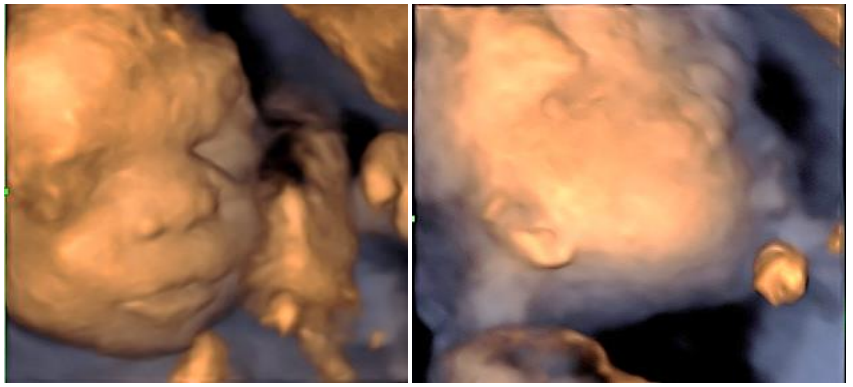
SOS1: Good developmental prognosis

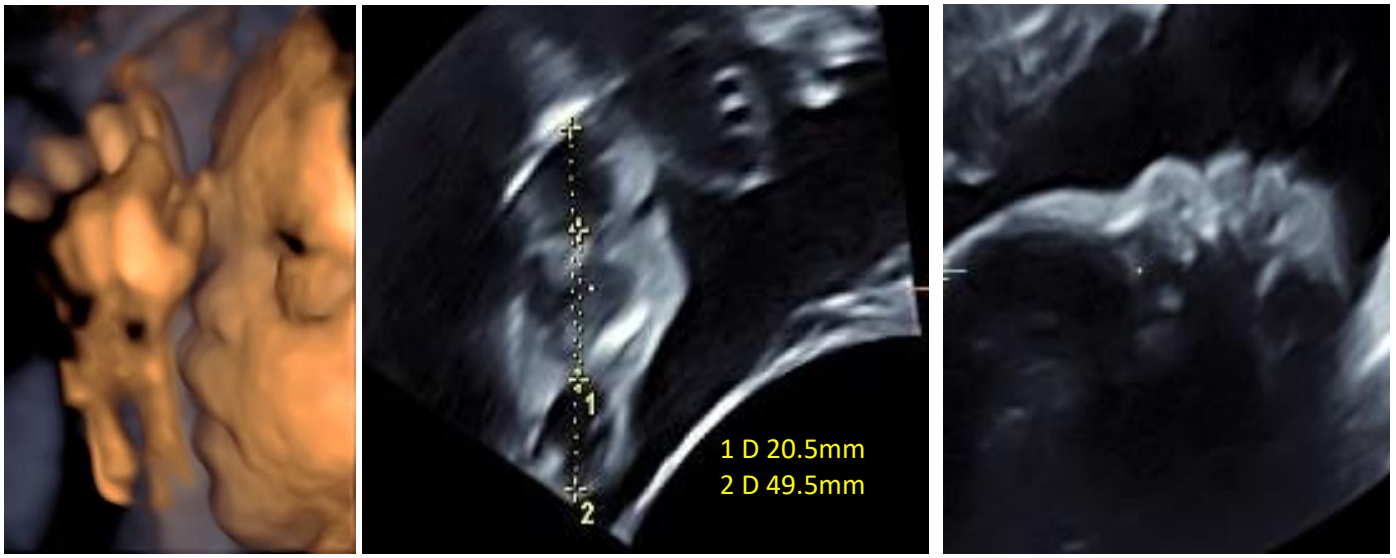




Case 2

- Healthy 32-year-old woman
- G3P2
- 22 GA ultrasound
  - Shortening of all long bones
  - Facial dysmorphism
  - ASD
- Amniocentesis:
  - Microarrays: normal, 46 XY
  - FGFR3: negative
  - RASopathies panel: negative
- 33 GA: US scan: all long bones <5th centile, delayed apparition of epiphyseal ossification centers. No axial skeletal anomaly





Suspicion of … Noonan? Skeletal dysplasia?

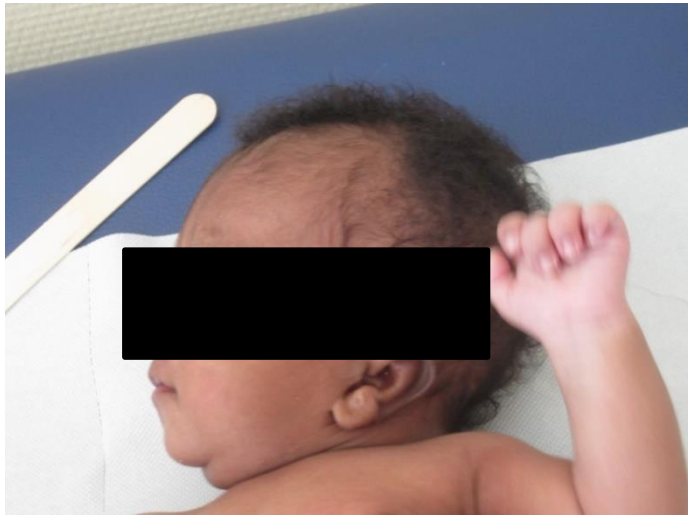
⇒ **Mendelioma**

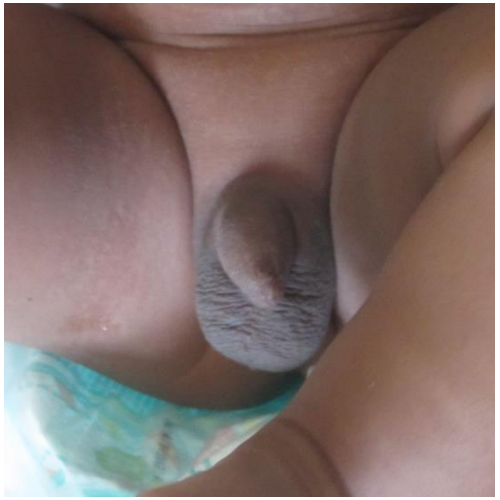
FGD1: c.527dupC (p.Leu177Thrfs*40)

- hemizygous (chr. X)
- inherited from a carrier mother

Aarskog-Scott syndrome

Good developmental prognosis

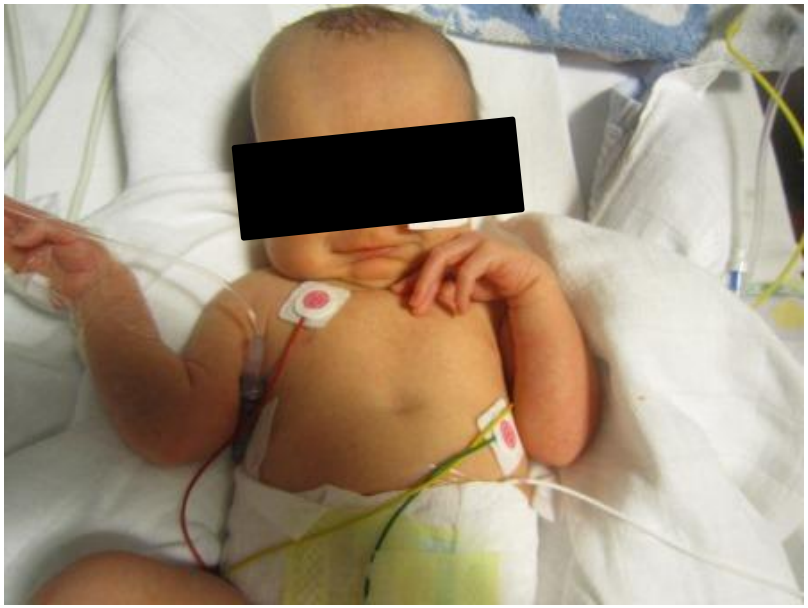


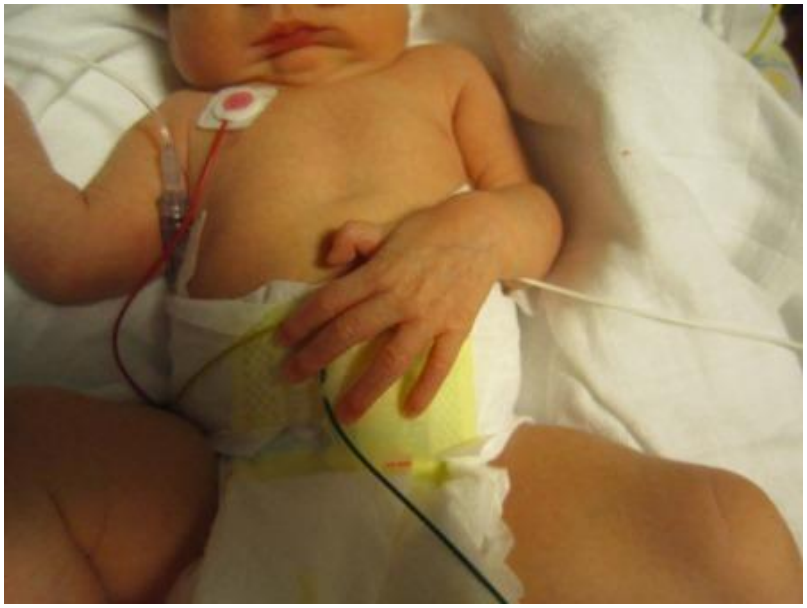


At birth: dysmorphism, height 42cm (-3SD)

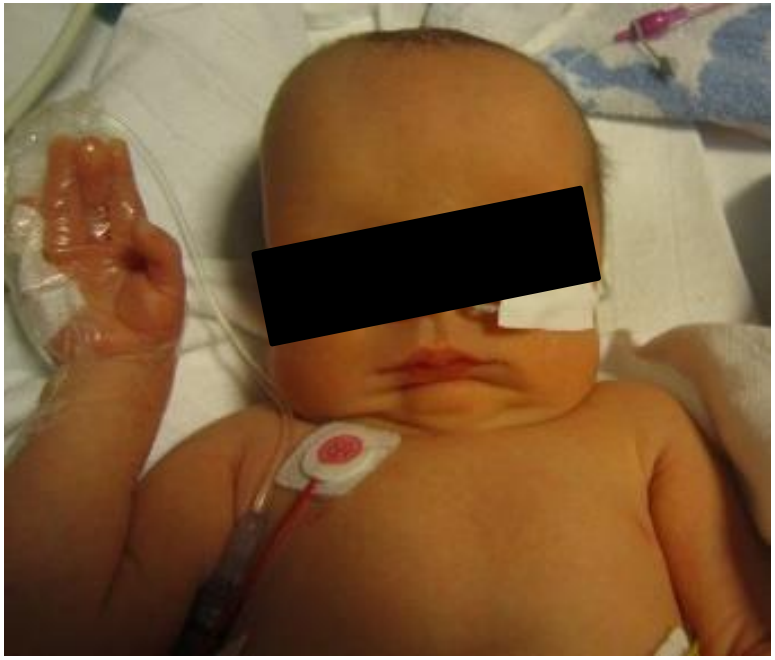
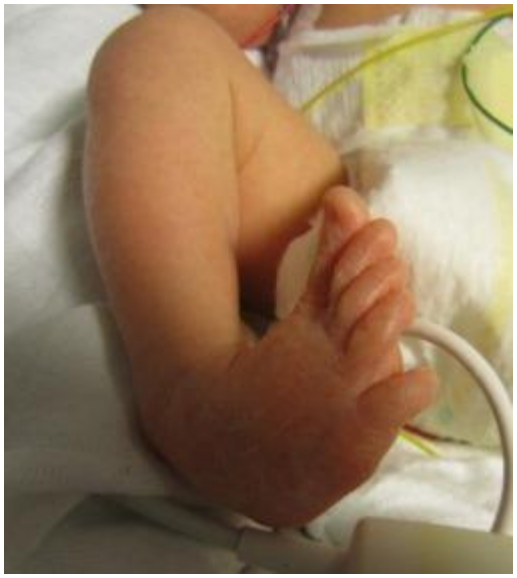
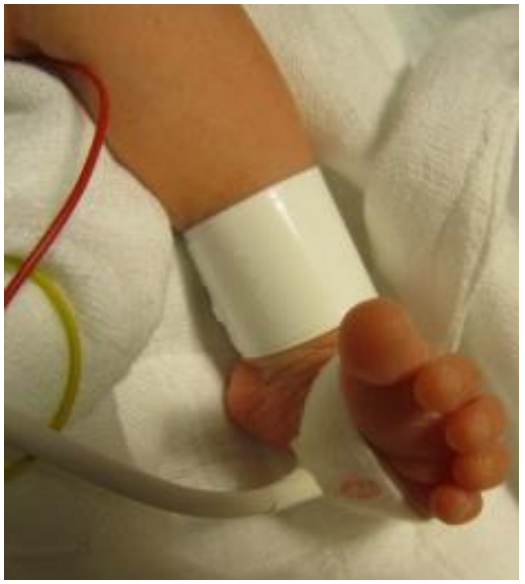
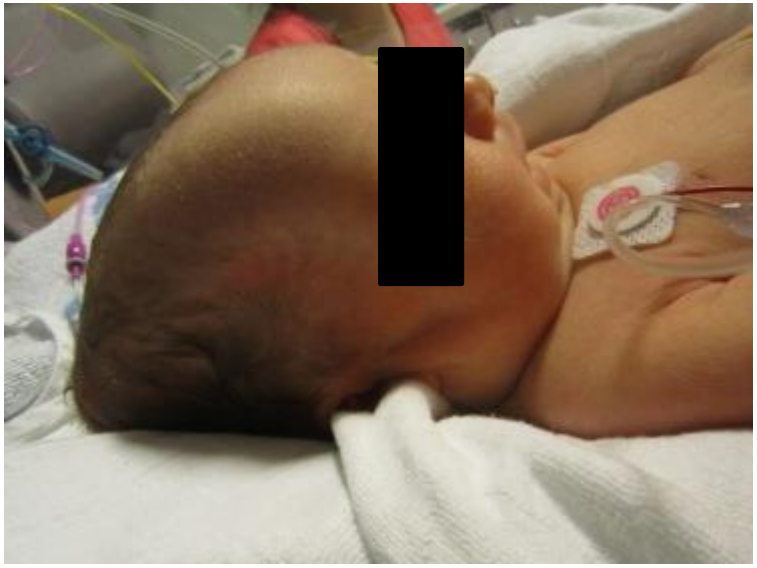
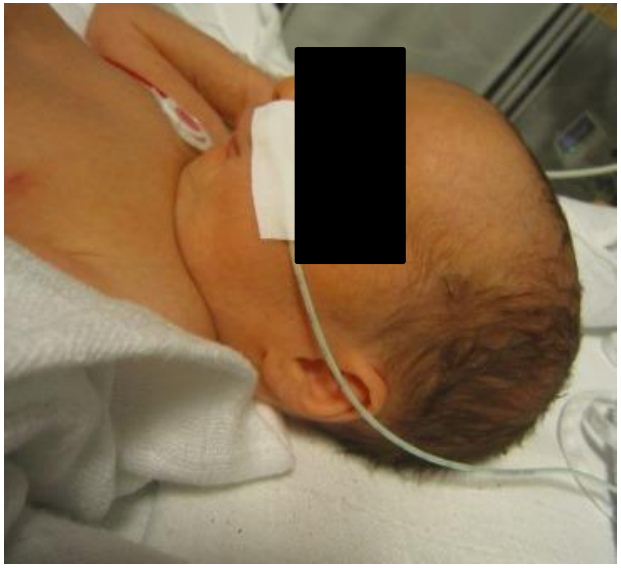
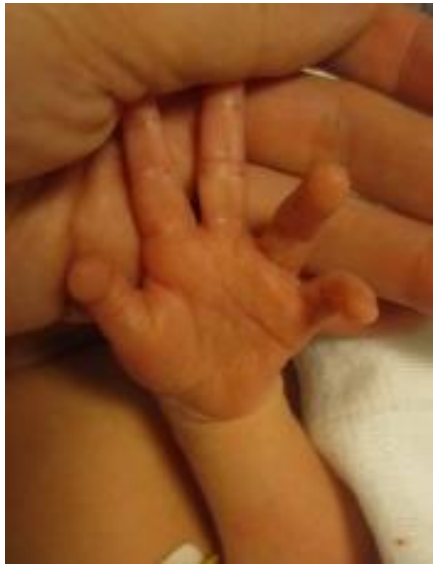
Diagnostic, prognosis and genetic counselling

Case 3

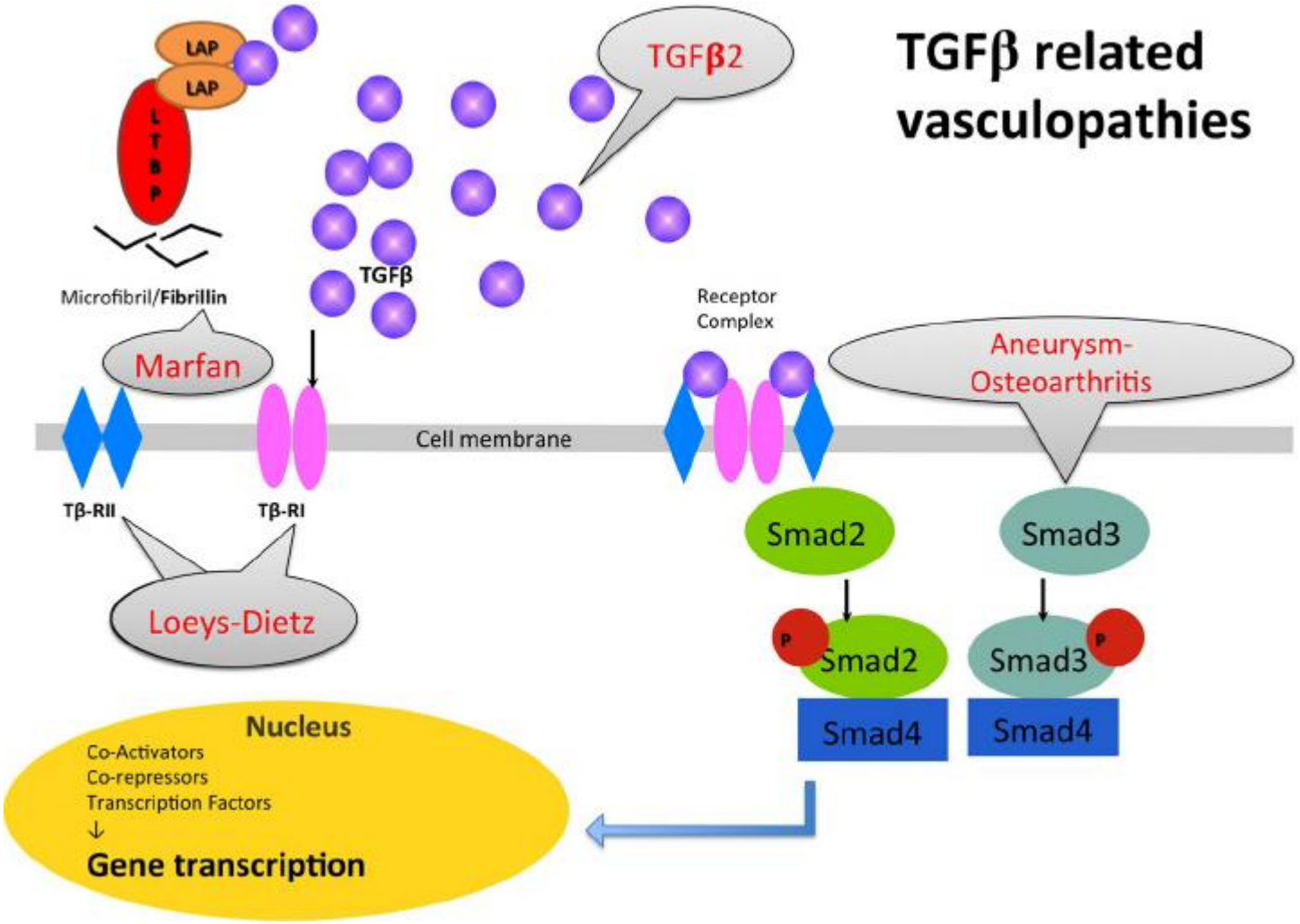




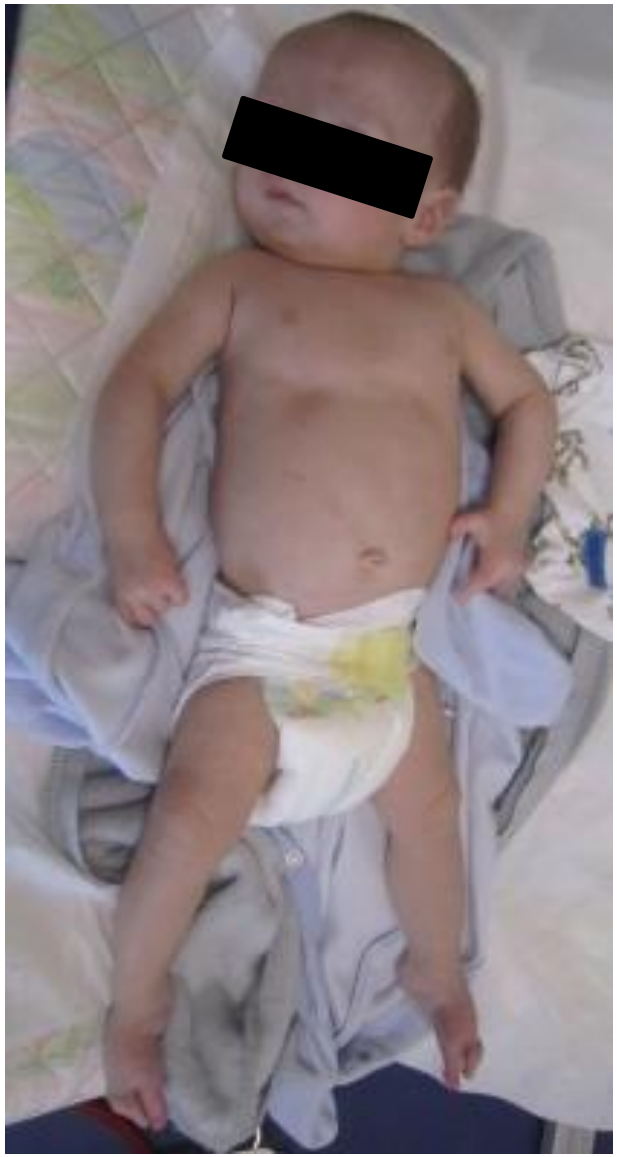
- Non consanguineous parents
- Pregnancy: polyhydramnios
- Birth (36w4/7):
  2kg600, 49 cm, **HC 37cm (>P97)**
- Neonatal hypotonia
- Frontotemporal caput succedaneum (right>left)
- Hypertelorism
- Posterior cleft of the soft palate, bifid uvula
- Retrognathia
- Pectus excavatum
- Long fingers, thumbs camptodactyly
- Bilateral single palmar crease
- Bilateral metatarsus varus

