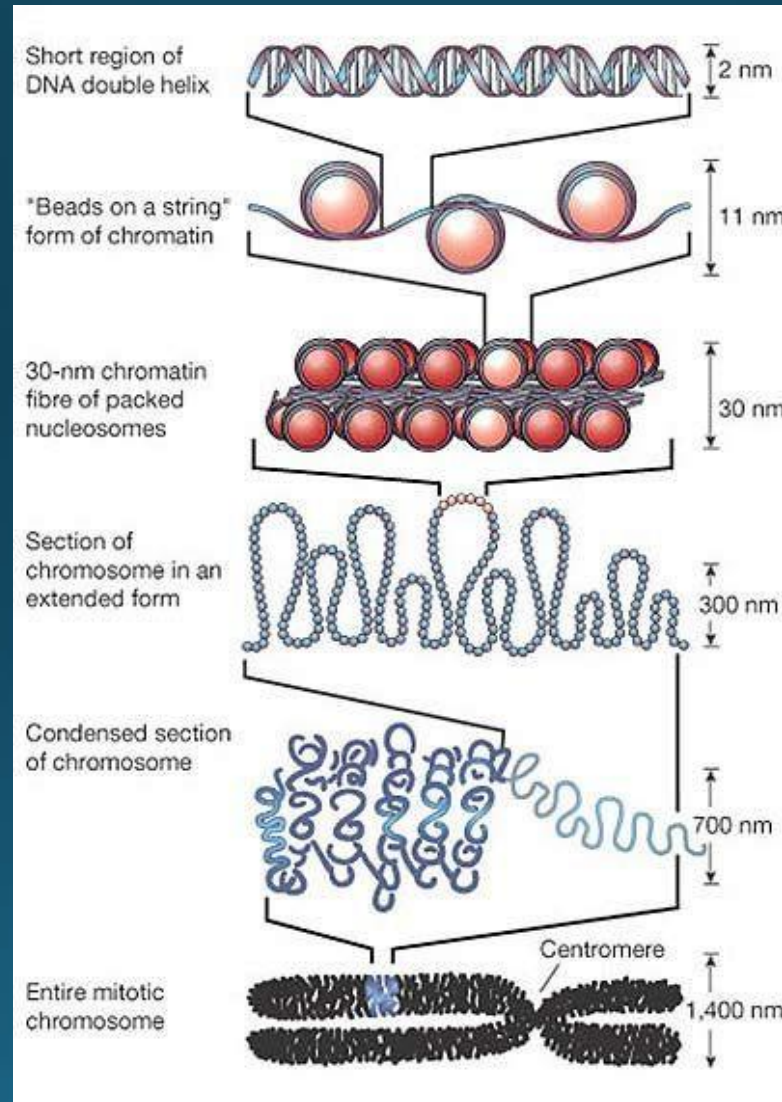


# Postnatal Cytogenetics

Jennifer Castaneda, MD, PhD  
WUM 2025

# Review: chromosome structure

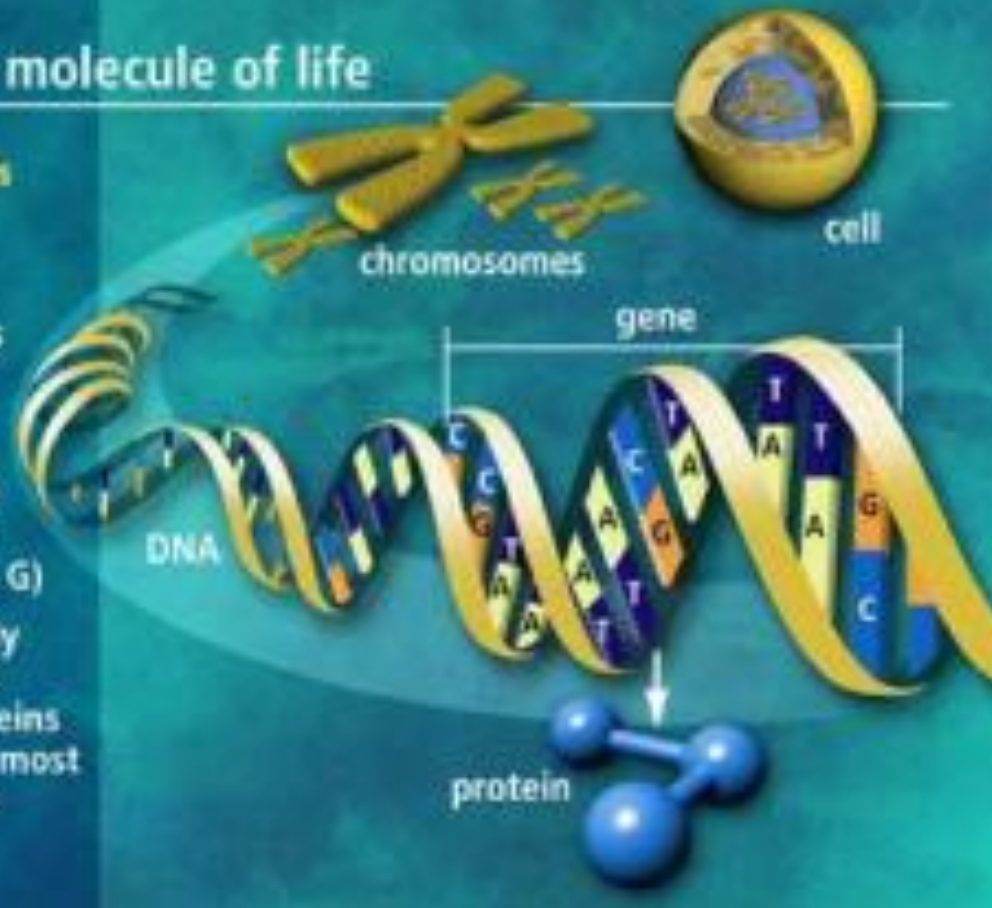


# DNA the molecule of life

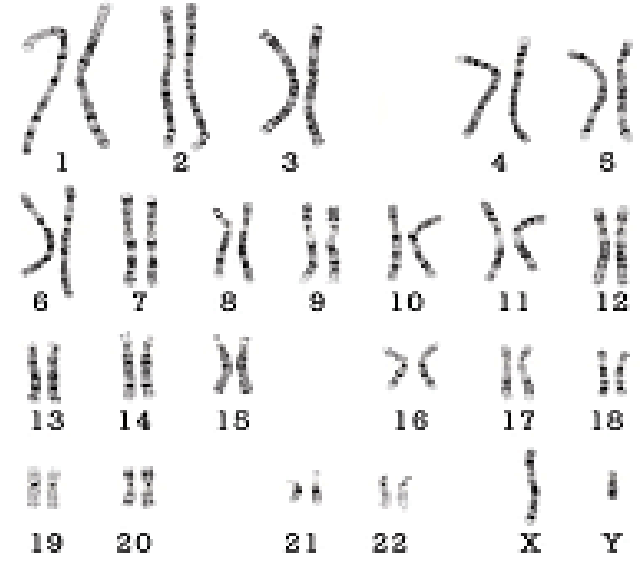
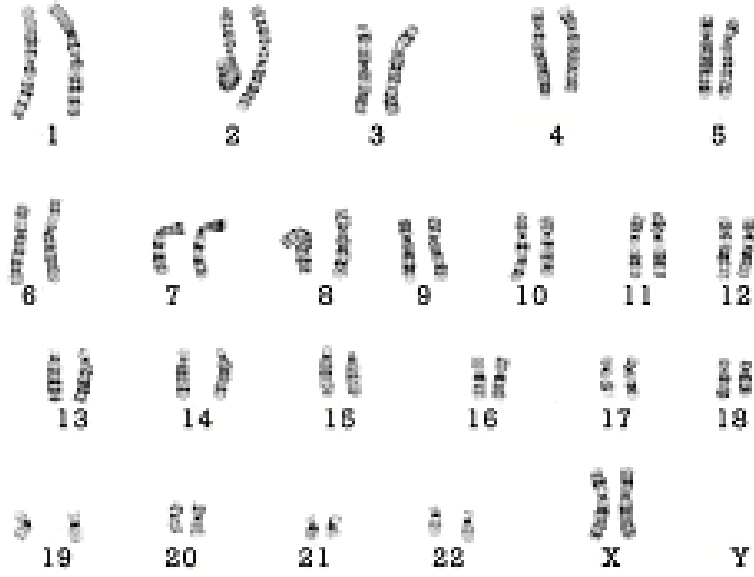
## Trillions of cells

Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions

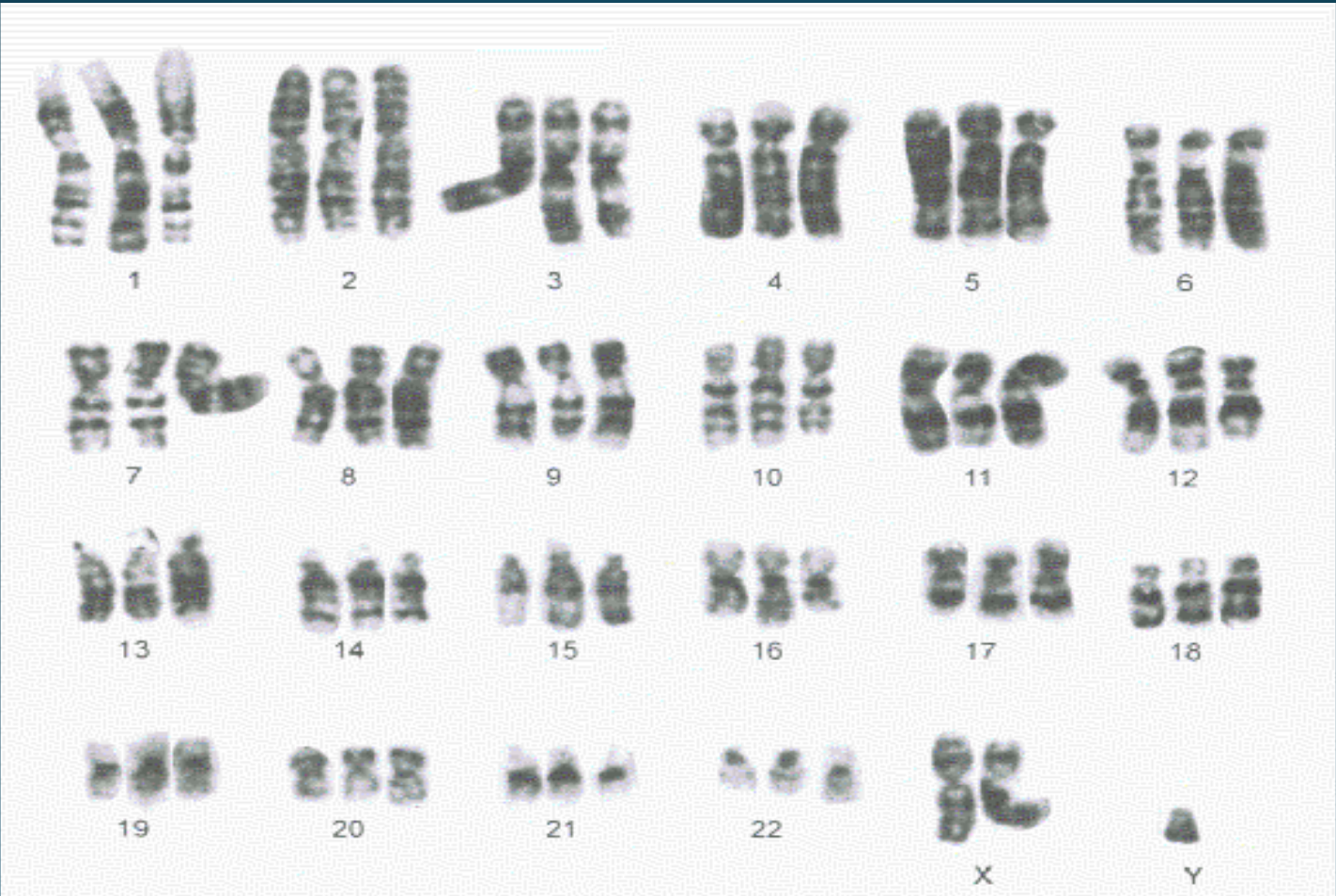


<https://thenaturalhistorian.com/2013/02/24/genetic-distance-humans-chimpanzees-divergence-baraminology/>



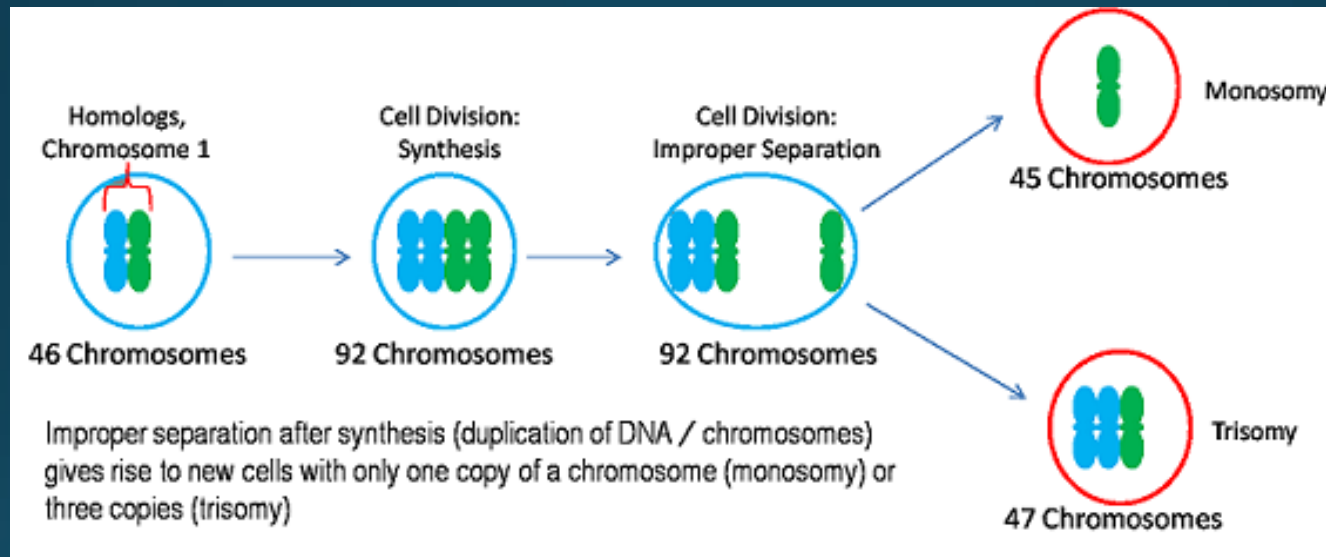
# Classification of chromosomal aberrations

- ▶ Autosomal vs sex chromosome aberrations
- ▶ Abnormalities of chromosome number:
  - ▶ Polyploidy
  - ▶ Autosomal aneuploidy
  - ▶ Sex chromosome aneuploidy
- ▶ Abnormalities of chromosome structure
  - ▶ Translocations
  - ▶ Deletions, microdeletions
  - ▶ Duplications
  - ▶ Inversions
  - ▶ Ring chromosomes





# Aneuploidy caused by meiotic disjunction



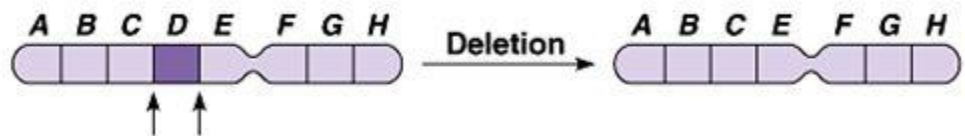


# Changes in chromosome structure

error of replication

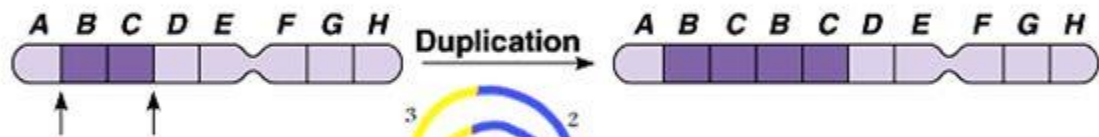
- deletion

– loss of a chromosomal segment



- duplication

– repeat a segment



error of crossing over

- inversion

– reverses a segment



- translocation

– move segment from one chromosome to another



# Chromosomal aberrations

- ▶ 1 of 150 live births
- ▶ The earliest (1959) identified chromosomal aberrations:

Trisomy 21 – Down syndrome

XXY – Klinefelter syndrome

Monosomy X – Turner syndrome

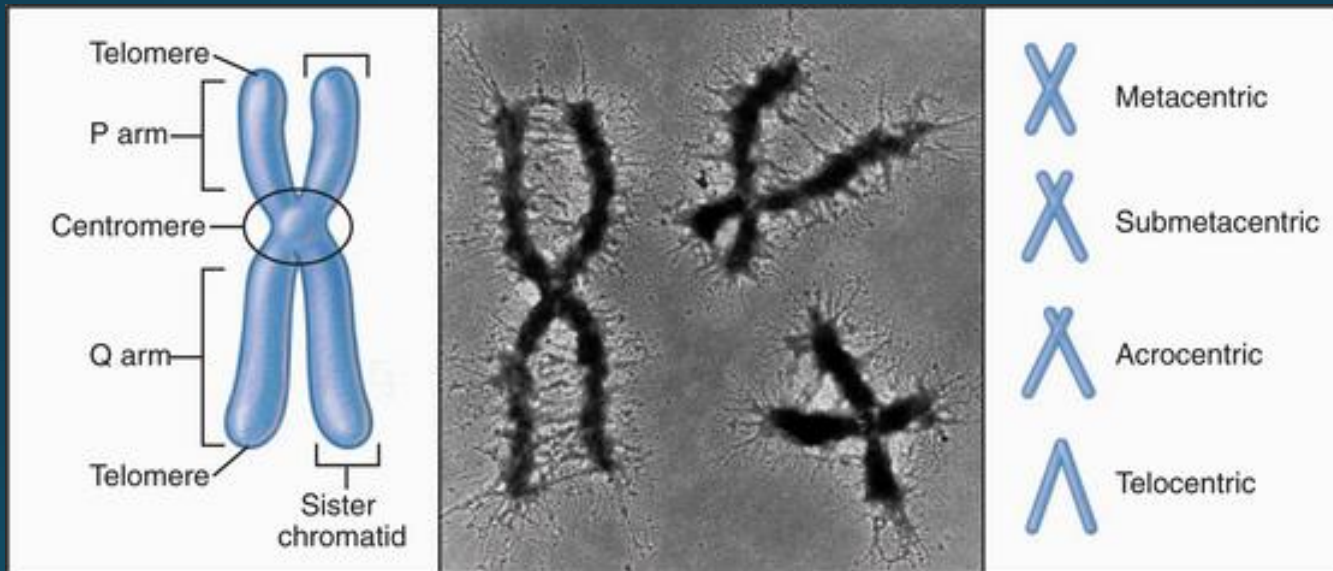
# Chromosomal aberrations - symptomatology

- ▶ Frequent miscarriage
- ▶ Sex chromosome aberrations – short/tall stature, ambiguous genitalia, infertility, primary amenorrhea, delayed development of secondary sexual characteristics

„chromosomal aberration phenotype“:

- ▶ Multiple congenital anomalies
- ▶ Developmental delay, mental retardation
- ▶ Dysmorphic features

# Chromosome morphology

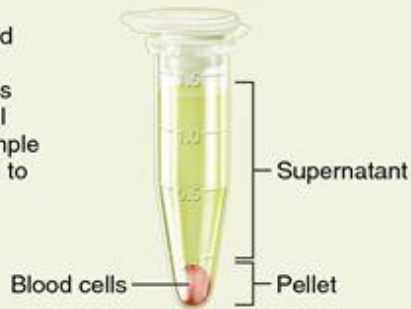


<http://clinicalgate.com/chromosome-organization/>

# Cytogenetic analysis methods

- ▶ Classic cytogenetics - karyotype
- ▶ Molecular cytogenetics
  - FISH (Fluorescence *in situ* hybridization)
  - CGH (Comparative genomic hybridization)
- ▶ MLPA (Multiplex ligase-dependent probe amplification)

1 A sample of blood is collected and treated with drugs that stimulate cell division. The sample is then subjected to centrifugation.



