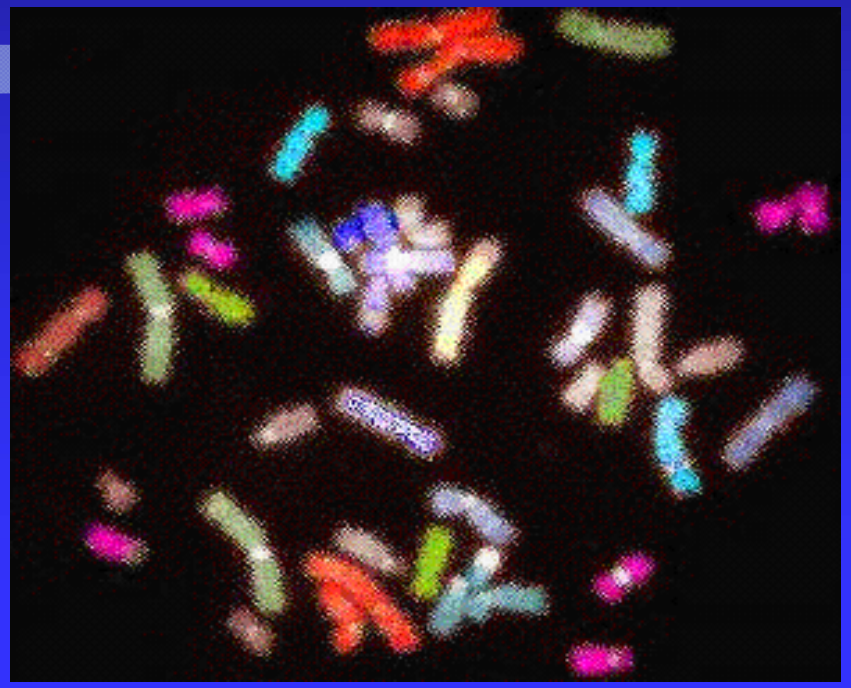
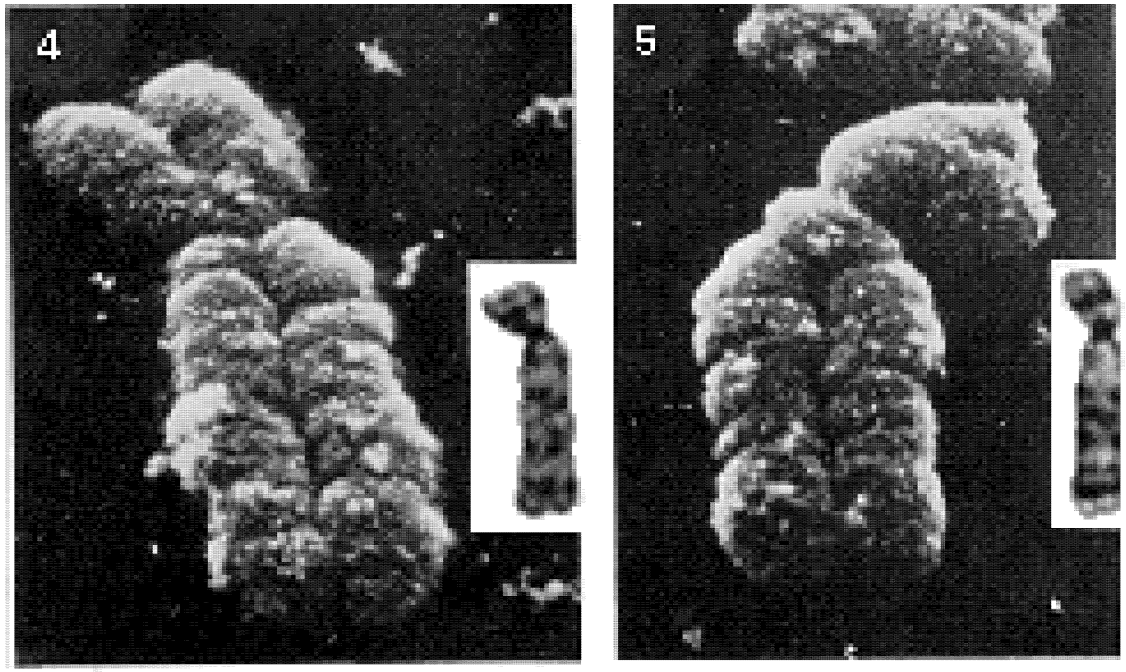
La patologia cromosomica

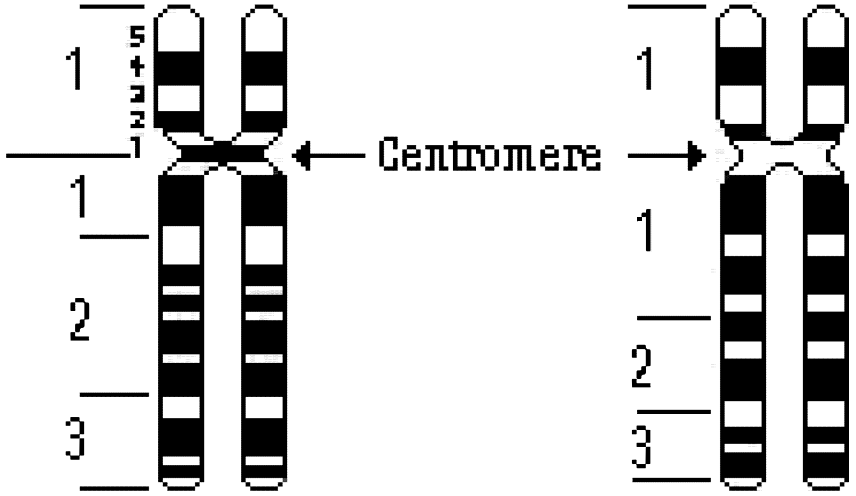


FISIOKINESITERAPIA-NEWS.IT

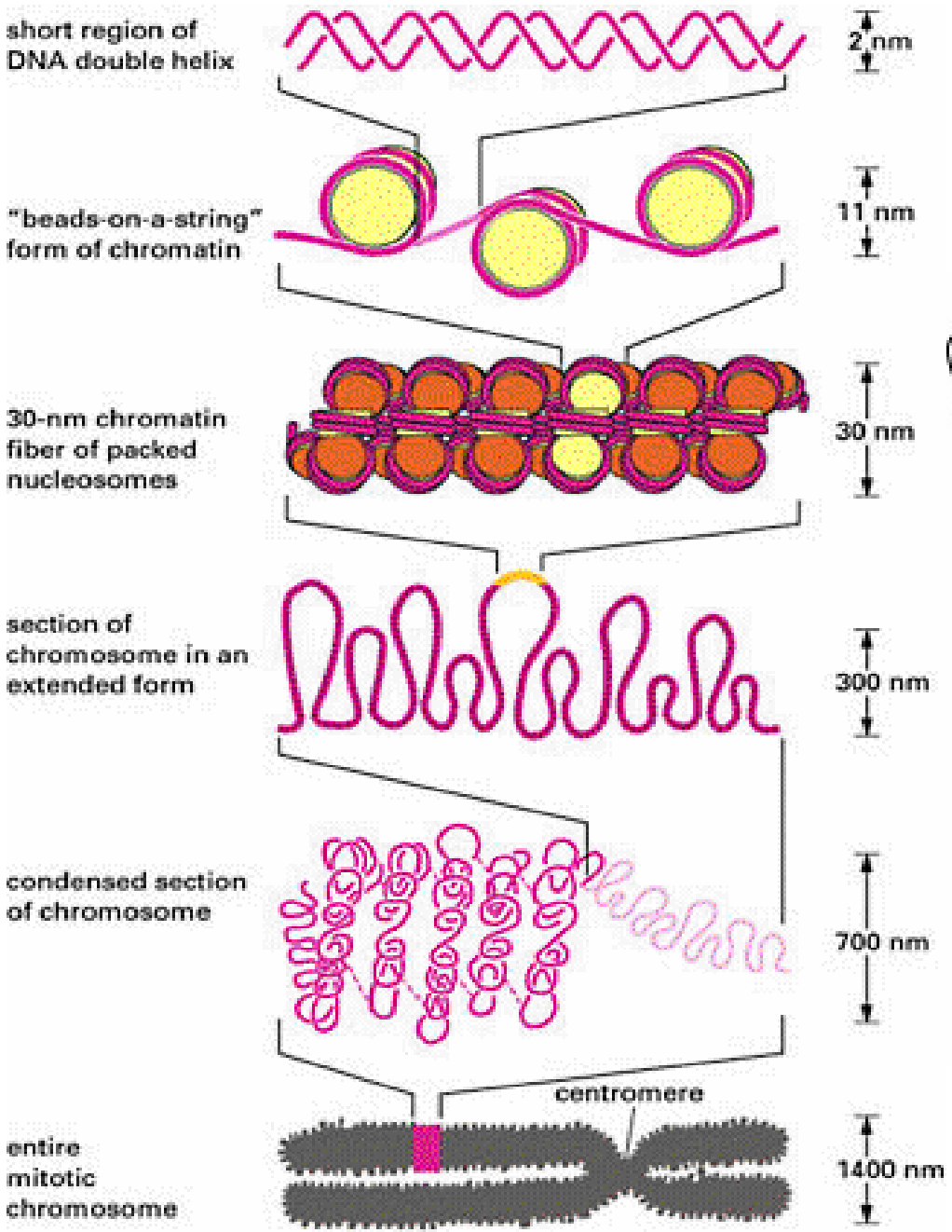
LO STUDIO DEI CROMOSOMI: CITOGENETICA

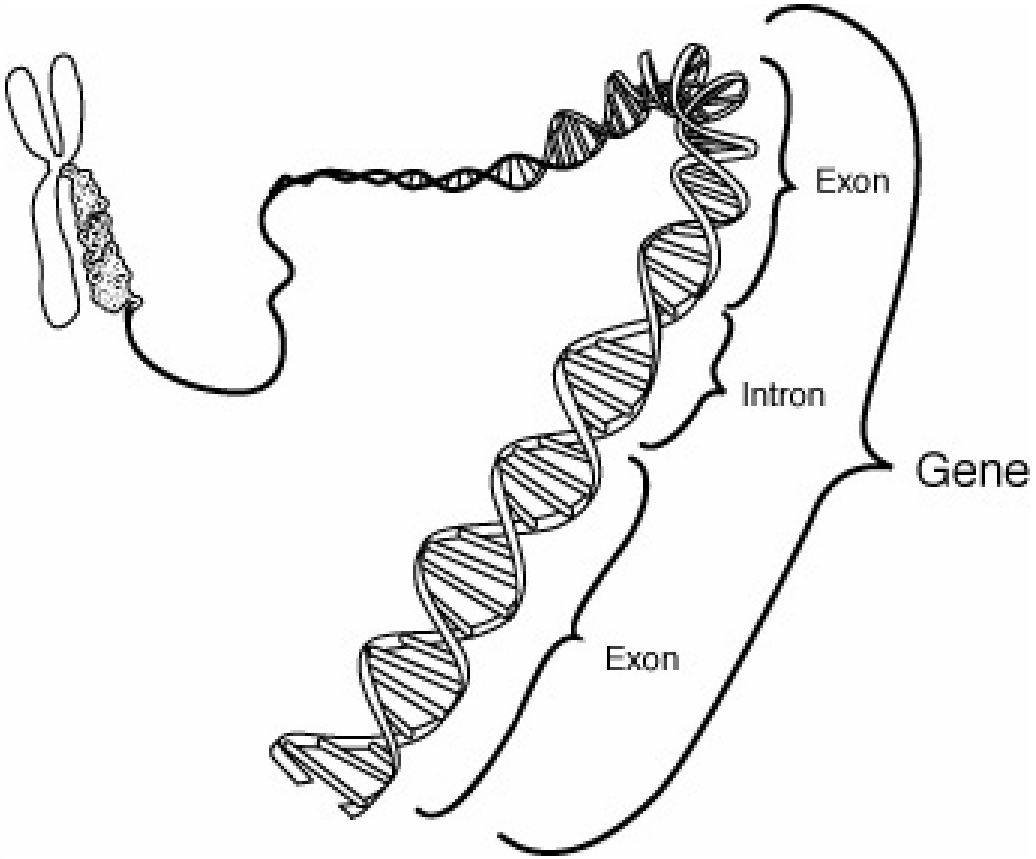
- alcuni concetti di citogenetica
- classificazione
- la frequenza delle malattie cromosomiche ed alcune patologie più frequenti
- quando la citogenetica tradizionale non ce la fa...





