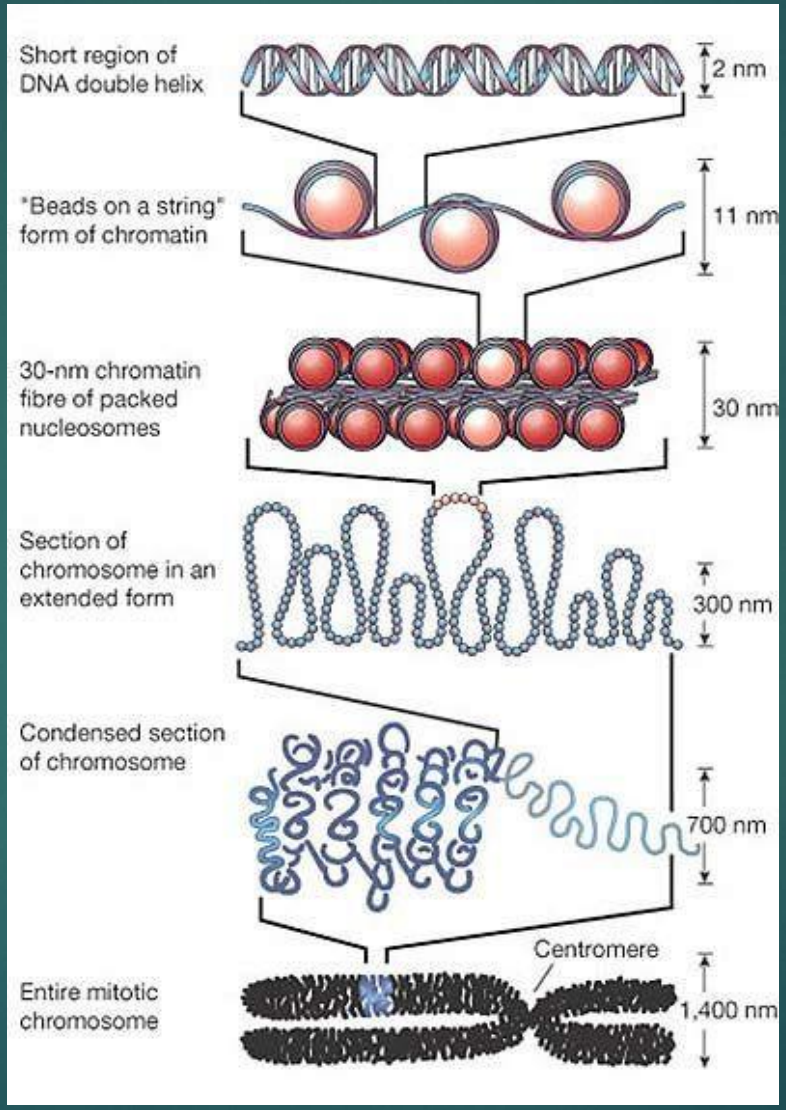
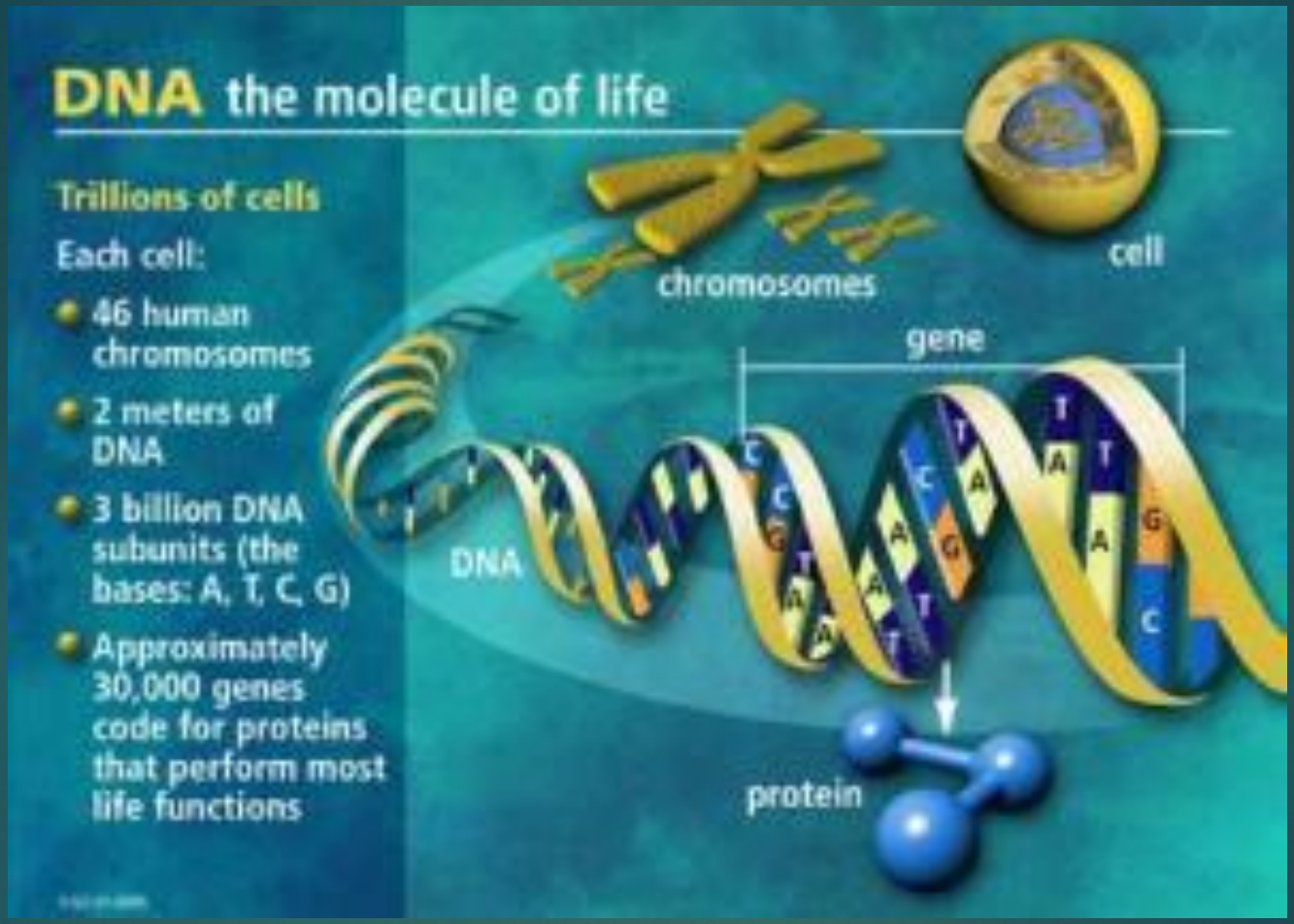
Postnatal Cytogenetics