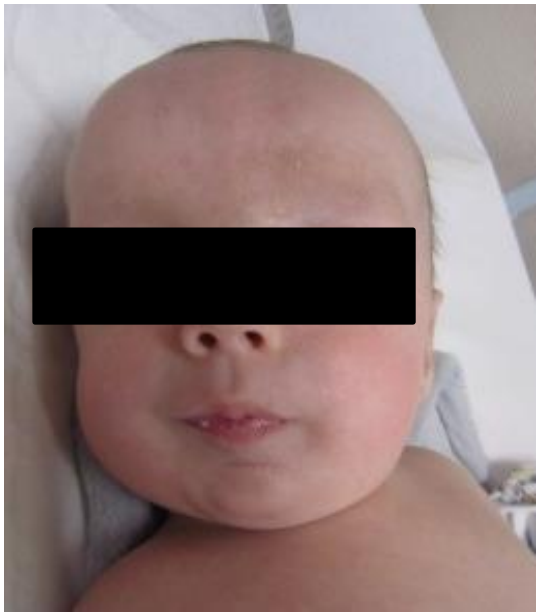
Suspicion of … Loeys-Dietz syndrome

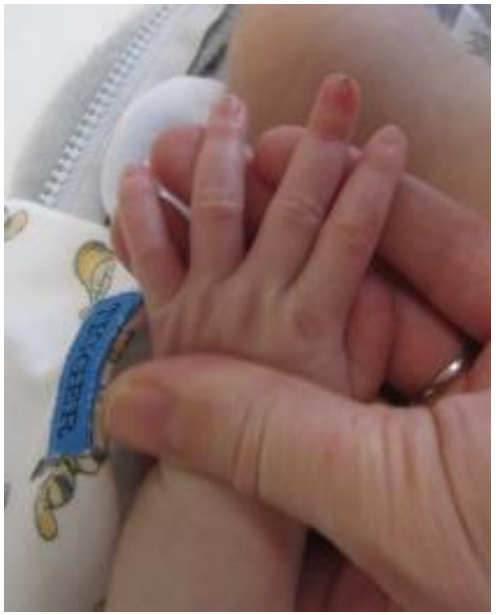


⇒ TAAD (Marfan and Marfan-like) gene panel



At 3 months





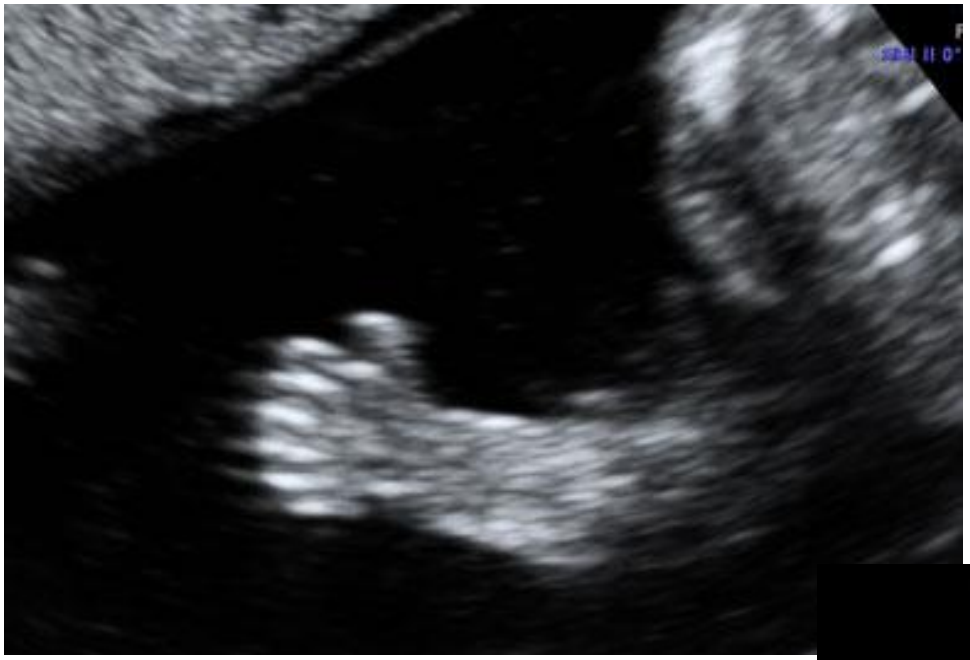
TGFβR2: Hz c.1346 C>T (p.Ser449Phe)

- de novo
- already described

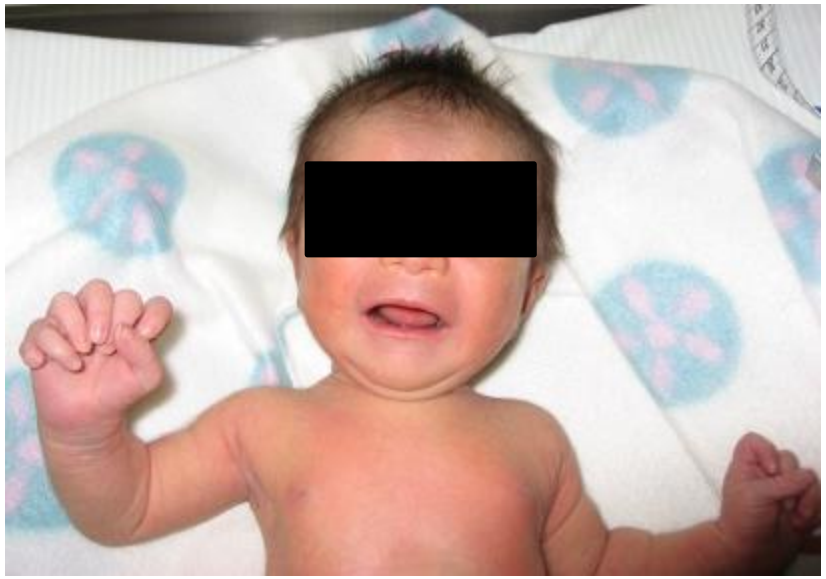
⇒ **Preventive follow up**
(arterial tortuosities, aortic and non-aortic arterial aneurysms,…)

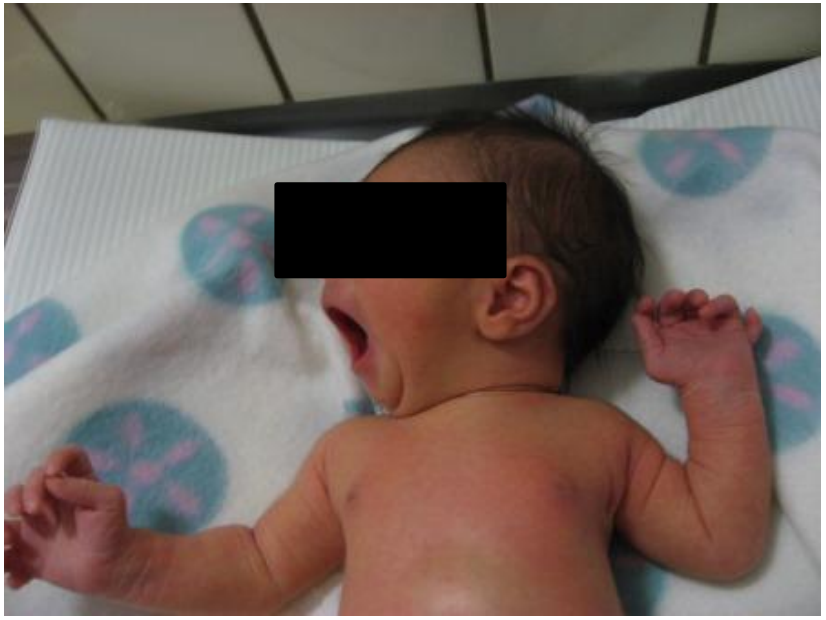
Diagnostic, preventive follow-up and genetic counselling

Case 4

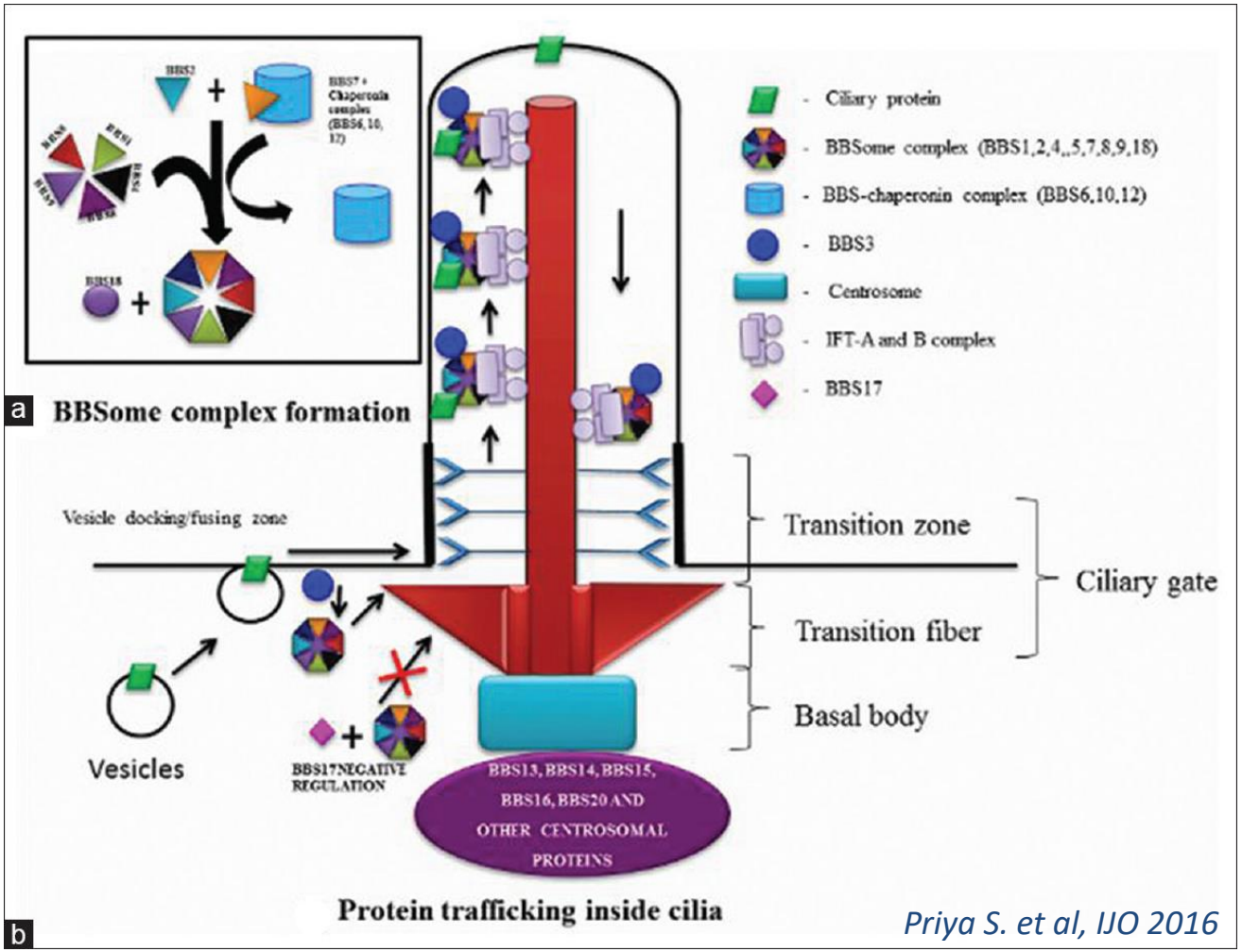


4-limbs polydactyly



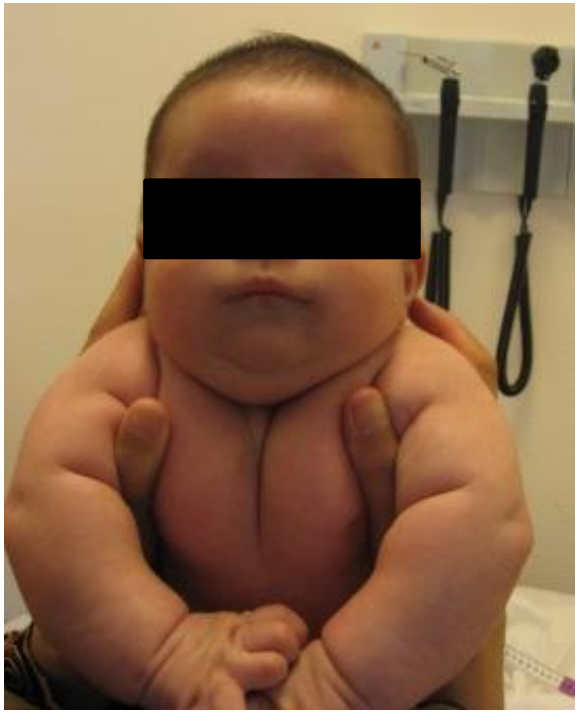



Suspicion of … Bardet Biedl syndrome



Priya S. et al, IJO 2016

⇒ **Ciliopathies gene panel**



At 18 months

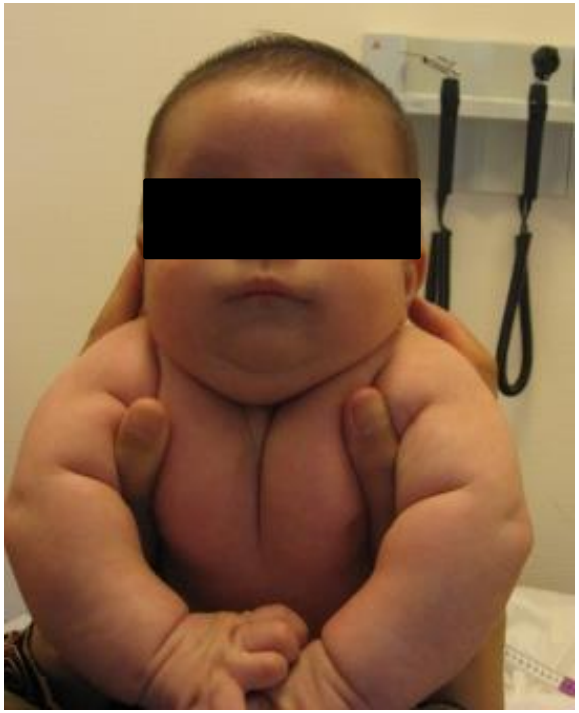
BBS5:

Homo c.123del (p.Gly42Glufs*11)

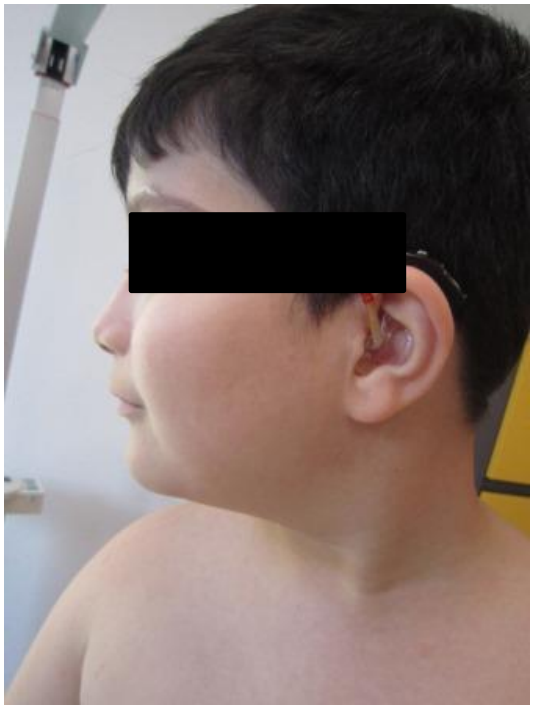
⇒ **Preventive follow up**

(cone-rod dystrophy, truncal obesity, abnormal renal function, intellectual disability, …)

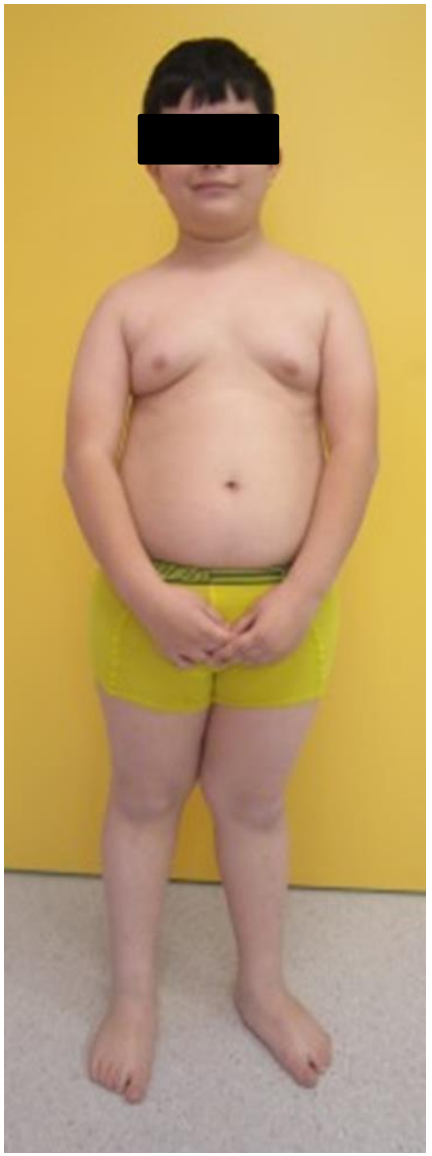
Diagnostic, preventive follow-up and genetic counselling



At 18 months



At 9 years: deafness



Ciliopathy gene panel:

BBS5: Homo c.123del (p.Gly42Glufs*11)

Mendelioma:

OTOG: Homo c.2527C>T (p.Gln843*)

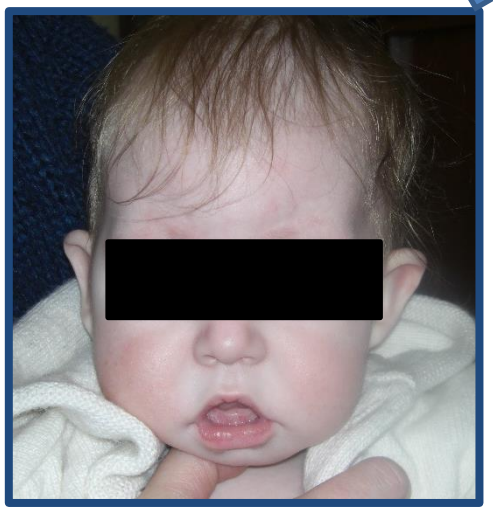
A diagnostic can hide another…

One test for all disorders?

KU LEUVEN

IPG

No diagnosis



5 months
Severe feeding problems
Delayed development
Cleft uvula
Hydronephrosis

Kabuki syndrome ?

MLL2
KDM6A

MLL2 mutation

1. SEQUENCING : massive and parallel

$\Rightarrow$ huge amount of data

2. IDENTIFICATION OF THE VARIANTS

- What do we detect?
- What do we miss?

3. INTERPRETATION OF THE VARIANTS

KU LEUVEN

IPG

Limitations of the NGS technology?

1. Probe coverage :

   Target DNA covered? Optimal capture?

Not covered

=> NO sequences obtained in this region

=> mutation will be missed!

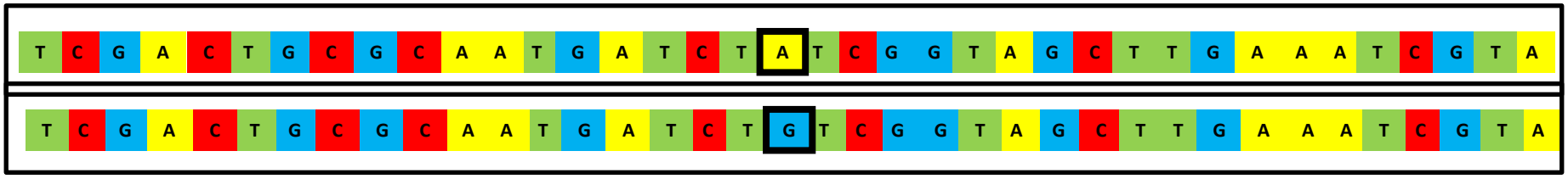
Technical difficulties:

GC-rich regions, repetitive sequences, pseudogenes, homologous regions,…

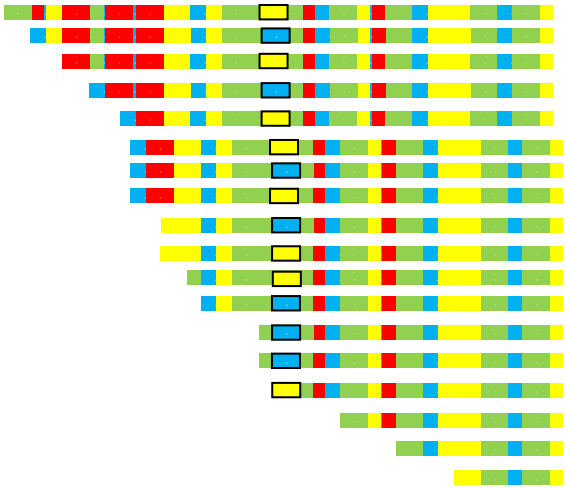
Limitations of the NGS technology?