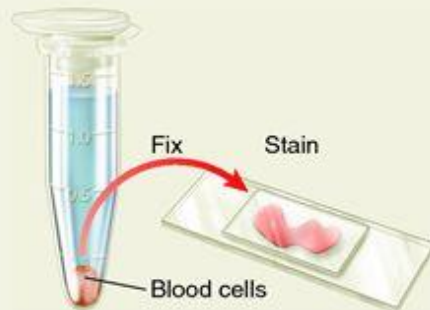
4 The slide is viewed by a light microscope equipped with a camera; the sample is seen on a computer screen. The chromosomes can be photographed and arranged electronically on the screen.



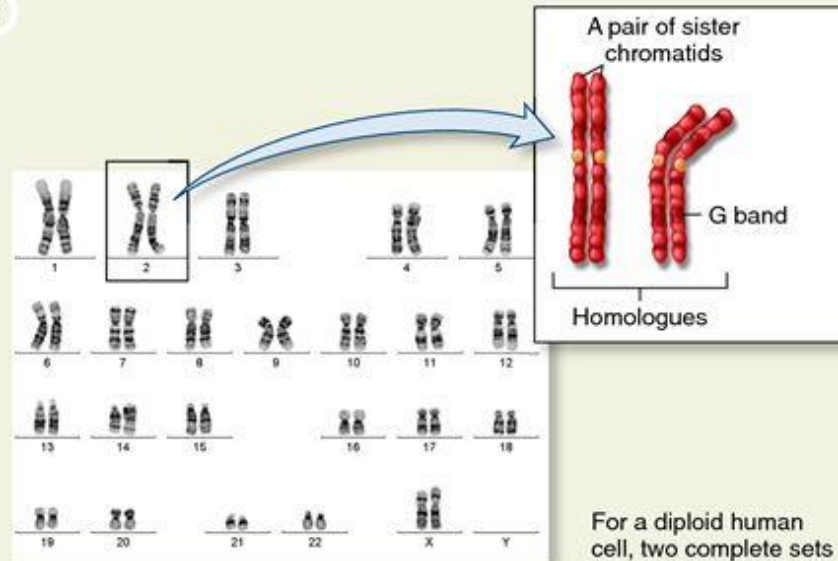
2 The supernatant is discarded, and the cell pellet is suspended in a hypotonic solution. This causes the cells to swell and the chromosomes to spread out from one another.



3 The sample is subjected to centrifugation a second time to concentrate the cells. The cells are suspended in a fixative, stained and placed on a slide.

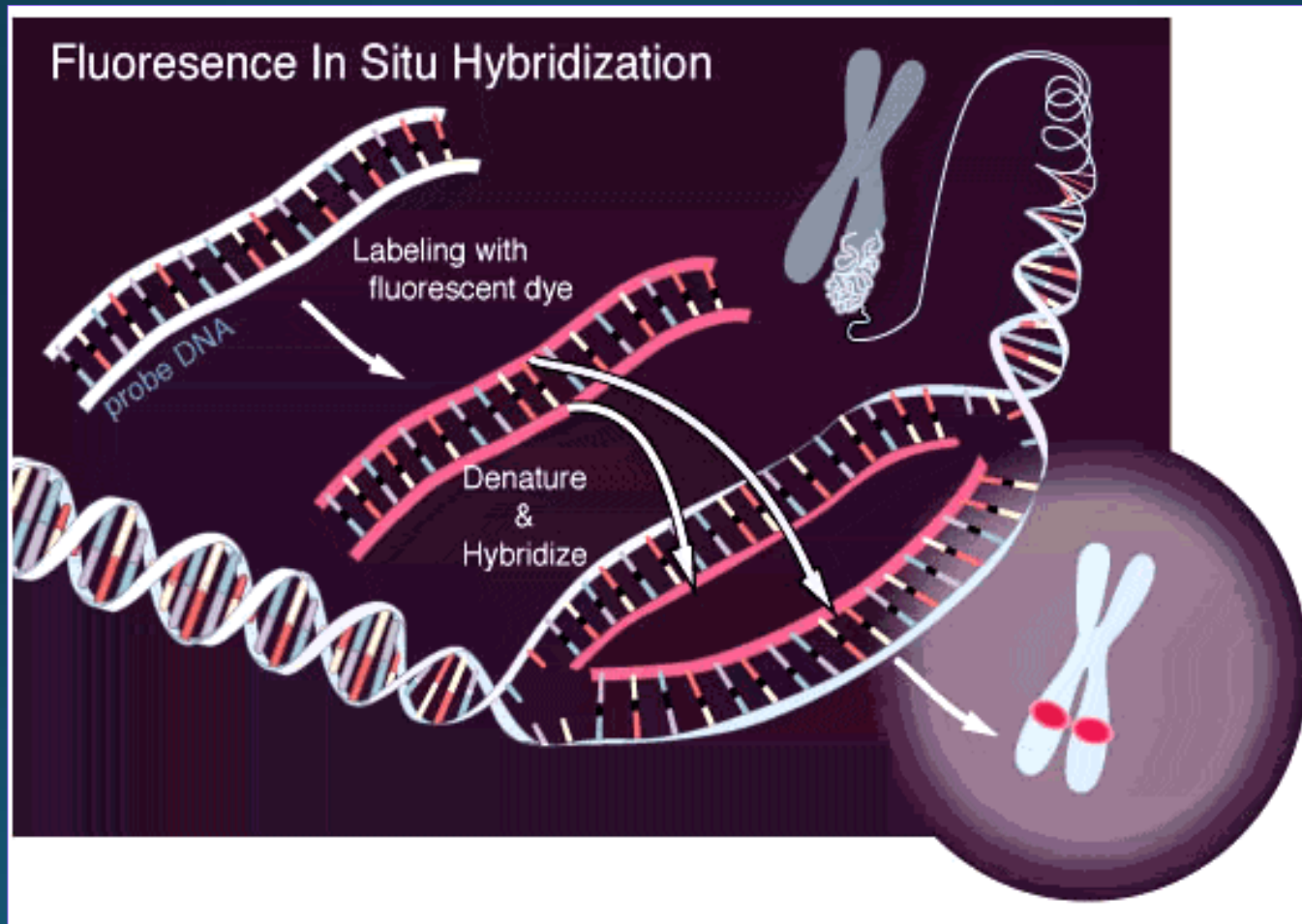


5

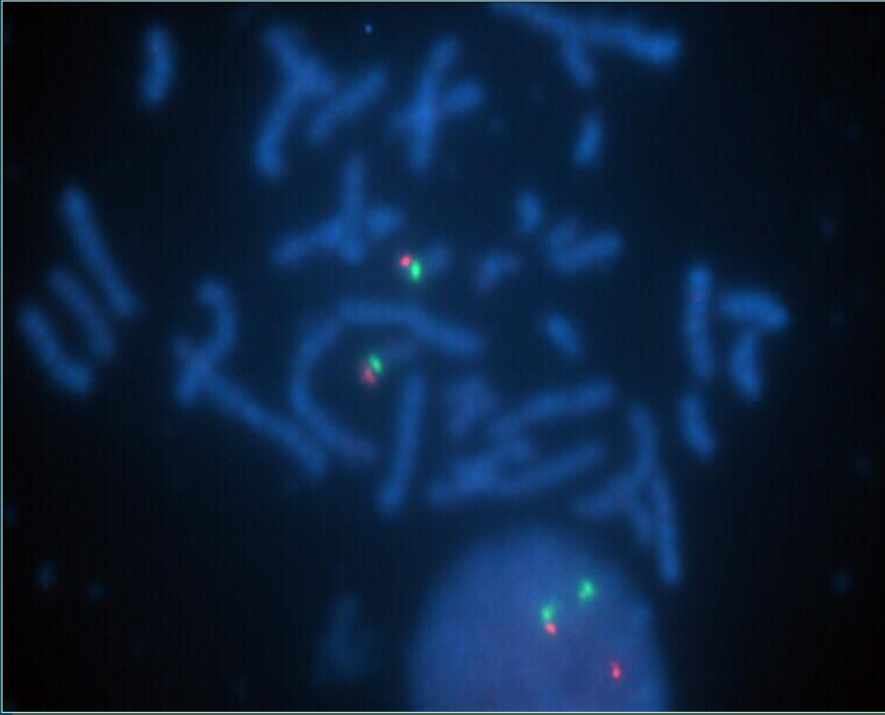


For a diploid human cell, two complete sets of chromosomes from a single cell constitute a karyotype of that cell.

# FISH



# FISH

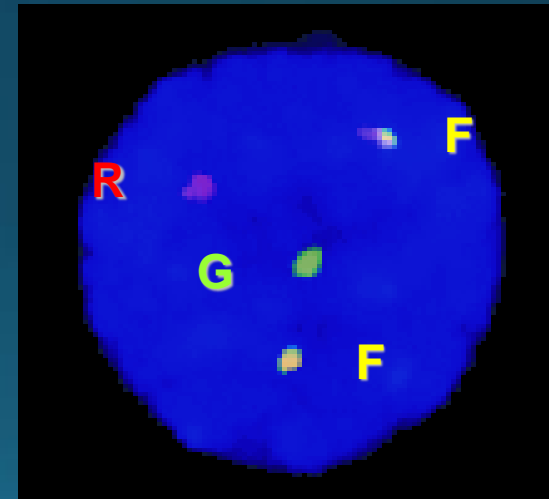
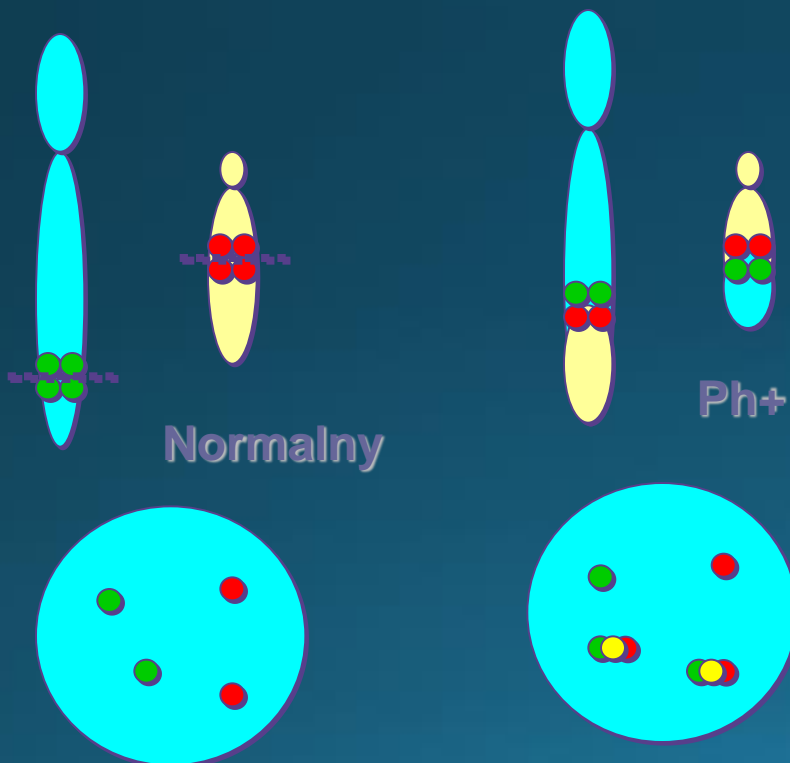