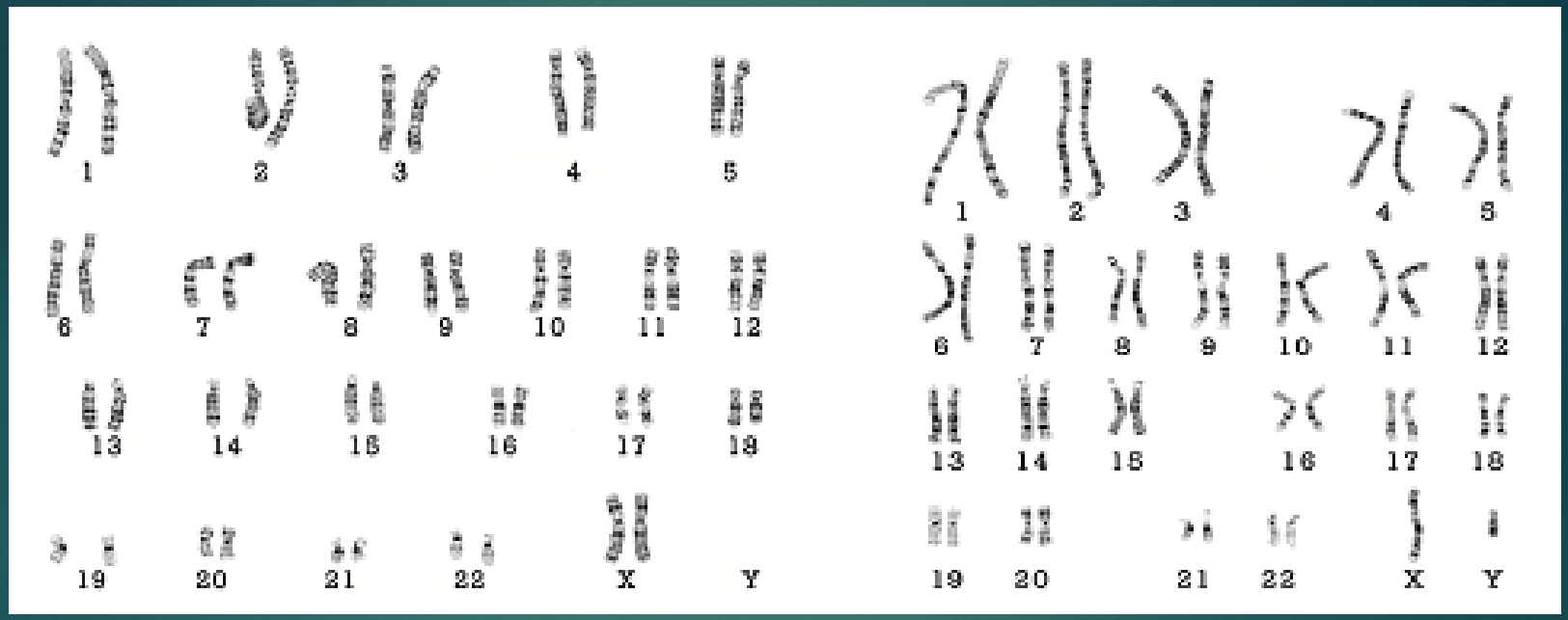
JENNIFER CASTANEDA, MD, PHD

Review: chromosome structure



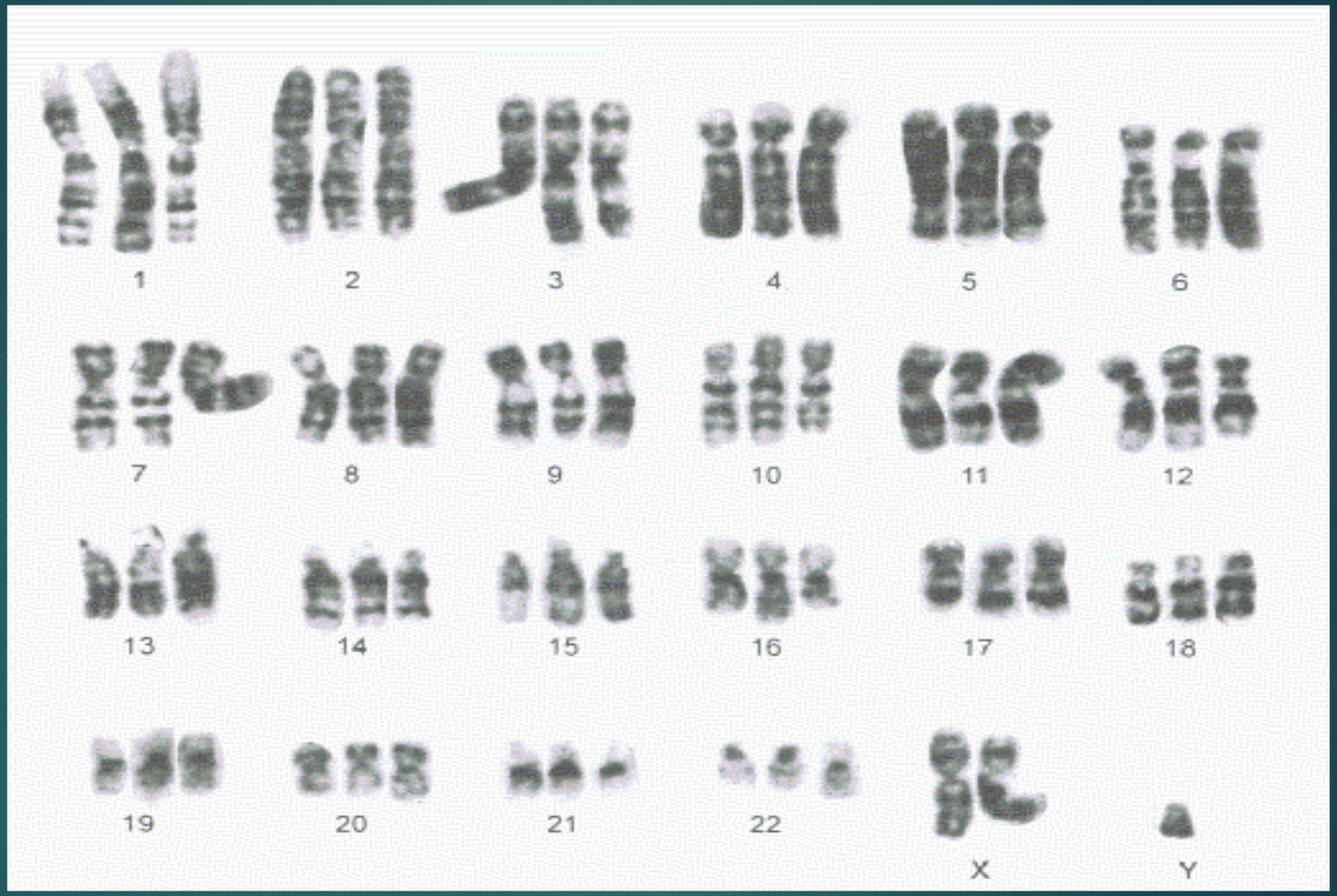


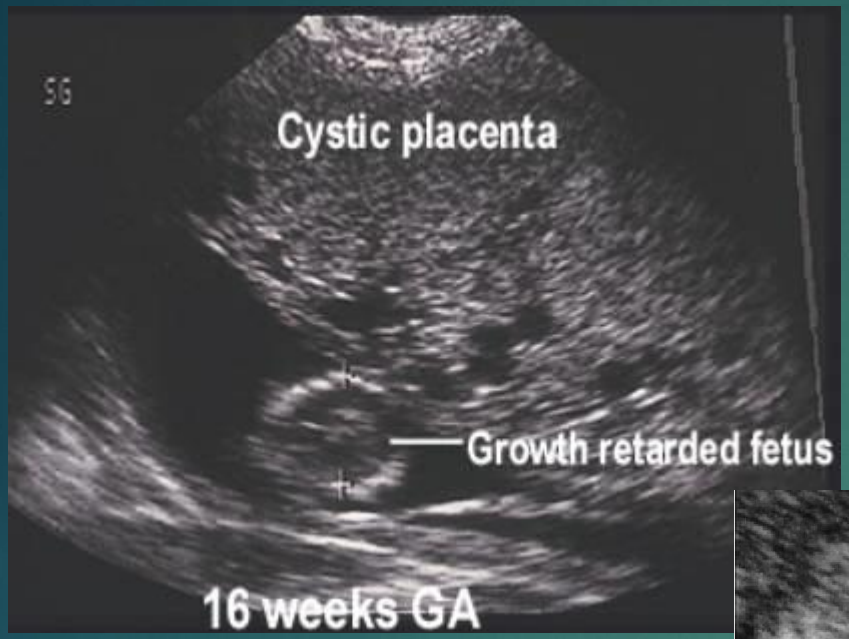
https://thenaturalhistorian.com/2013/02/24/genetic-distance-humans-chimpanzees-divergence-baraminology/

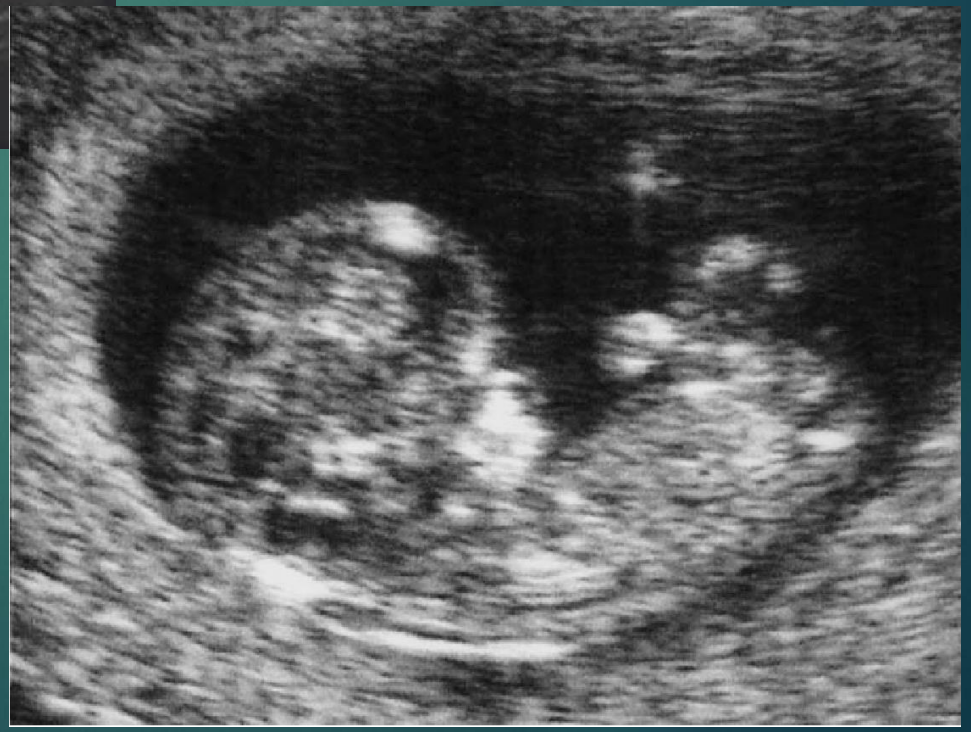

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Classification of chromosomal aberrations

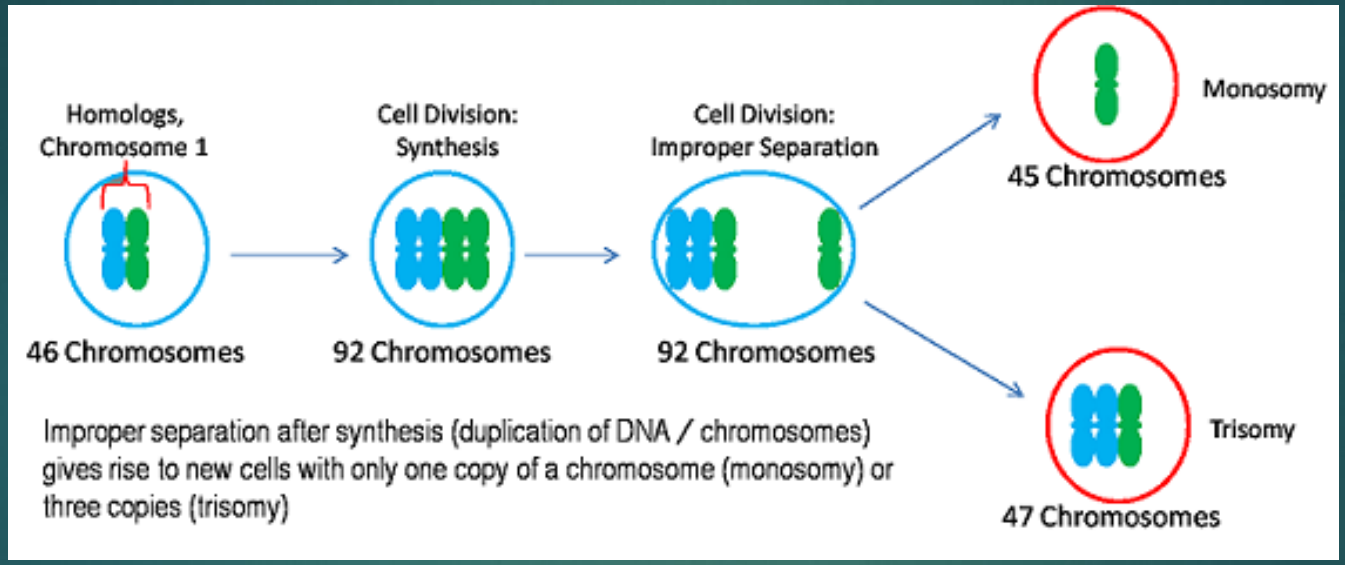
- Autosomal vs sex chromosome aberrations
- Abnormalities of chromosome number:
  - Polyploidy
  - Autosomal aneuploidy
  - Sex chromosome aneuploidy
- Abnormalities of chromosome structure
  - Translocations
  - Deletions, microdeletions
  - Duplications
  - Inversions
  - Ring chromosomes


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y


SG
Cystic placenta
Growth retarded fetus
16 weeks GA



Aneuploidy caused by meiotic disjunction



http://study.com/academy/lesson/aneuploidy-definition-disorders-quiz.html

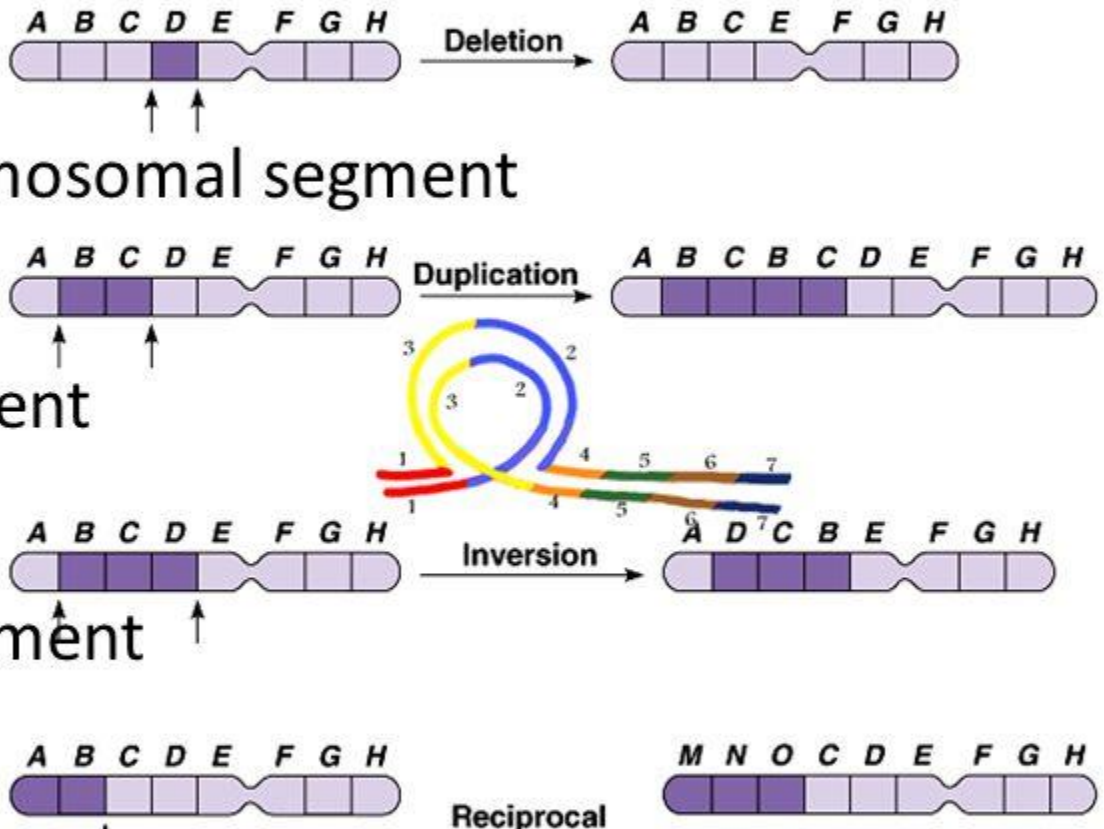
Changes in chromosome structure

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 first chromosomal aberrations identified (1959):