2. Sequencing depth :

Number of reads which cover a target sequence?



PATIENT

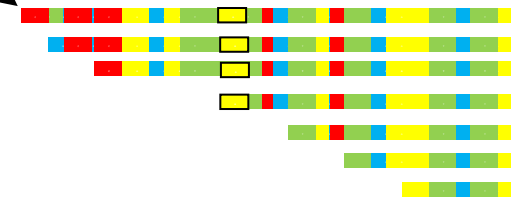


Sequencing depth = 15 x

8 = A

7 = G

⇒ **mutation**



Sequencing depth = 4 x

4 = A

G missed !

⇒ **false negative**

Limitations of the NGS technology?

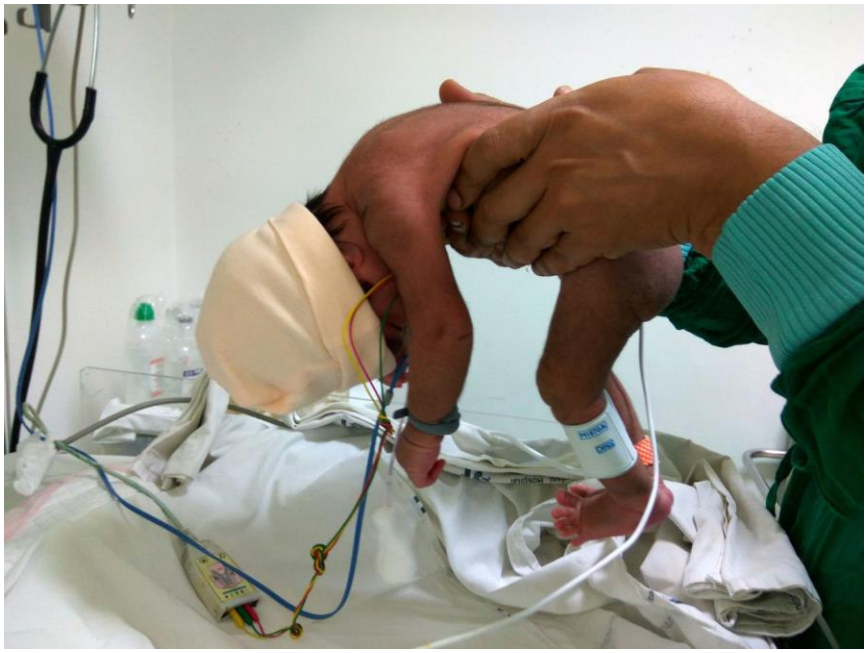
3. Alterations which does not alter the coding nuclear DNA sequence:

1. Deep intronic mutations
   Promotor and regulatory sequences mutations
2. Uniparental disomy
3. Epigenetic alterations (imprinting disorders)
4. Trinucleotide repeat expansion diseases
5. Mitochondrial diseases

Examples of NGS limitations in perinatal setting

KU LEUVEN

IPG

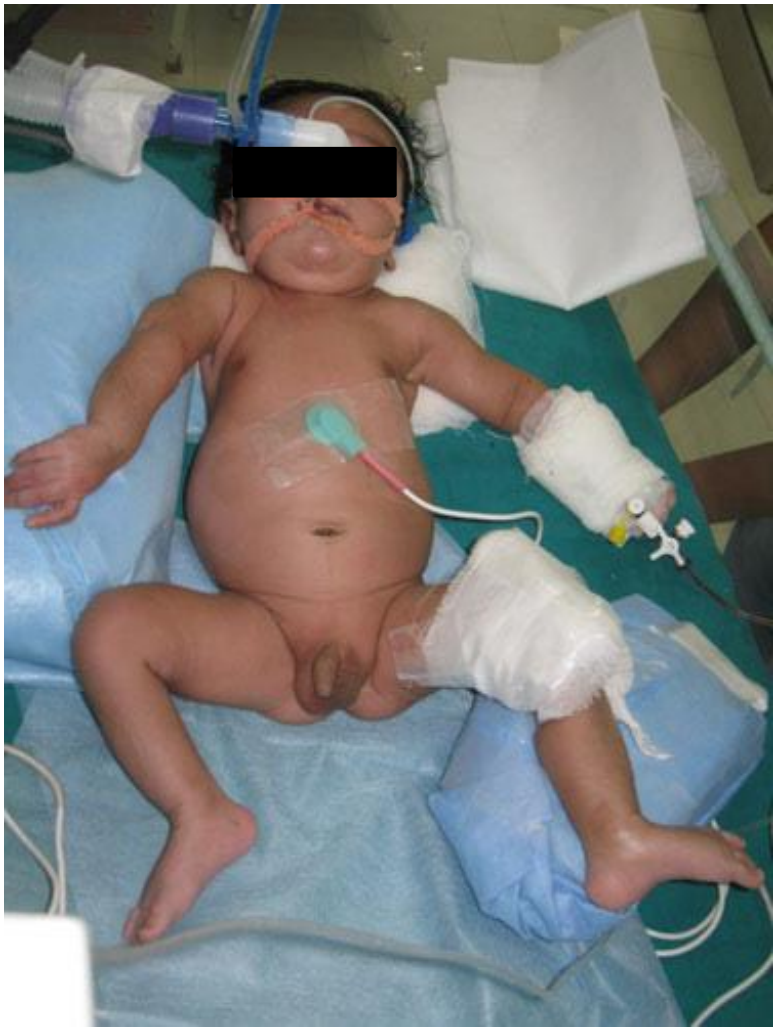


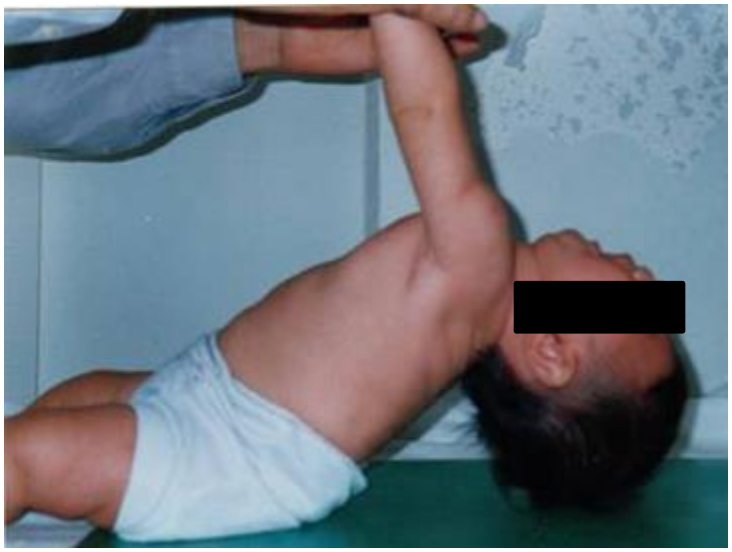
FLOPPY INFANT

EXOME IN TRIO IS NORMAL

What do you miss ?

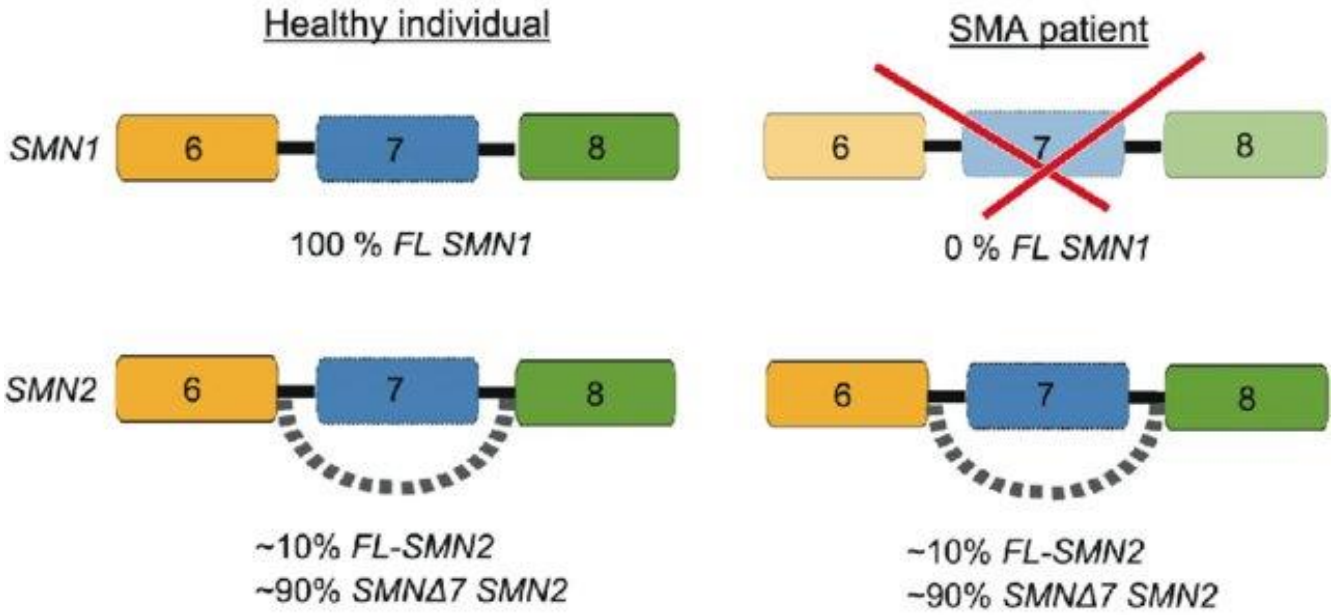
Case 1





- Severe hypotonia
- Severe respiratory distress
  with paradoxical breathing
- Good visual contact

Suspicion of … Spinal Muscular Amyotrophy



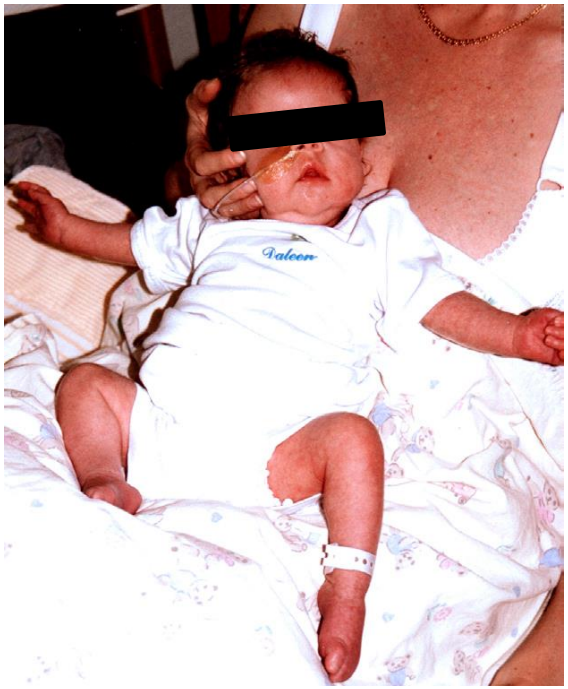
Bowerman M. et al, Disease Models and Mechanisms, 2017

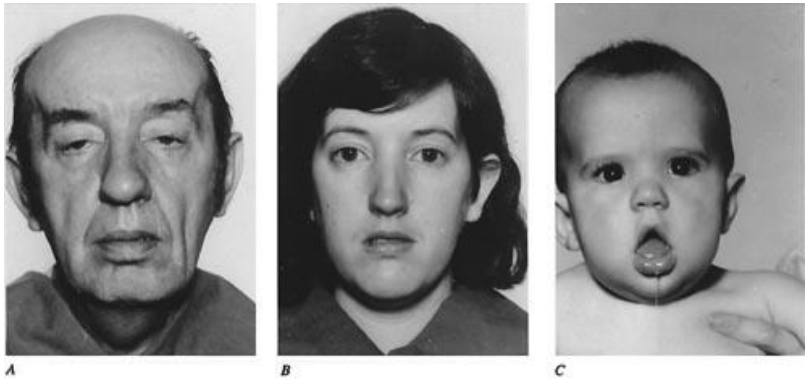
2 difficulties for NGS detection:

- Small deletion
- Pseudogene SMN2

Treatment !!!

Case 2

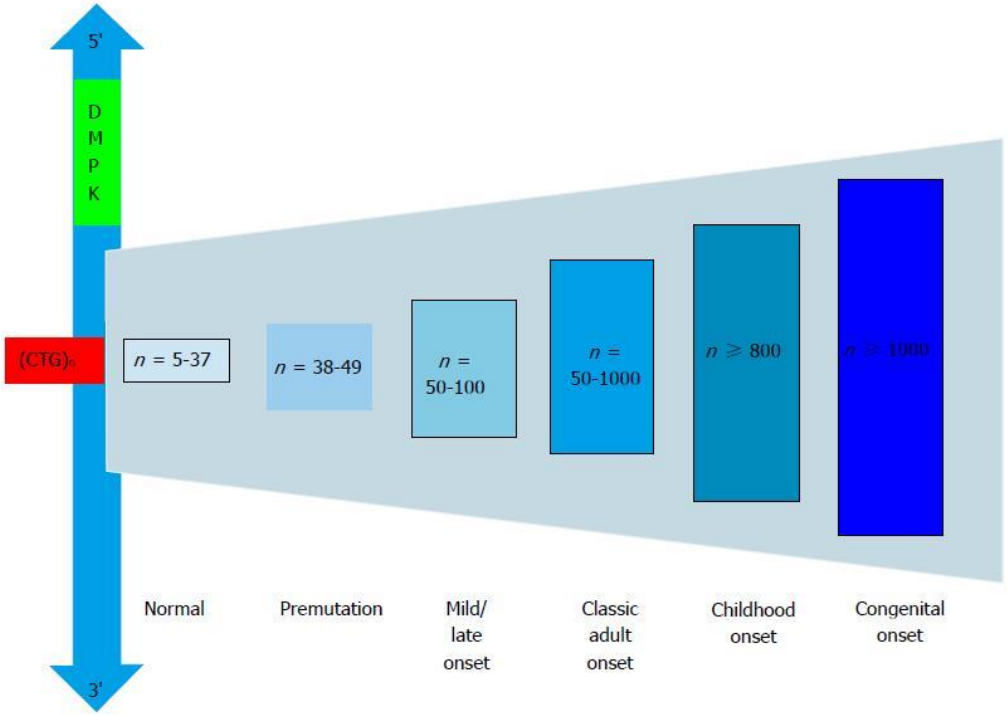


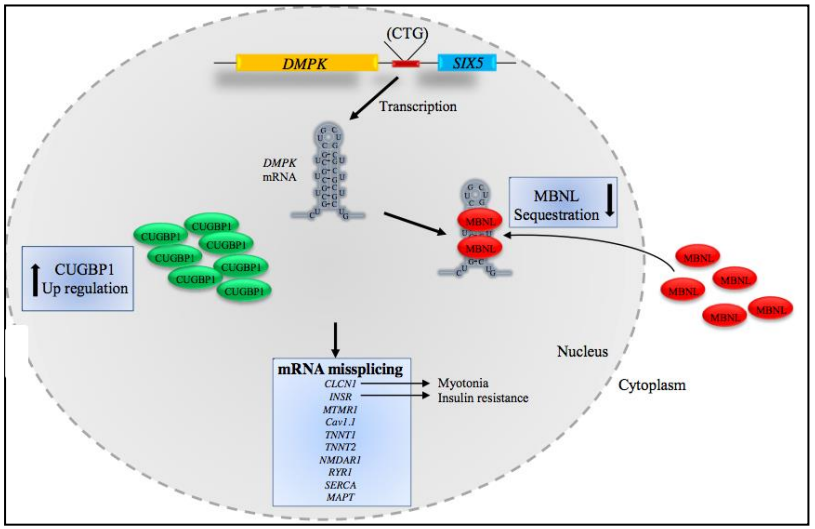


Anticipation in maternal transmission

- Severe hypotonia
- Feeding difficulties
- Respiratory deficiency
- Feet equinus deformity
- Familial history of muscle weakness, myotonia, balding and cataract

Suspicion of … Myotonic dystrophy type1 (congenital Steinert disease)





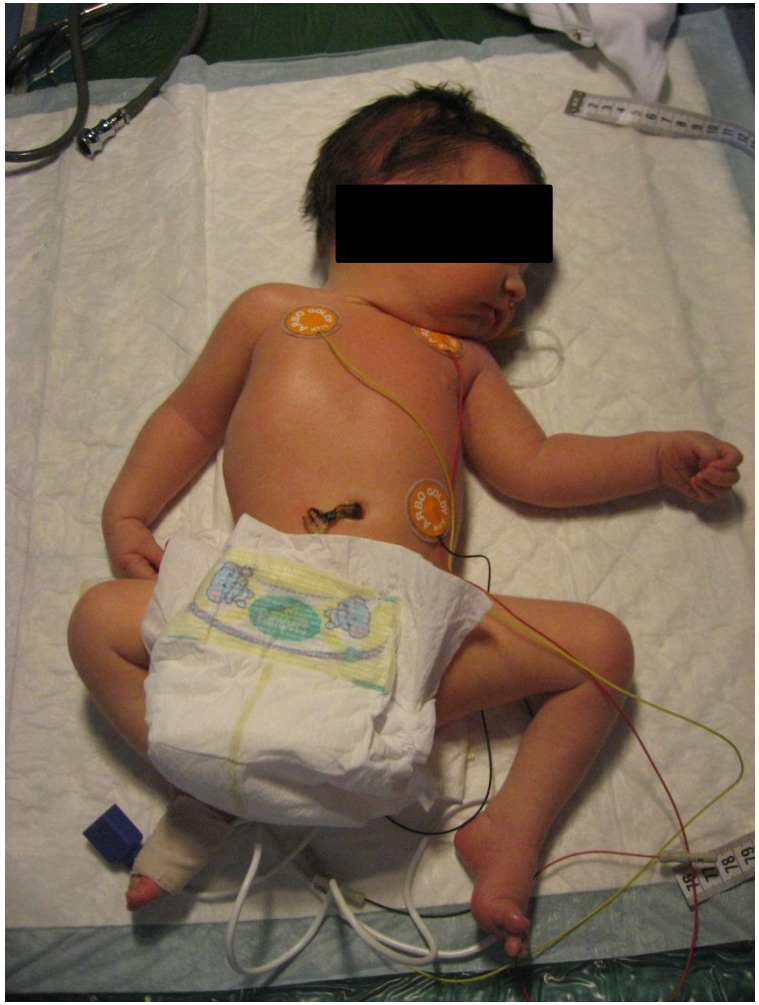
Santoro M. et al, 2015

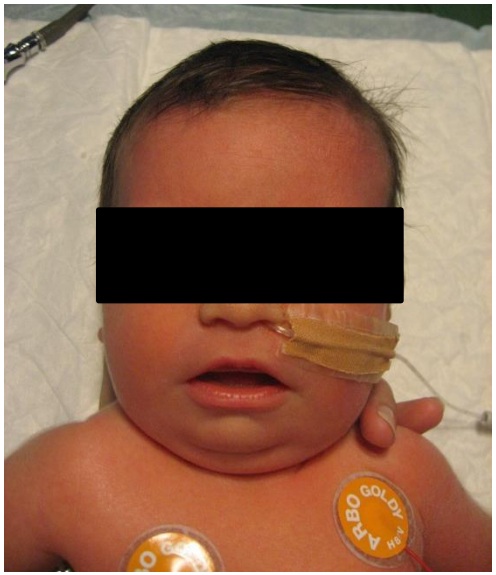
Difficulty for NGS detection:

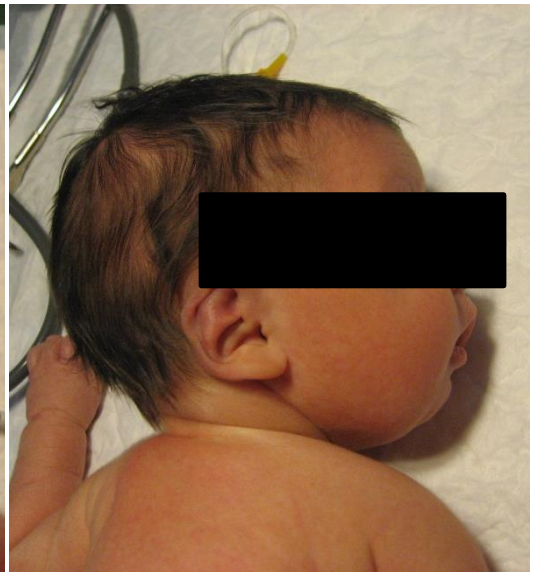
- Trinucleotides repeat expansion

Diagnosis, prognosis,
preventive follow-up and genetic counselling

Case 3

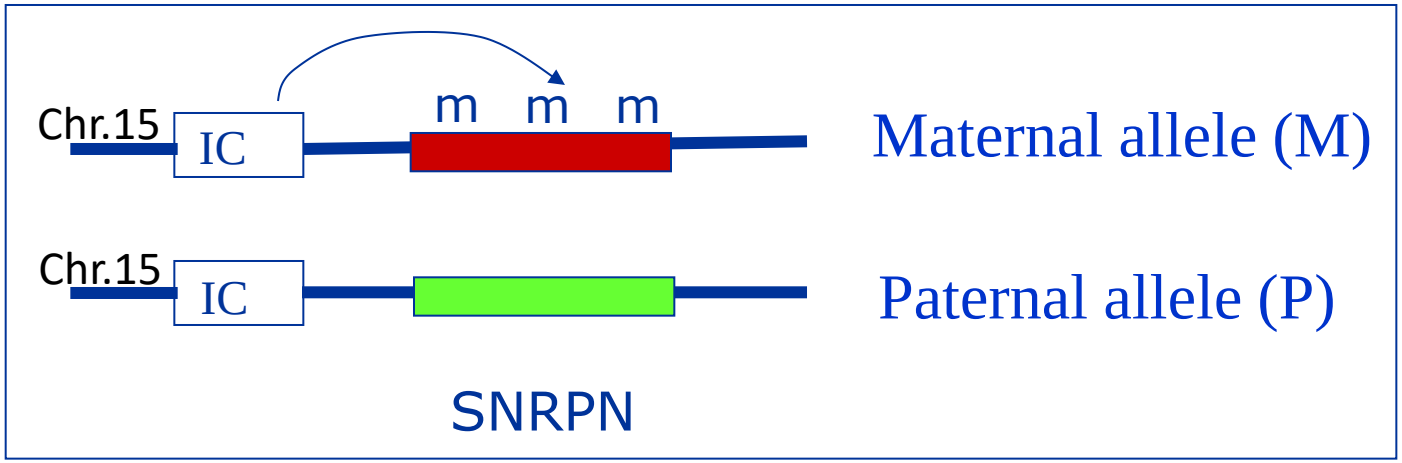




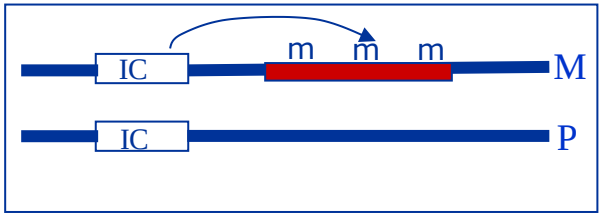


- Severe hypotonia
- Feeding difficulties
- No familial history

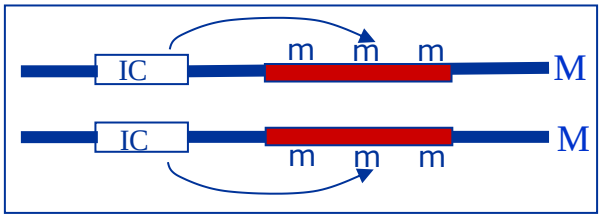
Suspicion of … Prader-Willi syndrome



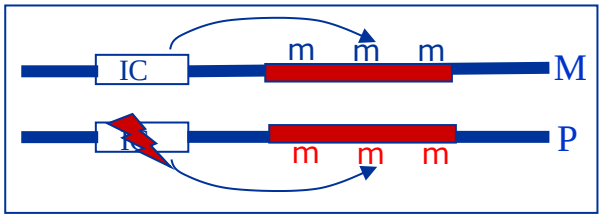
Paternal allele deletion **70%**



Maternal uniparental disomy **25-30%**

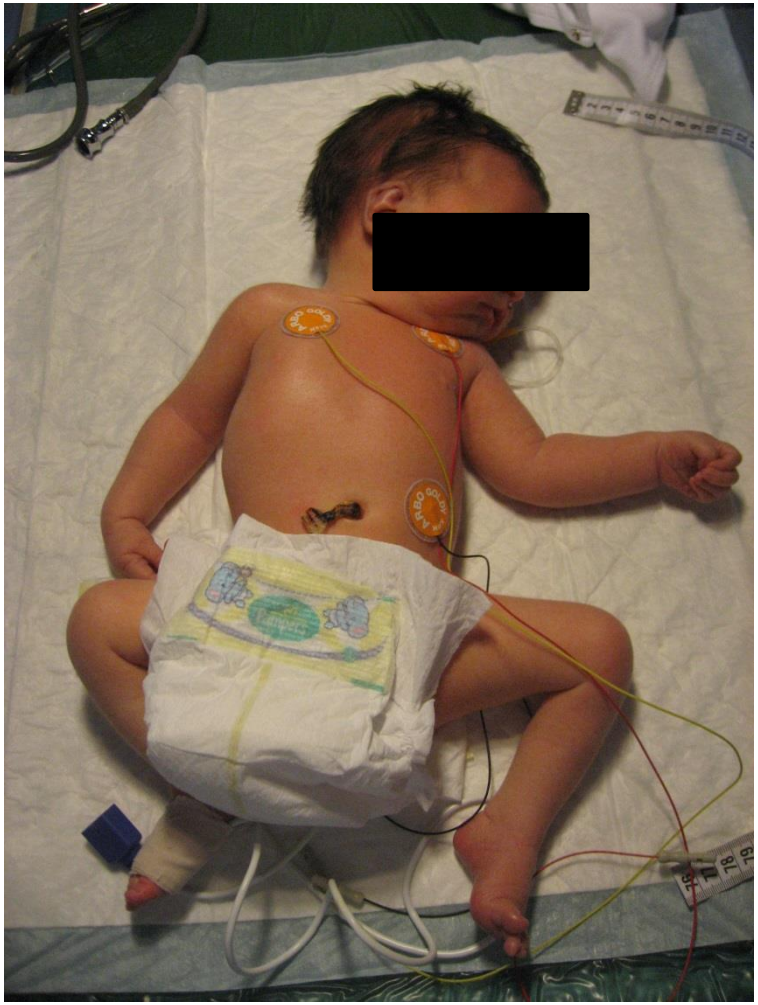


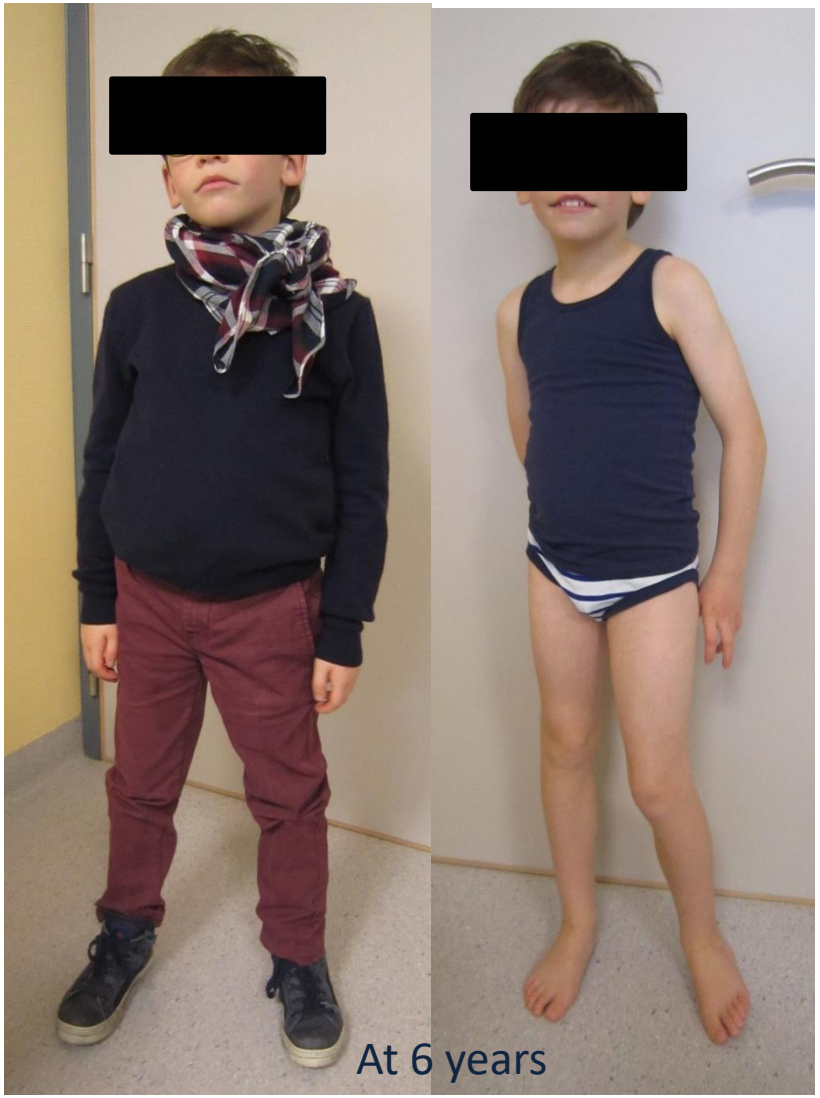
Imprinting center or epigenetic defect **<5%**



Difficulties for NGS detection:

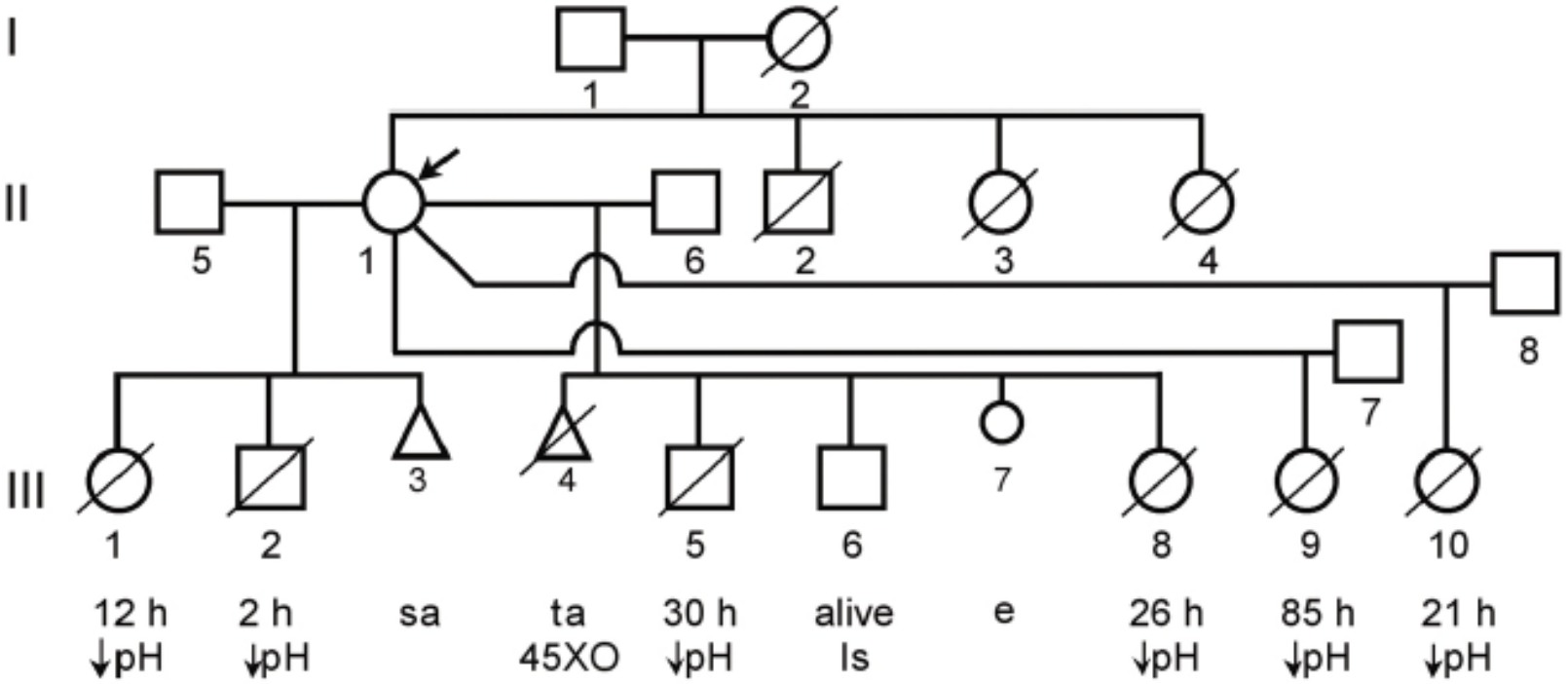
- Small deletions (CNVs detection)
- Uniparental disomies
- Epigenetic defects (imprinting disorder)





Diagnosis, <u>preventive follow-up</u>
and genetic counselling

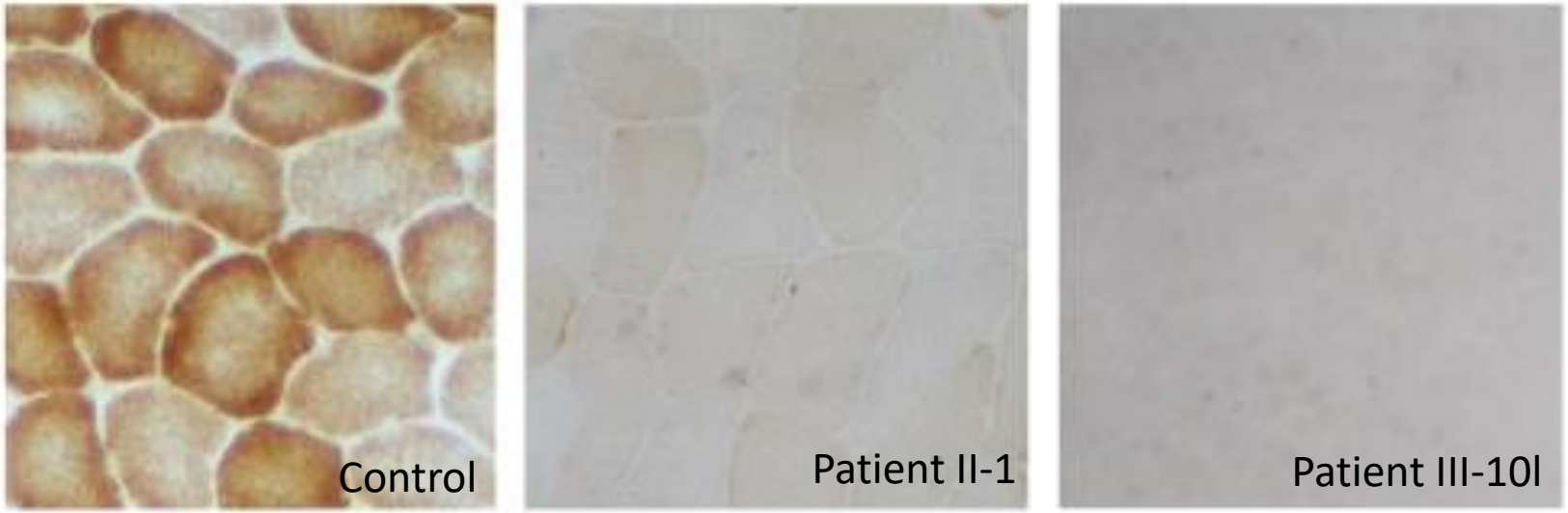
Case 4



McFarland R. et al, Nat Genet 2002

- 6 neonatal deaths and 1 surviving child with Leigh syndrome
- Severe hypotonia
- Lactic acidosis
- Multiple organe failure

Suspicion of … Mitochondrial disease



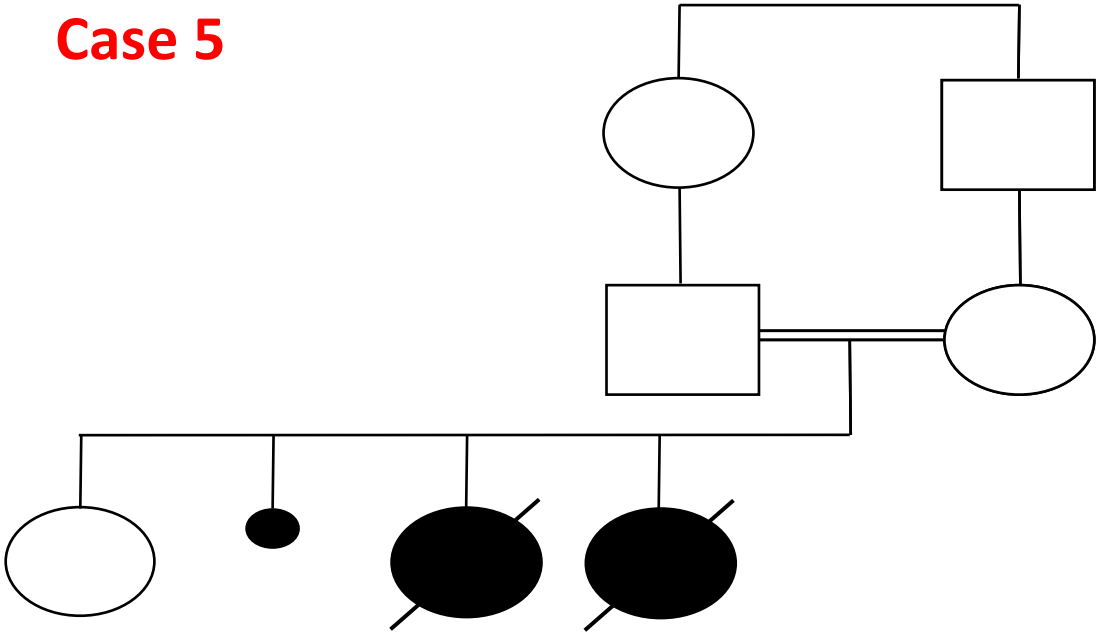
Low cytochrom c oxidase activity of skeletal muscle sections of affected patients
Homoplasmic C1624T within the mt-tRNA^{Val} gene

McFarland R. et al, Nat Genet 2002

Difficulty for NGS detection:

- mtDNA mutations are not well covered
- mtDNA mutations may be absent in a blood sample

Case 5



week 24
delivery wk 31
no tests

week 20
delivery wk 32
AC: array-CGH normal
muscle biopsy not contributive

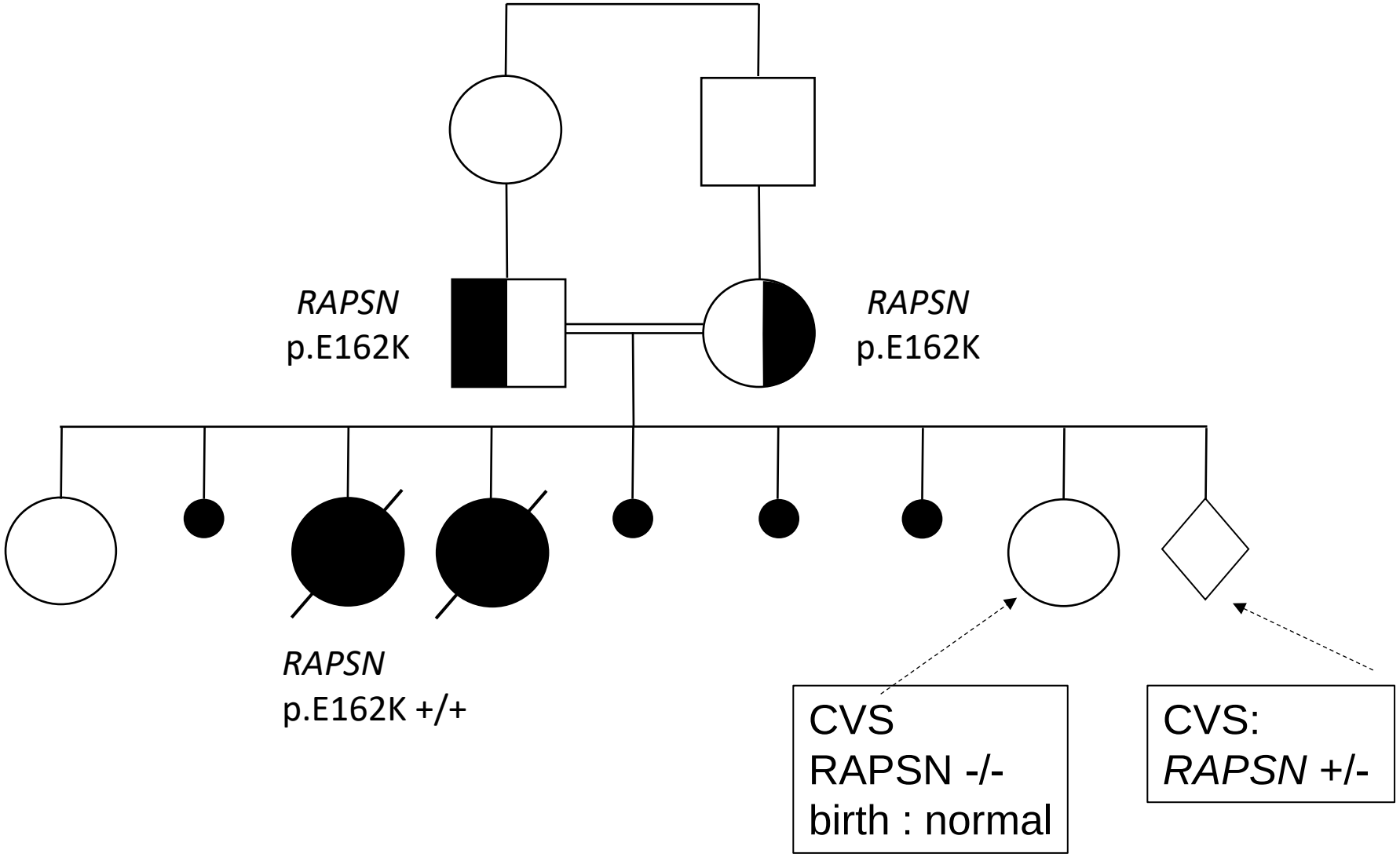
polyhydramnios,
hydrops fetalis,
clenched hands,
clubfeet

Diagnosis ?