- Metaphase and interphase
- Detection of identified sequence
- Identification of chromosome

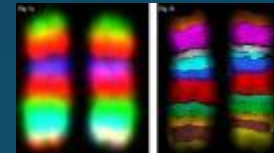
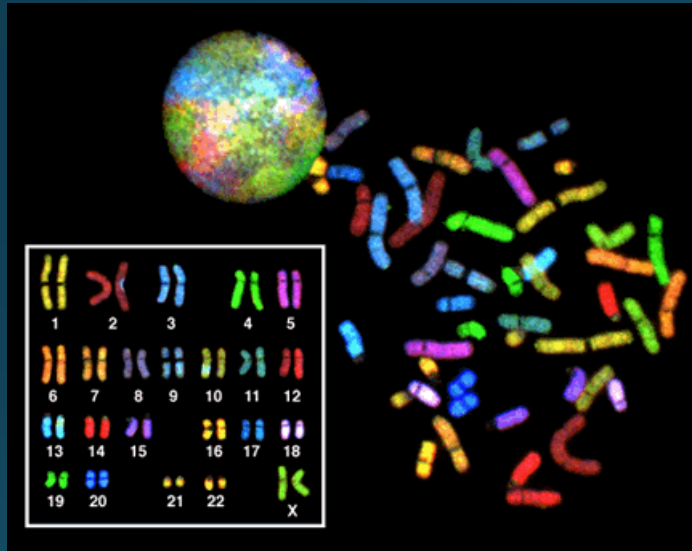


# FISH ANALYSIS IN CHRONIC MYELOID LEUKEMIA

- Philadelphia chromosome t(9;22)

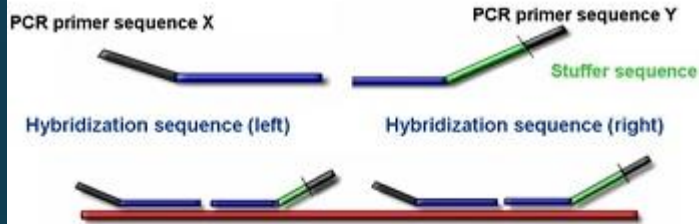


# M-FISH, SKY-FISH



# MLPA – Multiplex Ligase-Dependent Probe Amplification

## 1. Denaturation and Hybridization



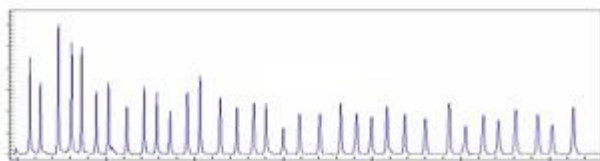
## 2. Ligation



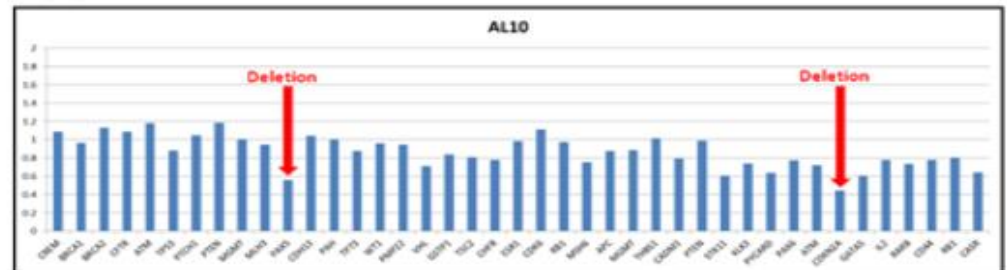
## 3. PCR with universal primers X and Y exponential amplification of ligated probes only



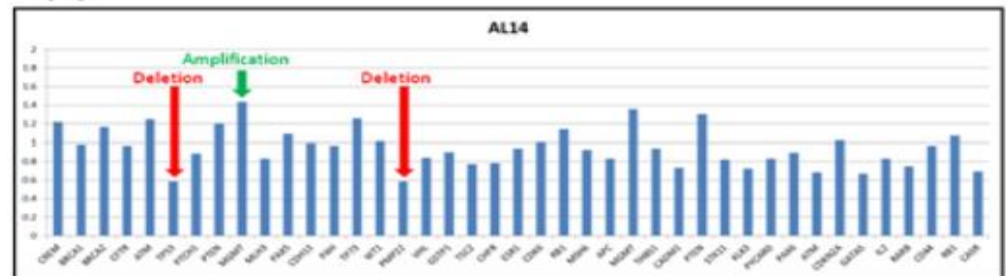
## 4. Fragment analysis



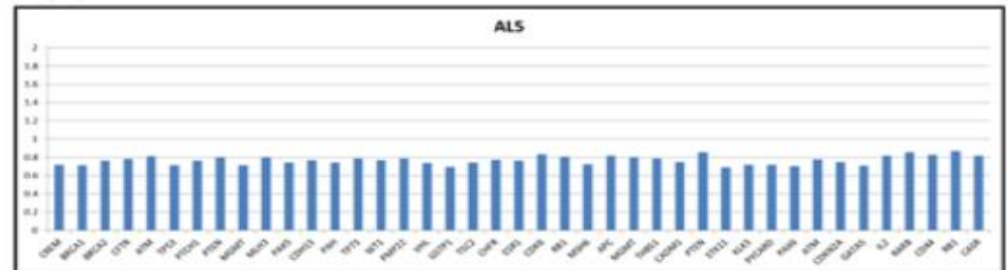
## (A)