  Trisomy 21 – Down syndrome

  XXY – Klinefelter syndrome

  Monosomy X – Turner syndrome

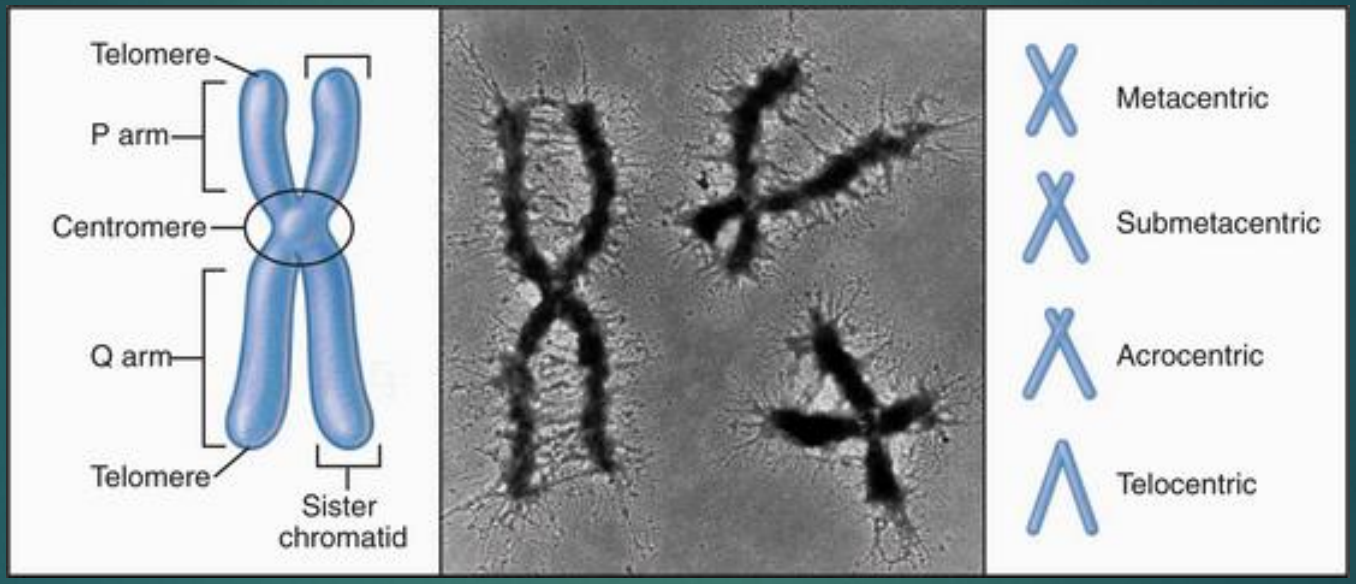
Chromosomal aberrations - symptomatology

- 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/

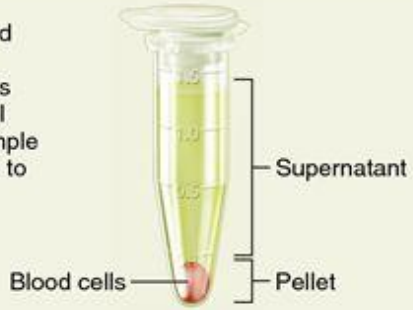
Cytogenetic analysis methods

- Classic cytogenetics - kariotype
- Molecular cytogenetics

  - FISH (Fluorescence *in situ* hybridization)

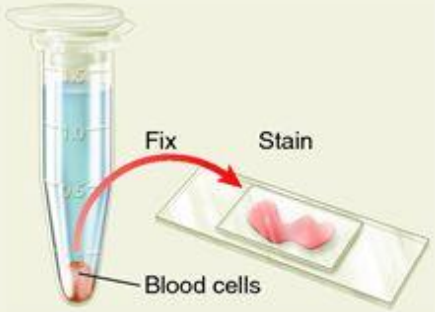
  - CGH (Comparative genomic hybridization)
- MLPA (Multiplex ligase-dependent probe amplification)

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



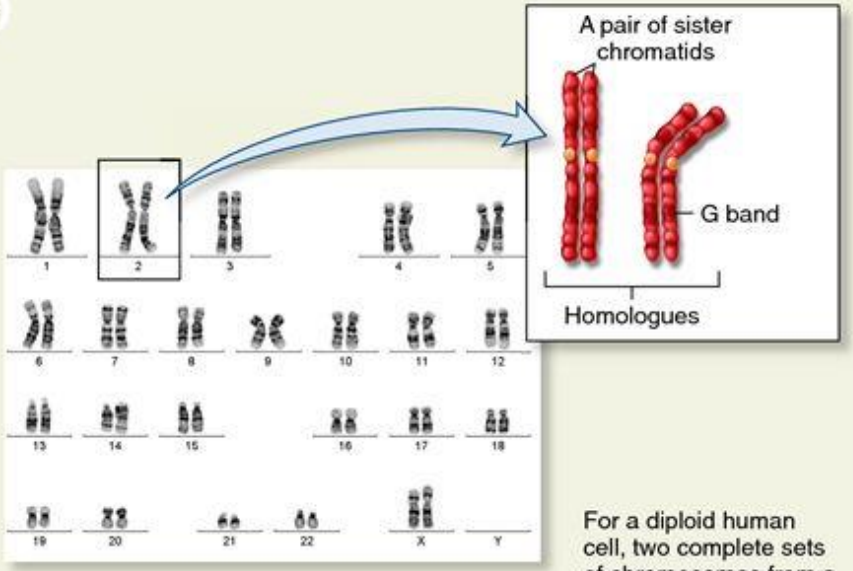
2 The supernatant is discarded, and the cell pellet is suspended in a hypotonic solution. This causes the cells to swell and the chromosomes to spread out from one another.

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.



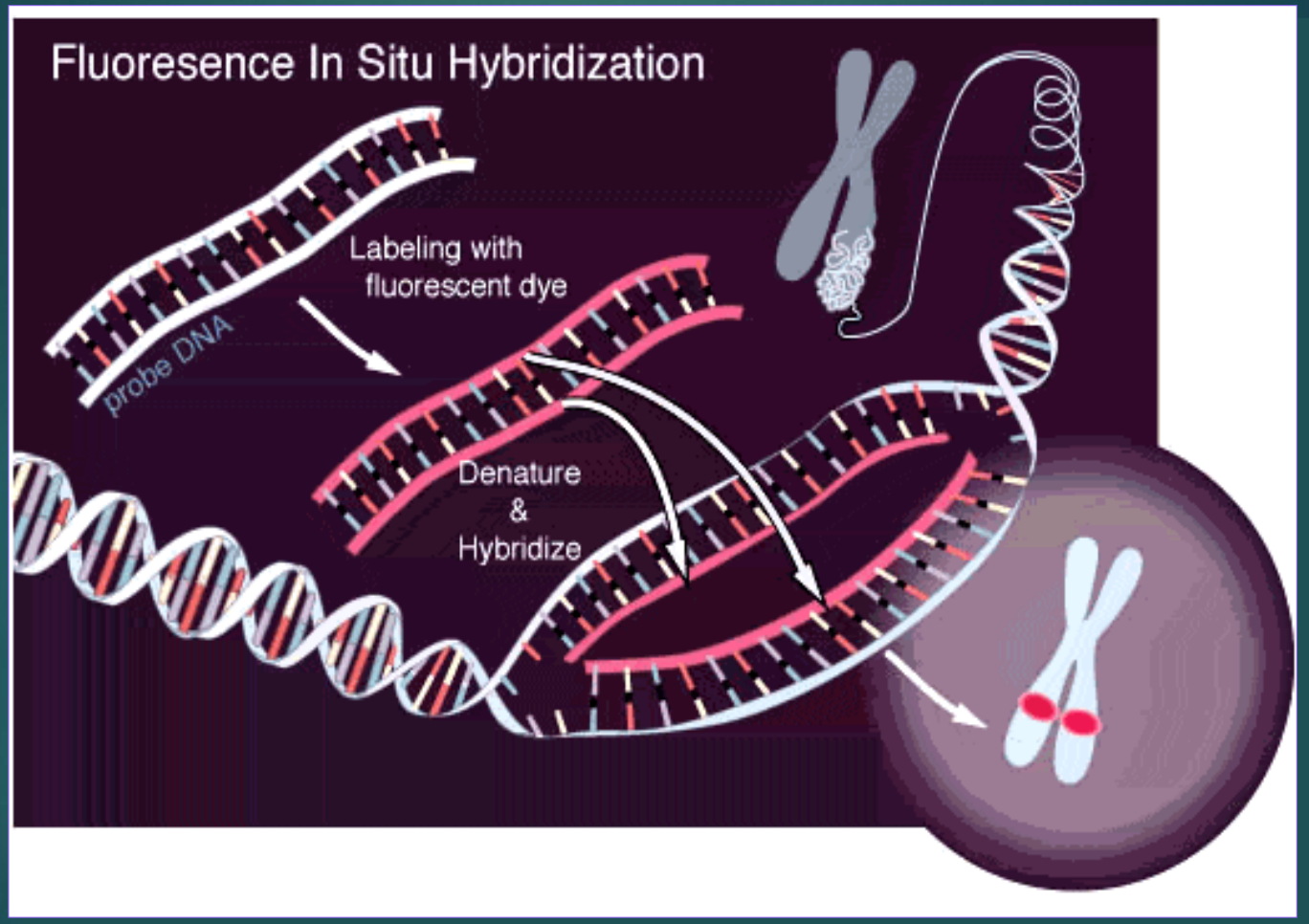
4 The slide is viewed by a light microscope equipped with a camera; the sample is seen on a computer screen. The chromosomes can be photographed and arranged electronically on the screen.

5

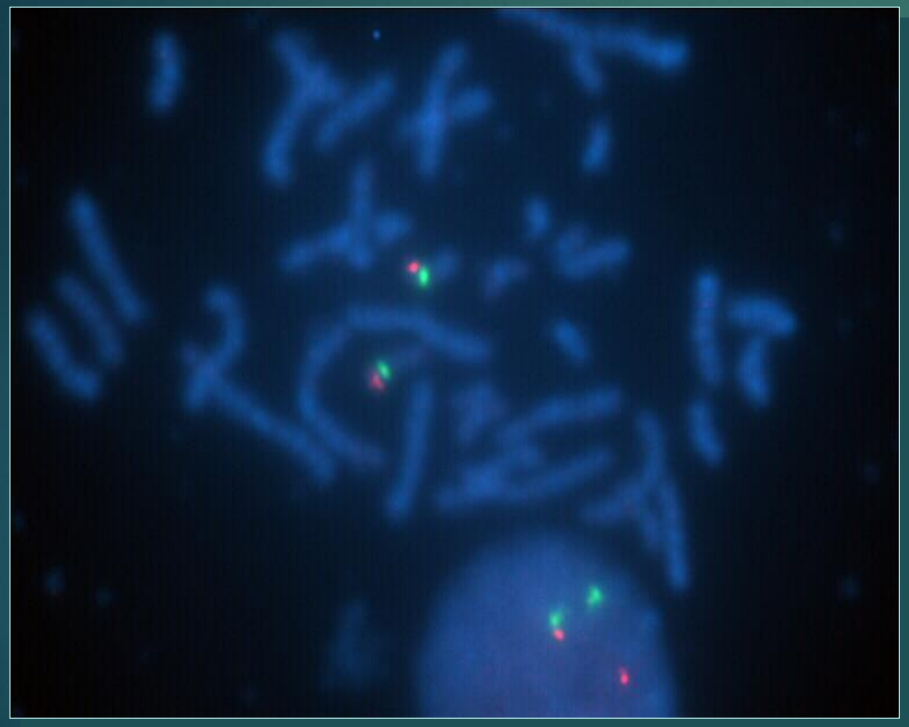


For a diploid human cell, two complete sets of chromosomes from a single cell constitute a karyotype of that cell.