4 5

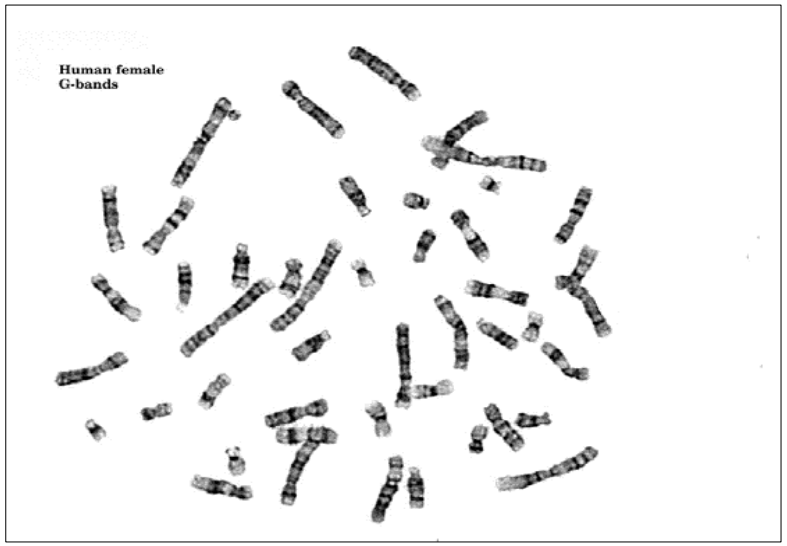


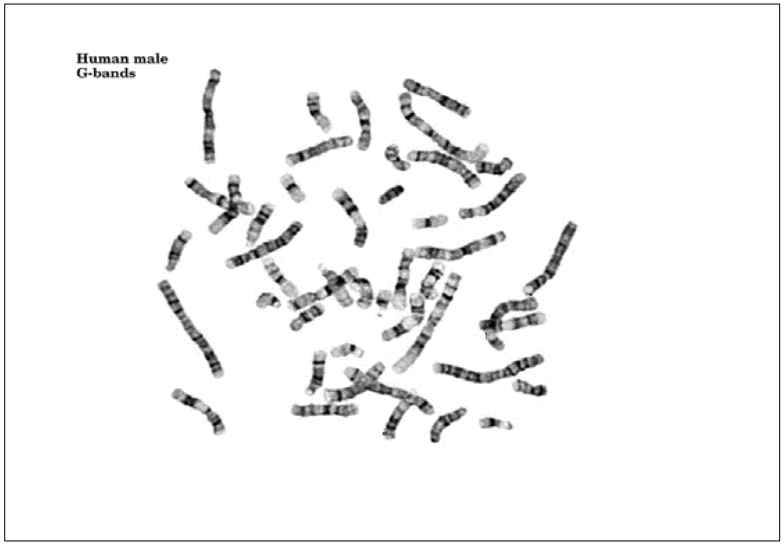


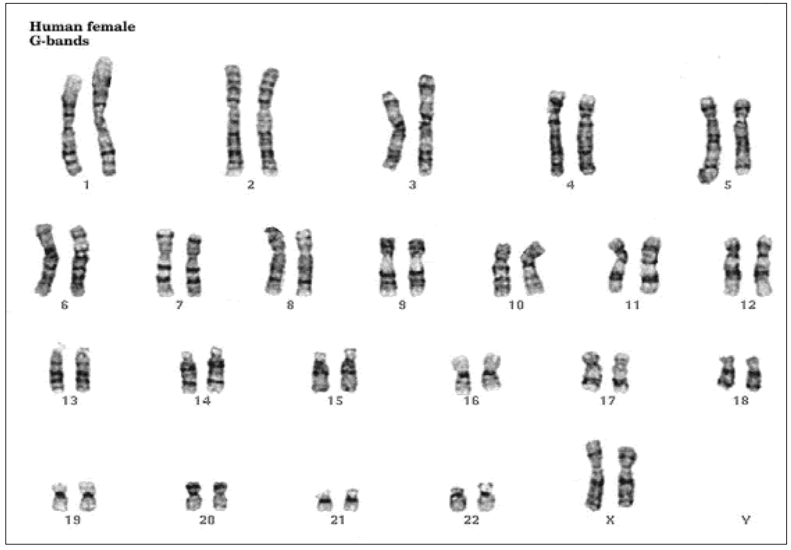
NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

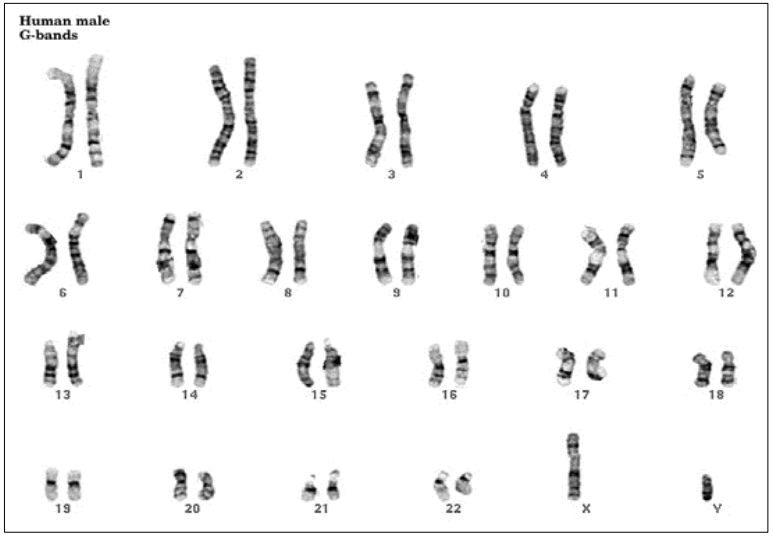
Harvest Procedures

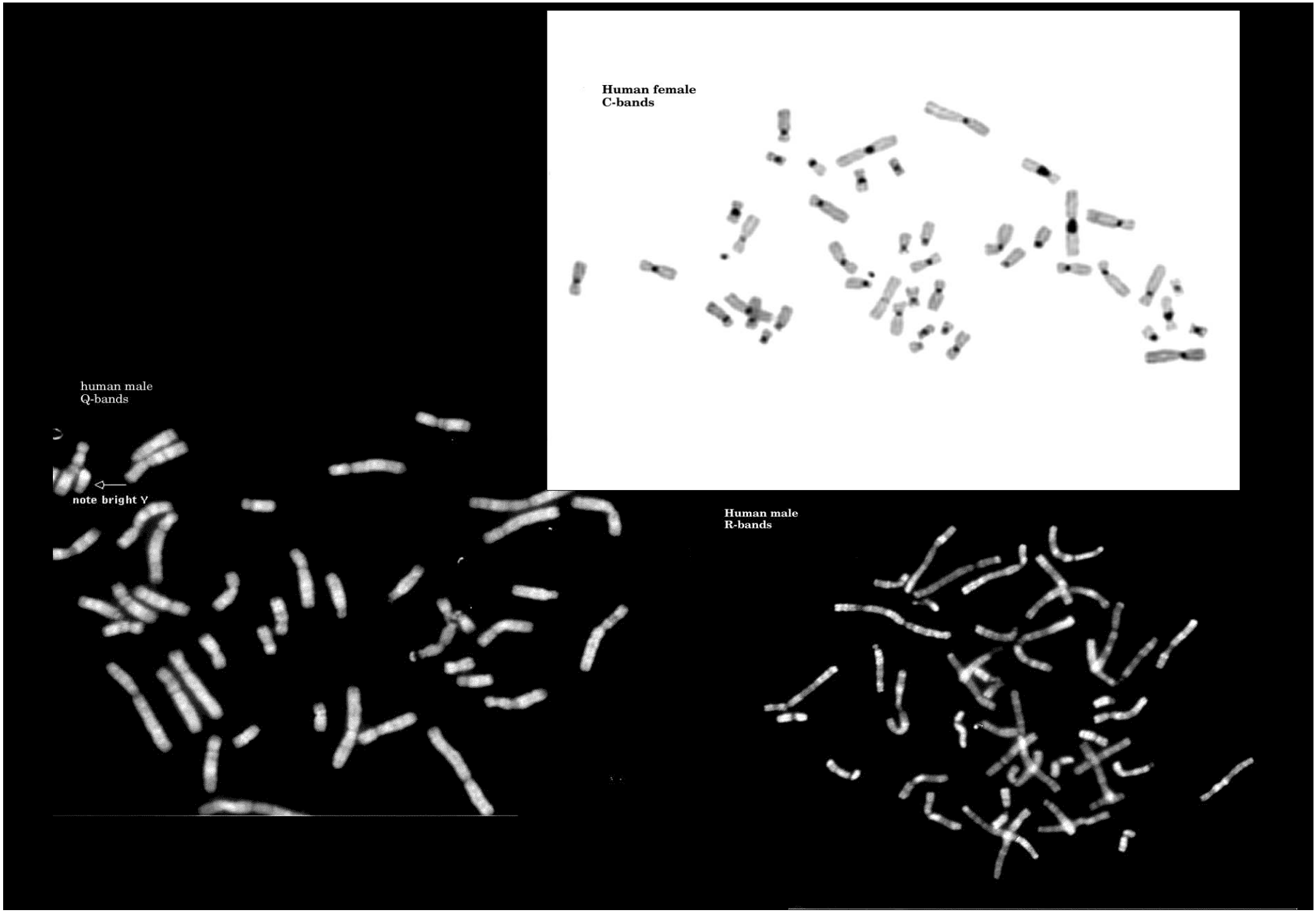
- Mitotic Activity
  - Stimulation
  - Tissue Culture
  - Direct harvest
  - Unstimulated Culture
- Metaphase Inhibition
  - Colchicine, Colcemid
- Hypotonic Treatment
  - KCl
- Fixation-Wash
  - 3:1 methanol:glacial acetic acid (Carnoy's)
- Slide Preparation
- Banded Staining
  - GTG-G bands (trypsin-Giemsa)