## (B)



## (C)

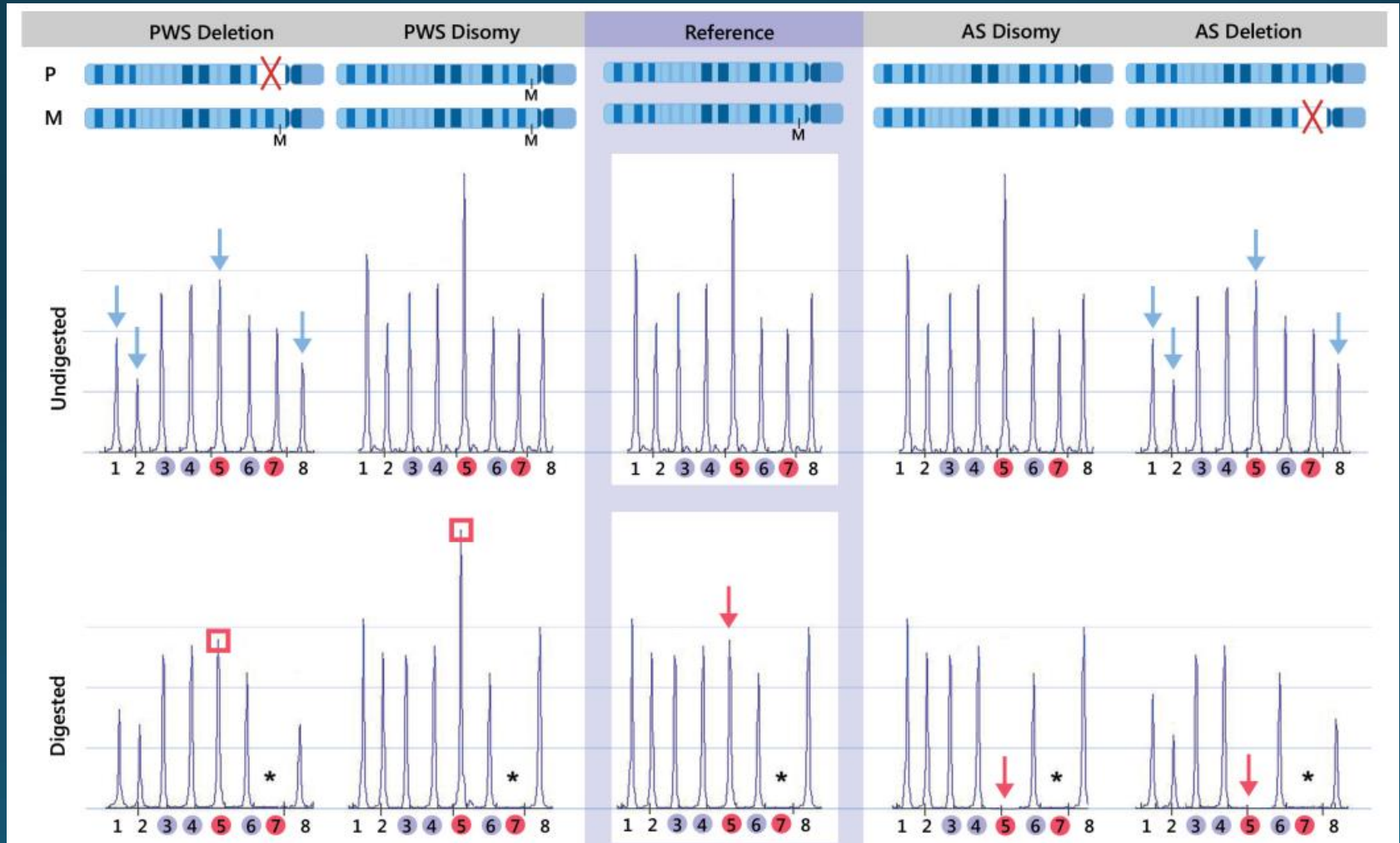


# Methylation-specific MLPA (MS-MLPA)

MRC-Holland

MLPA®

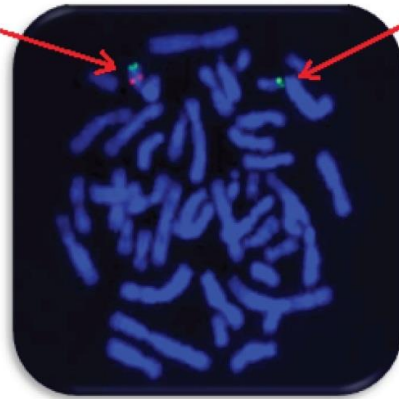
ME028 Prader-Willi/Angelman



# 22q11.2 microdeletion – Di George syndrome

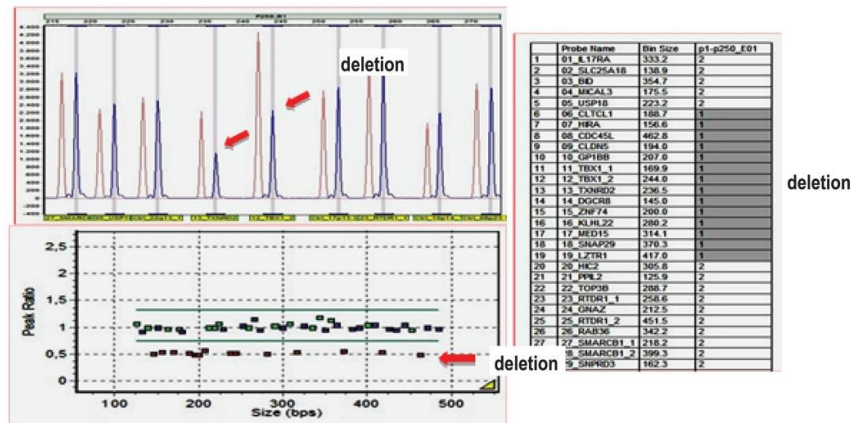
**A**

Normal: presence of two probes.

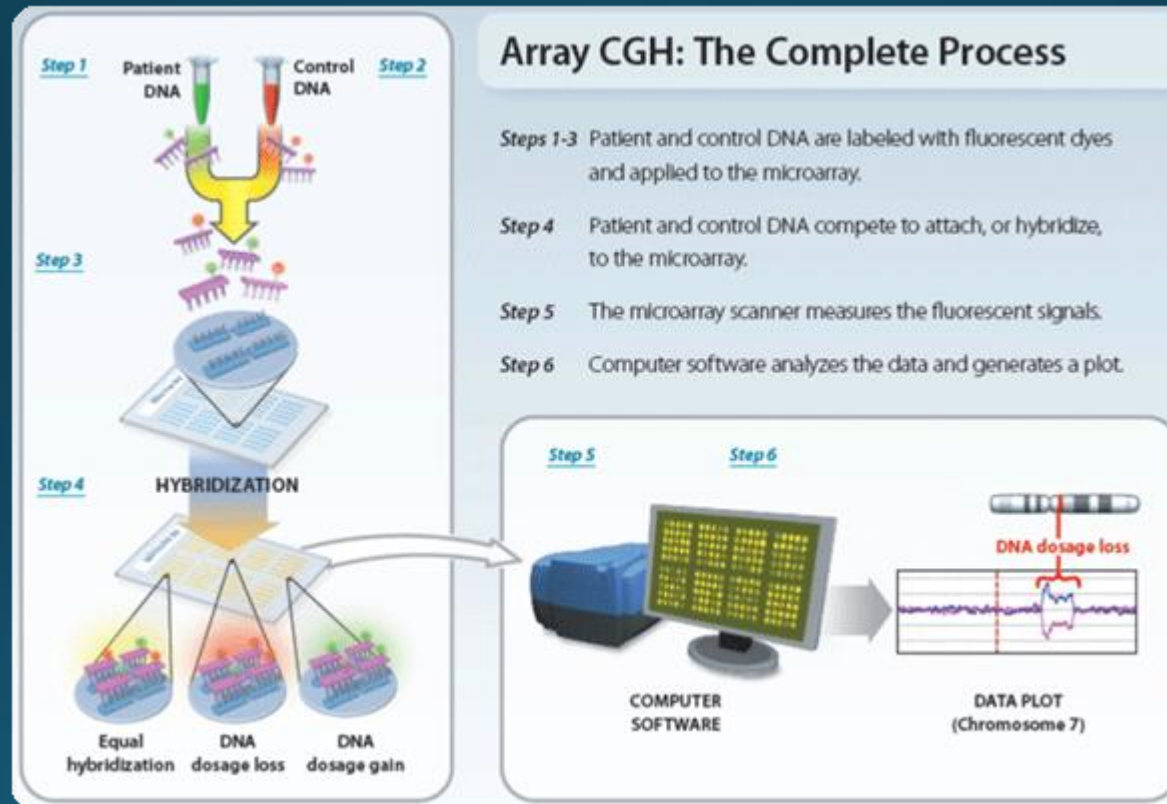


Deleted: absence of hybridization of the TUPLE1 probe

**B**



# Array CGH



# Material for cytogenetic analyses

- ▶ Lymphocytes
- ▶ Skin fibroblasts
- ▶ Bone marrow
- ▶ Amniotic fluid / umbilical blood
  - prenatal diagnosis
- ▶ Cancer cells from pleural effusion
- ▶ Somatic cancer cells

# Standard nomenclature

ISCN 2016 - *International System for Human Cytogenic Nomenclature*

Chromosome, arm, region, band

1q32.1

Chromosome 1, long arm, region 3, band 2, sub-band 1



# ISCN symbols

+	Additional chromosome
-	Absence of chromosome
cen	Centromere
del	Deletion
der	Derivative
dic	Dicentric
dup	Duplication

i	Isochromosome
inv	Inversion
mar	marker
mat	Maternal origin
pat	Paternal origin
t	Translocation
?	Doubtful or questionable

# Standard Nomenclature for Chromosome Karyotypes

Karyotype	Description
46,XY	Normal male chromosome constitution
47,XX,+21	Female with trisomy 21, Down syndrome
47,XY,+21[10]/46,XY[10]	Male who is a mosaic of trisomy 21 cells and normal cells (10 cells scored for each karyotype)
46,XY,del(4)(p14)	Male with distal and terminal deletion of the short arm of chromosome 4 from band p14 to terminus
46,XX,dup(5)(p14p15.3)	Female with a duplication within the short arm of chromosome 5 from bands p14 to p15.3
45,XY,der(13;14)(q10;q10)	A male with a balanced Robertsonian translocation of chromosomes 13 and 14. Karyotype shows that one normal 13 and one normal 14 are missing and replaced with a derivative chromosome composed of the long arms of chromosomes 13 and 14
46,XY,t(11;22)(q23;q22)	A male with a balanced reciprocal translocation between chromosomes 11 and 22. The breakpoints are at 11q23 and 22q22
46,XX,inv(3)(p21q13)	An inversion on chromosome 3 that extends from p21 to q13; because it includes the centromere, this is a pericentric inversion
46,X,r(X)(p22.3q28)	A female with one normal X chromosome and one ring X chromosome formed by breakage at bands p22.3 and q28 with subsequent fusion
46,X,i(Xq)	A female with one normal X chromosome and an isochromosome of the long arm of the X chromosome

# Overview of chromosomal disorders

## „CHROMOSOME PHENOTYPE“

- developmental delay, behavioral disturbances
- congenital anomalies
- dysmorphic features



# FREQUENCY OF CHROMOSOMAL ABERRATIONS

- **0,9 %** live births, abnormal phenotype in half of them
- **23 – 34%** of cases of ID + multiple congenital defects
- **50 - 60%** of 1st trimester miscarriages
- **6%** of fetal deaths

# MICRODELETIONS/MICRODUPLICATIONS

- Contiguous gene syndromes
- 0,7 – 1/1000, mostly *de novo*, AD
- More severe phenotype effects in microdeletions than in microduplications
- Microduplications: frequent familial occurrence

# Groupwork – microdeletion/microduplication syndromes

On the basis of the given cytogenetic result:

- What is the diagnosis / syndrome?
- Frequency, Clinical symptoms, Health supervision
- Prognosis

Group A: 46,XY,del22q11.2

Group B: 46,XY, del7q11.23

Group C: 46,XX, del(5p)

Group D: 46,XX,del22q13.3



# DiGeorge syndrome

- More frequent cleft lip/palate
- Small jaw
- Small upper lip/mouth
- Eyes slanted upward or downward
- Low-set and/or abnormal folding of ears
- Short stature, mild to moderate learning difficulties
- Underdeveloped parathyroid and thymus
- Cardiac malformations



Digilio et al., 2005

Facial  
modules

Development  
over time

Neural crest  
contributions

Facial  
malformations

Summary

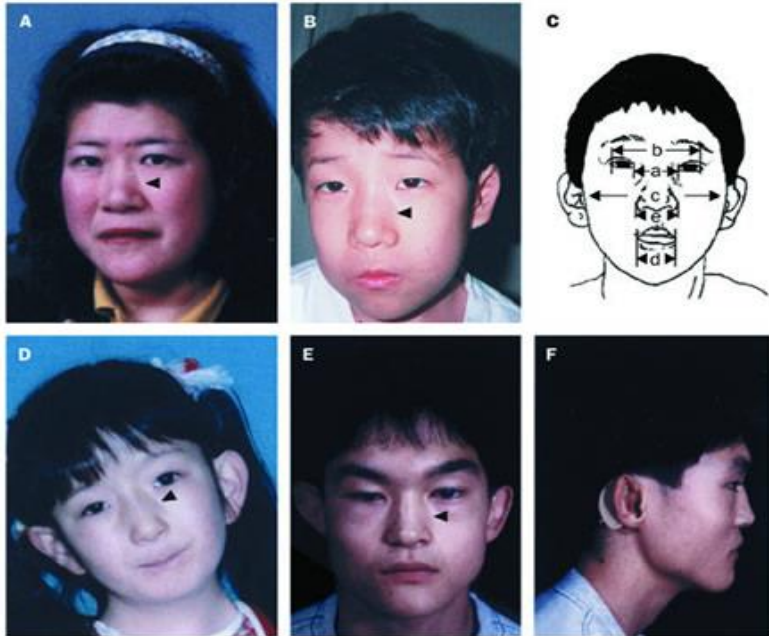
Acronym "CATCH 22"