FISH


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

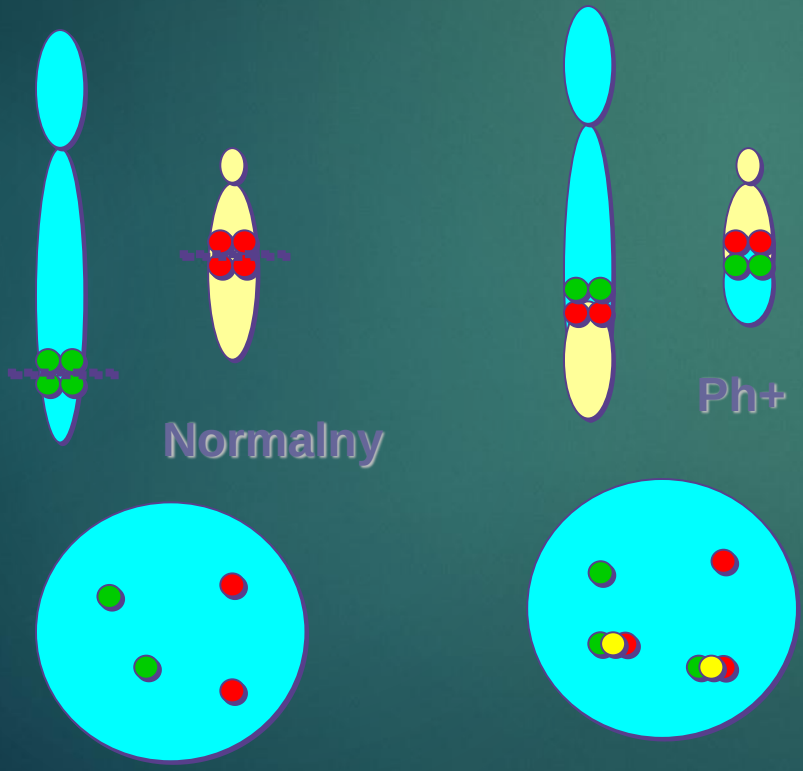
FISH

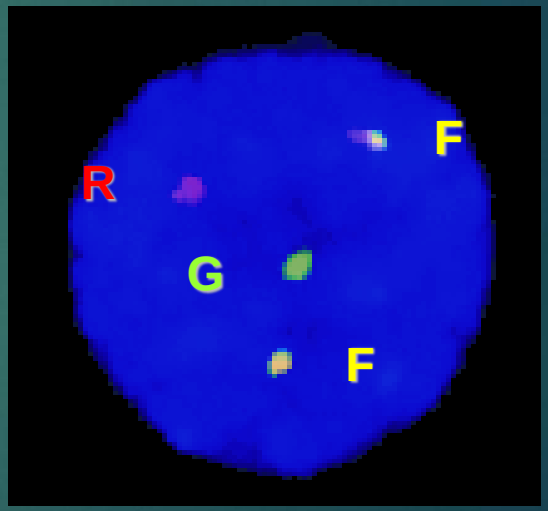


- Metaphase and interphase
- Detection of identified sequence
- Identification of chromosome

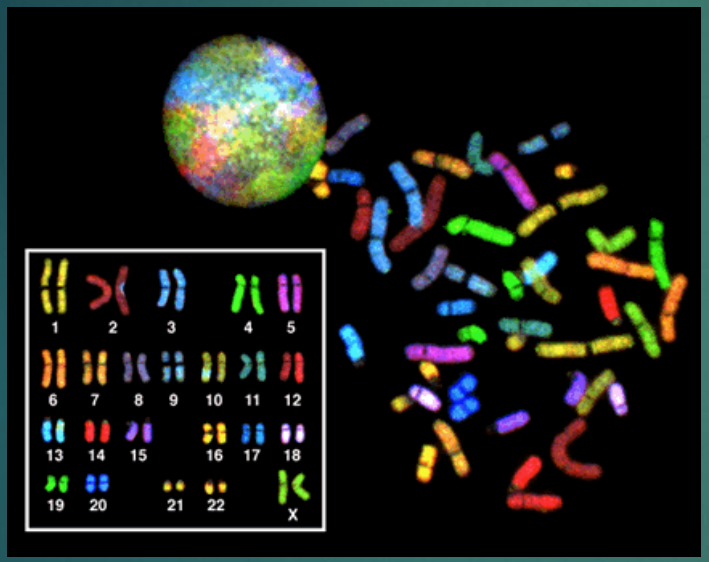
FISH ANALYSIS IN CHRONIC MYELOID LEUKEMIA

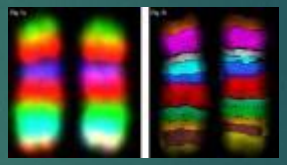
- Philadelphia chromosome t(9;22)