autosomal recessive
fetal akinesie

Clinical exome
filtering for fetal akinesia panel

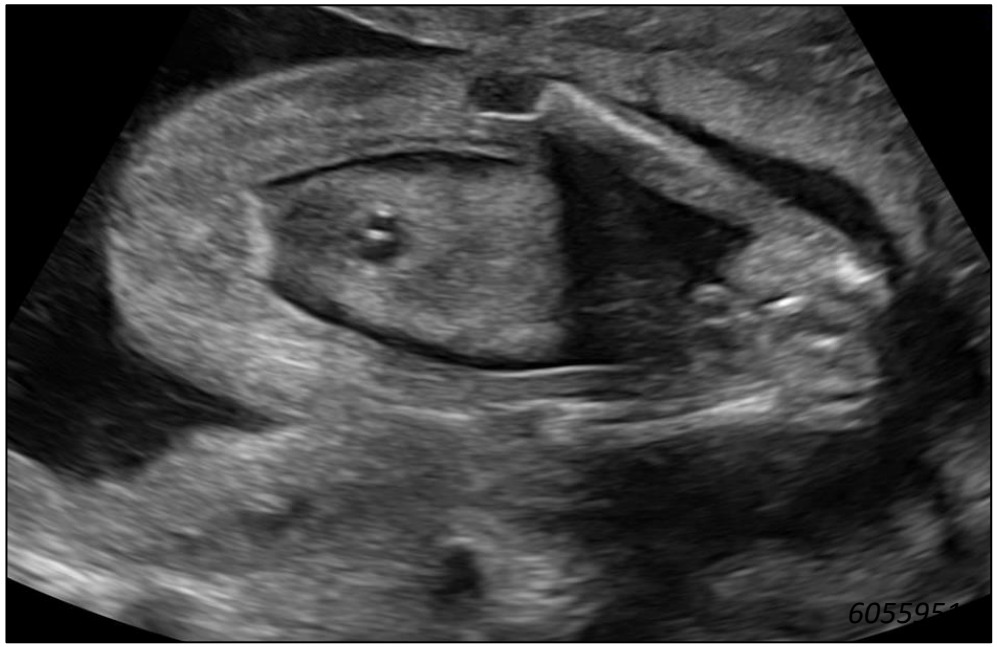
Homozygous RAPSN
c.484G>A p.E162K


RAPSN
p.E162K
RAPSN
p.E162K
RAPSN
p.E162K +/+
CVS
RAPSN -/-
birth : normal
CVS:
RAPSN +/-

BUT …

Gestational age 17+6 weeks :

- No hands movements
- Fixed hips and knees
- Bilateral clubfeet
- *No polyhydramnios*
- *No hydrops fetalis*



- **error at CVS?**
  - no maternal contamination
  - no sample swaps
- **Different type of fetal akinesia ?**
  - different manifestations:

    earlier, no polyhydramnios, no hydrops

Re-analysis of parental sequences

KLHL41
Homozygous mutation c.171C>A p.Tyr57*

Identification of KLHL41 Mutations Implicates BTB-Kelch-Mediated Ubiquitination as an Alternate Pathway to Myofibrillar Disruption in Nemaline Myopathy

Nemaline myopathy (NM) is a rare congenital muscle disorder primarily affecting skeletal muscles that results in neonatal death in severe cases as a result of associated respiratory insufficiency. NM is thought to be a disease of sarcomeric thin filaments as six of eight known genes whose mutation can cause NM encode components of that structure, however, recent discoveries of mutations in non-thin filament genes has called this model in question. We performed whole-exome sequencing and have identified recessive small deletions and missense changes in the Kelch-like family member 41 gene (KLHL41) in four individuals from unrelated NM families. Sanger sequencing of 116 unrelated individuals with NM identified compound heterozygous changes in KLHL41 in a fifth family. Mutations in KLHL41 showed a clear phenotype-genotype correlation: Frameshift mutations resulted in severe phenotypes with neonatal death, whereas missense changes resulted in impaired motor function with survival into late childhood and/or early adulthood. Functional studies in zebrafish showed that loss of Klhl41 results in highly diminished motor function and myofibrillar disorganization, with nemaline body formation, the pathological hallmark of NM. These studies expand the genetic heterogeneity of NM and implicate a critical role of BTB-Kelch family members in maintenance of sarcomeric integrity in NM.

KLHL41
Homozygous mutation c.171C>A p.Tyr57*

This gene was not yet in the fetal akinesia panel at the time of testing !


UN TRAIN PEUT
EN CACHER
UN AUTRE
Journées BMW EfficientDy
Du 20 au 31 mars

1. SEQUENCING : massive and parallel

$\Rightarrow$ huge amount of data

2. IDENTIFICATION OF THE VARIANTS

- What do we detect?
- What do we miss?

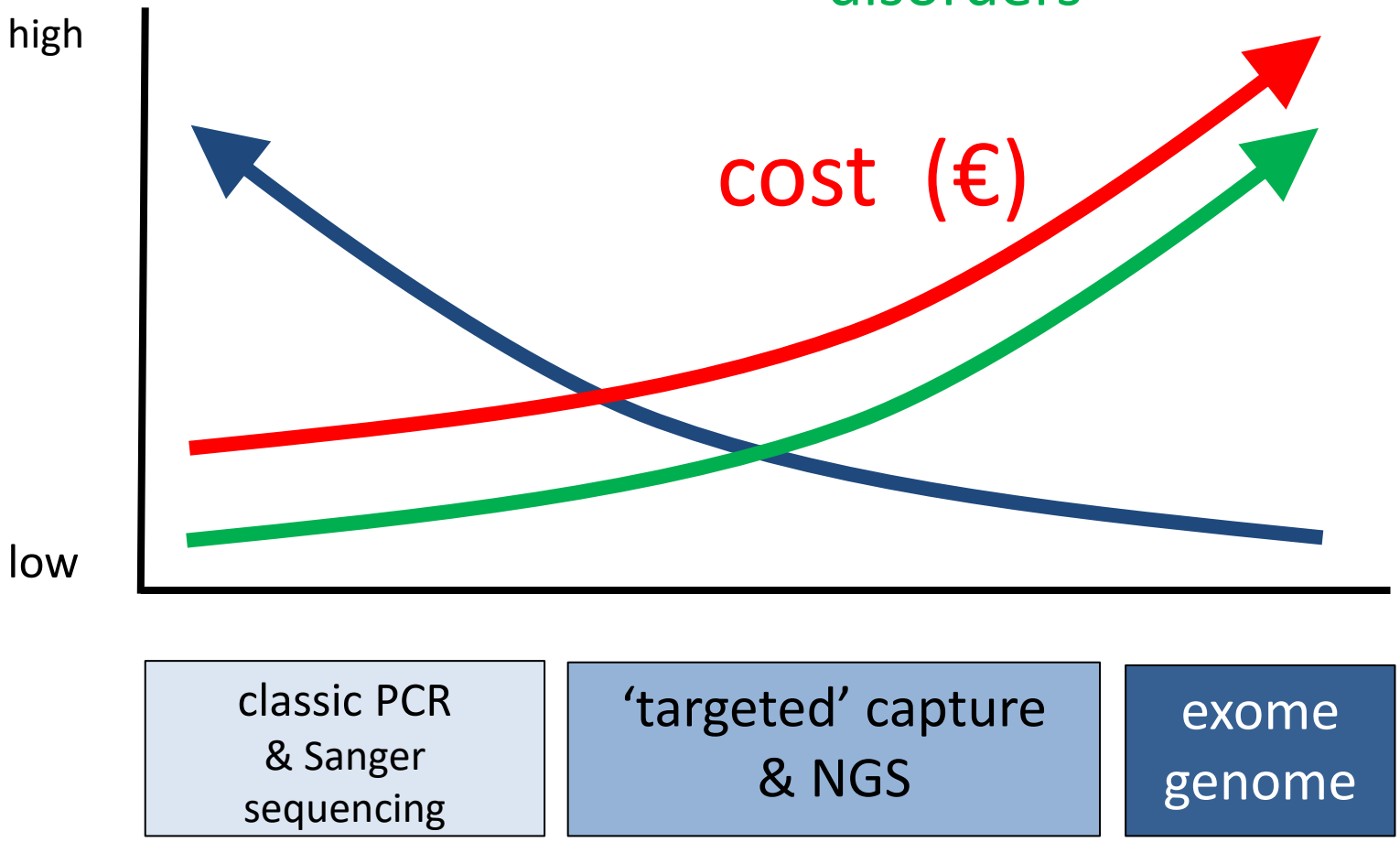
3. INTERPRETATION OF THE VARIANTS

KU LEUVEN

IPG

Detection of a mutation in a specific gene

Detection of mutations in genetic heterogeneous disorders



Achondroplasia *Cystic fibrosis* *Kabuki* *Noonan* *deafness* *epilepsy* *Intellectual deficiency*



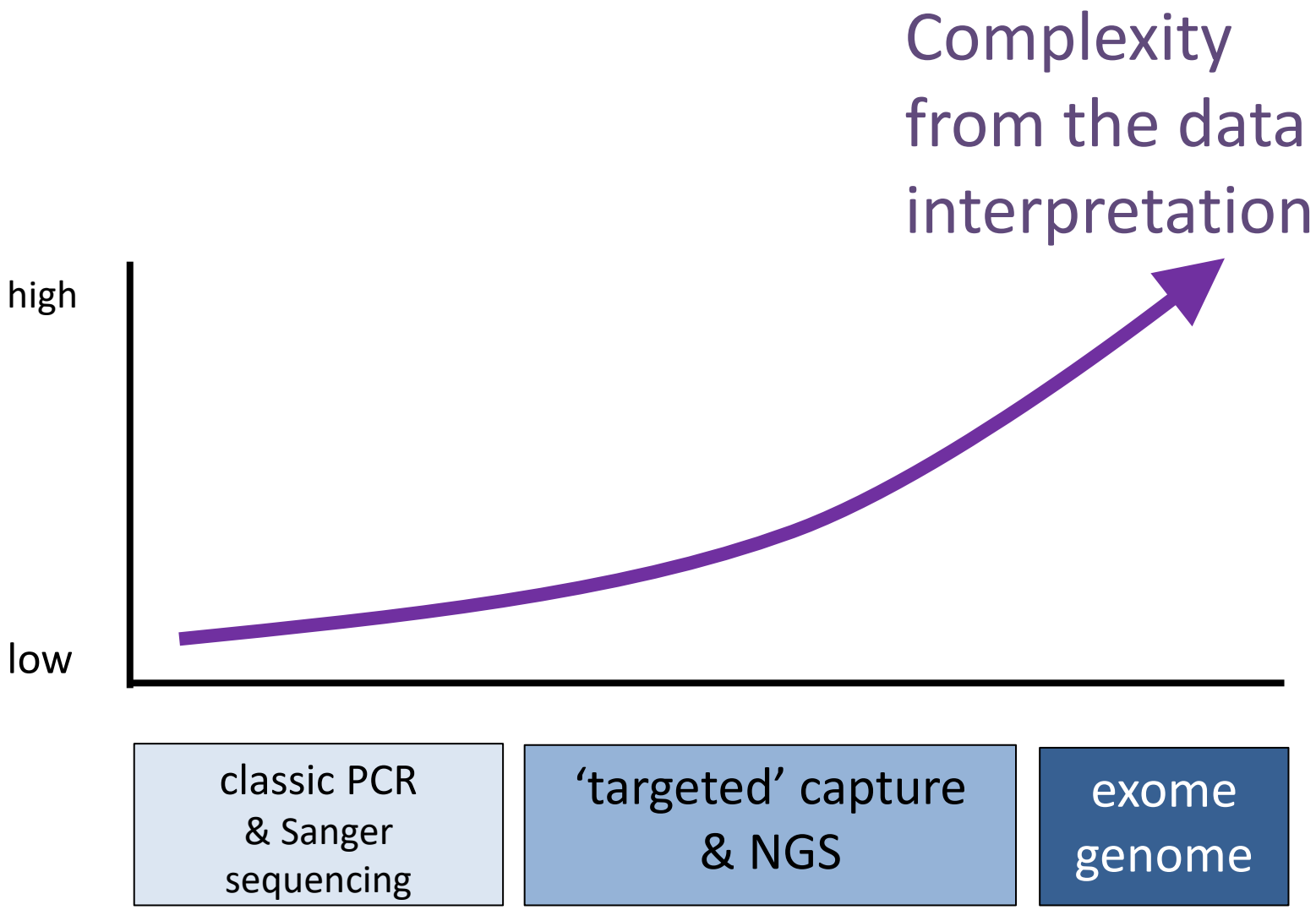




1 mutation | 1 gene | 2 genes | 7 genes | 65 genes | 85 genes | >500 genes

1 of a few genes (1-5) | **panel of genes (5-100)** | **All genes**

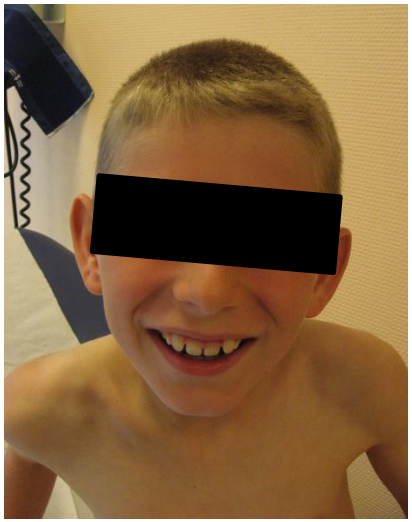
PCR + Sanger sequencing | **targeted capture (gene panels)** | Mendeliome exome genome


Complexity
from the data
interpretation
high
low
classic PCR
& Sanger
sequencing
‘targeted’ capture
& NGS
exome
genome

«Reverse phenotyping »

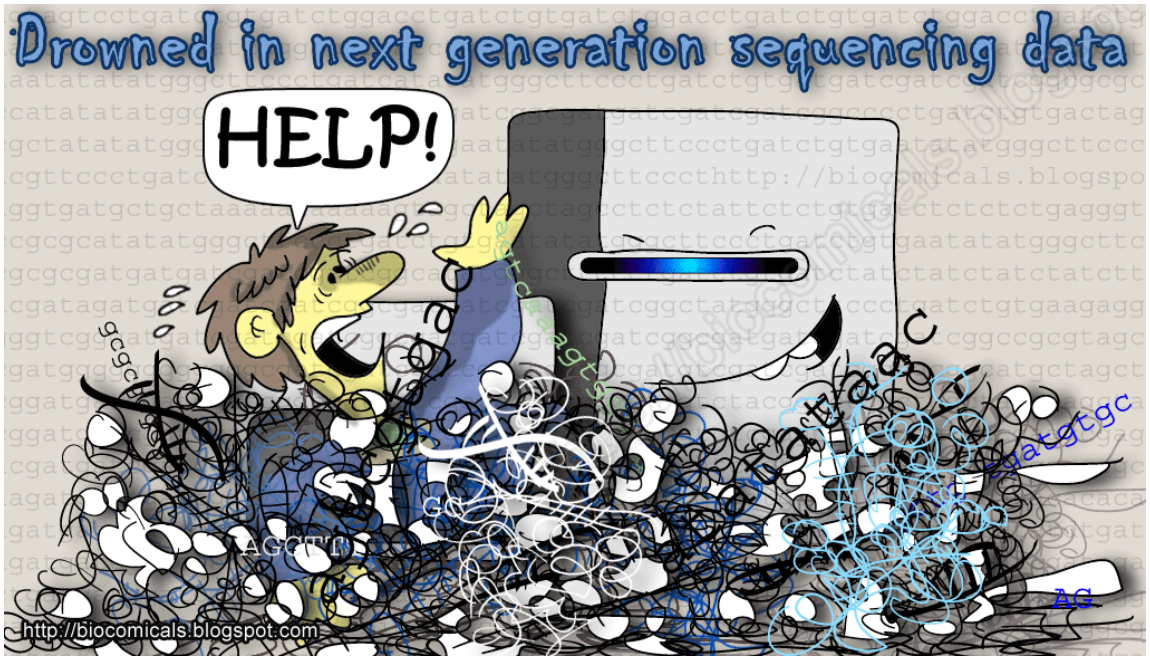
Genotype

Variants interpretation



genotype-phenotype correlations

Next generation sequencing :
from **variant detection**
to **variant interpretation**



Just spelling errors in DNA …

Patient’s DNA sequence

ATTGTACGTGATGACCAGTGGAAT
ACCGTAAGGTAAAGTACCGTGTAC
TTGGTTGGAACGTAGACTGAATGC
CAACCCTGGTATTGGTGTCCCGTG
TACAAGGTTAGTAATGTACCATTG
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
mistake ? GTGACGTAAC**C**TATATGACACACG
TCAGTACGGTCAGTACACACATGC
TGTGGTGCAGTACAGATACAGTAC
AGATTAGCAGAAATGCAGATTTAG
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC

“normale” DNA sequence

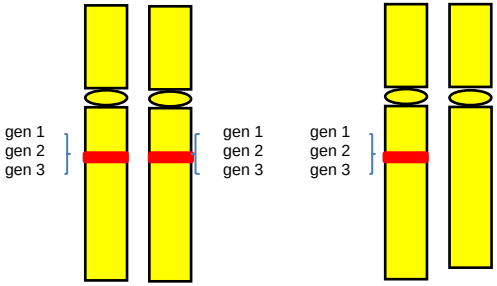
ATTGTACGTGATGACCAGTGGAAT
ACCGTAAGGTAAAGTACCGTGTAC
TTGGTTGGAACGTAGACTGAATGC
CAACCCTGGTATTGGTGTCCCGTG
TACAAGGTTAGTAATGTACCATTG
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAAC**T**TATATGACACACG
TCAGTACGGTCAGTACACACATGC
TGTGGTGCAGTACAGATACAGTAC
AGATTAGCAGAAATGCAGATTTAG
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC
TTCCGTAATACGTGTGGCGCGTGC
GTAACACACTGACTGACCATCCTG
GTAGCTAGTCAAGTCGTAGCTGTC
GTGACGTAACGTATATGACACACG
TCAGTACGGTCAGTACACACATGC

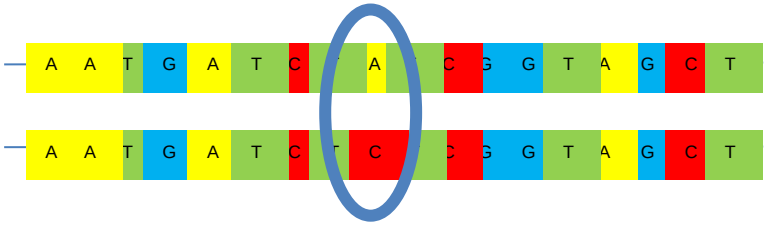
23 chromosomes
20,000 genes

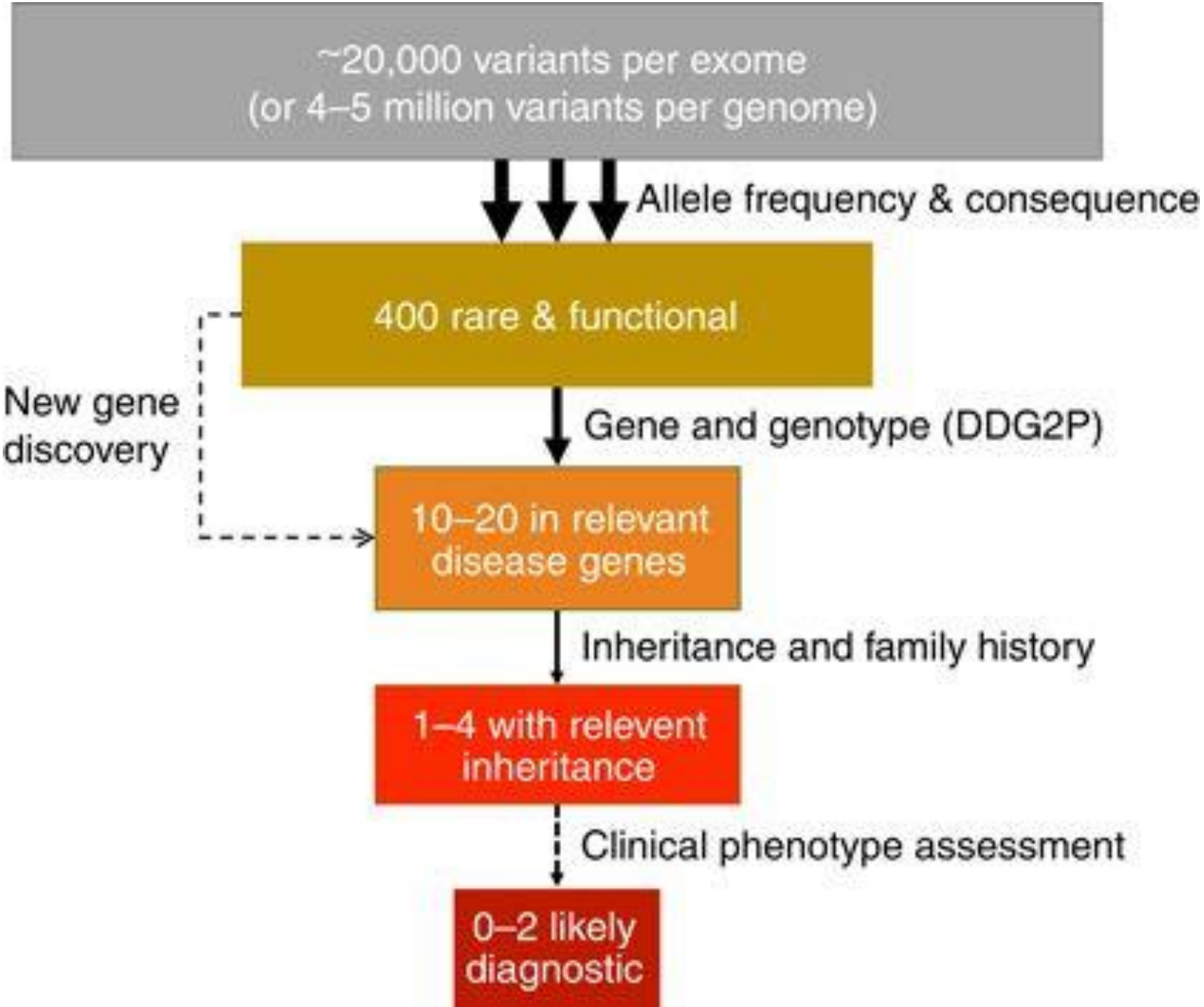
30 million nucleotides (exome)
3,2 billion nucleotides (genome)

chapters (CNV's):
+/- 150

letters (SNV's):
+/- 4-5 million (1/1000)