Human female
G-bands


Human male
G-bands

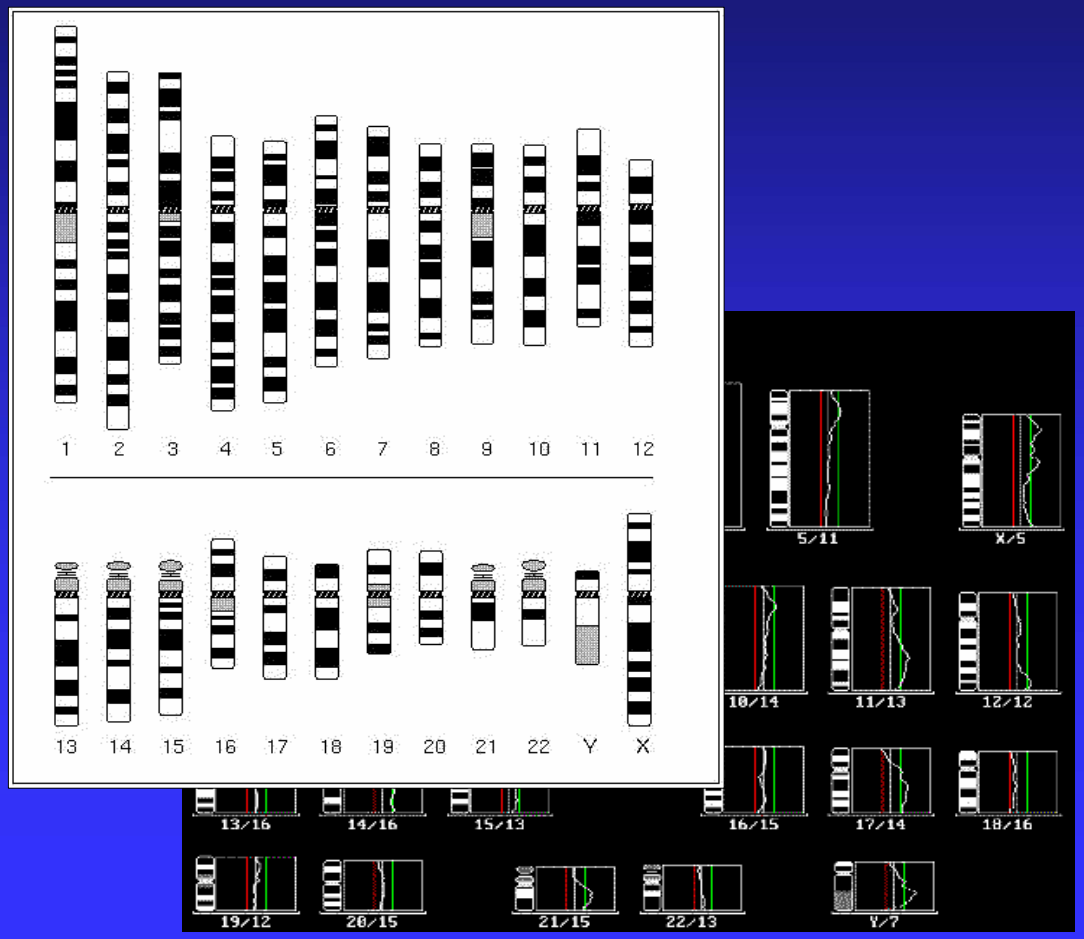

Human female
G-bands
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y


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


Human female
C-bands
human male
Q-bands
note bright Y
Human male
R-bands

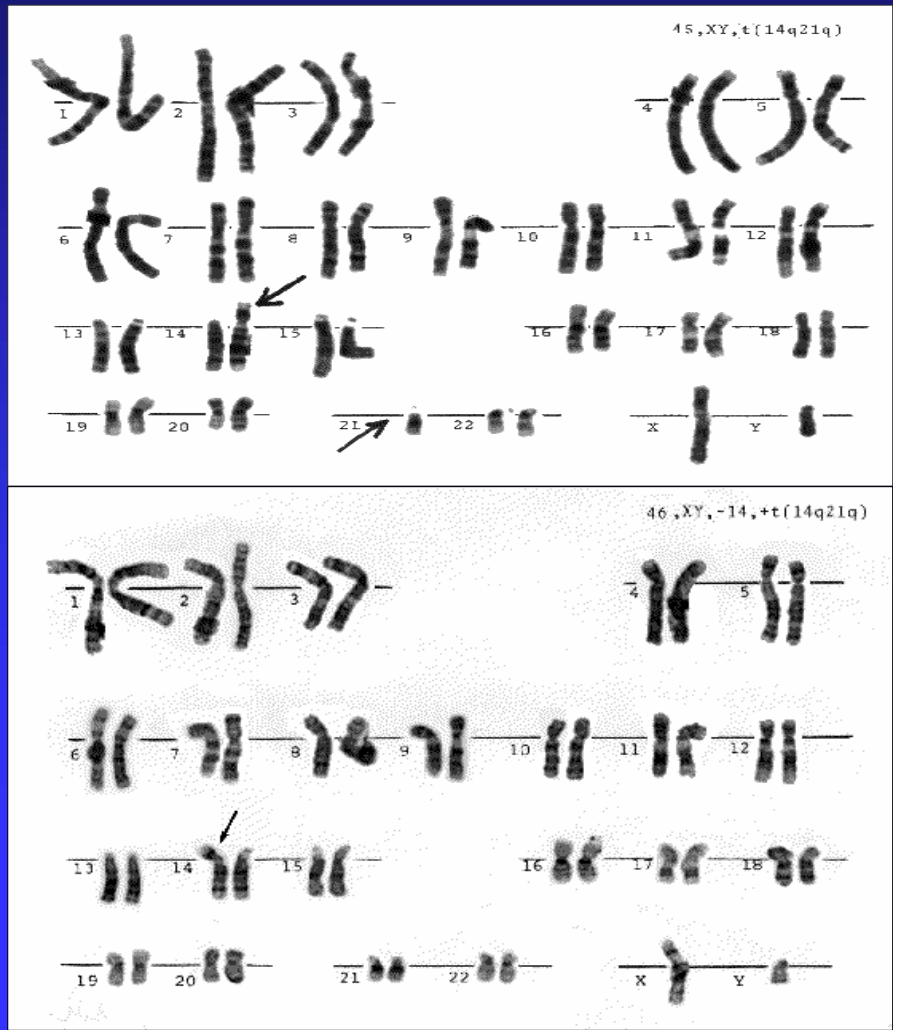
Anomalie cromosomiche

- **Come classificarle**
- **la loro frequenza**

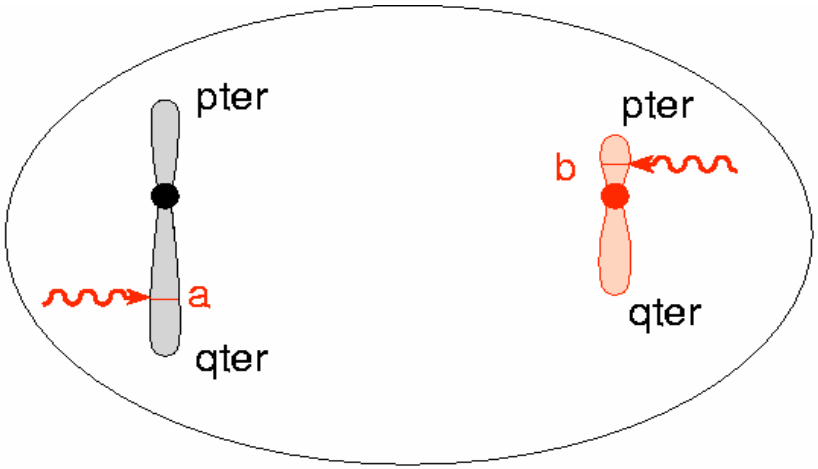


Anomalie cromosomiche

- **Bilanciate:**
- **nella maggioranza dei casi non sono correlate ad un fenotipo anomalo**
- **Sbilanciate:**
- **sono correlate ad un fenotipo anomalo (malformazioni e/o ritardo mentale)**



TRASLOCAZIONI CROMOSOMICHE:
ROTTURE SU PIU'
DI UN CROMOSOMA

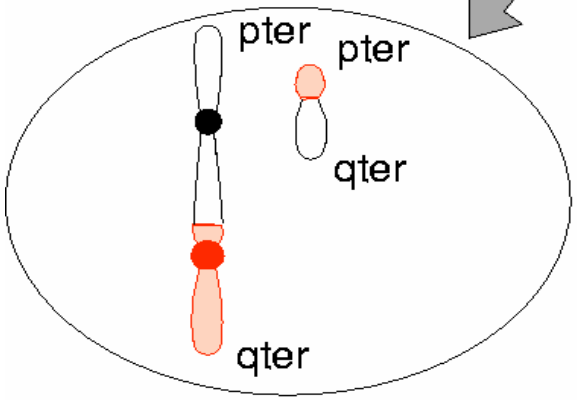


TRASLOCAZIONE RECIPROCA

Exchange of centric fragment for acentric fragment

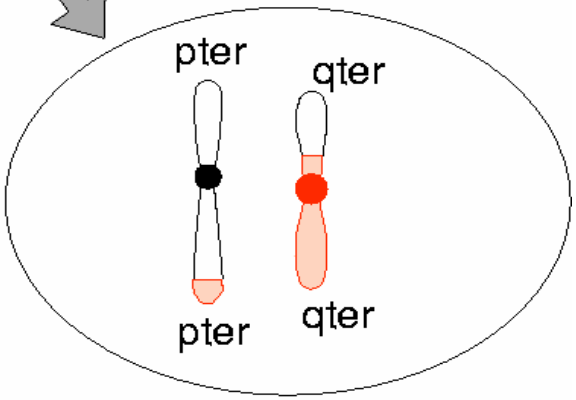
Exchange of acentric fragments a–qter and b–pter

SBILANCIATA



Nonviable

BILANCIATA



Often viable

ANOMALIE CROMOSOMICHE

- **ANOMALIE COSTITUZIONALI: presenti in cellule di tutto il corpo. Spermatozoo e ovulo normali, fecondazione anomala o evento anomalo nelle prime fasi di sviluppo embrionale**
- **ANOMALIE SOMATICHE: presenti in un piccolo sottogruppo di cellule o tessuti. Diverse costituzioni cromosomiche pur derivando tutte le cellule dallo stesso zigote. MOSAICO GENETICO.**