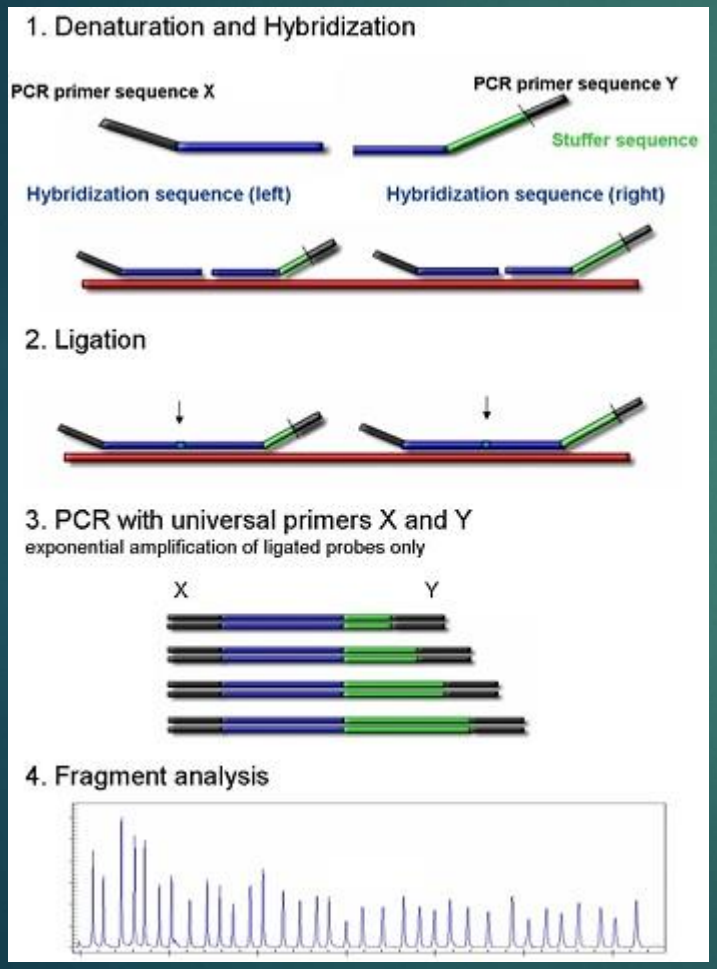


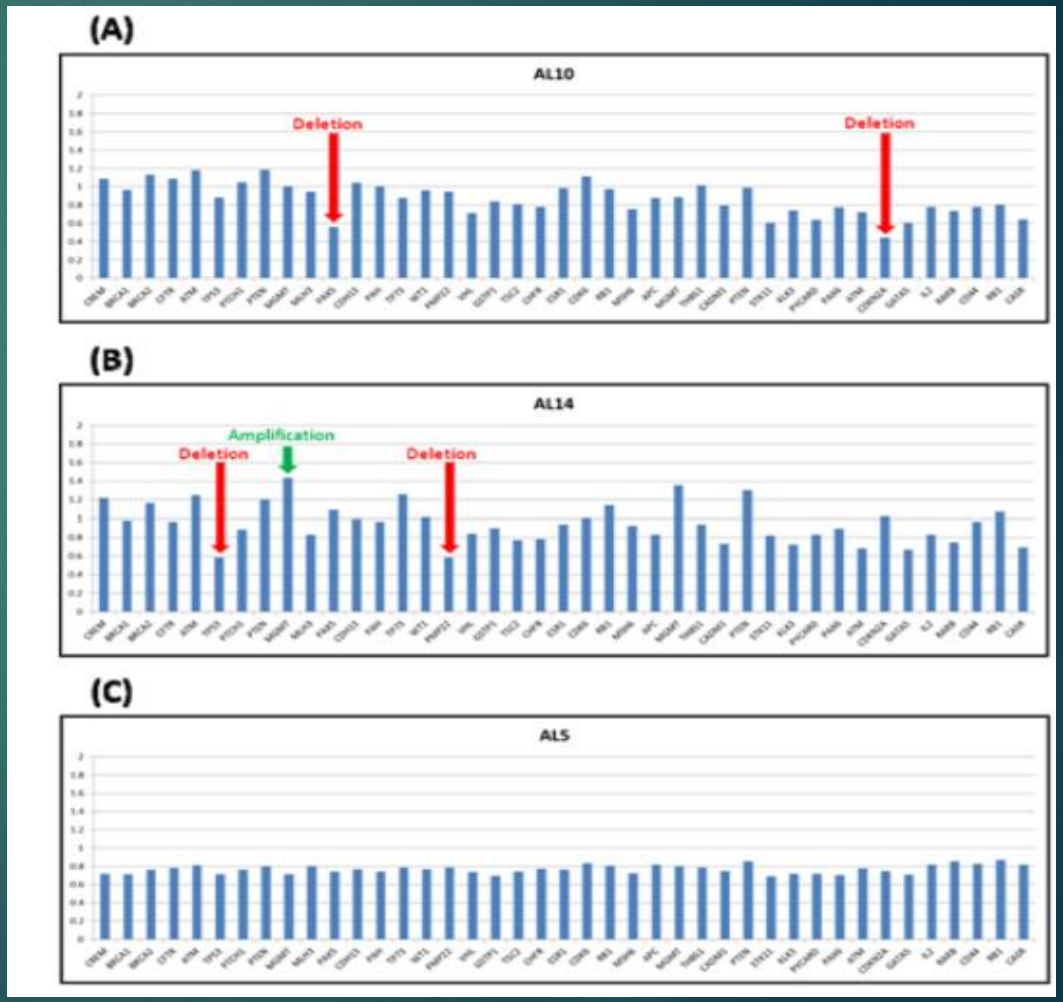
M-FISH, SKY-FISH





MLPA – Multiplex Ligase-Dependent Probe Amplification



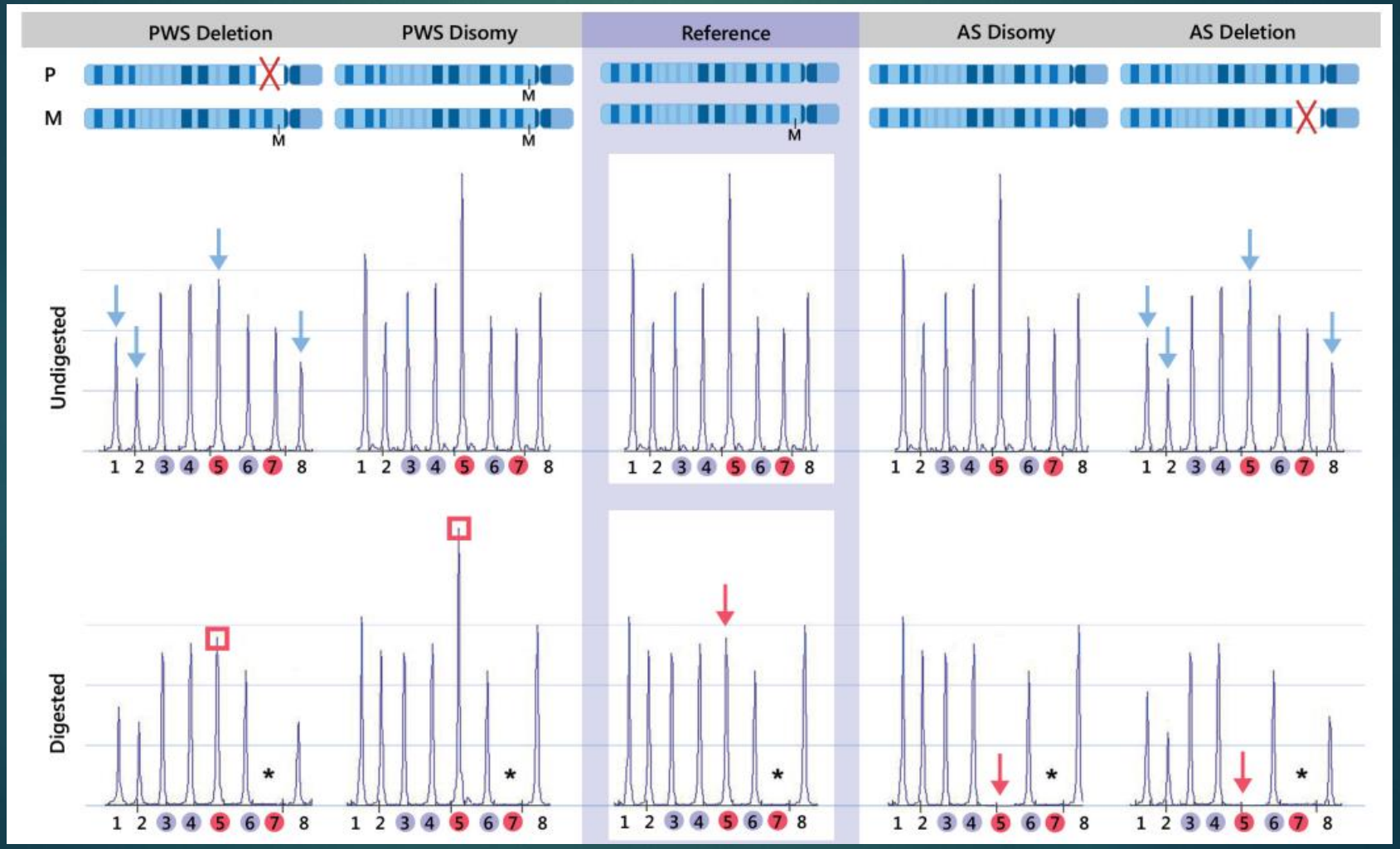


http://mlpa.com/WebForms/WebFormMain.aspx

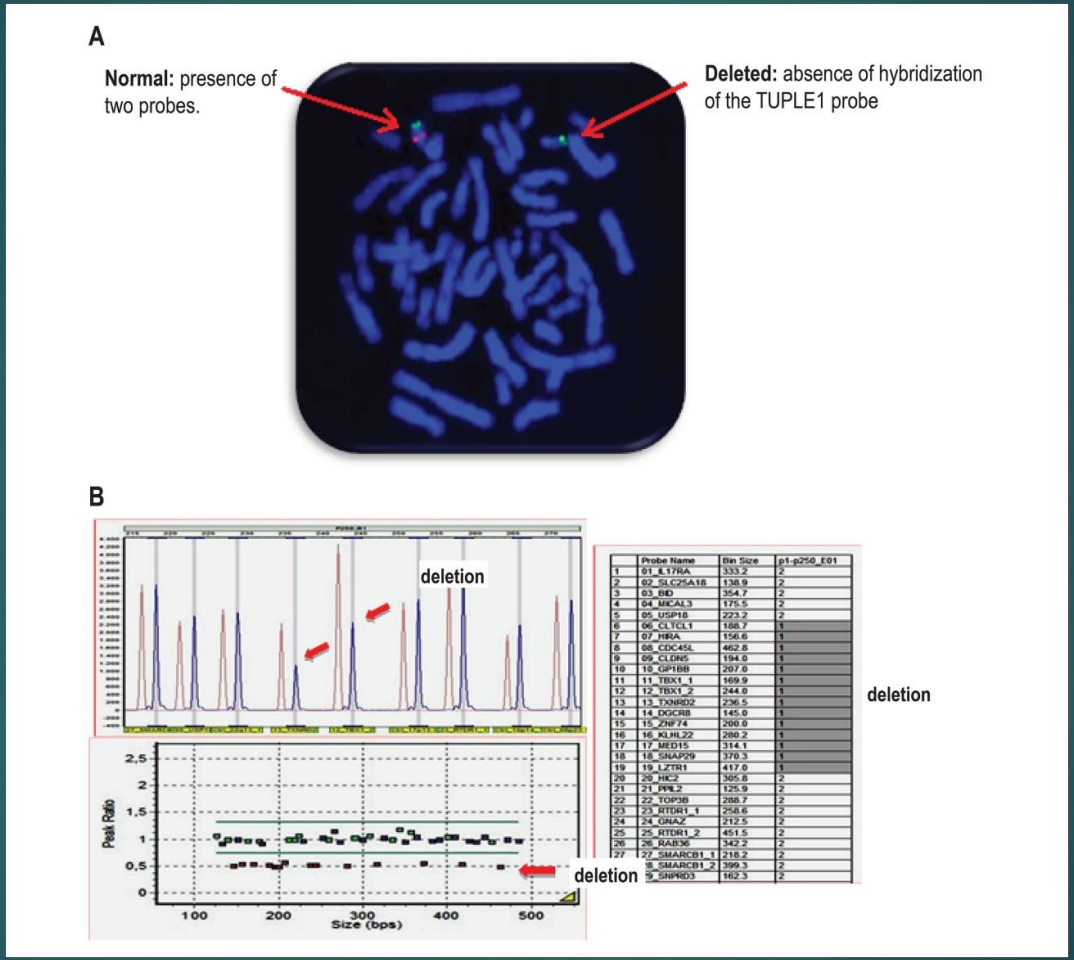
Methylation-specific MLPA (MS-MLPA)

MRC-Holland MLPA®

ME028 Prader-Willi/Angelman

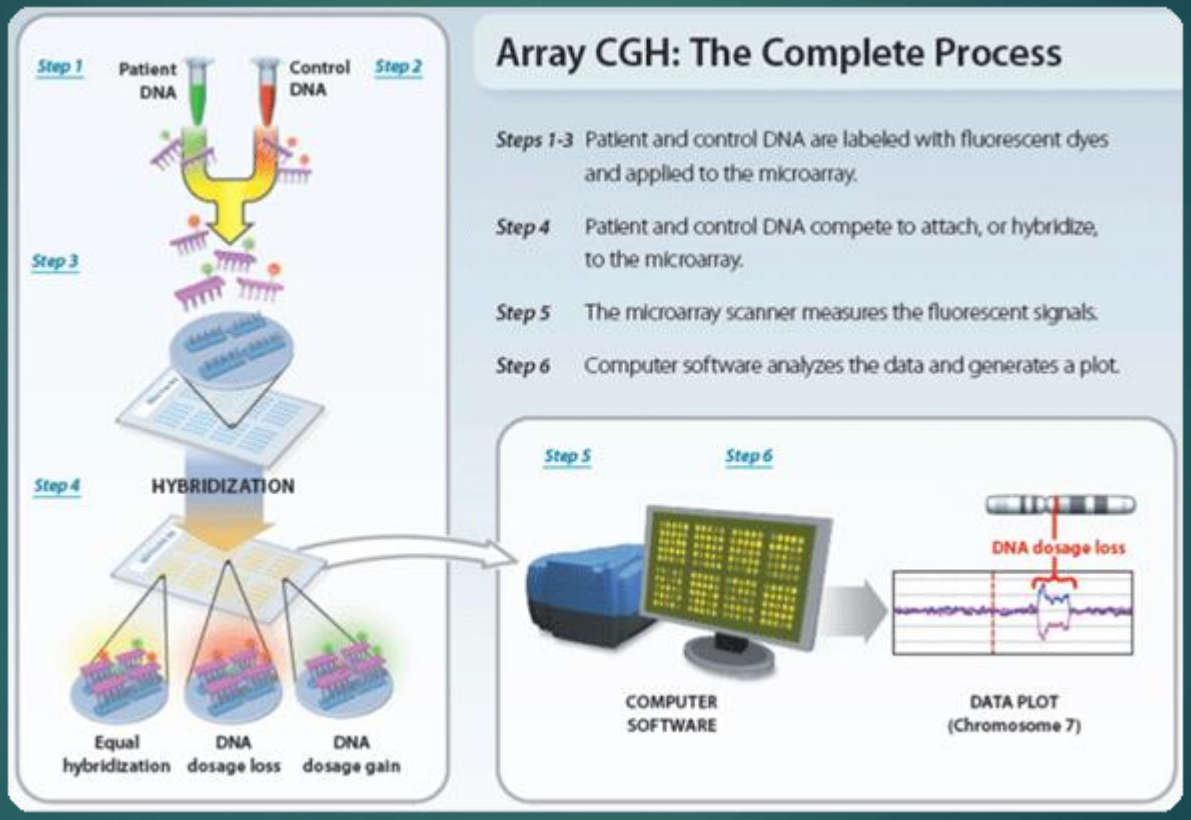


22q11.2 microdeletion – Di George syndrome



http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2014002300004

Array CGH



http://www.nggthailand.com/array-comparative-genomic-hybridisation-acgh/

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

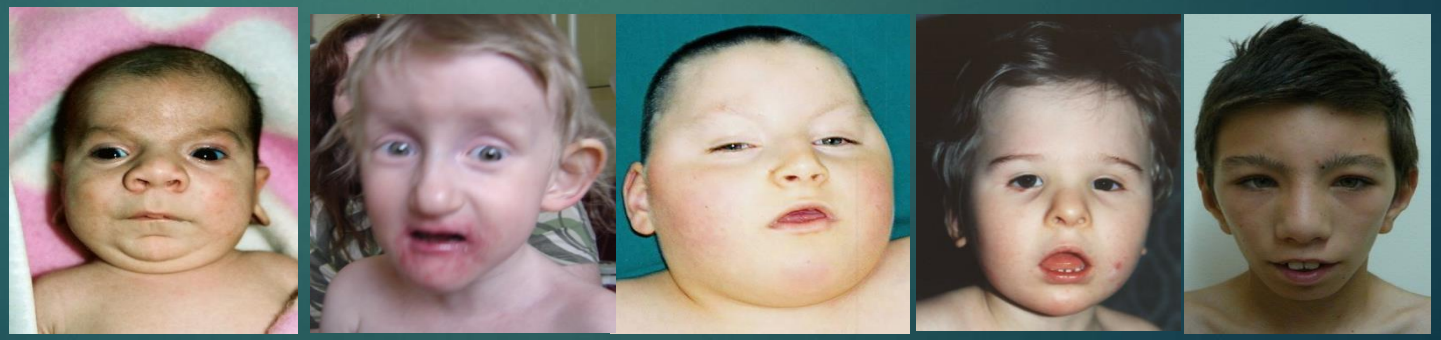
Standard Nomenclature for Chromosome Karyotypes

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%** 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 occurence

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

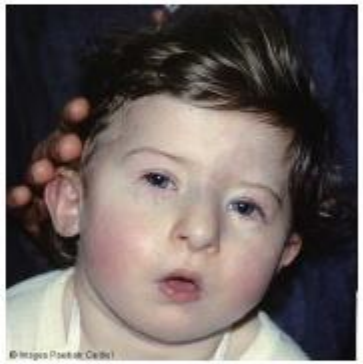
Group C: 46,XY, del17p11.2

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

Group E: 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

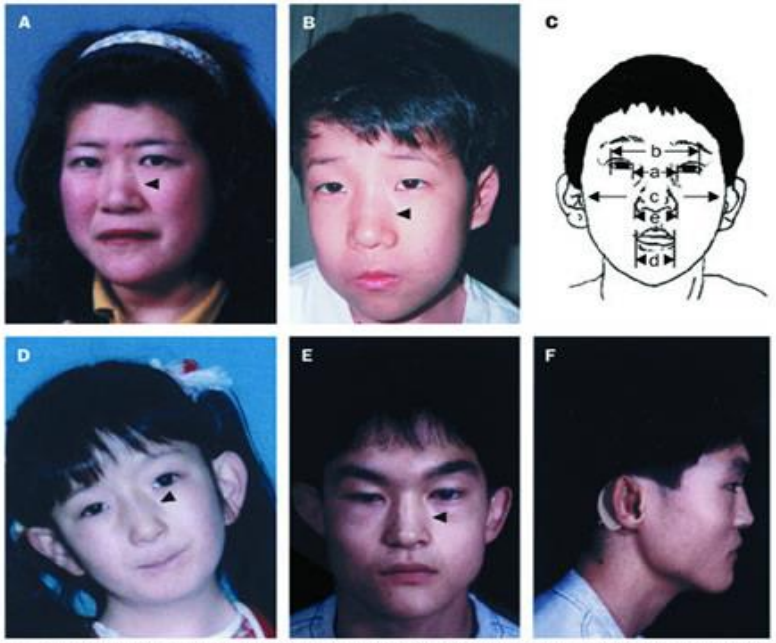
Acronym "CATCH 22"

www.philipcaruso-story.com

DiGEORGE syndrome del22q11.2

Figure

Characteristic facial appearance of the patients

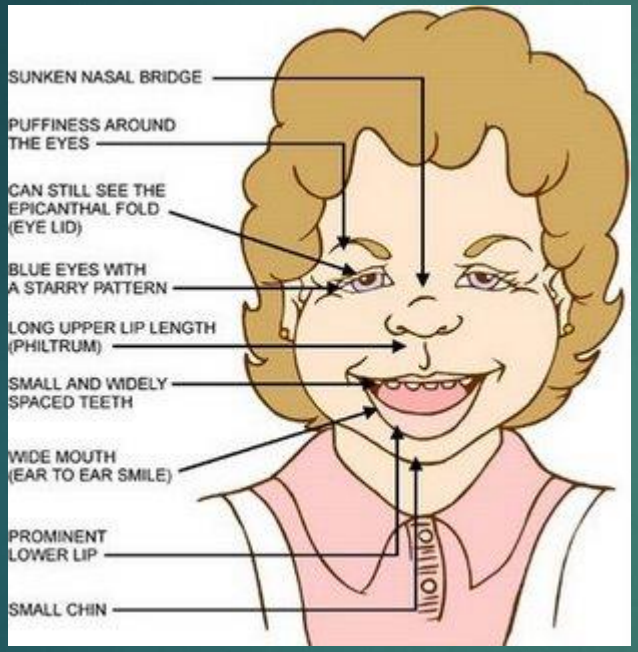


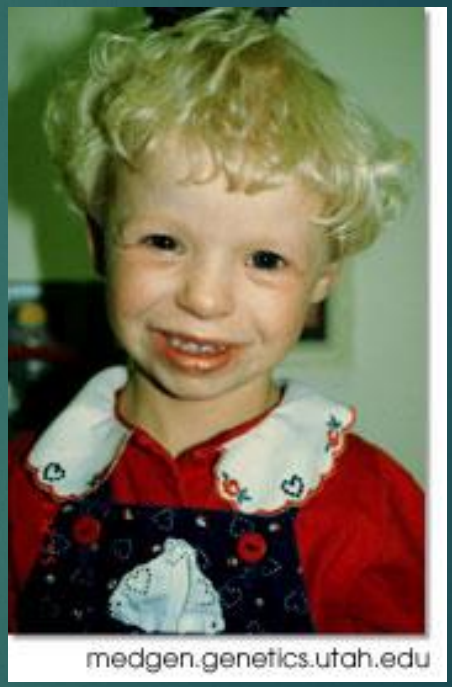
(A, B) Typical conotruncal anomaly face of del22q11.2 syndrome in familial cases (mother and son). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides. (C) Items of anthropometric measurement—a ÷ c (wide ocular hypertelorism), (b–a) ÷ b (short palpebral fissures), d ÷ c, and d ÷ 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 anomoly 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