[www.philipcaruso-story.com](http://www.philipcaruso-story.com)



# DiGEORGE syndrome del22q11.2

Figure  
Characteristic facial appearance of the patients



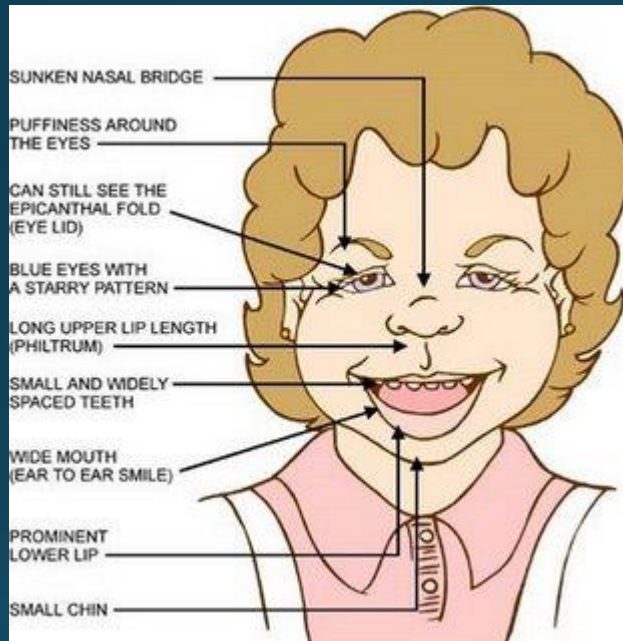
(A, B) Typical conotruncal anomaly face of del22q11.2 syndrome in familial cases (mother and son). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides. (C) Items of anthropometric measurement— $a \div c$  (wide ocular hypertelorism),  $(b-a) \div b$  (short palpebral fissures),  $d \div c$ , and  $d \div e$  (small mouth).  $a$ =inner canthal distance.  $b$ =outer canthal distance.  $c$ =transverse facial width.  $d$ =oral width.  $e$ =nasal width. (D) Facial appearance of a patient with conotruncal anomaly face syndrome without the 22q11.2 deletion. (E, F) Facial appearance of a patient with DiGeorge's syndrome without the 22q11.2 deletion (E, frontal view; F, side view). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides.

Reproduced from *Lancet*, Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet*. 2003;362:1366-1373.



DiGeorge syndrome - Thymic aplasia and right aortic arch - Anterior view. Neonatal death by laryngeal atresia

# William's syndrome del7q11.23



<http://odlarmed.com/?p=833>

# WILLIAMS SYNDROME

*ELN* gene

- IUGR, postnatal hypotrophy, short stature
- feeding difficulties, gastro-esophageal reflux
- congenital heart defect: **SVAS (75%)**, PPS; hypertension, arrhythmia, sudden cardiac death, mitral valve prolapse in adults
- connective tissue symptoms: rough voice, inguinal hernia, hypermobile joints, delicate, elastic skin
- **idiopathic hypercalcemia (30%)**, **hypercalciuria (15%)**
- constipation
- **behavioral phenotype** – „cocktail party manner”, **empathy**, **overfriendly behavior**, oversensitivity, anxiety, phobias
- **facial dysmorphism**
- average IQ or mild ID
- chronic ear infection
- ocular defects (50%)
- renal disorders – stenosis of renal arteries, kidney stones (5%)



Williams syndrome

<https://www.youtube.com/watch?v=BlexMOZCSVQ>

## 22q11.2 microduplication s.

### **Frequent symptoms:**

Abnormal skull shape

Flat facial profile

Prominent ears

Ear tags

Schooling difficulties, global developmental delay

### **Less frequently:**

Palatal, laryngeal insufficiency  
hypocalcemia

Autistic behavior



Syndrom overlap with  
DiGeorge s./VCFS

Frequent familial cases

Pedigree analysis,  
Clinical assessment of relatives

# Microduplication syndromes

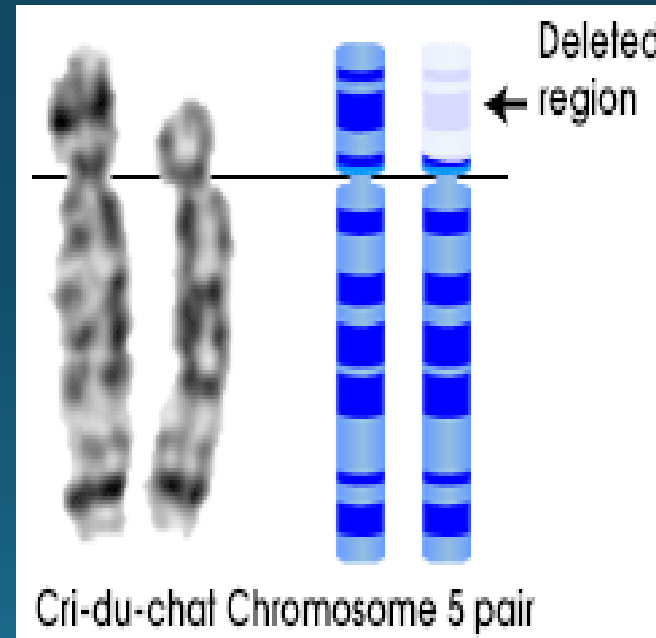
- 22q11.2** clinical features similar to microdeletion  
(DiGeorge's/VCFS)
- 7q11.23** severe speech delay  
(WBSCR)
- 15q11-13** autism, speech delay  
(PWS/AS)
- Xq28** ID, speech defect, hypotonia, frequent infections  
(Rett s.)  
*MECP2*
- 17p11.2** Potocki – Lupski syndrome  
(Smith Magenis s. –del17p11)
- dup6q24 - 27** ID, obesity

# *cri du chat syndrome*

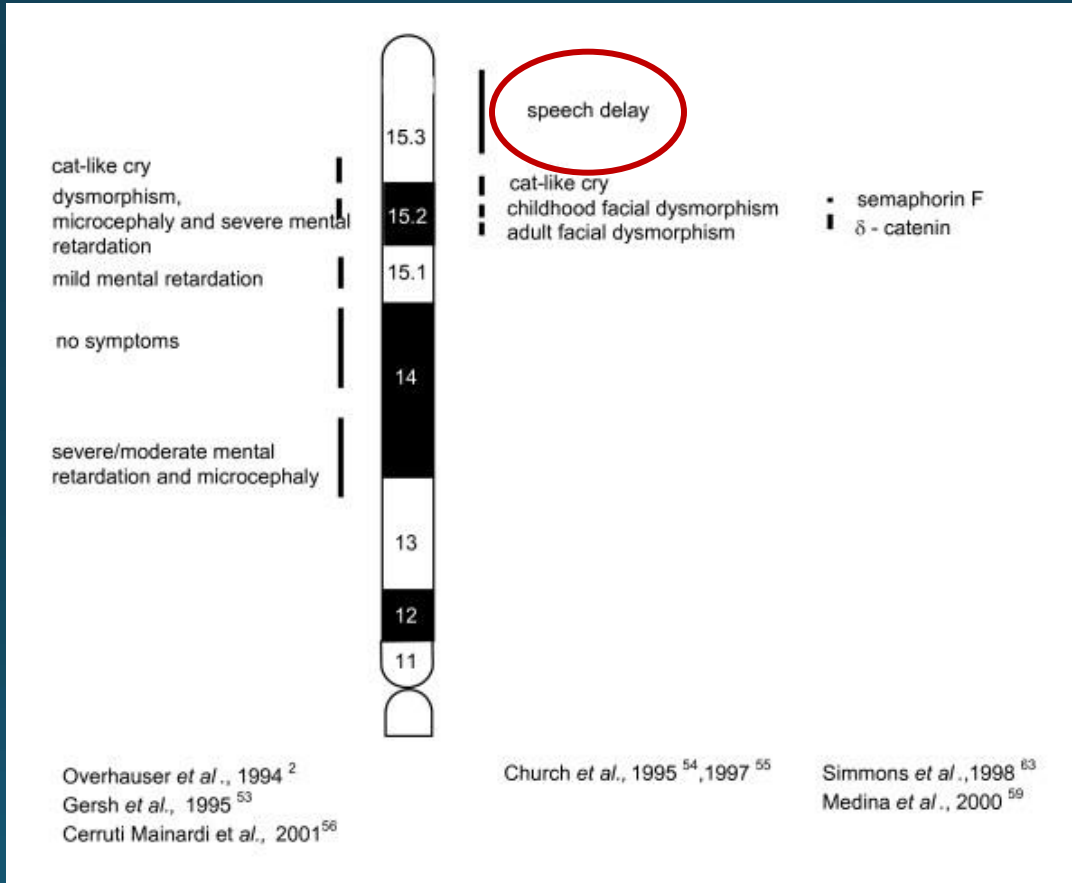
- 46, XX, del 5p      46, XY, del 5p
- Round face in newborn, cat's cry (disappears around age 2), congenital heart defect, ID



CdC Support Group  
[www.cridchat.u-net.com](http://www.cridchat.u-net.com)



# CDC – CONTIGUOUS GENE SYNDROME



Cerruti Mainardi, Paola. (2006). Cri du Chat syndrome. Orphanet journal of rare diseases. 1. 33. 10.1186/1750-1172-1-33.



# PHELAN-MCDERMID SYNDROME – DEL 22Q13.3



- Developmental delay, autistic behavior, increased pain threshold
- Hypotonia
- Dysmorphic features: dolichocephaly, long eyelashes, dysplastic ears, rounded nose tip, pointed chin, large hands, dysplastic nails

Koolen A, et al (2005). Molecular characterisation of patients with subtelomeric 22q abnormalities using chromosome specific array-based comparative genomic hybridisation. *European journal of human genetics* : EJHG. 13. 1019-24. 10.1038/sj.ejhg.5201456.