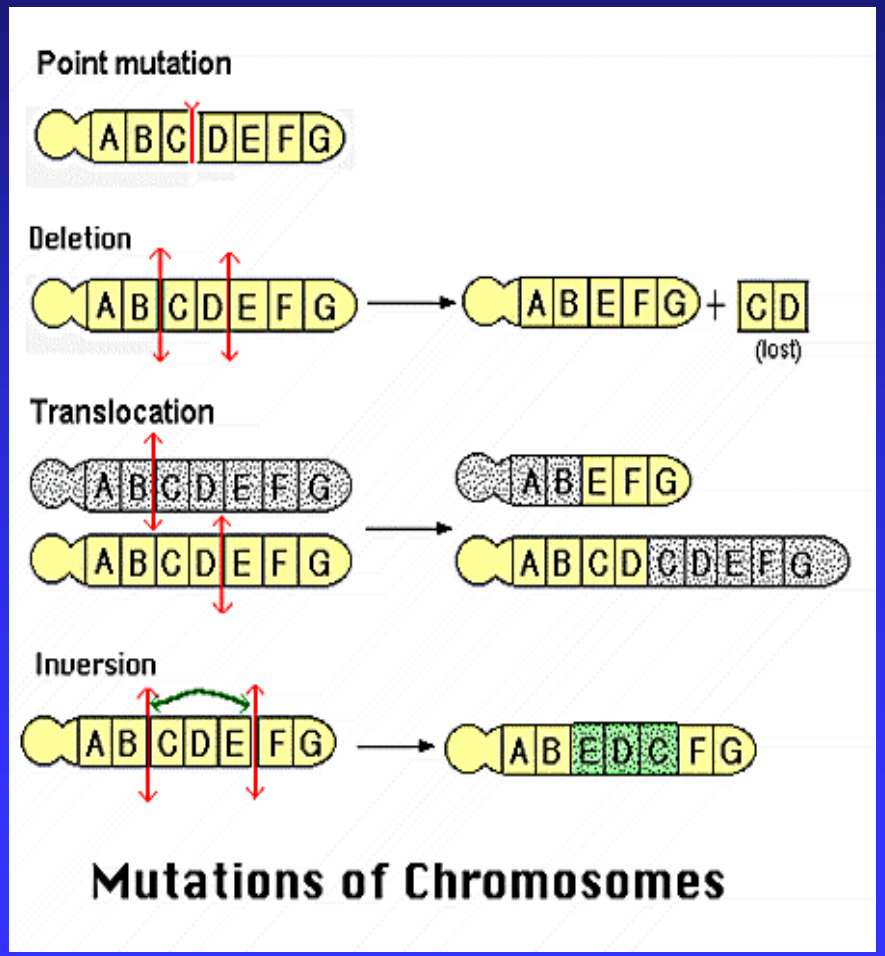
Quali sono le anomalie cromosomiche

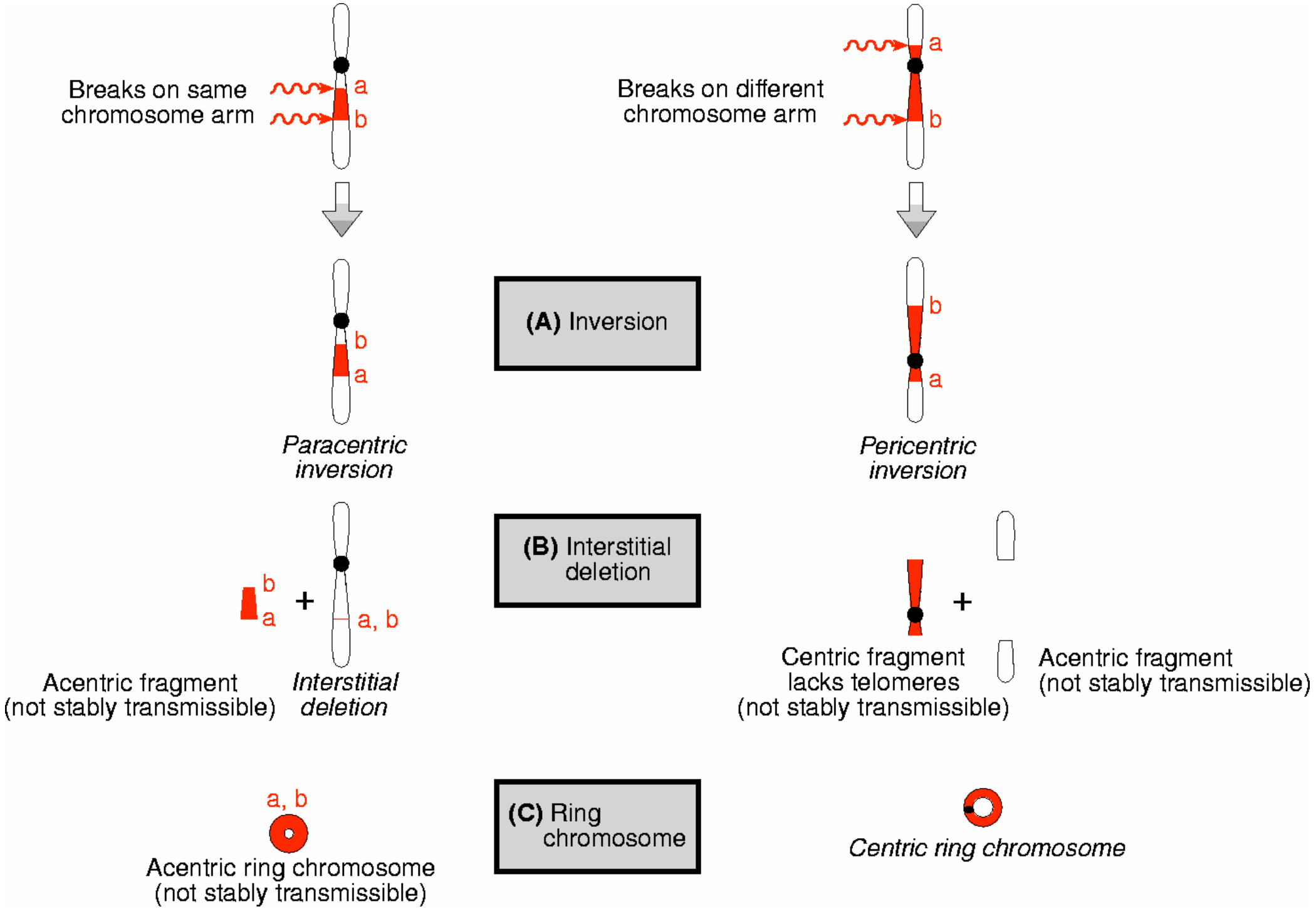
Di numero

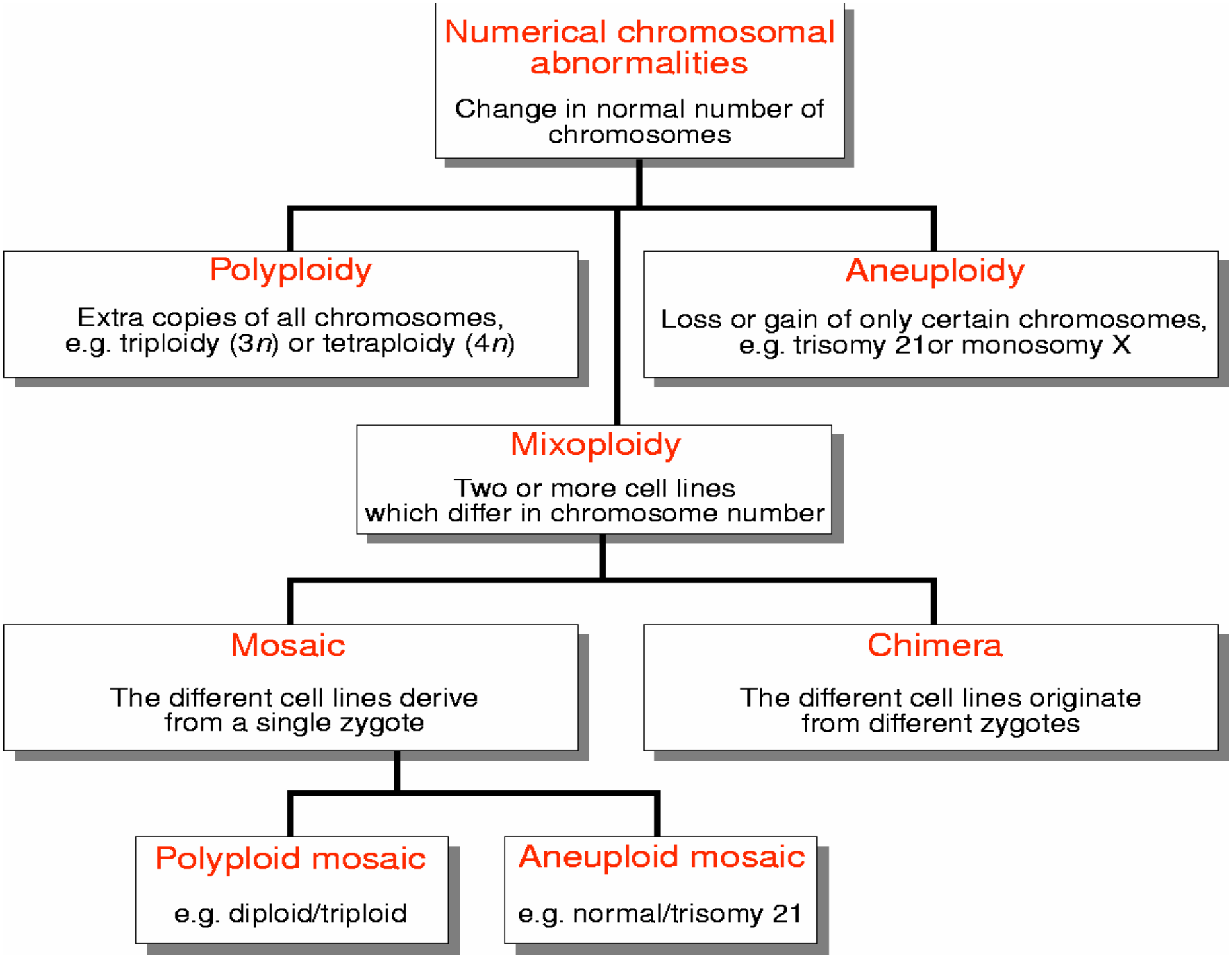
- trisomie
- monosomie
- triploidie
- tetraploidie

Di struttura

- traslocazioni
- inversioni
- delezioni
- duplicazioni




Breaks on same chromosome arm
a
b
Breaks on different chromosome arm
a
b
(A) Inversion
b
a
Paracentric inversion
b
a
Pericentric inversion
(B) Interstitial deletion
b
a
+
a, b
Acentric fragment (not stably transmissible)
Interstitial deletion
+
Centric fragment lacks telomeres (not stably transmissible)
Acentric fragment (not stably transmissible)
(C) Ring chromosome
a, b
Acentric ring chromosome (not stably transmissible)
Centric ring chromosome

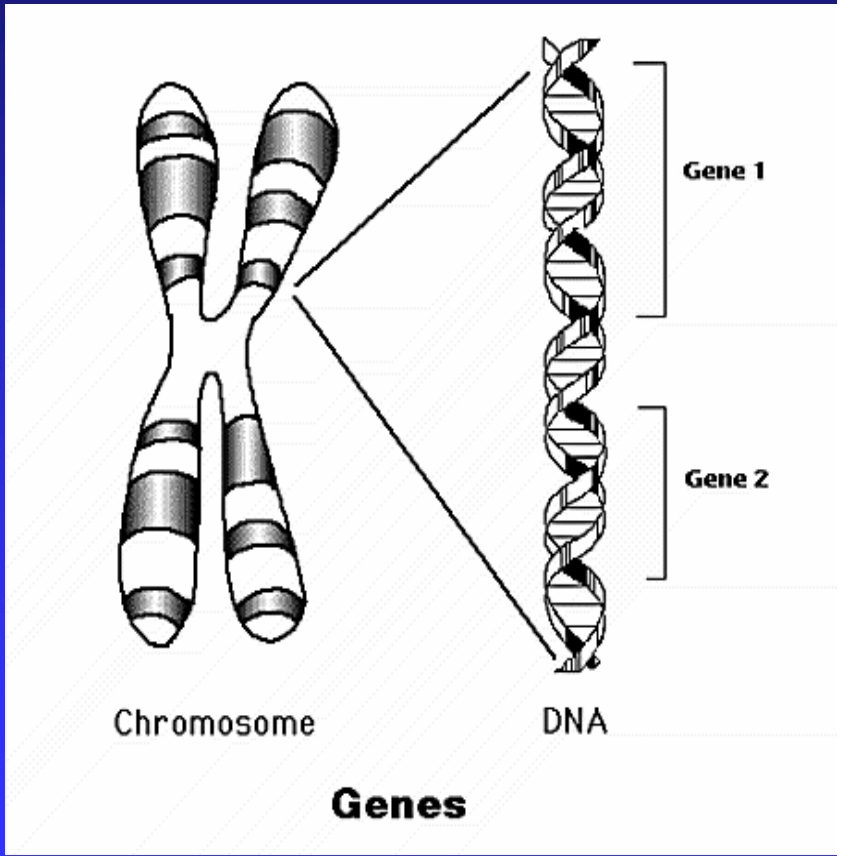

Numerical chromosomal abnormalities
Change in normal number of chromosomes
Polyploidy
Extra copies of all chromosomes, e.g. triploidy (3n) or tetraploidy (4n)
Aneuploidy
Loss or gain of only certain chromosomes, e.g. trisomy 21or monosomy X
Mixoploidy
Two or more cell lines which differ in chromosome number
Mosaic
The different cell lines derive from a single zygote
Chimera
The different cell lines originate from different zygotes
Polyploid mosaic
e.g. diploid/triploid
Aneuploid mosaic
e.g. normal/trisomy 21

ANEUPLOIDIA

- MONOMIE e TRISOMIE
- CELLULE NEOPLASTICHE: aneuploidia estrema, con anomalie cromosomiche multiple
- **CAUSE DI ANEUPLOIDIA**:
- **NON-DISGIUNZIONE**: incapacità di cromosomi separati di appaiarsi durante la prima divisione meiotica, o dei cromatidi fratelli appaiati di separarsi nella seconda divisione meiotica. I due cromosomi o cromatidi congiunti migrano ad un polo e vengono inclusi in una sola cellula figlia, mentre l'altra avrà materiale genetico in meno
- **RITARDO ANAFASICO**: ritardata migrazione del cromosoma durante l'anafase, conseguente perdita del cromosoma. Mancata incorporazione di un cromosoma nel nucleo di una delle cellule figlie.