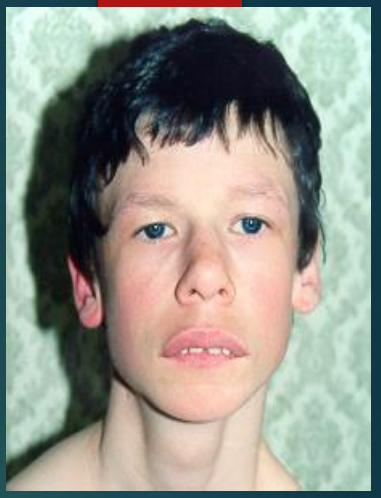


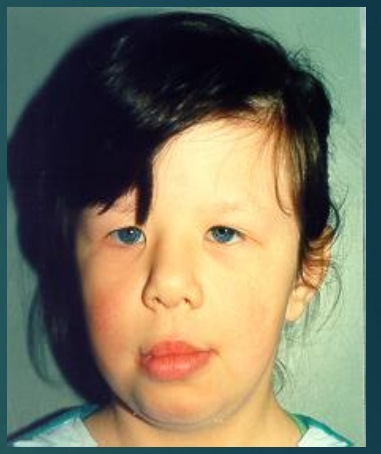
http://odlarmed.com/?p=833

WILLIAM's SYNDROME

ELN gene

- IUGR, postnatal hypotrophy, short stature
- feeding difficulties, gastro-esophageal reflux
- congenital heart defect: SVAS (75%), PPS; hypertension, arrythmia, 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 dysmorphy
- average IQ or mild ID
- chronić ear infection
- ocular defects (50%)
- renal disorders – stenosis of renal arteries, kidney stones (5%)

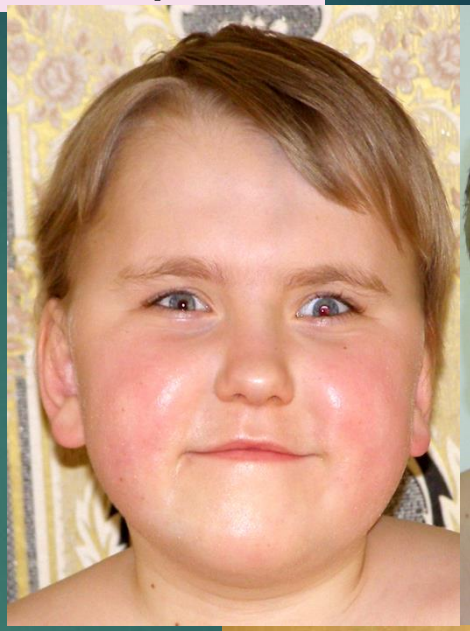


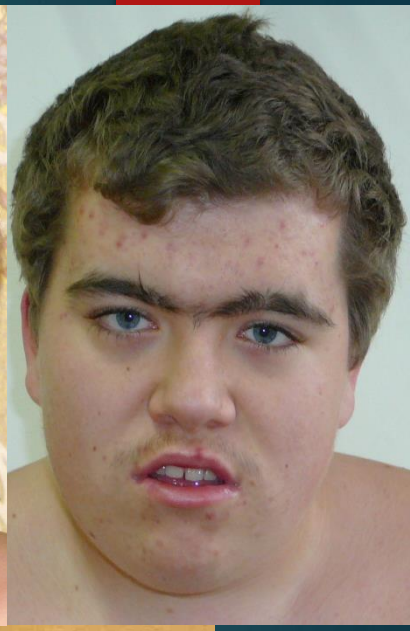


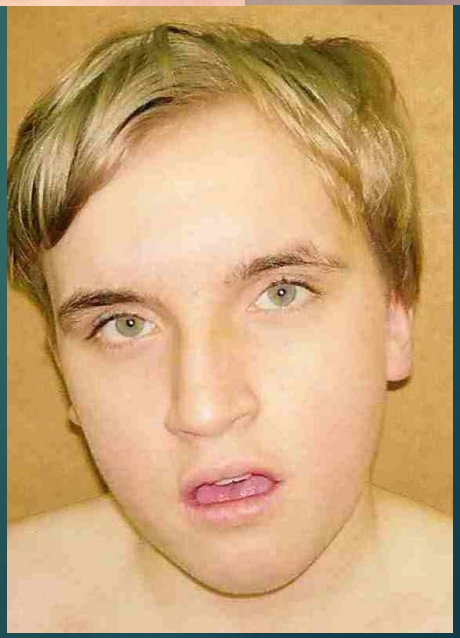
Williams syndrome
https://www.youtube.com/watch?v=BlexMOZCSVQ

SMITH – MAGENIS SYNDROME del 17p11.2

- brachycephaly
- prognatism
- wide face, deepset eyes
- short stature
- short, stubby fingers
- **hoarse voice**
- **frequent ear infections**
- **hypercholesterolemia**
- **hypotonia in infancy**
- **sleep disturbances**
- stereotype movements
- **autoagression**
- ID, severe speech delay
- peripheral neuropathy



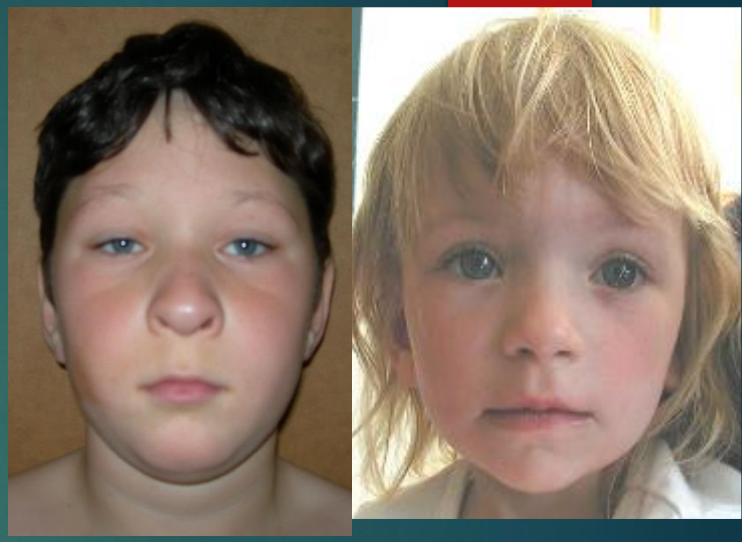




22q11.2 microduplication s.

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



Symdrome overlap with DiGeorge s./VCFS

Frequent familial cases

Pedigree analysis,
Clinical assessment of relatives

Microduplication syndromes

22q11.2 clinical features similar to microdeletion (DiGeorge'a/VCFS)

7q11.23 (WBSCR) severe speech delay

15q11-13 (PWS/AS) autism, speech delay

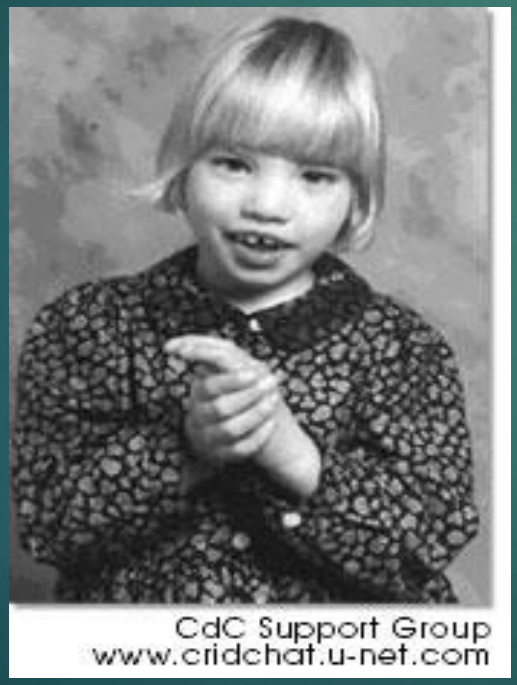
Xq28 (Rett s.) *MECP2* ID, speech defect, hypotonia, frequent infections

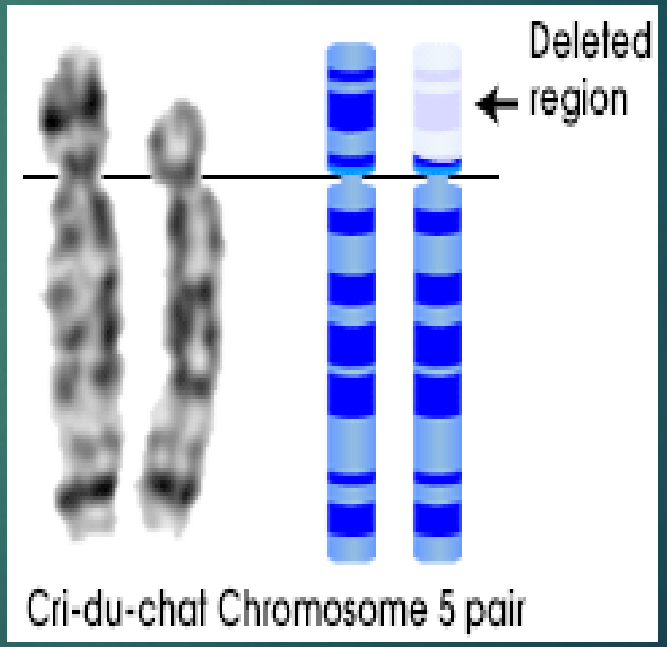
17p11.2 (Smith Magenis s. –del17p11) Potocki – Lupski syndrome