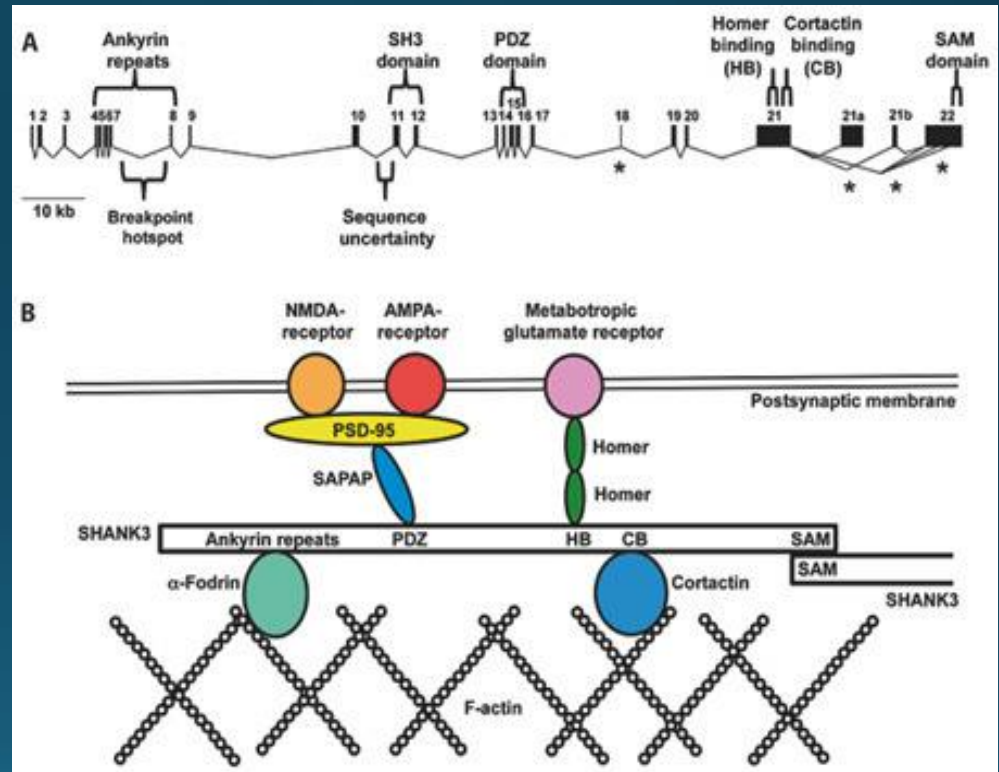
**Table 1.** Features associated with 22q13.3 deletion syndrome [Cusmano-Orog et al., 2007; Dhar et al., 2010; Phelan et al., 2010]

>75% cases	>50% cases	>25% cases	<25% cases
global developmental delay absent or severely delayed speech normal or accelerated growth neonatal hypotonia	large, fleshy hands dysplastic toenails long eyelashes dolicocephaly poorly formed/large ears wide brow full/puffy cheeks full/puffy eyelids deep-set eyes flat midface wide nasal bridge bulbous nose pointed chin sacral dimple decreased perspiration autism/autistic like behavior decreased perception of pain mouthing/chewing non-food items	strabismus ptosis renal abnormalities epicanthal folds long philtrum high arched palate malocclusion/widely spaced teeth 2-3 syndactyly of the toes seizures cardiac defects lymphedema gastroesophageal reflux cyclic vomiting precocious or delayed puberty	teeth grinding (24%) arachnoid cyst (15%) tongue thrusting (15%) 5th finger clinodactyly (14%) cortical visual impairment (6%) hypothyroidism (5%)



# PMS – SHANK3 GENE

- 22q13.3 deletion (80-85%)
- Ring chromosome
- Unbalanced translocation (15-20%)



Phelan, Katy & McDermid, Heather. (2012). The 22q13.3 Deletion Syndrome (Phelan-McDermid Syndrome). *Molecular syndromology*. 2. 186-201. 10.1159/000334260.

# BECKWITH – WIEDEMANN syndrome (BWS)

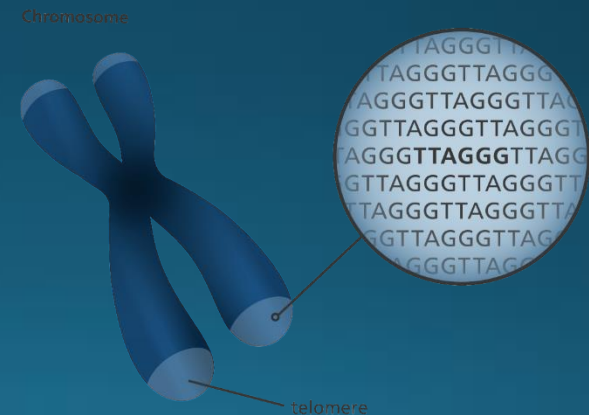
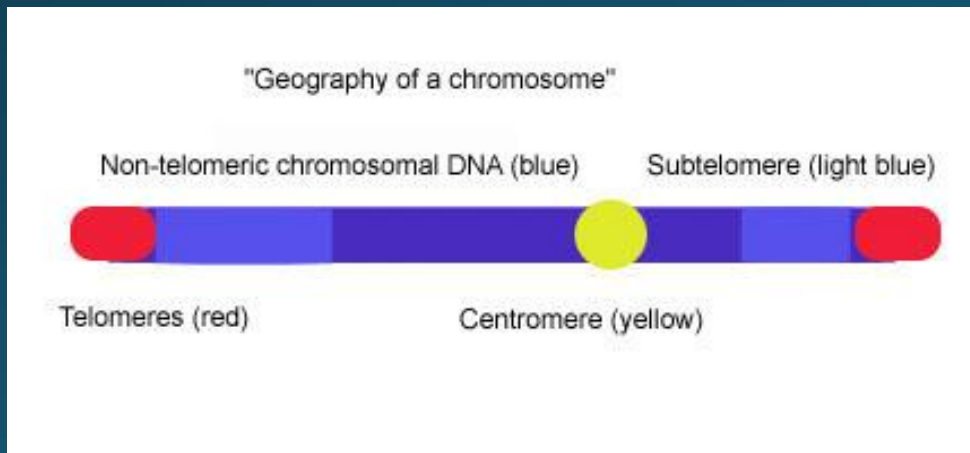
- Duplication / Deletion of 11p15 (*IGF2* gene)
- macroglossia, omphalocele, high birth weight, ear pits, hemihyperplasia
- hypoglycemia in neonatal period (could cause developmental delay), high tumor risk (Wilms tumor, hepatoblastoma, rhabdomyosarcoma, adrenal tumor)



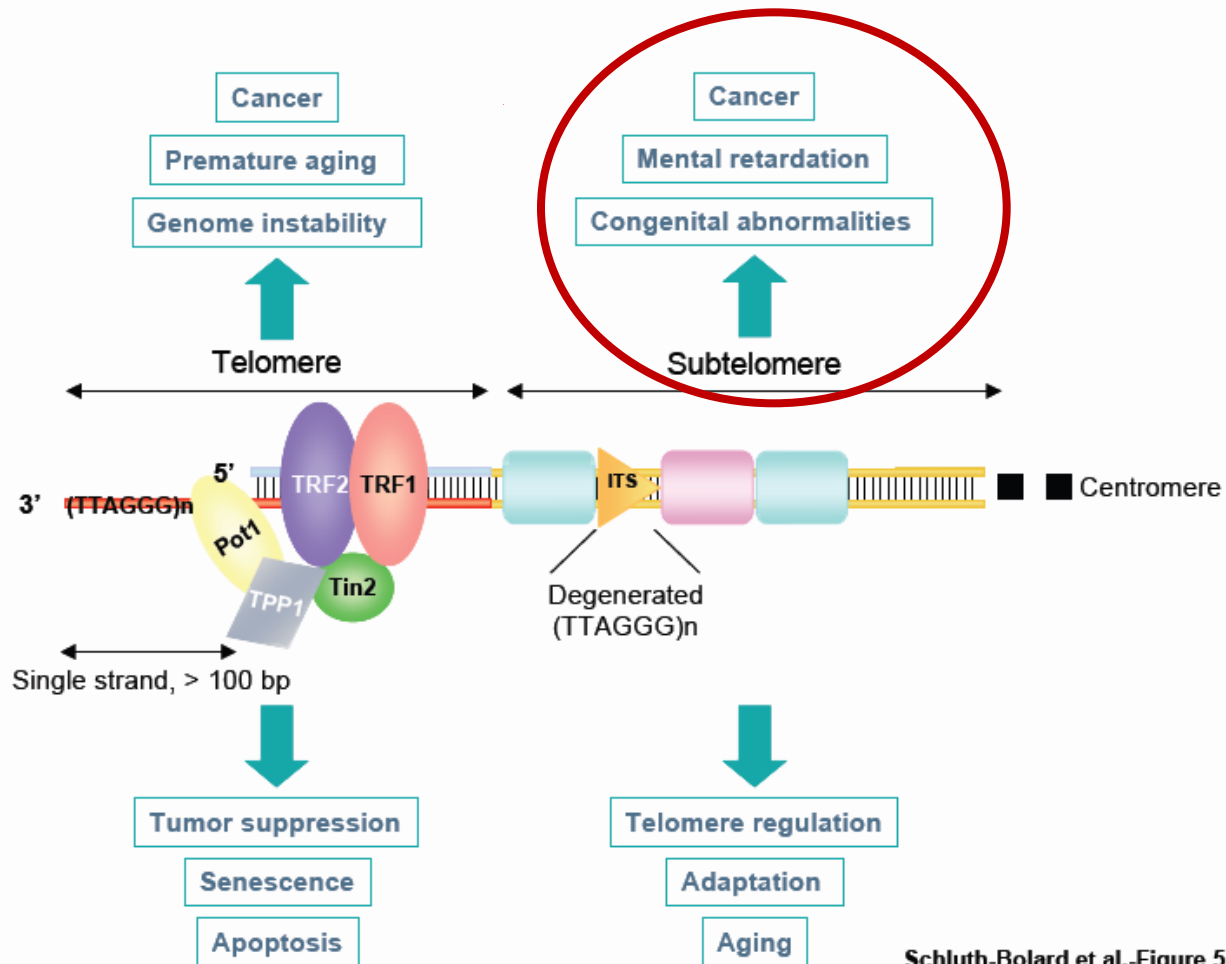
# Subtelomeric rearrangements

# TELOMERES

- Protect chromosomes from deterioration during cell replication
- Chromosomal organization in the nucleus
- Sequence: **TTAGGG**