Gravità delle anomalie cromosomiche

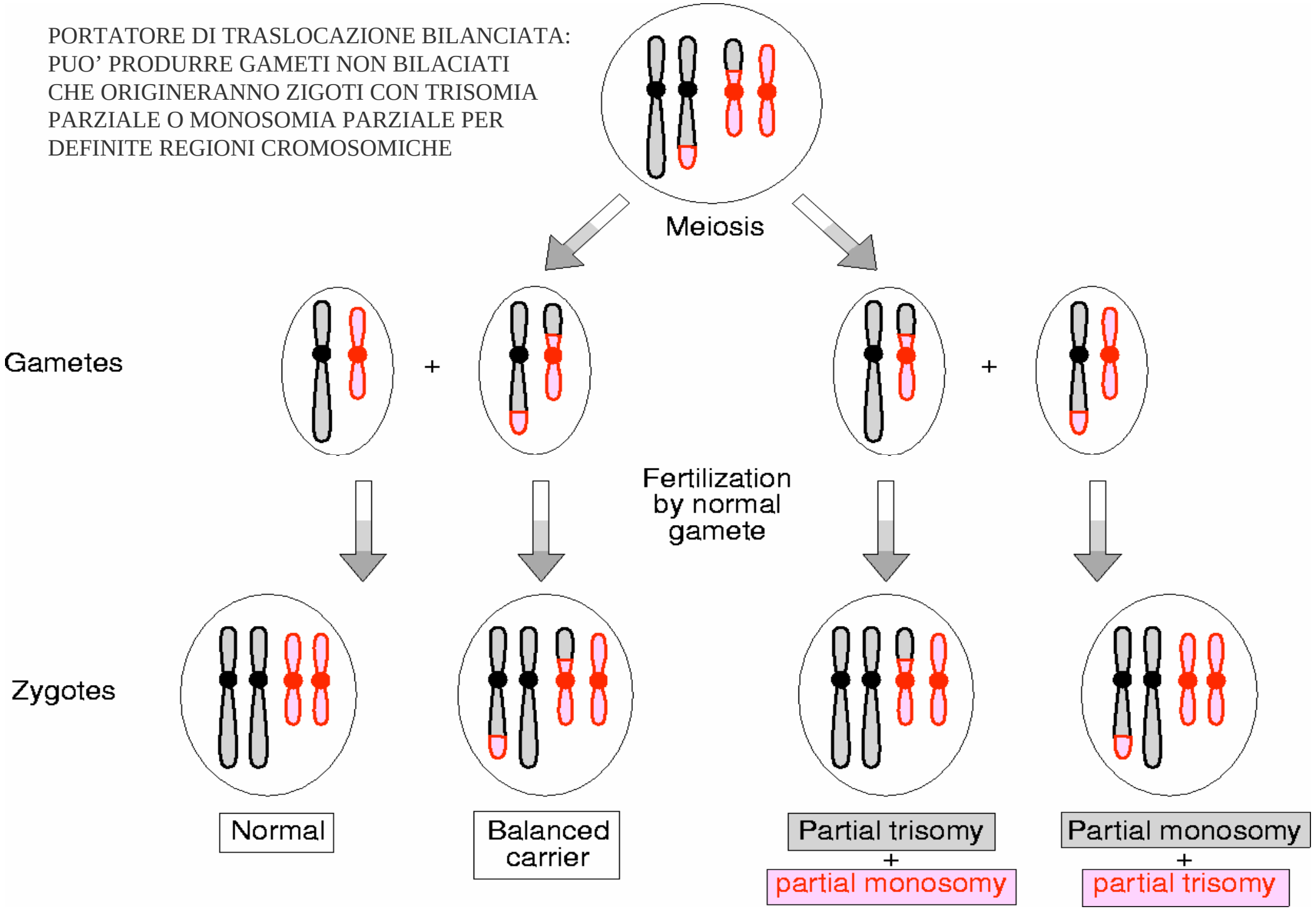
- La gravità è correlata al tipo di cromosoma e alla quantità di geni interessati
- Tanto più grave è lo sbilanciamento cromosomico tanto più precoce sarà l'interruzione di gravidanza



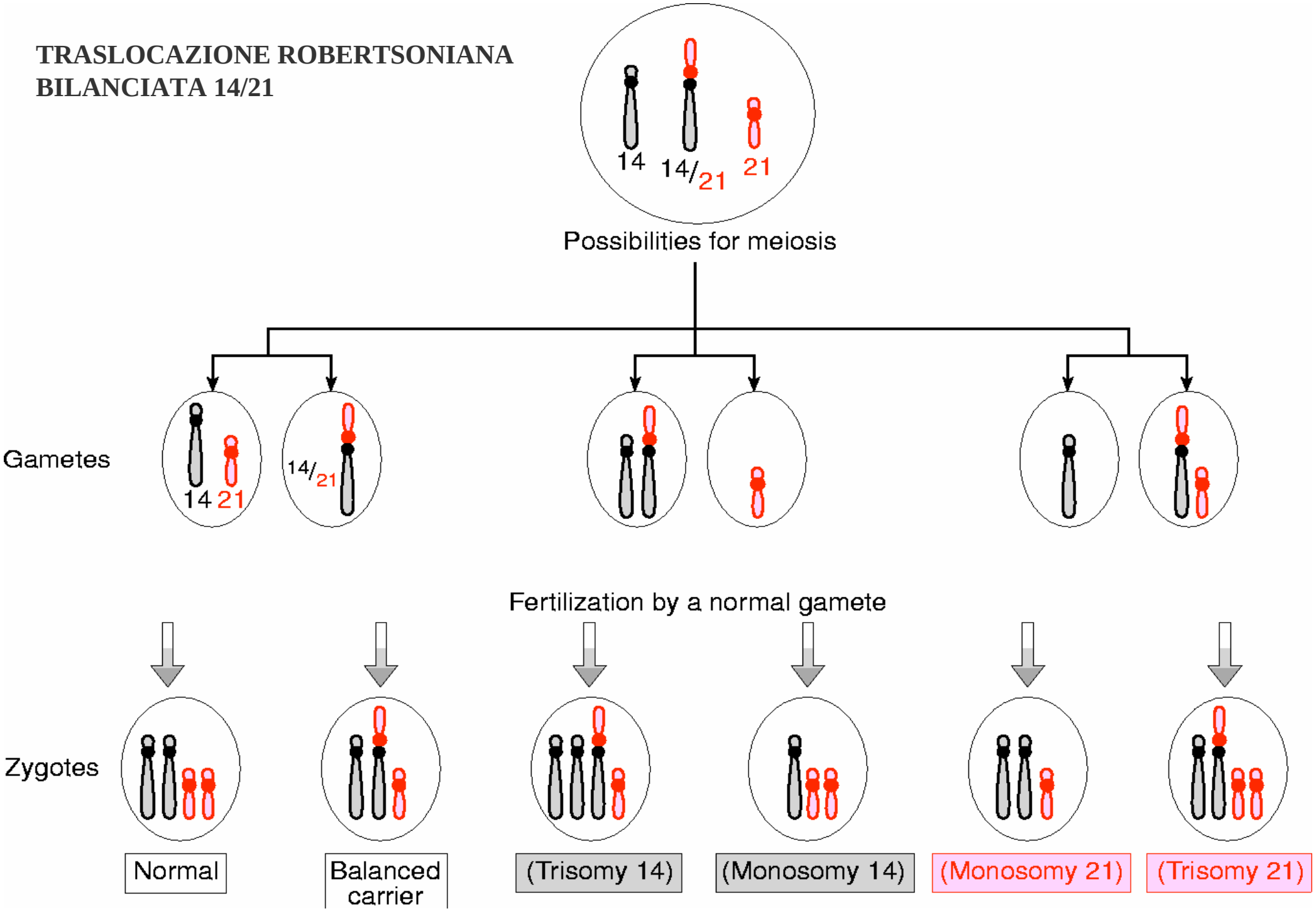
E nei casi di anomalie bilanciate?

- Il problema non si pone per il soggetto portatore
- ma riguarda la sua discendenza...

PORTATORE DI TRASLOCAZIONE BILANCIATA:
PUO' PRODURRE GAMETI NON BILACIATI
CHE ORIGINERANNO ZIGOTI CON TRISOMIA
PARZIALE O MONOSOMIA PARZIALE PER
DEFINITE REGIONI CROMOSOMICHE

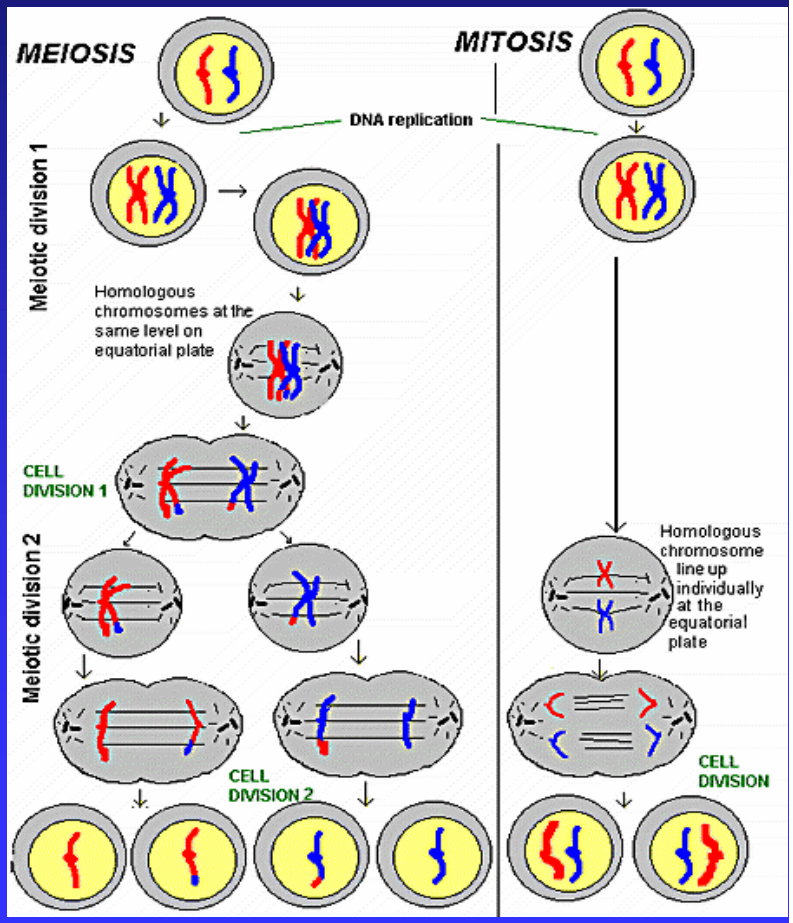


TRASLOCAZIONE ROBERTSONIANA BILANCIATA 14/21



La frequenza delle anomalie cromosomiche è:

- **Direttamente correlata con l'età materna**
- **Inversamente correlata con l'epoca gestazionale**