dup6q24 - 27 ID, obesity

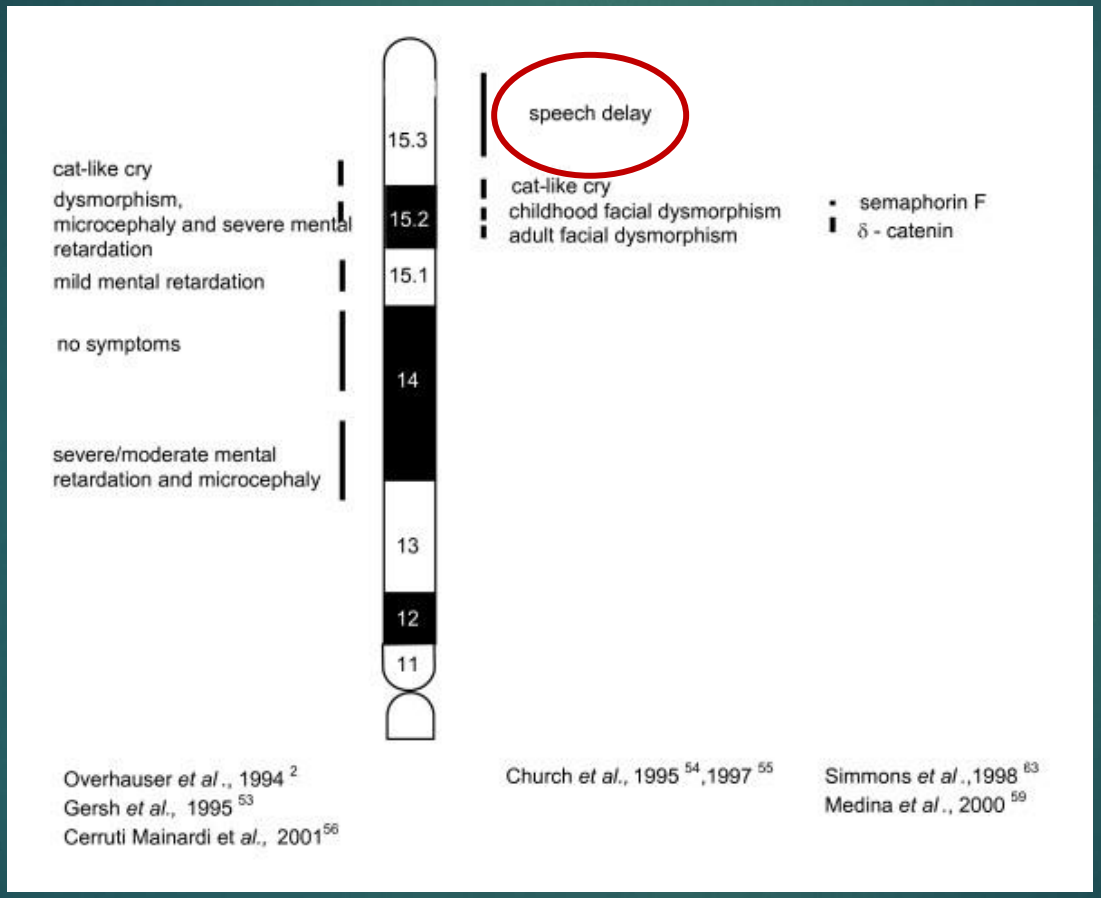
cri du chat syndrome

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



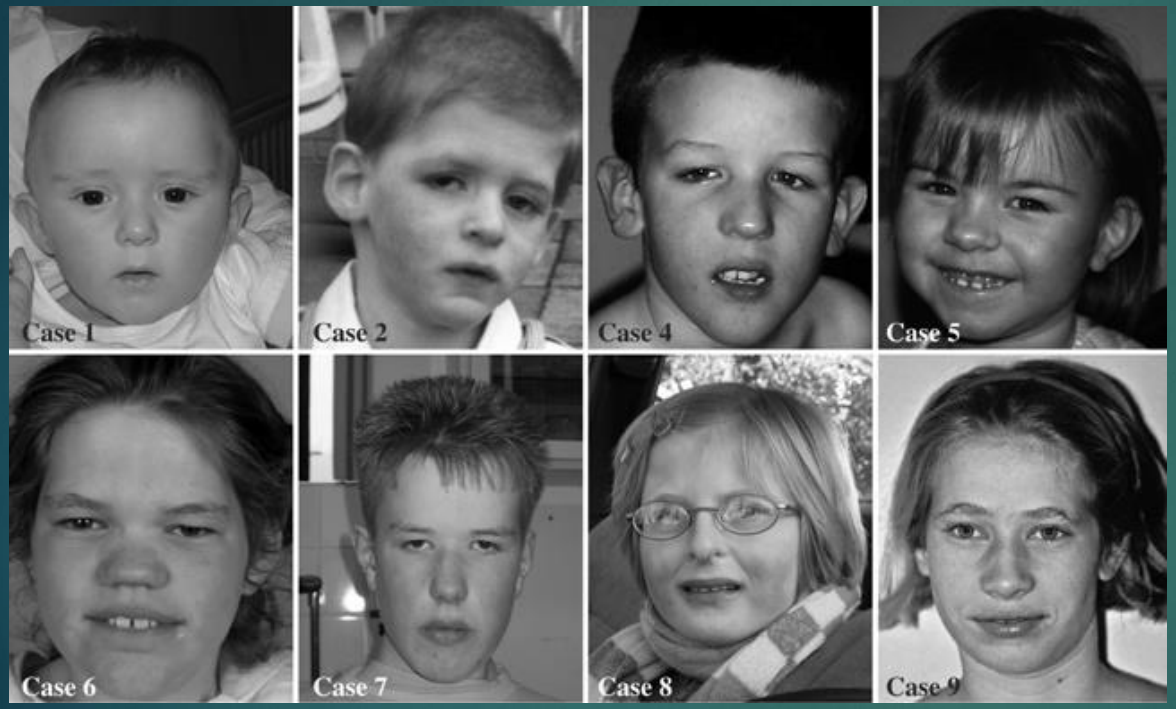


CDC – CONTIGUOUS GENE SYNDROME



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.

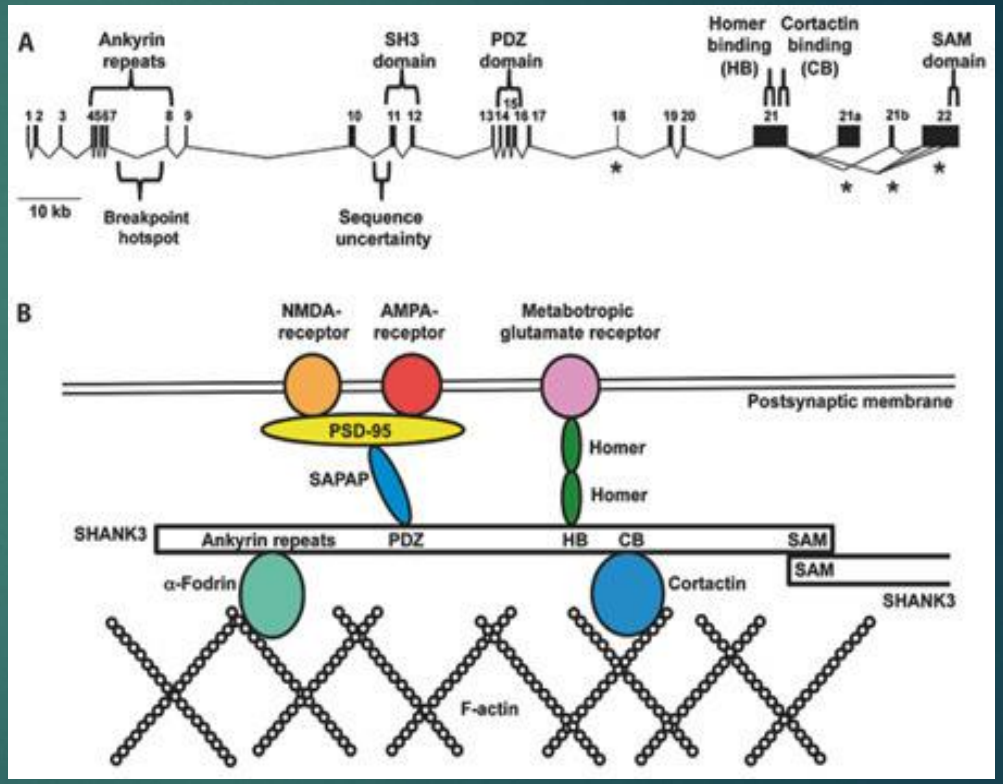
Table 1. Features associated with 22q13.3 deletion syndrome [Cusmano-Orog et al., 2007; Dhar et al., 2010; Phelan et al., 2010]

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

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

PMS – *SHANK3* GENE

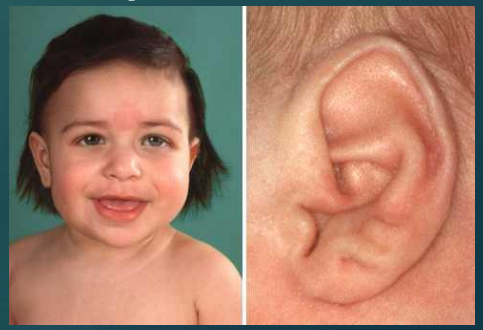
- 22q13.3 deletion (80-85%)
- Ring chromosome
- Unbalanced translocation (15-20%)



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)

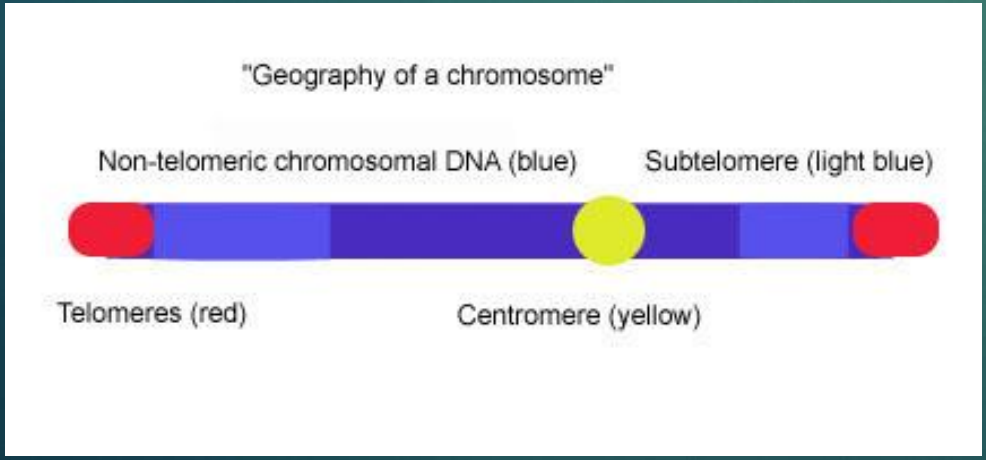
- **Duplication / Deletion of 11p15 (*IGF2* gene)**
- macroglossia, omphalocoele, high birth weight, ear pits, hemihyperplasia
- hypoglycemia in neonatal period (could cause developmental delay), high tumor risk (Wilms tumor, hepatoblastoma, rhabdomyosarcoma, adrenal tumor)