~20,000 variants per exome
(or 4–5 million variants per genome)
Allele frequency & consequence
400 rare & functional
New gene discovery
Gene and genotype (DDG2P)
10–20 in relevant disease genes
Inheritance and family history
1–4 with relevent inheritance
Clinical phenotype assessment
0–2 likely diagnostic

The “NORMAL” DNA sequence does not exist…

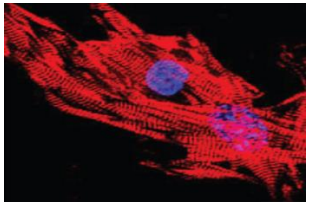
… just REFERENCE sequence

How to separate the wheat from the chaff ?

classification and **interpretation** of genetic variants

In silico

In vivo



In vitro

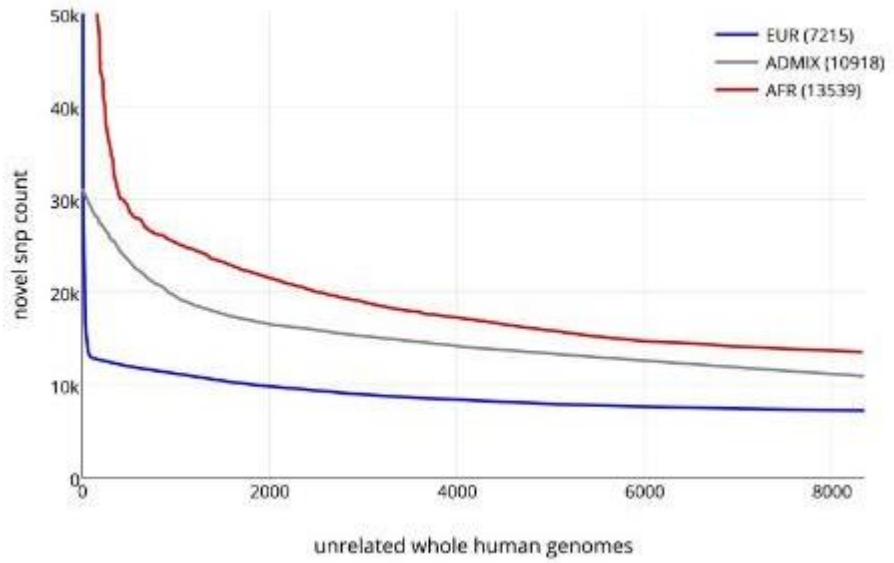
pathogenic **??** **benign**

+ does the variant explain the phenotype?

In silico

DATABASE

* **Normal variants** : variant is too frequently identified in the general population



Africa
13,539

Europa
7,215

- each new sequenced genome: an average of 8,579 new variants
- each genome: an average of 700,000 bp absent in the reference genome

Telenti et al. PNAS, 2016;113:11901–11906

In silico

DATABASE

* **<u>Known pathogenic mutation</u>** : described in persons with the disease

EJHG Open

European Journal of Human Genetics (2012) 20, 905–908

High prevalence of genetic variants previously associated with LQT syndrome in new exome data

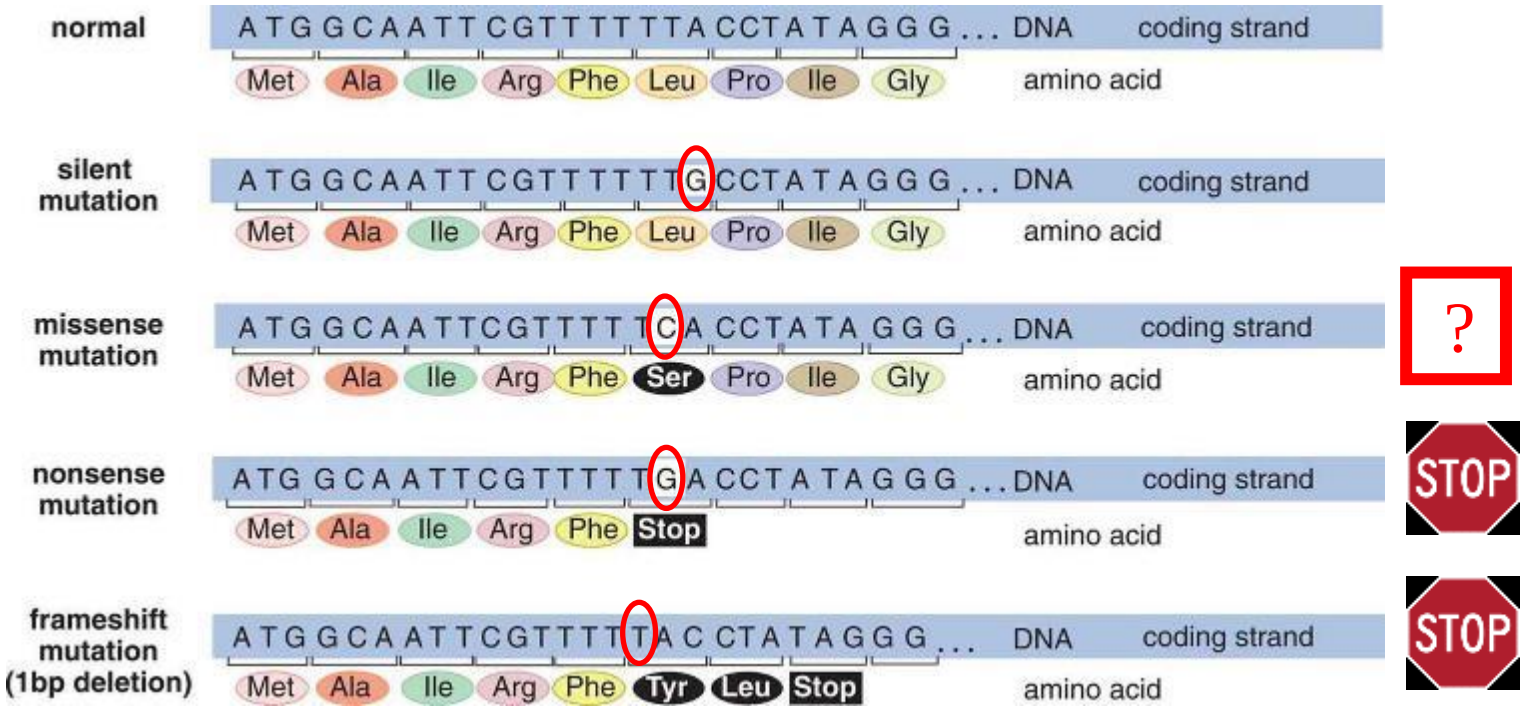
Clin Genet 2013

High prevalence of genetic variants previously associated with Brugada syndrome in new exome data

In silico

PREDICTION PROGRAMS

- Charge of amino acid
- Amino acid conservation
- Protein domain structure
- Effect on splicing
- Effect on protein interactions



In silico

PREDICTION PROGRAMS

14819 missense variants in CLINVAR

		CORRECT CONCORDANCE ?		FALSE CONCORDANCE ?	
		18/18 tools	**3/3 freq. tools***	**18/18 tools**	**3/3 freq. tools***
BENIGN	n = 7346	5,2%	46,2%	0,8%	18,2%
PATHOGENIC	n = 7473	39,2%	84,9%	0,003%	2,1%

* 3 frequently used tools: Polyphen, SIFT,CADD

How to simplify mutation identification ?

1. CLINICAL INFORMATION => gene-panels

= prioritization of genes that are analyzed

= based on clinical information

=> reduction of variant “noise”

epilepsy genes

Arthrogryposis genes

How to simplify mutation identification ?

In vivo

2. TAKE (likely) INHERITANCE MECHANISM INTO ACCOUNT

- de novo dominant
- autosomal recessive
- X-linked

DDD study : yield +/- 40%

1. Vast majority
= de novo dominant

⇒TRIO ANALYSIS

Will also pick up de novo X-linked !

2. **Quantifying the contribution of recessive coding variation to developmental disorders**

Martin et al., Science 362, 1161–1164 (2018)

6040 children with intellectual disability

N= 5684

European

3,6%

Mostly de novo dominant
also X-linked

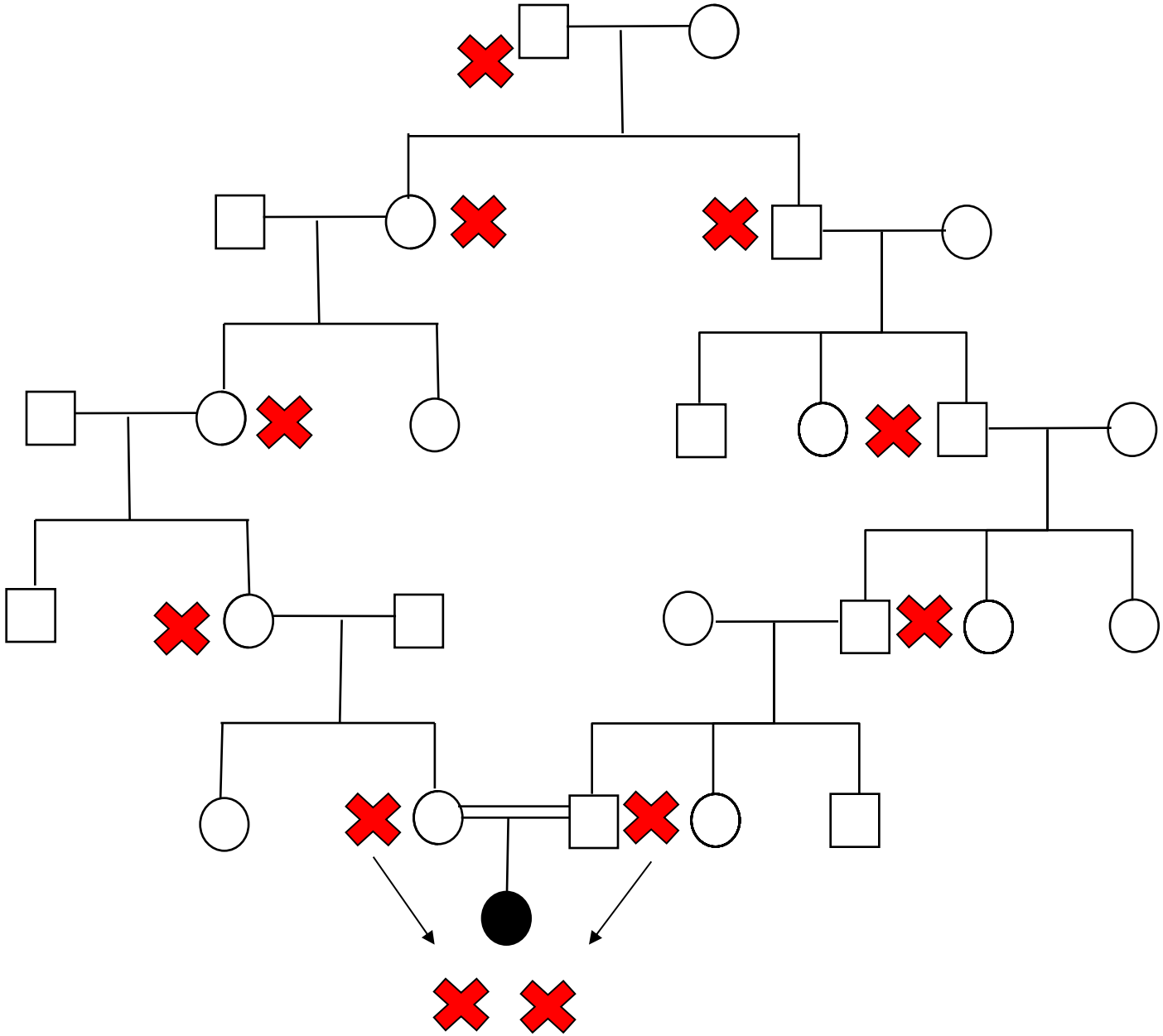
N= 356

Pakistani

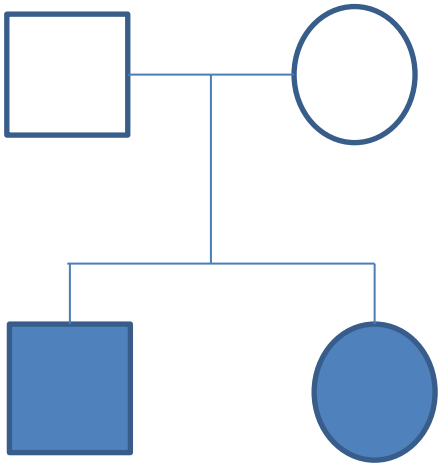
31%

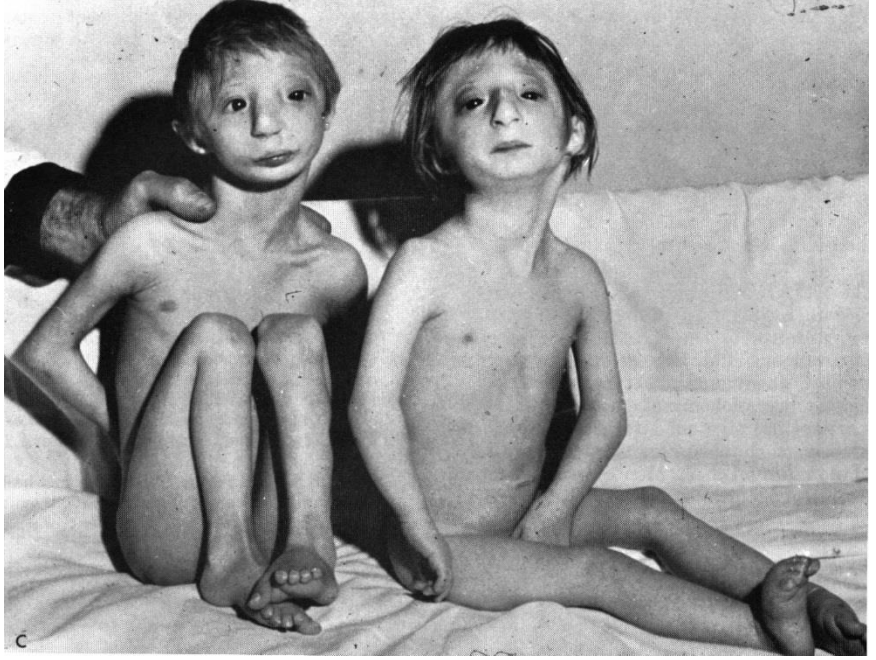
autosomal recessive
= homozygous variants
consanguinity

% children with autosomal recessive condition



HOMOZYGOTE FOR THE MUTATION

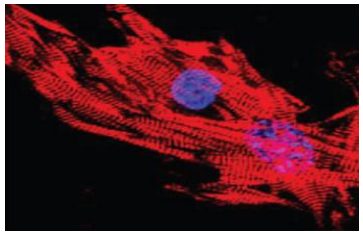




UNRELATED PARENTS

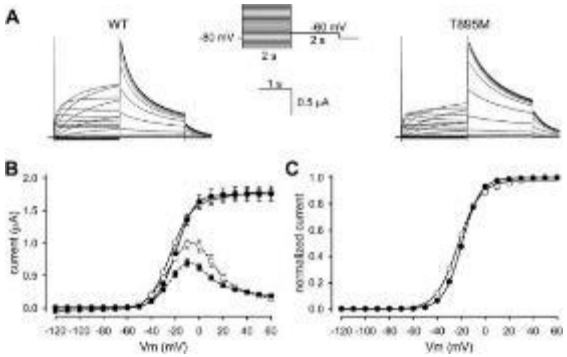
=> Two mutations in 1 gene?

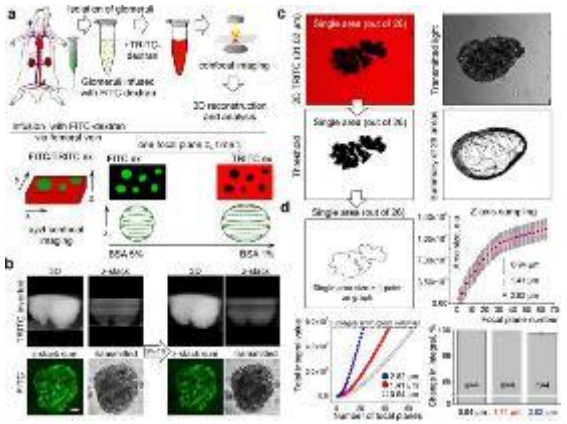
- Can be different mutations or the same
- One from each parent
- QUAD ANALYSIS

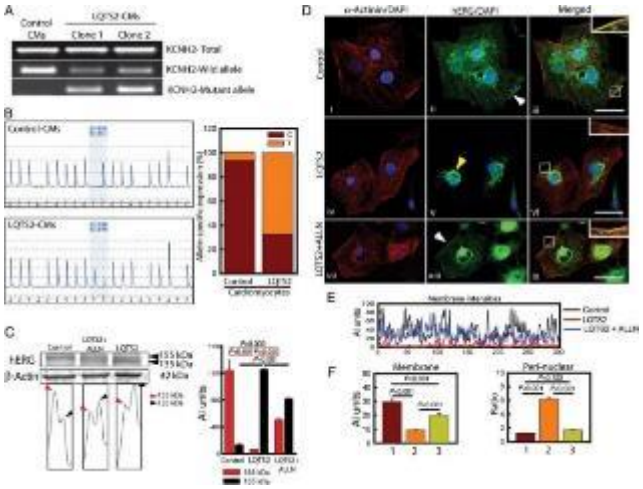


In vitro

MOSTLY RESEARCH !



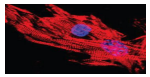




SYNTHESIS OF CLASSIFICATION?