# SUBTELOMERIC REGION



# WHAT WE KNOW ABOUT SUBTELOMERES

- Dynamic patchworks of multichromosomal blocks
- Homologous sequences prone to rearrangements
- Subtelomeres include encoding regions





# SUBTELOMERIC REARRANGEMENTS

- 1p36 deletion
- Fascioscapulohumeral muscular dystrophy (FSHD)
- Wolf-Hirschhorn syndrome (4p-)
- Cri-du-chat syndrome (5p-)
- 9q34 deletion
- Miller-Dieker syndrome
- Phelan-McDermid syndrome

## SUBTELOMERIC REARRANGEMENTS

- 5,1% of children with ID – second most frequent cause next to Down syndrome
- 7,4% of children with moderate or severe ID
- 0,5% of children with mild ID

# 1p36 deletion

## Symptoms (~100%)

- Severe ID
- Absent speech
- Hypotonia
- Facial dysmorphism
- Gait disturbances
- Abnormal behavior

## Frequent (50 - 80%)

- Growth delay
- Heart defect
- Epilepsy
- Deafness

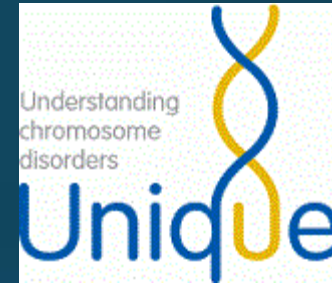
## Less frequent (<50%)

- Cardiomyopathy
- Cleft lip / palate
- Hypothyroidism
- Obesity



*Prominent forehead, deepset eyes, flat nose, pointed chin, horizontal eyebrows*

[WWW.RARECHROMO.ORG](http://WWW.RARECHROMO.ORG)

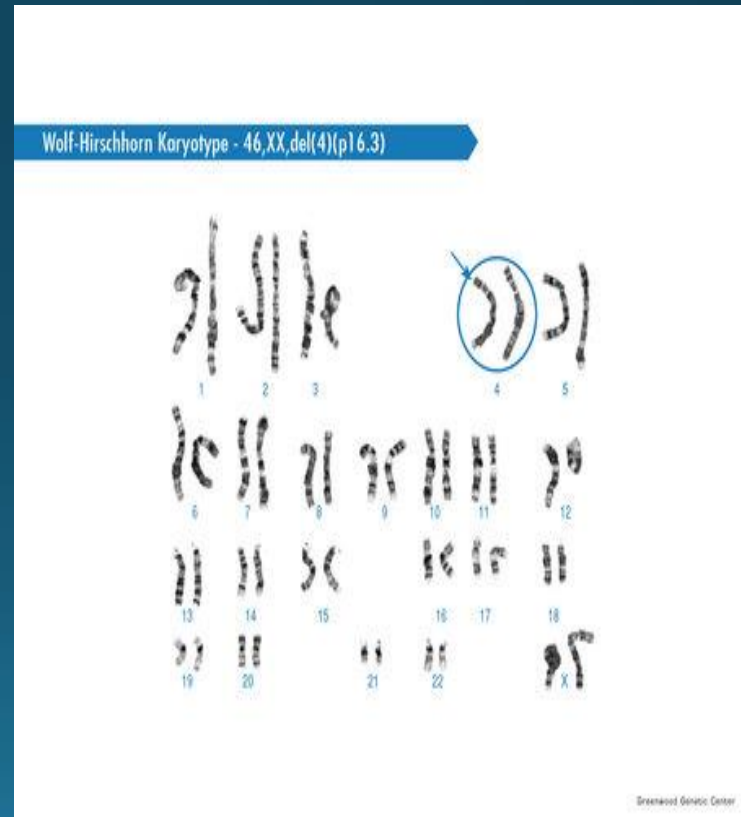


## 1p36 deletion syndrome



# WOLF-HIRSCHHORN SYNDROME

- ▶ del 4p
- ▶ Hypertelorism, prominent glabella, wide nose („greek helmet face“), thick lower lip, *iris coloboma*, cleft palate, ID



# FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY (FSHD) – del4q35

- Third most frequent muscular dystrophy, after DMD/BMD, myotonic dystrophy type I
- Muscle weakness of face, shoulder girdle
- Lower limbs rarely affected
- Hyperlordosis
- Deafness (60% of patients)
- Cardiologic symptoms - RBBB

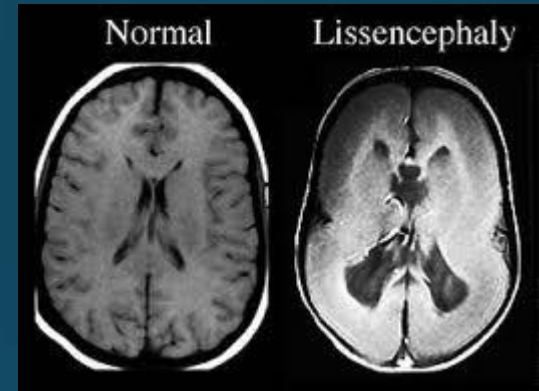


## FSHD - DIAGNOSIS

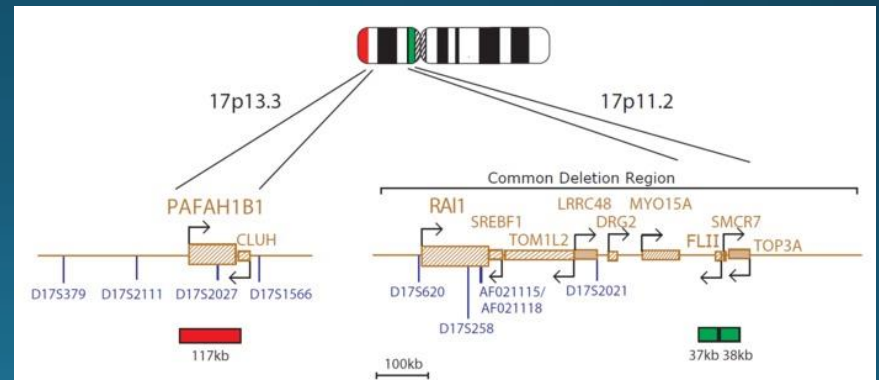
- Typical clinical features
- CK – normal or slightly elevated
- EMG – nonspecific myopathic changes
- Muscle biopsy – dystrophic changes
- MR – evaluation of affected muscles

# MILLER-DIEKER SYNDROME – del17p13.3

- Lissencephaly
- Severe ID
- Hypotonia
- Epilepsy before 6 mos
- Microcephaly
- Growth delay



<https://prezi.com/hyphafzgrzel/miller-dieker-syndrom/>



<http://www.cytocell.com/probes/123-smithmagenis-flimillerdieker-probe-combination>



# MDS – DYSMORPHIC FEATURES



- Prominent forehead
- Midface hypoplasia
- Short nose
- Lowset, dysplastic ears
- Micrognathia

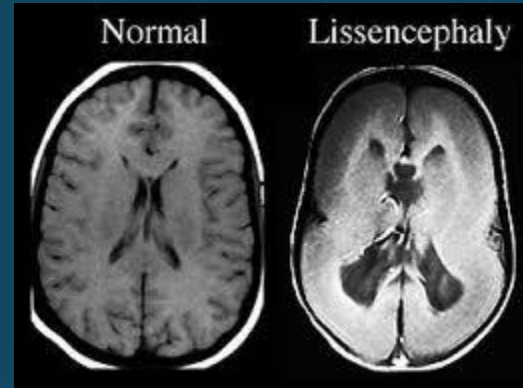
<https://www.ncbi.nlm.nih.gov/books/NBK5189/figure/chrom17-lis.F2/?report=objectonly>

# GENES AND SYNDROMES - LISSENCEPHALY

Syndrome/Disease	Gene symbol	Protein function	Region	OMIM number	Inheritance pattern
<b>Lissencephaly classic Type I</b>					
Miller-Dieker type	PAFAH1B1	Nuclear migration factor	17p13.3	601545	AD
Sub-band heterotopia type	DCX	microtubule regulator	Xq22.3	300121	XL
	RELN	Neuronal migration factor	7q22	600514	AR
	ARX	Homeobox, neuron function	Xp22.13	300382	XL
<b>Cobblestone type lissencephaly Type II</b>					
Muscle-Eye-Brain	POMGNT1	O-mannosyl glycan synthesis	1p34	253280	AR
Fukuyama	FCMD	role in o-mannosyl glycosylation	9q31	607440	AR
Walker-Warburg Syndrome (WWS)*	POMT1	o-mannoyl transferase 1	9q34.1	607423	AR
Walker-Warburg Syndrome (WWS)	POMT2	o-mannoyl transferase 2	14q24.3	607439	AR
Muscular Dystrophy, Congenital, MDC1C	FKRP	role in o-mannosyl glycosylation	19q13.3	606596	AR
Muscular Dystrophy, Congenital, MDC1D	LARGE	role in o-mannosyl glycosylation	22q12.3	603590	AR
* Note: all of the above 6 genes have been noted in WWS, POMT1 is most common					



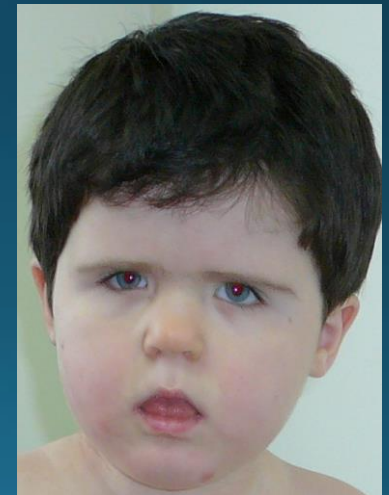
Wolf-Hirschhorn s.



Miller-Dieker s.



FSHD



del1p36

# GENETIC COUNSELLING

- Estimation of family genetic risks
- Referral to specialists
- Periodic genetic consultations (new diagnostic tests, healthcare surveillance)
- Genetic counselling at age of maturity
- Information on support groups, family networks, associations