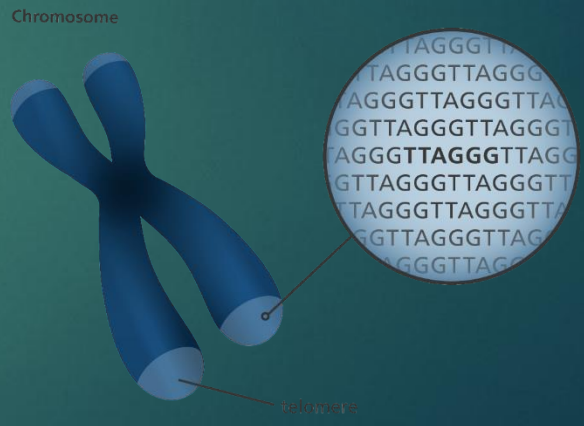


Subtelomeric rearrangements

TELOMERES

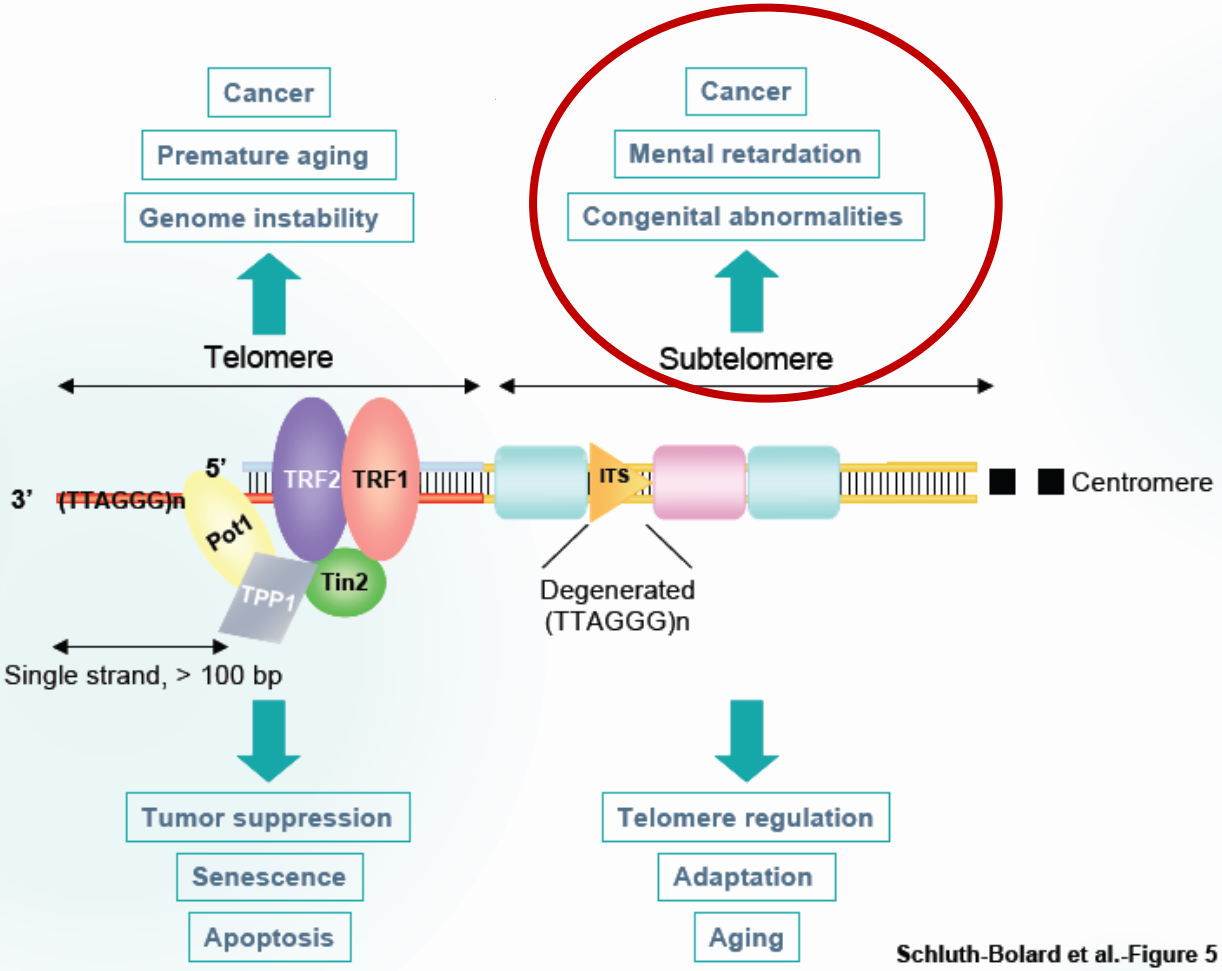
- Protect chromosomes from deterioration during cell replication
- Chromosomal organization in the nucleus
- Sequence: TTAGGG





https://www.yourgenome.org/facts/what-is-a-telomere

SUBTELOMERIC REGION



Schluth-Bolard et al.-Figure 5

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 dysmorphy
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

Understanding chromosome disorders Unique

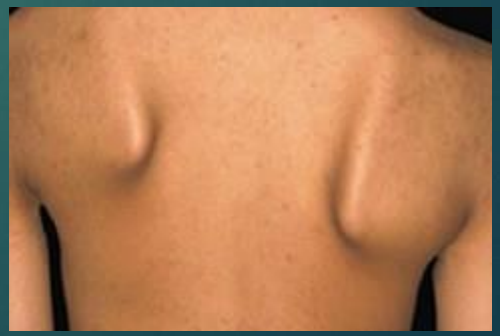
1p36 deletion syndrome

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



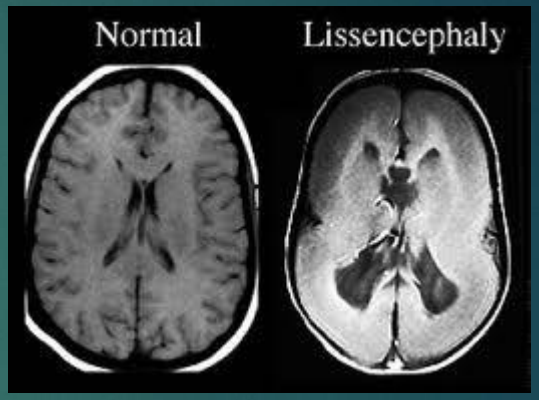
https://www.fshsociety.org/facioscapulohumeral-description/

FSHD - DIAGNOSIS

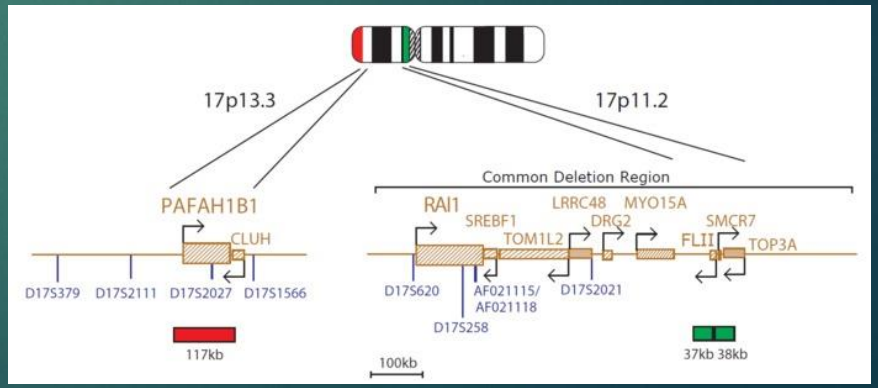
- Typical clinical features
- CK – normal or slightly elevated
- EMG – nonspefic myopathic changes
- Muscle biopsy – dystrophic changes
- MR – evaluation of affected muscles

MILLER-DIEKER SYNDROME – del17p13.3

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

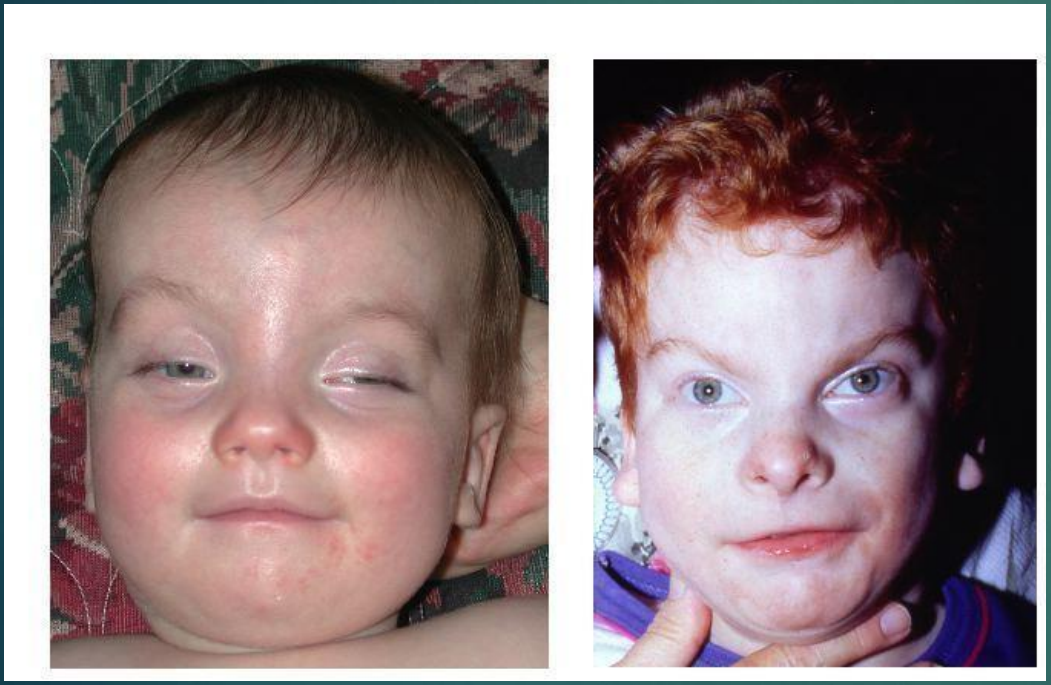


https://prezi.com/hyphafzgrzel/miller-dieker-syndrom/



http://www.cytocell.com/probes/123-smithmagenis-fliimillerdieker-probe-combination

MDS – DYSMORPHIC FEATURES

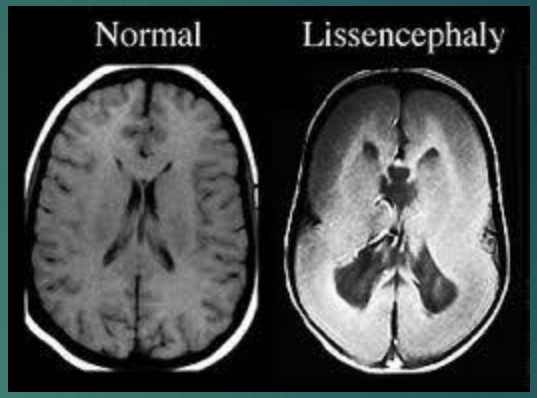


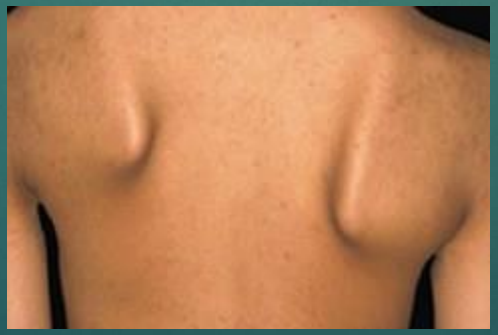
- Prominent forehead
- Midface hypoplasia
- Short nose
- Lowset, dysplastic ears
- Micrognathia

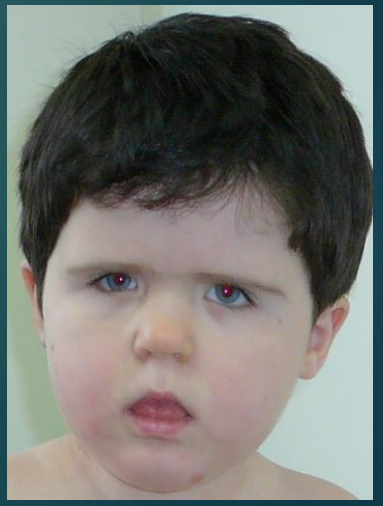
https://www.ncbi.nlm.nih.gov/books/NBK5189/figure/chrom17-lis.F2/?report=objectonly

GENES AND SYNDROMES - LISSENCEPHALY

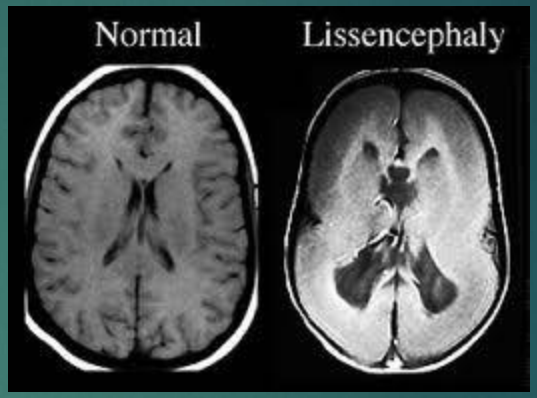
Syndrome/Disease	Gene symbol	Protein function	Region	OMIM number	Inheritance pattern
Lissencephaly classic Type I					
Miller-Dieker type	PAFAH1B1	Nuclear migration factor	17p13.3	601545	AD
Sub-band heterotopia type	DCX	microtubule regulator	Xq22.3	300121	XL
	RELN	Neuronal migration factor	7q22	600514	AR
	ARX	Homeobox, neuron function	Xp22.13	300382	XL
Cobblestone type lissencephaly Type II					
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					


Normal
Lissencephaly

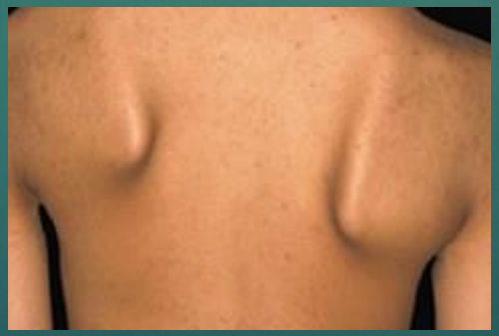




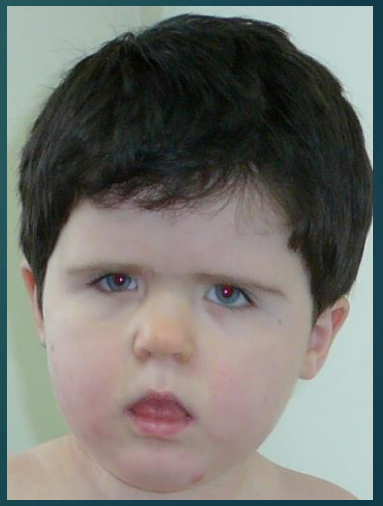
Wolfa-Hirschhorn s.



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