In silico

In vivo



In vitro

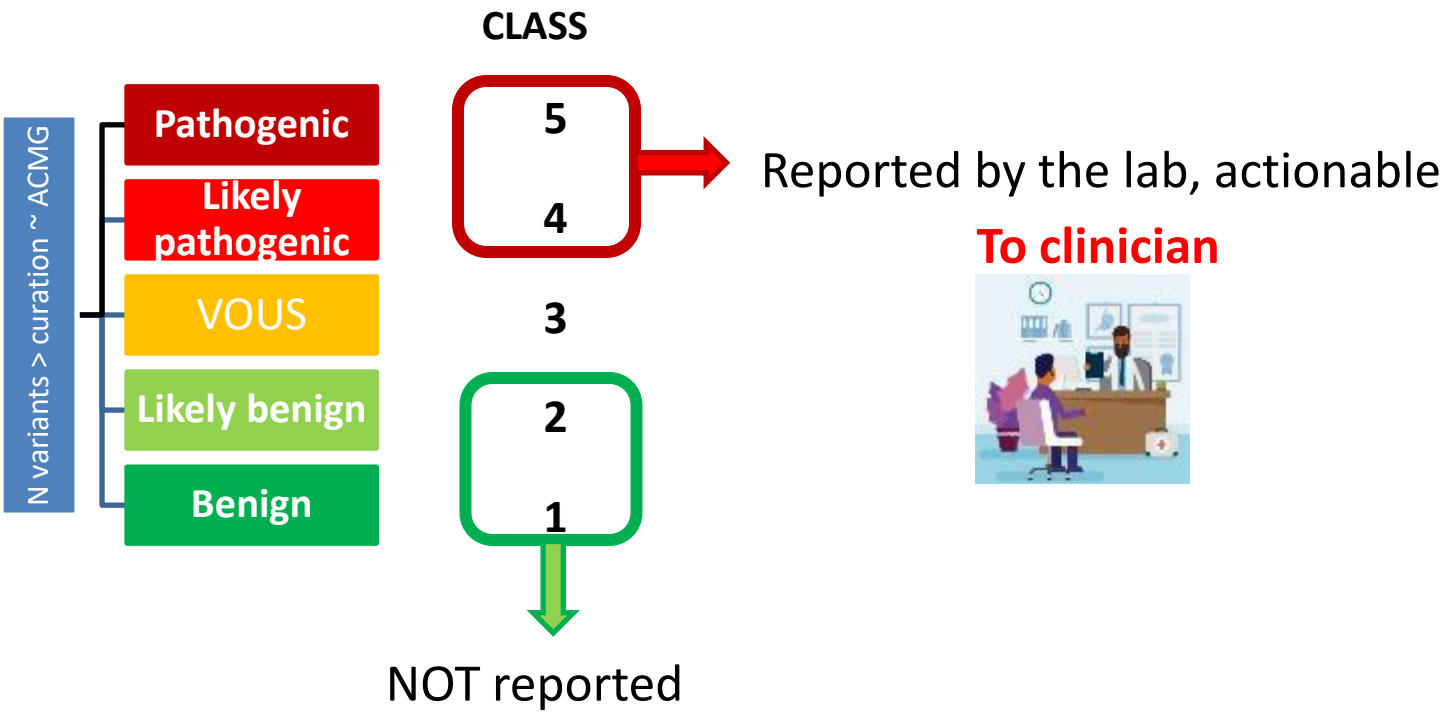
ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

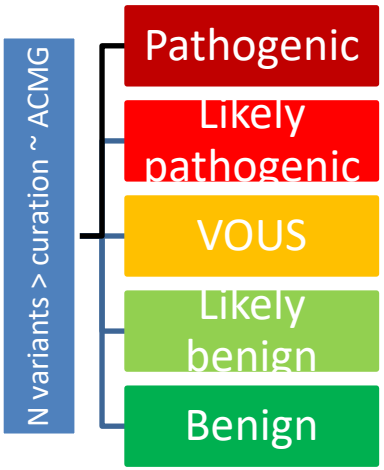
Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Disclaimer: These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Standards and Guidelines. They also are advised to take notice of the date any particular guideline was adopted and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Genomic Variation – report



Genomic Variation – report



CLASS

5

4

3

2

1

Usually 'NOT' reported unclassified variants

However sometimes reported

RESEARCH

SEGREGATION

Class 5: pathogenic > 99%
Class 4: likely pathogenic 95 tot 99%
Class 3: unclassified variant 5 - 94.9%
Class 2: likely not pathogenic 1-0.49%
Class 1: no functional effect <1%

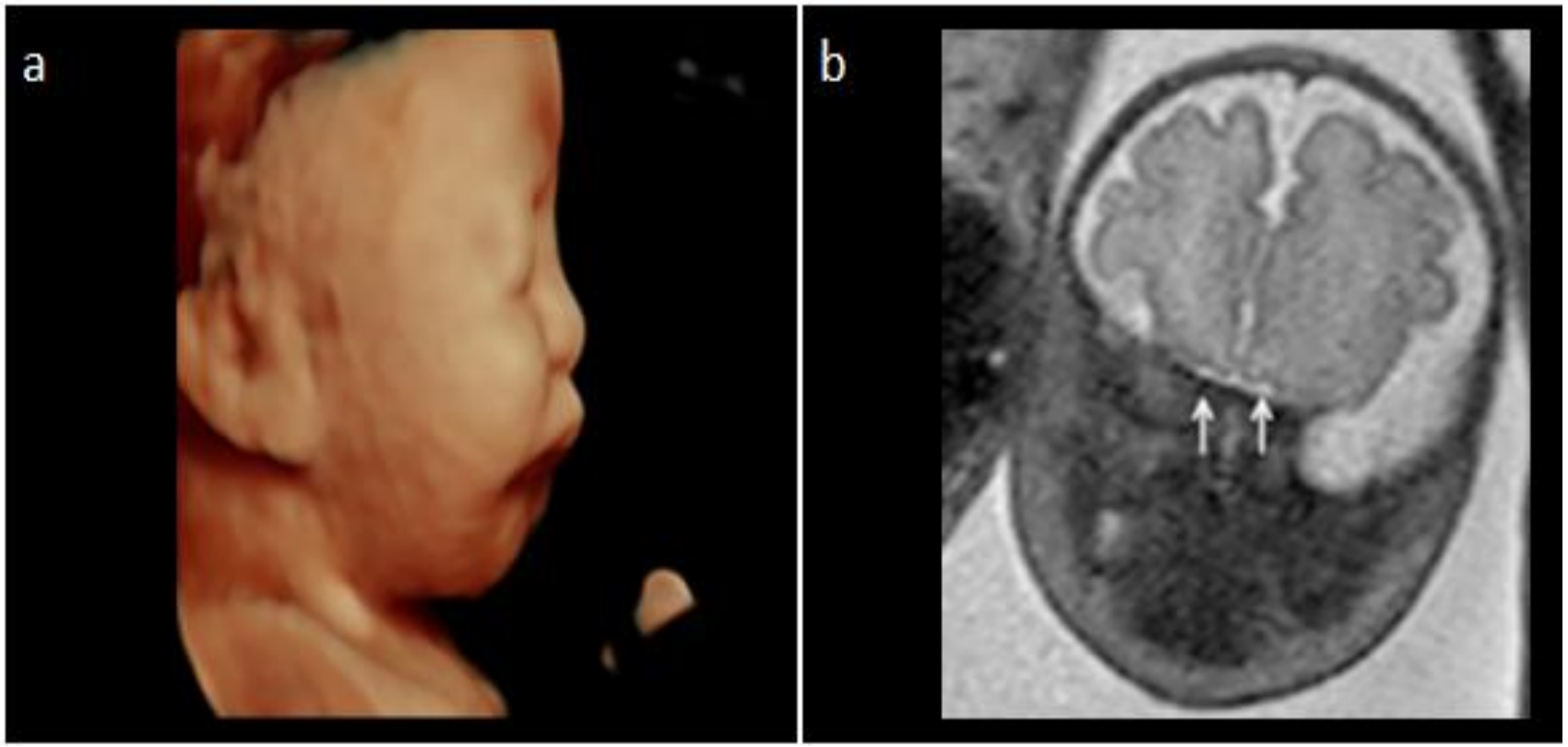
Probability

Examples of « touchy » variant interpretations

KU LEUVEN

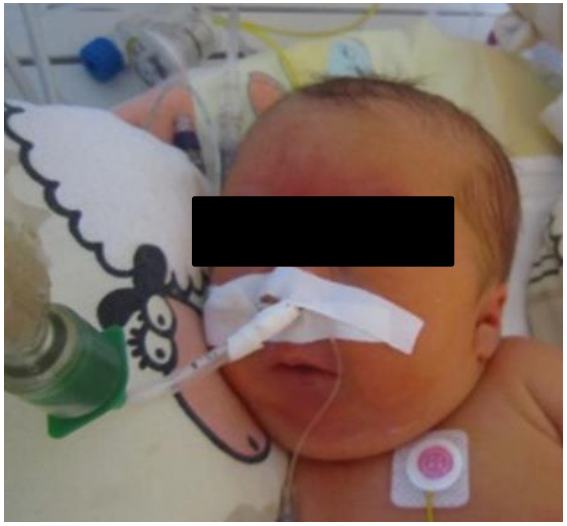
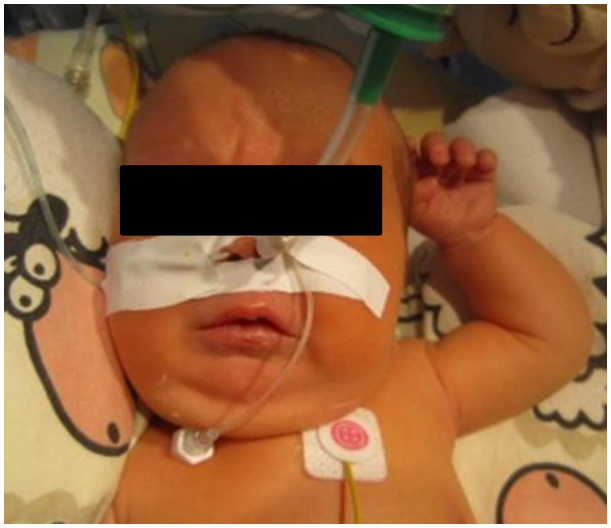
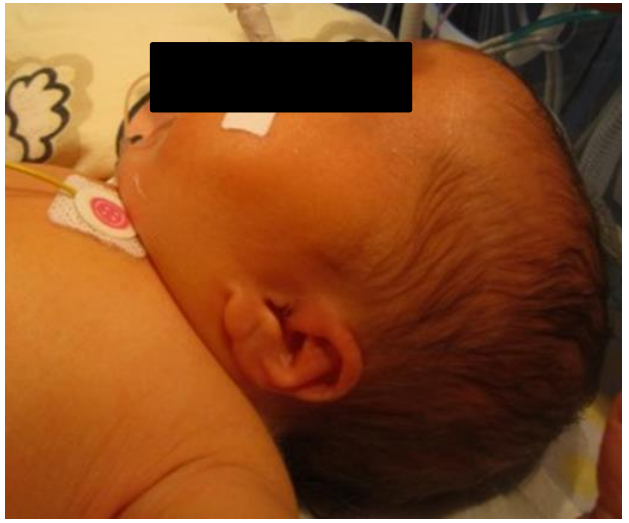
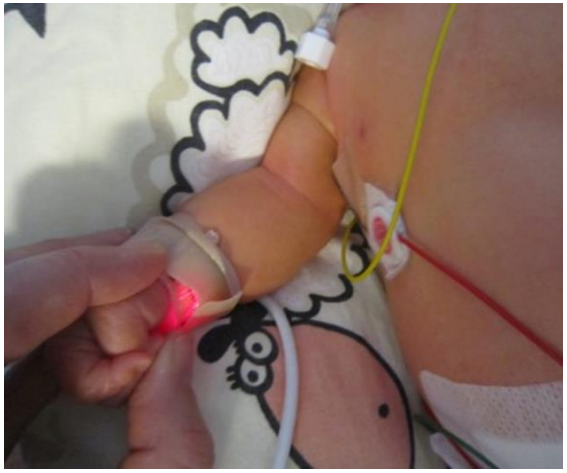
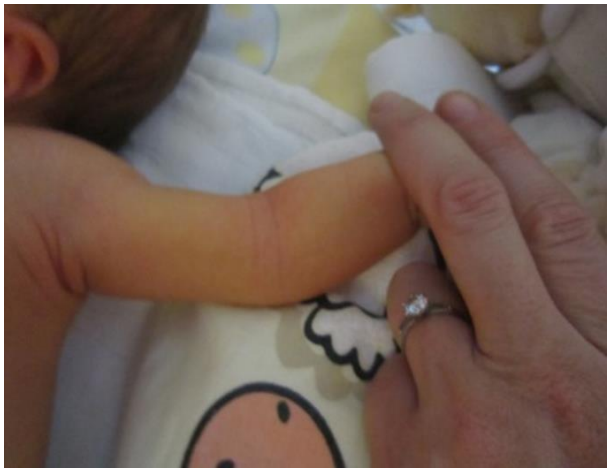
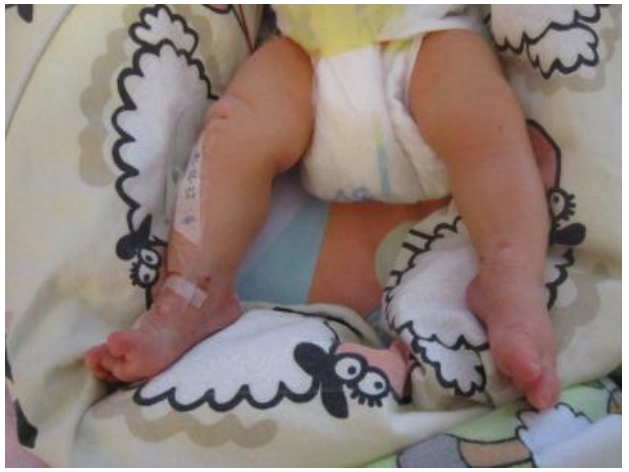
IPG

Case 1



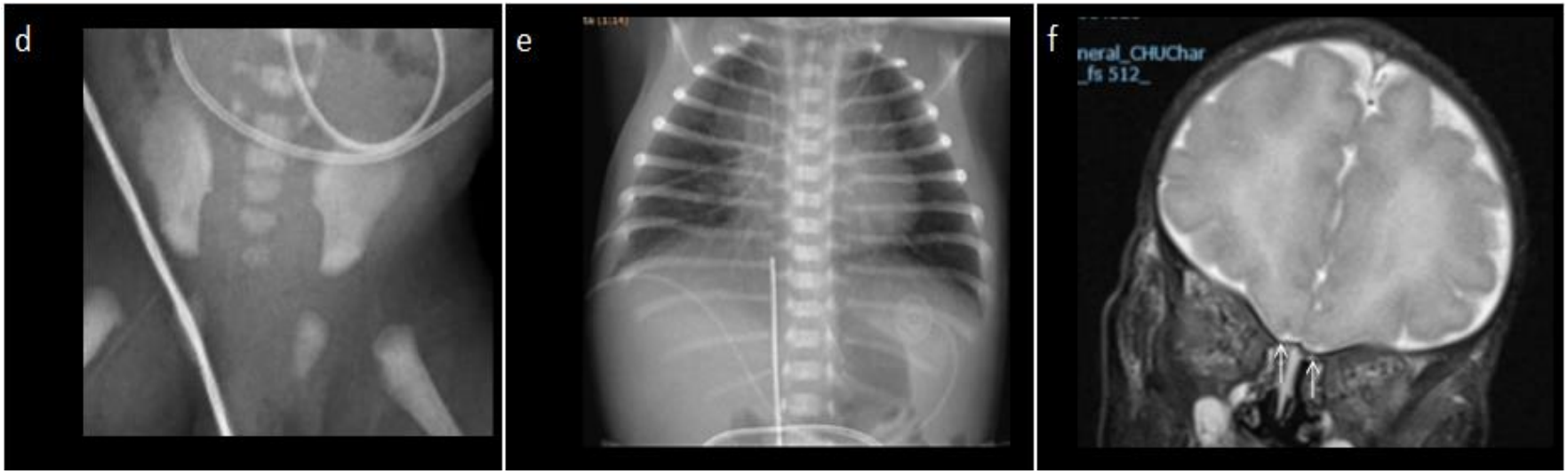
Antenatal signs

- Flat face, retrognathia
- Cleft palate
- Polyhydramnios
- Thin corpus callosum
- Suspicion of olfactory bulbs agenesis

At birth (37w)

- 2kg690 (P35), 46cm (P25), 35cm (P90)
- Flat face, retrognathia, low set ears, posterior cleft palate, short neck
- Rhizomelic shortening, elbow contractures, short and large hands, feet equinus deformity
- Severe respiratory distress



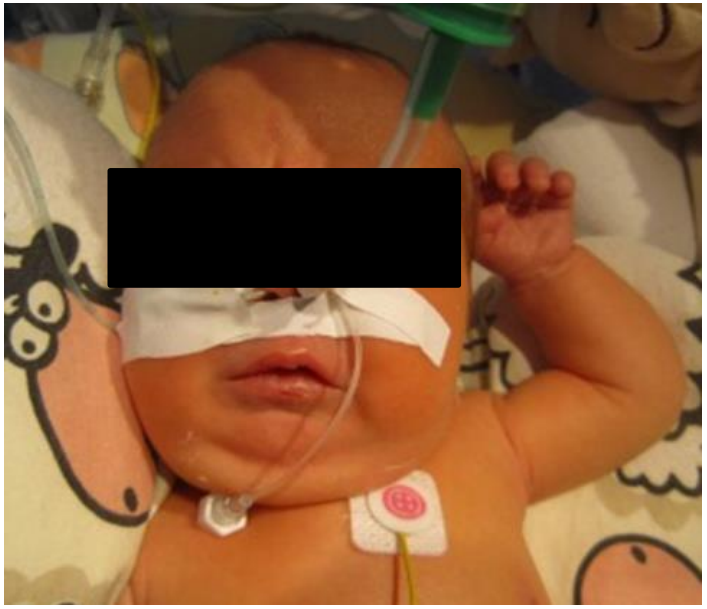
- Heart US : normal
- X-rays: hypoplastic narrow iliac wings, dislocated hips, verticalization of the ischions and lack of ossification of the pubis, gracile ribs

- Death at 8 days from severe respiratory failure

- Post-mortem MRI: global simplified gyral pattern and agenesis of olfactory bulbs

Suspicion of …

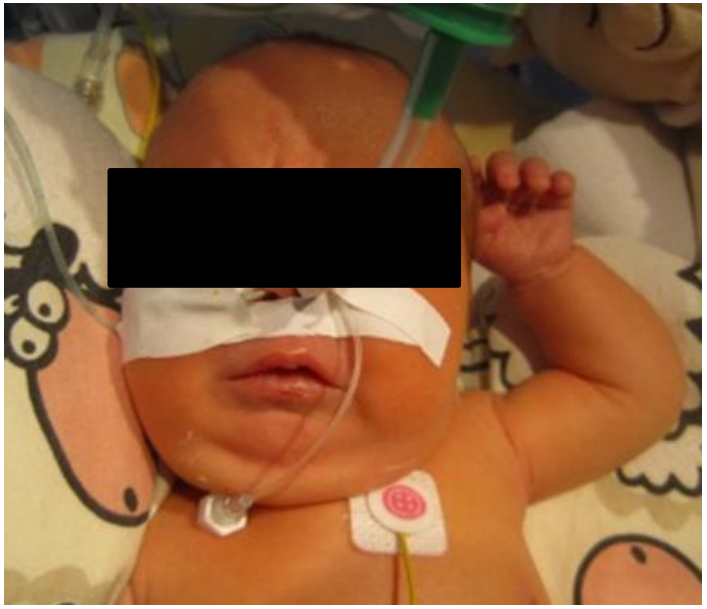
⇒ **Mendelioma**



SOX9: Hz c.250T>G p.(Tyr84Asp)

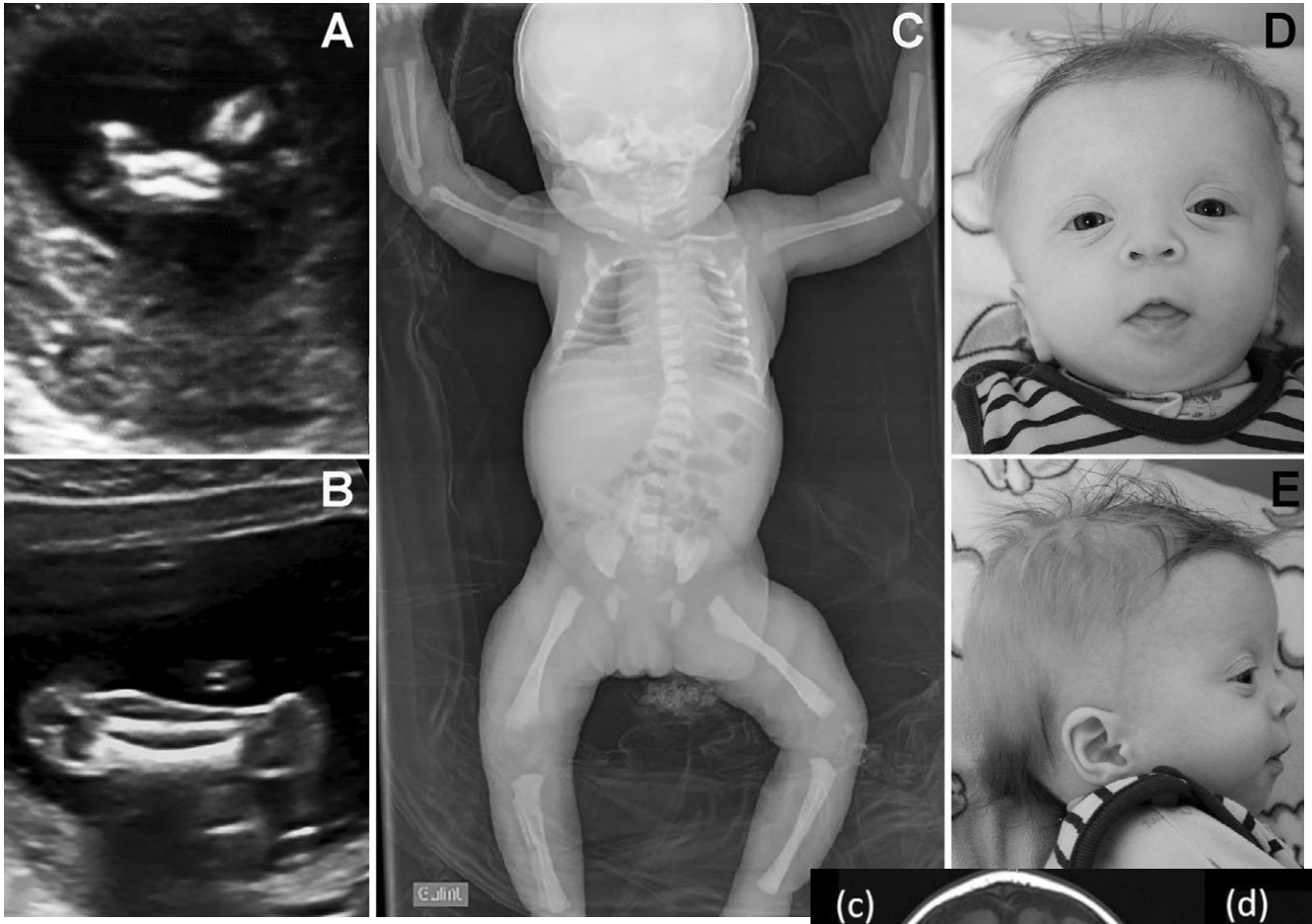
1. Trio analysis: de novo variant 👍
2. General population database (GnomAD): variant not known 👍
3. Literature: not yet reported in other affected patients 👎
4. Prediction programs: likely pathogenic 👍

- **Very conserved nucleotide (phyloP: 4,73 [-14,1;6,4]**
- **Very conserved amino acid to drosophilia (through 12 species)**
- **Important physico-chemical change from Tyr to Asp (Dist.Grantham, 121[0-215]**
- **Align GVGD (v2007): C0 (GV:227.53-GD:59.88)**
- **SIFT (v6,2,0): Deleterious (score:0.01, media,:3.32)**
- **Mutation Taster (v2013): disease causing (p-value: 1)**

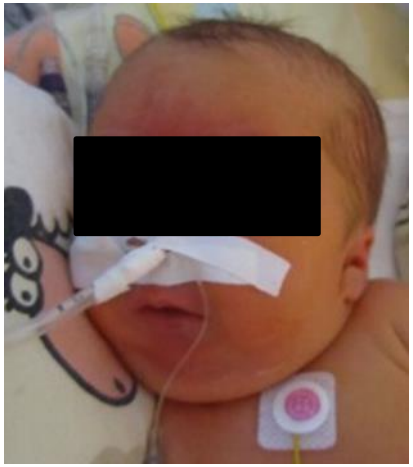


SOX9: Hz c.250T>G p.(Tyr84Asp)

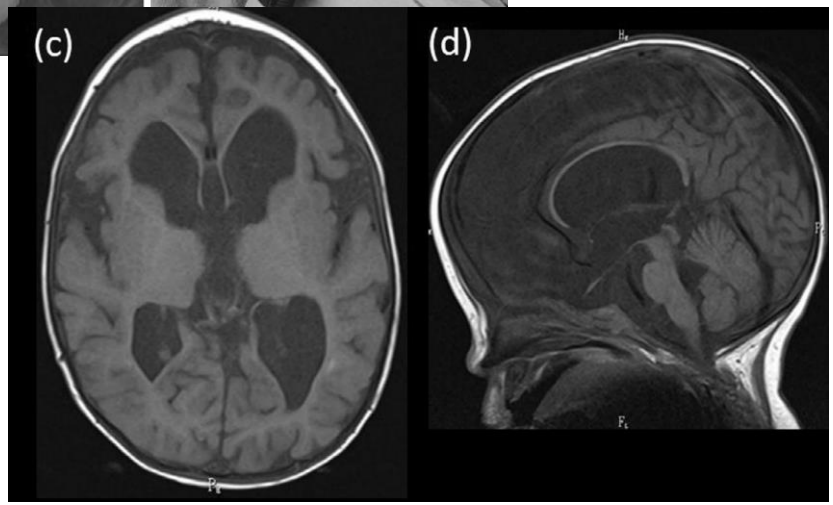
1. Trio analysis: de novo variant 👍
2. General population database (GnomAD): variant not known 👍
3. Literature: not yet reported in other affected patients 👎
4. Prediction programs: likely pathogenic (7/8) 👍
5. Could SOX9 mutation be related to the phenotype?