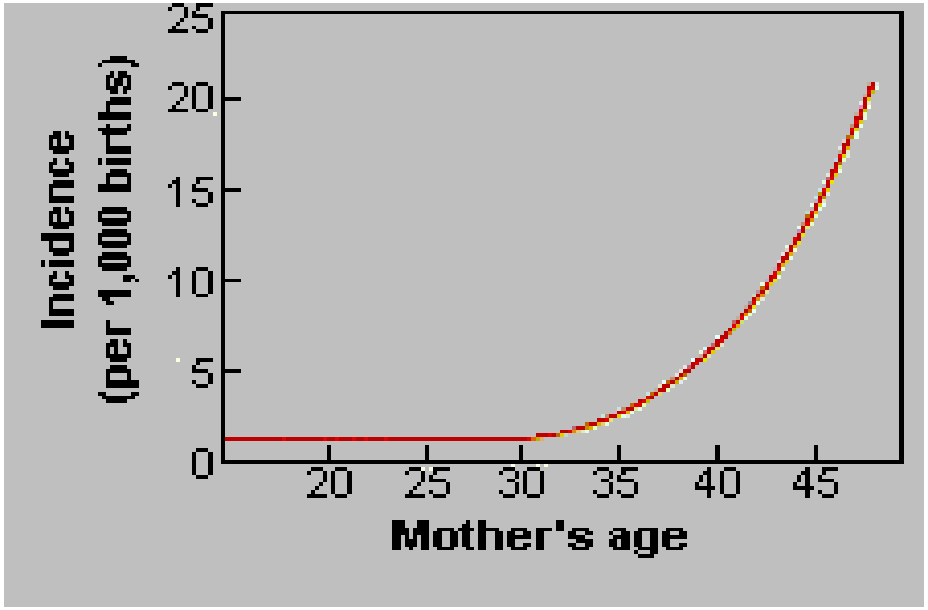
La non disgiunzione

- ***Esistono fattori che influenzano la non disgiunzione ?***

  Non ben conosciuti

- ***Dove e quando avviene la non disgiunzione ?***

  Più frequentemente nella I° meiosi materna

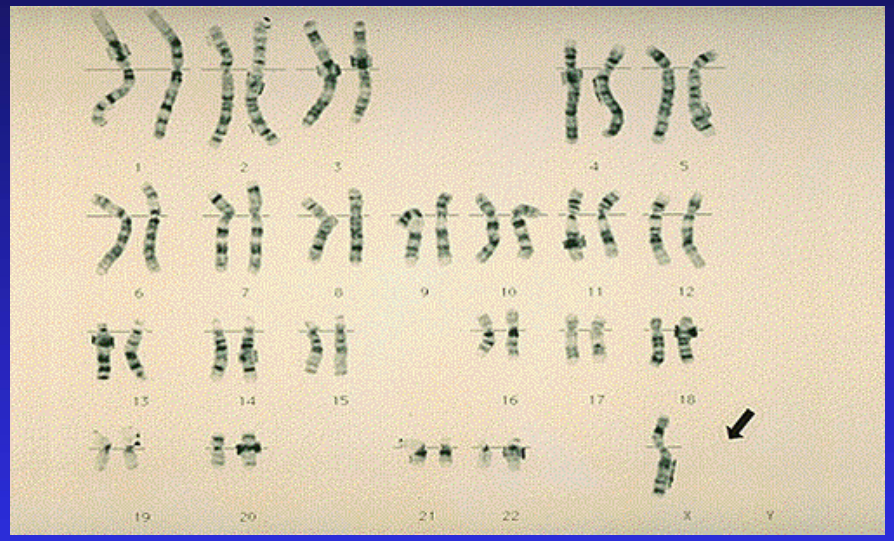


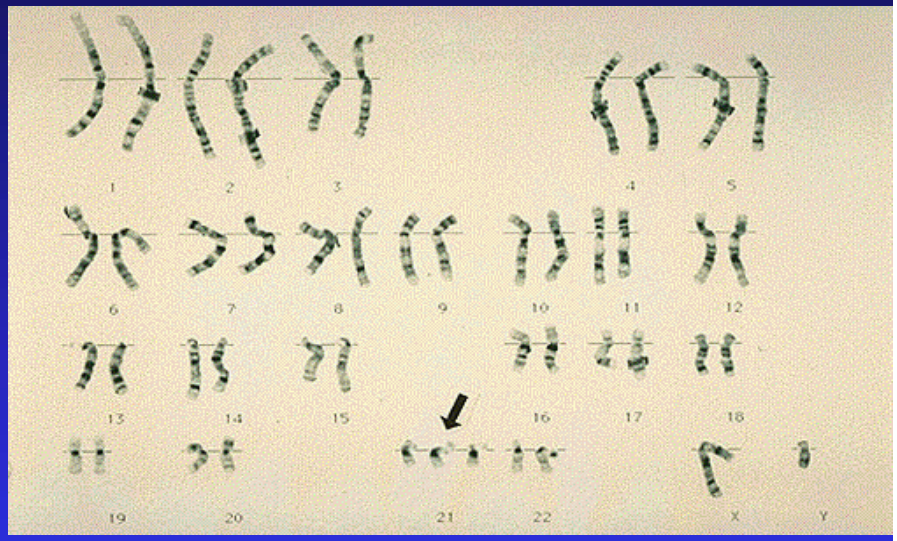
La frequenza delle anomalie cromosomiche alla nascita è *0.65%*

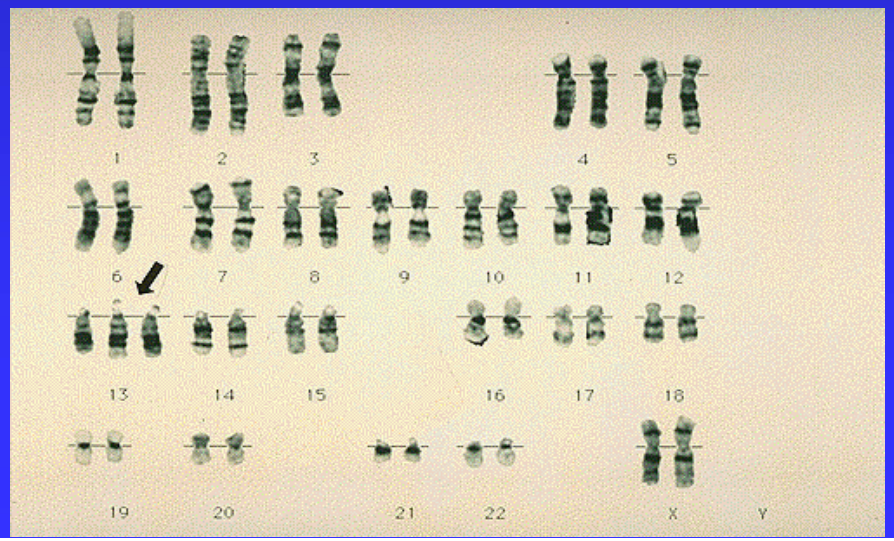
Trisomie	**+21**	**0.12%**	**1 su 833**
	+18	**0.013%**	
	+13	**0.004%**	
Monosomie	**45,X**	**0.024%**	
Tr. bilanciate		**0.2%**	**1 su 500**
Tr. sbilanciate		**0.05%**	

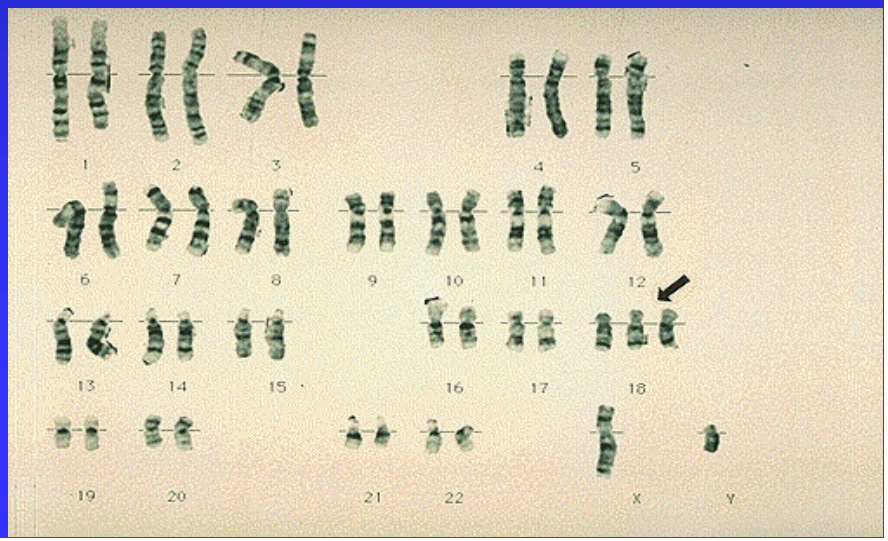
Nielsen et al. Human Genet. 1982 ; 61 : 98

Aneuploidie più frequenti alla nascita

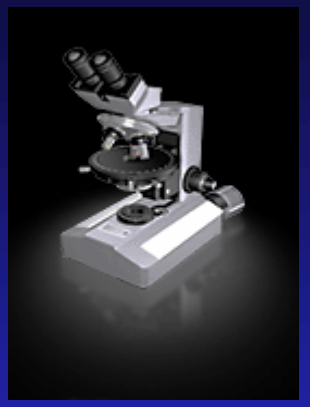








Malattie dovute ad aberrazioni cromosomiche.

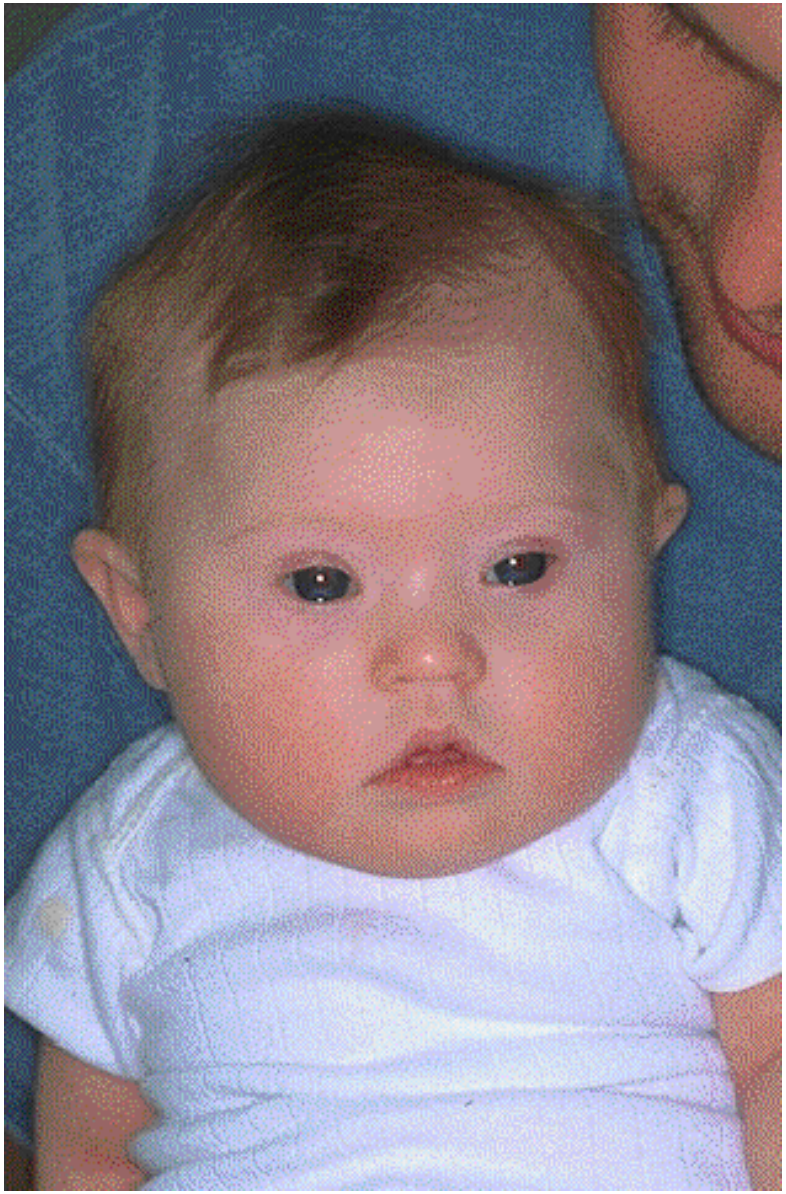


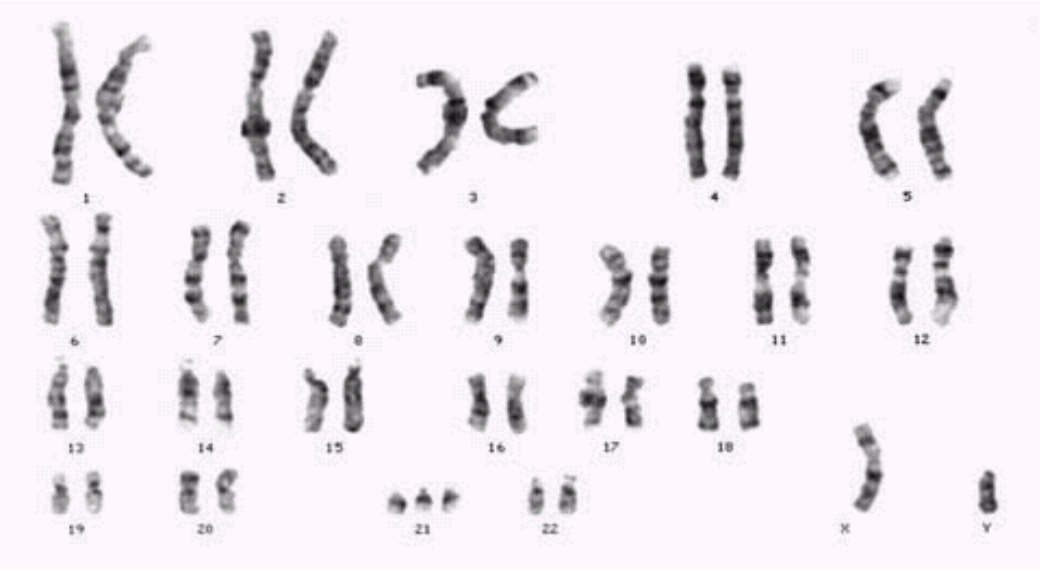
1. ANEUPLOIDIE (anomalie numeriche)