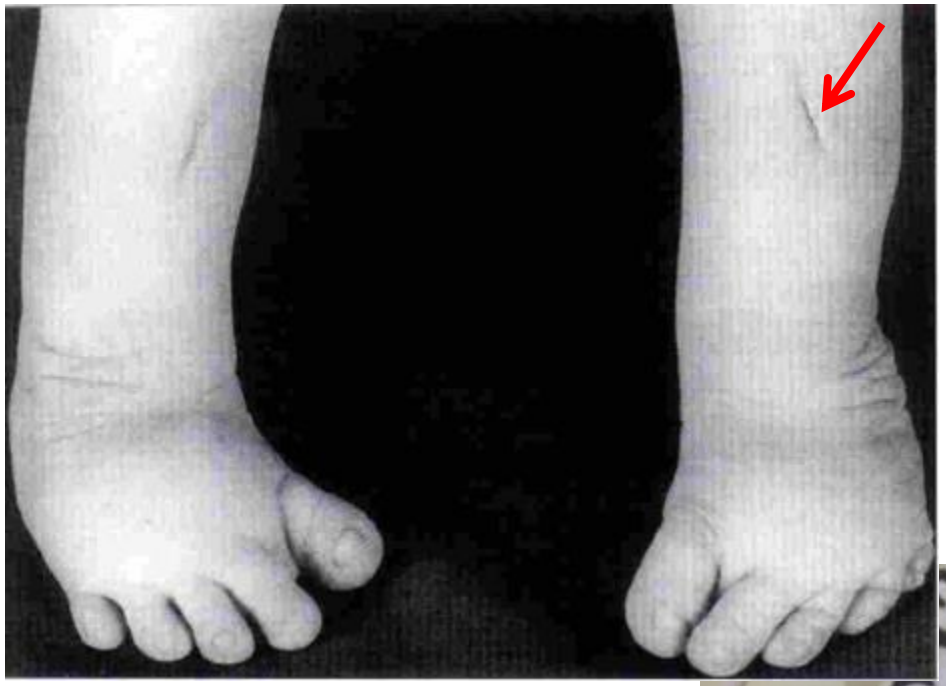
Preiksaitiene E. et al, Am J Med Genet 2015

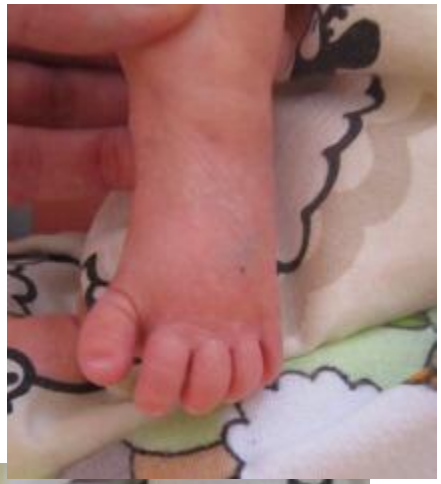


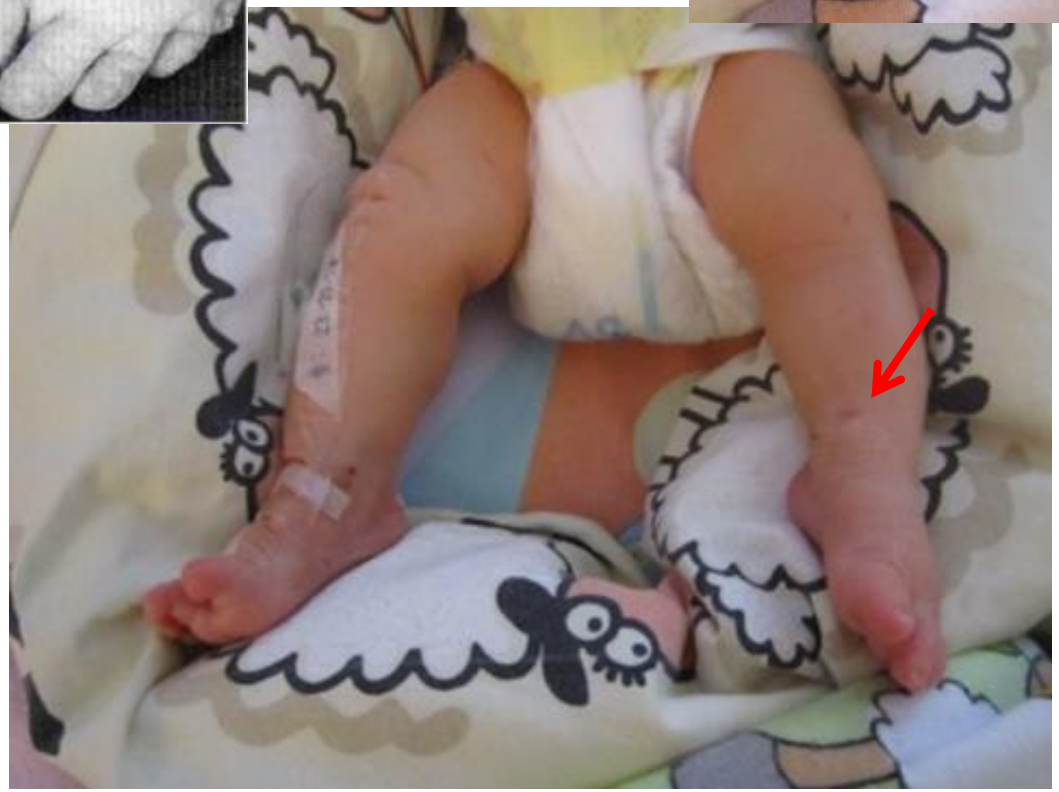
Cases of acampomelic campomelic dysplasia with SOX9 mutations



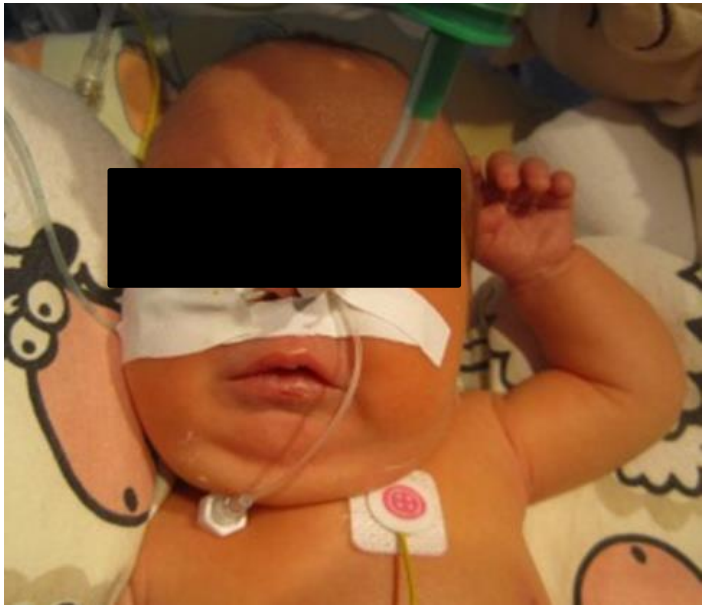
Matsumoto A. et al, Brain and dvpt 2018





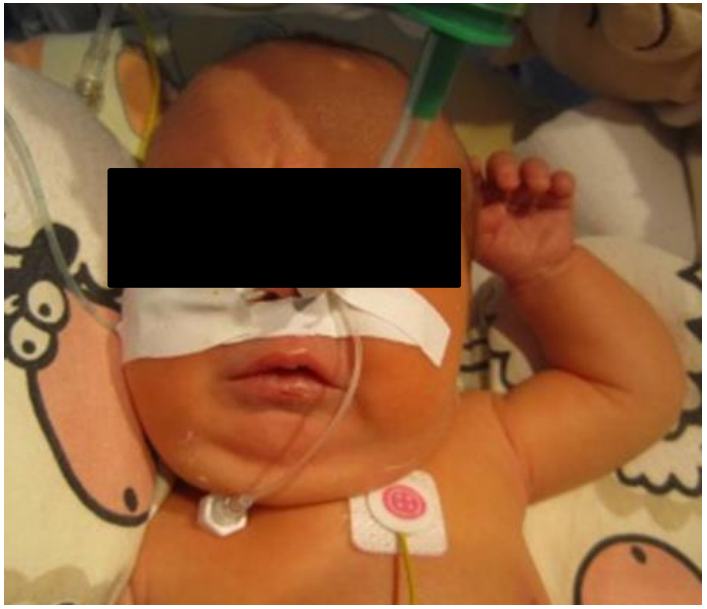


Savarirayan et al, Pediatr Pathol Mol Med, 2003



SOX9: Hz c.250T>G p.(Tyr84Asp)

1. Trio analysis: de novo variant 👍
2. General population database (GnomAD): variant not known 👍
3. Literature: not yet reported in other affected patients 👎
4. Prediction programs: likely pathogenic (7/8) 👍
5. Could SOX9 mutation be related to the phenotype? Yes 👍



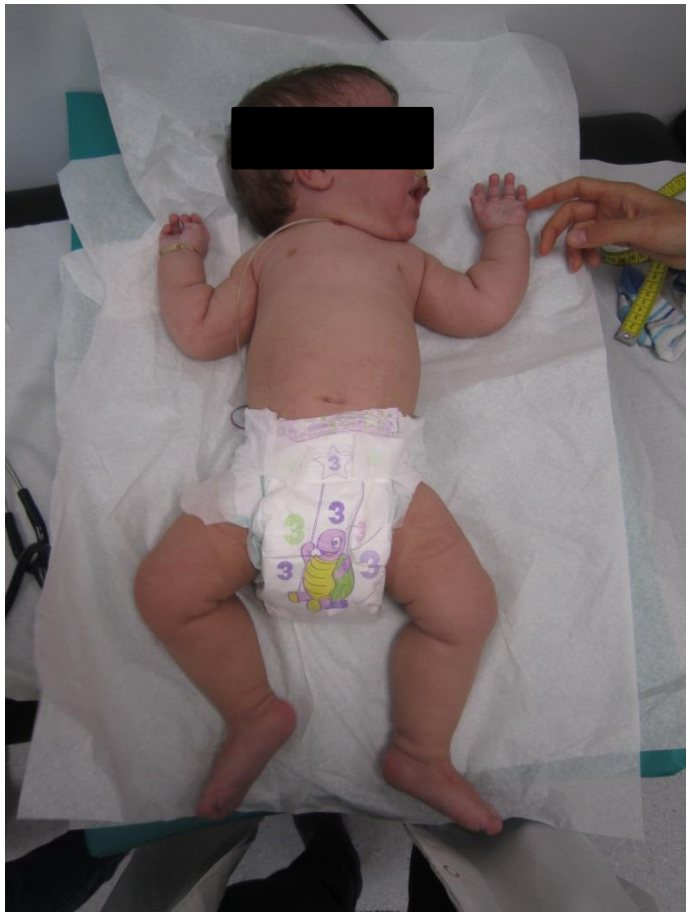
SOX9: Hz c.250T>G p.(Tyr84Asp)

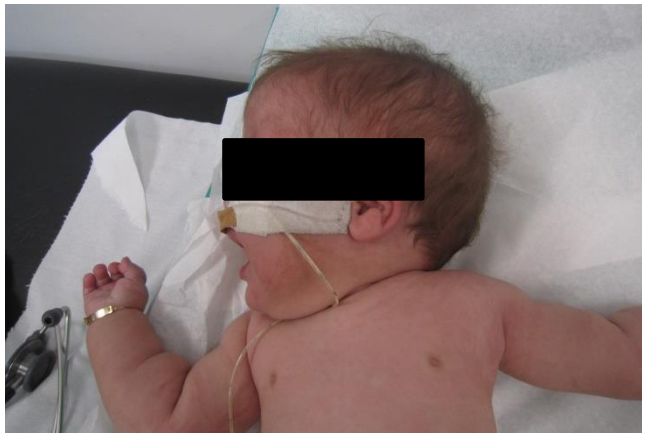
ACAMPOMELIC CAMPOMELIC DYSPLASIA

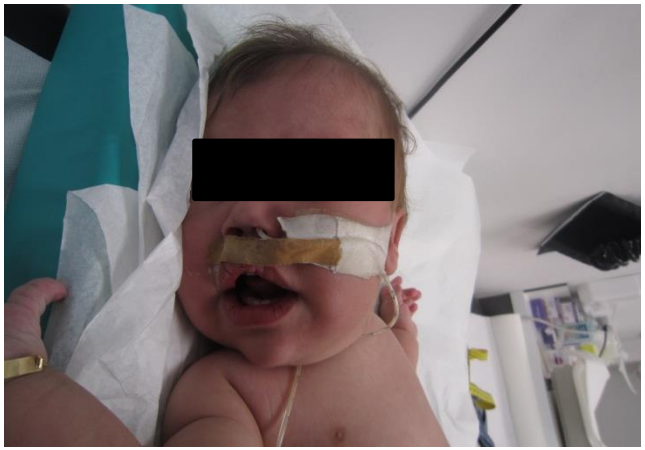
- poor respiratory prognosis
- low recurrence risk

Diagnosis, prognosis
and
genetic counselling

Case 2







- Consanguineous parents
- Severe hypotonia, feeding difficulties
- Hepatomegaly
- Coarse face
- Bilateral optic atrophy
- Severe developmental delay
- Death at 26 months (multiple organe failure)

Suspicion of … Autosomal recessive metabolic disease?

- **Trio mendeliome : no etiology**
- **Simplex exome (Nijmegen): no etiology**
- **Trio exome (research purpose):**

  homozygous c.452C>A=p.Pro151His in NAA30

1. Trio analysis: recessive inheritence
2. General population database (GnomAD): variant not known
3. Literature: NAA30 mutations never reported in human disease
4. Prediction programs: VOUS (3 pathogenic, 4 benign)

MOLECULAR & CELLULAR
PROTEOMICS

Published by the American Society for Biochemistry and Molecular Biology

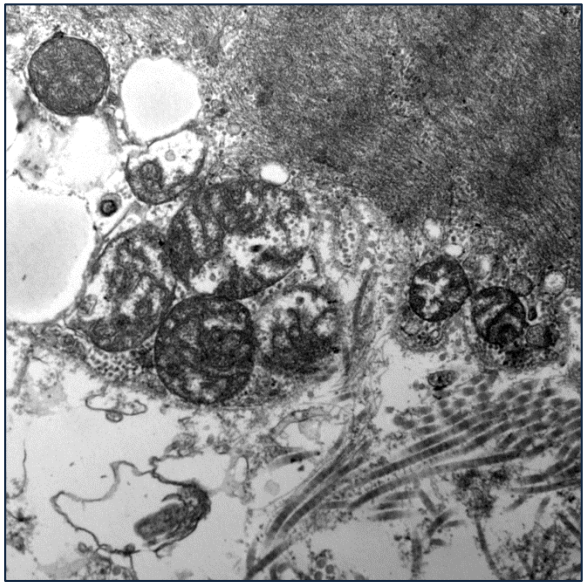
Mol Cell Proteomics. 2016 Nov; 15(11): 3361–3372.
Published online 2016 Sep 30. doi: 10.1074/mcp.M116.061010

PMCID: PMC5098035
PMID: 27694331

A Role for Human N-alpha Acetyltransferase 30 (Naa30) in Maintaining Mitochondrial Integrity[*] [S]

Petra Van Damme,[‡§¶¶||] Thomas V. Kalvik,[¶||] Kristian K. Starheim,[¶**||] Veronique Jonckheere,[‡§] Line M. Myklebust,[¶] Gerben Menschaert,[‡‡] Jan Erik Varhaug,[||§§] Kris Gevaert,[‡§] and Thomas Arnesen[¶§§]

Research studies on drosophila



Patient's muscle biopsy:
Abnormal mitochondrial structure

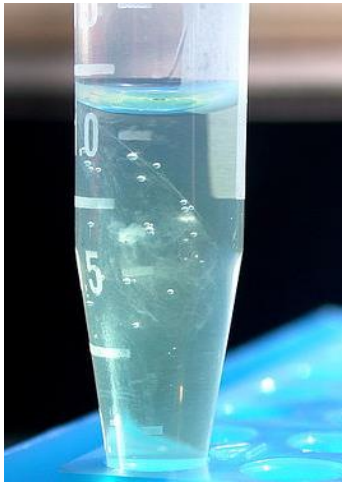
Variant signification???

International research collaboration

Genetic diagnostics
Still time-consuming and labor-intensive

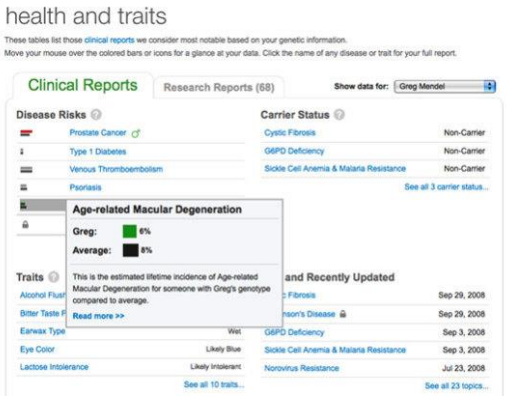
Golden rule:

Nice to know
Need to know



DNA extraction
& long term storage
30 €

Whole-genome
sequencing
1500 € (incl. BTW)



Complete analysis
Report
Pre/posttest counselling
4500 €

Take-home message

1. **NGS can be a good diagnostic tool**
   - Good clinical information is crucial
   - Trio strategy and familial segregation studies
   - Parental consent (! Incidental findings)

   But interpretation of the variants remains challenging

   (international databases, collaborative research, …)

2. **Screening tool?**

   ↑ therapeutic perspectives in the future…

3. **Poor predictive value for multifactorial diseases**

   (autism spectrum disorders, psychiatric diseases,…)

KU LEUVEN

IPG

☹ Commercial abuse !!!

Character Traits Inborn Talent Gene Test (1397$)

Ability1 Optimism2 Risk-taking3 Persistence4 Shyness5 Composure6 Split personality7 Hyper Activeness 8 Depression9 Impulsive10 Attentiveness11 Adaptability
IQ12 Intelligence 13 Comprehension14 Analytical15 Memory16 Creativity17 Reading Ability18 Imagination
Artistic 19 Performing20 Musical21 Drawing22 Dancing23 Literature24 Linguistic
EQ25 Affectionate26 Faithfulness27 Passion28 Propensity for Teenage Romance29 Sentimentality30 Sociability31 Self Reflection 32 Self Control
Sports33 Endurance34 Sprint 35 Technique 36 Training Sensitivity37 Tendency of Sport Injuries 38 Sport Psychology
Physical Fitness39 Height 40 General Wellness
Health41 Obesity42 Sensitivity to 2nd-hand Smoke 43 Insensitivity to 2nd-hand Smoke
Addiction44 Alcoholism45 Smoking 46 General addiction

AATDGTCGCGTTAACGTACTGACTTGACCATTT
AAATTGGTTCHGTAGACGAAGTCTATGGCTGG
CCGTCTGCATTGAAATGATGAAACGCGCGTGC
CGATACGATAGGAGGTCACTGCCGNCTGNCAT
TGAAACACCCATTGCAAGTCTATGGTTGGCCK
TCTGCATAAAACGUGCGTGCCGATAGCGCGTG
CCGATAGCGCGTGCCGATACGATAGGAGGTCA
CTAATTGGTTCCGYAGACGCGTAGACTGTAATC
GTGGTTGAAATGAATTGGTOCCGTAGACTGATA
CAGTAAATTGGTTCCGTAGACGGTACUGTAGG
CATGACCATGGTACGATGACGTCAATTGGTTCC
GTAGACTTAACGGTCATTGTCCGTACGGTTAAA
CCTGTAAATACCGTCGCGCGTTGACGTATTGTC
GTGGTCGAATGTACTATATAGACAATTGGTTCC

KU LEUVEN

IPG

KU LEUVEN

IPG