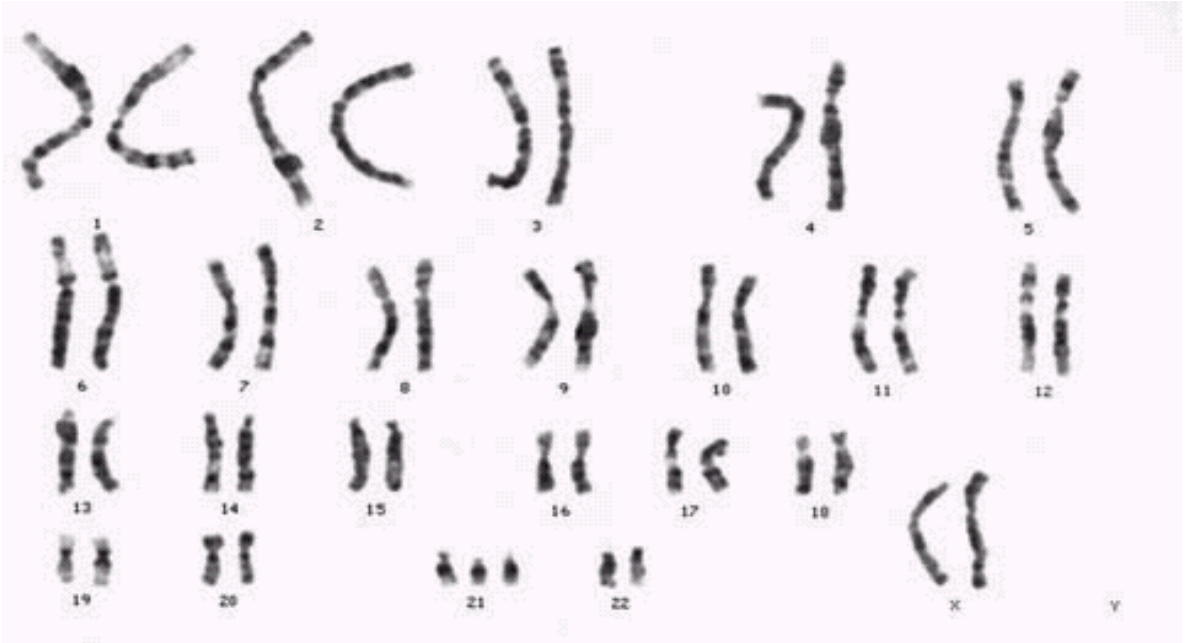
- sindrome di Down

- sindrome di Klinefelter

- sindrome di Turner




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

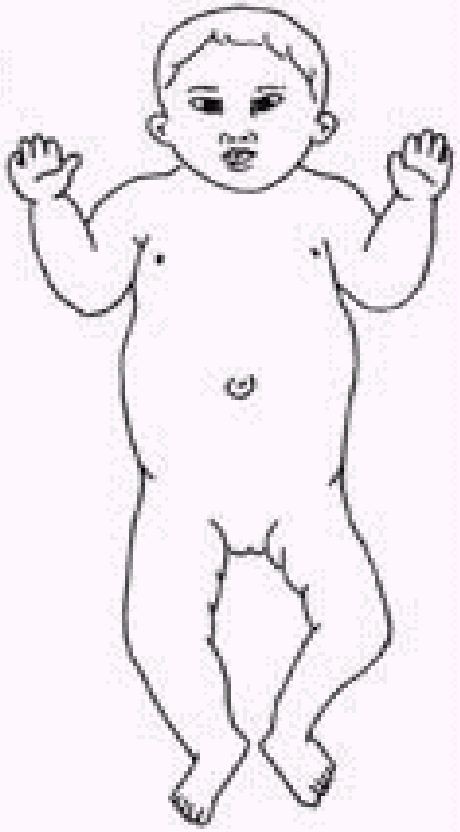

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

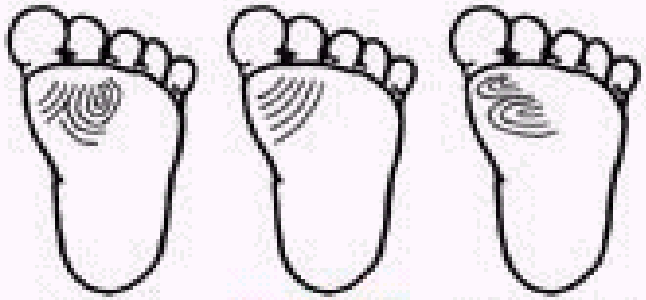
21 trisomy = Down syndrome

短頸，平板な顔貌，瞼裂斜上，
内眼角贅皮，鞍鼻，低位耳介，
折れ曲がった小さな耳介，
舌挺出，小さな口

発育不全，筋緊張低下

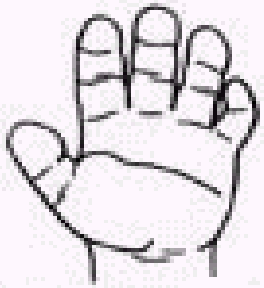
先天性心疾患，臍ヘルニア，
巨大結腸，骨盤変形，
十二指腸・小腸閉鎖





normal　21 trisomy　13trisomy など

足底母趾球部弓状紋



短い指，幅広い手
猿線・第Ｖ指内彎
第Ｖ指単一屈曲線

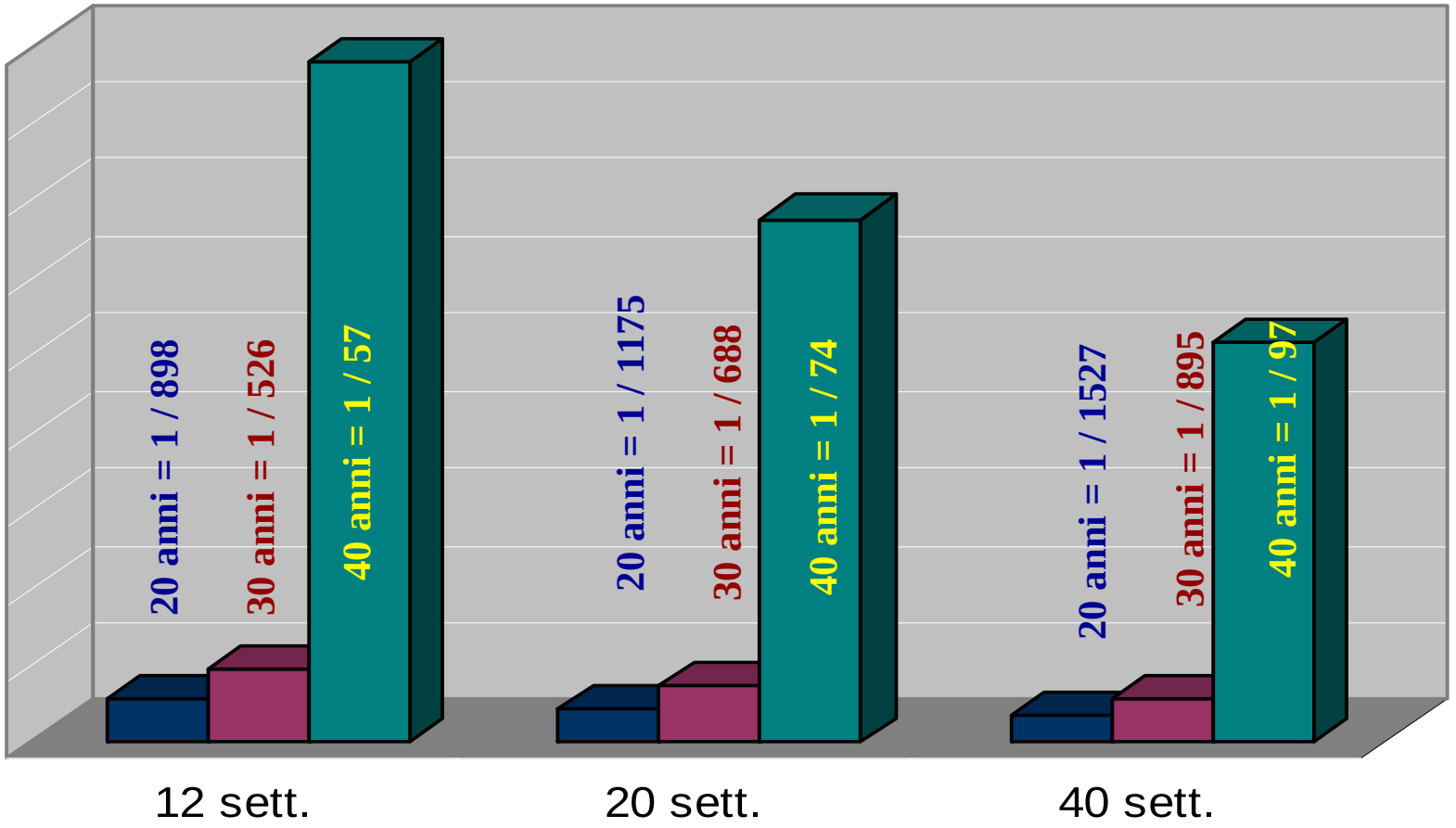
Sindrome di Down.

ETA' MATERNA	TIPO DI ANOMALIA (in percentuale)		
ANNI	47, +21	MOSAICO	TRASLOCAZIONE
15 -19	85	5	10
20 - 24	90	1	9
25 -29	91	2	7
30 -34	93	3	4
34 - 40	97	1	2
oltre i 40	97	2	1

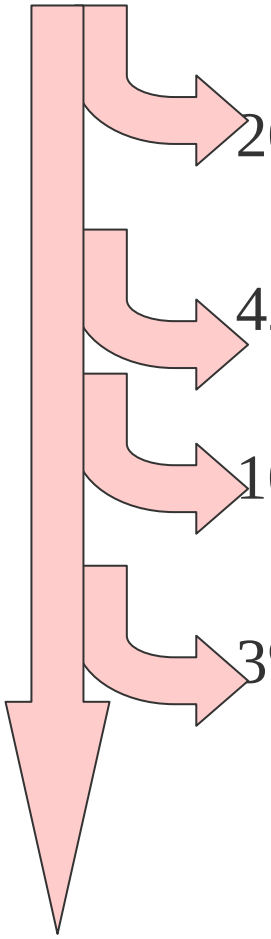
Rischio di trisomia 21

In relazione all'età materna e all'epoca gestazionale

Modificato da Snijders, 1994



Concepimento



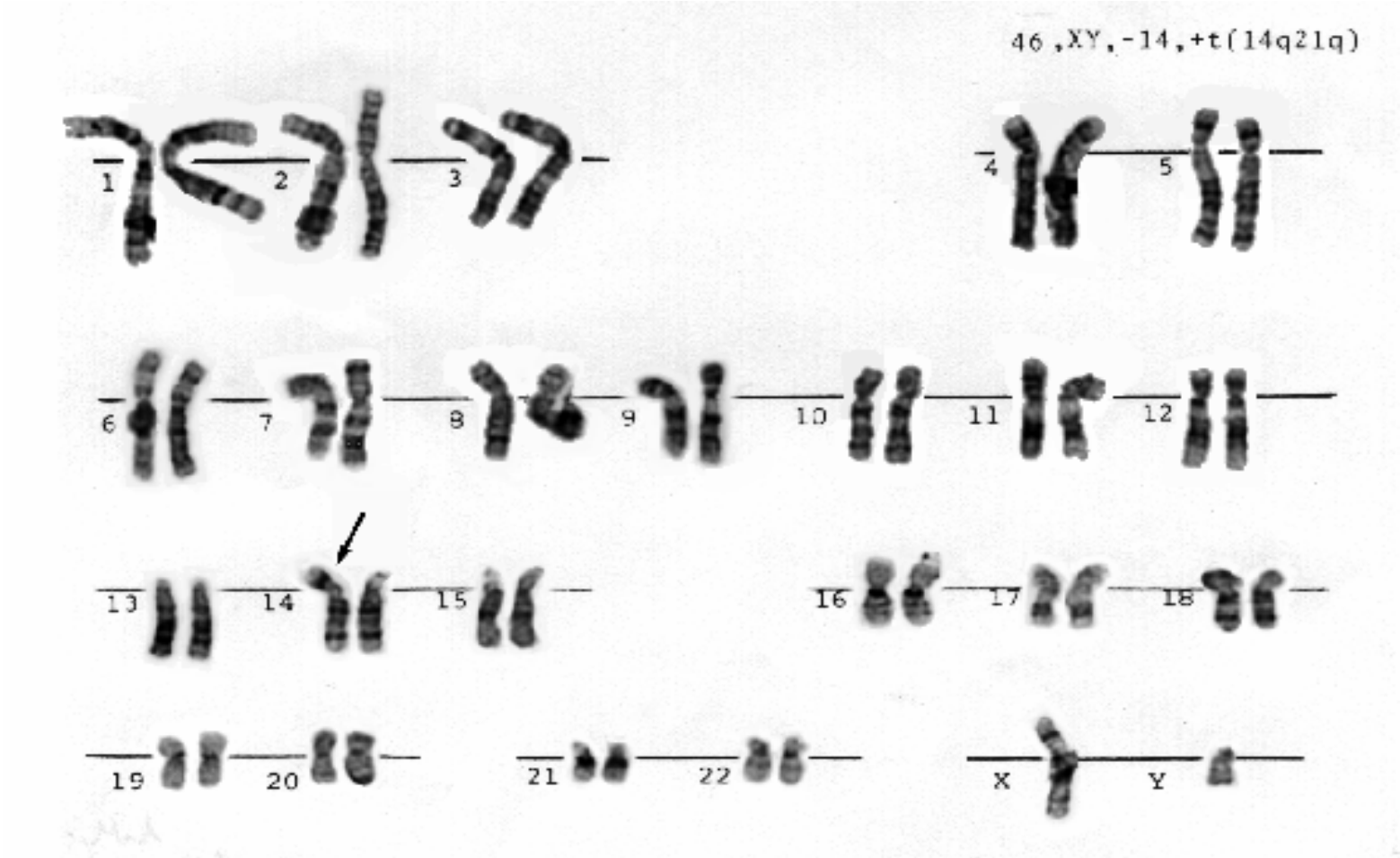
20% vengono abortiti prima dell’impianto

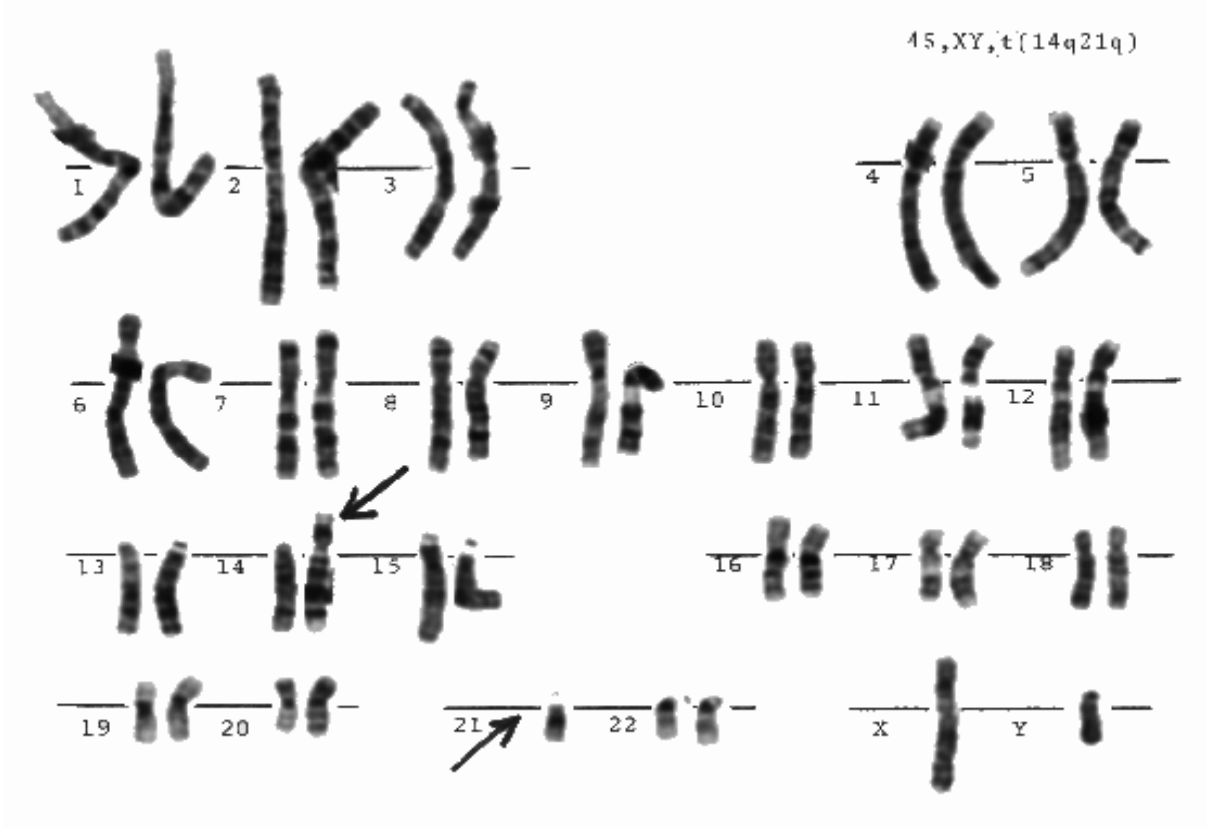
42% vengono abortiti prima della 7ª sett. di gest.

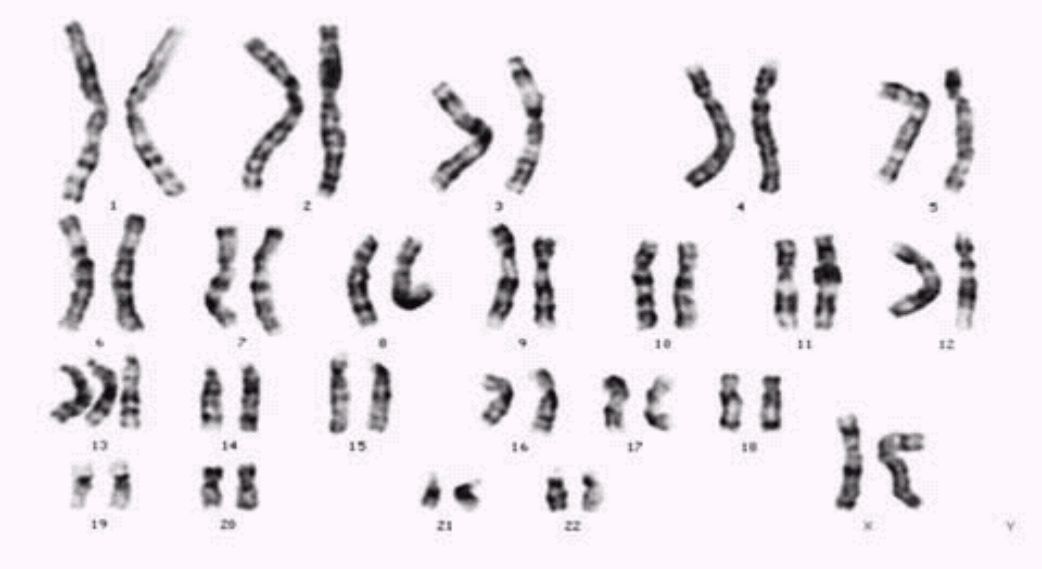
10% vengono abortiti tra la 7ª e la 12ª sett. di gest.

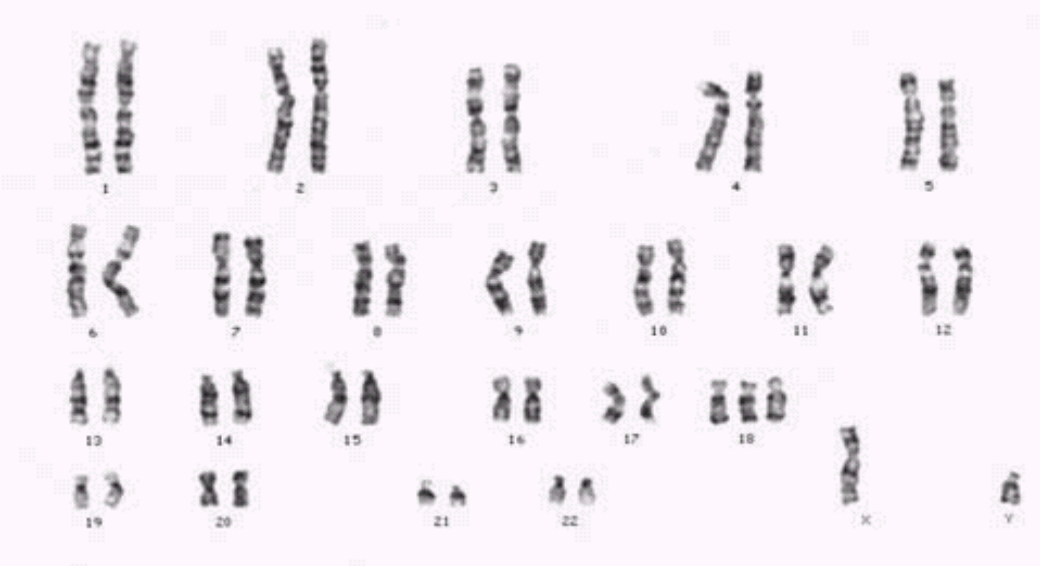
3% vengono abortiti dopo la 12ª sett. di gest.

NASCITA solo il 25% dei concepiti arrivano alla nascita


46,XY,-14,+t(14q21q)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

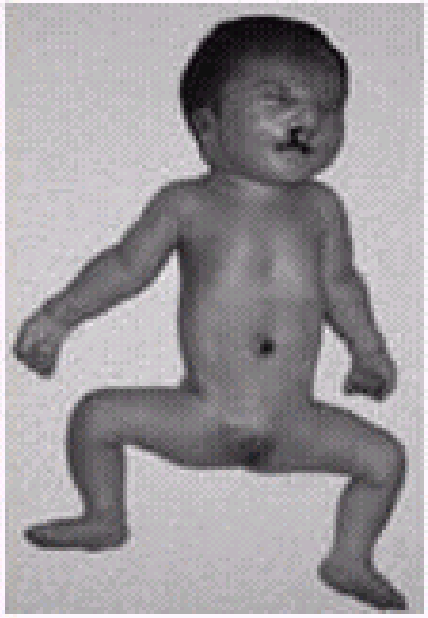

45,XY,t(14q21q)

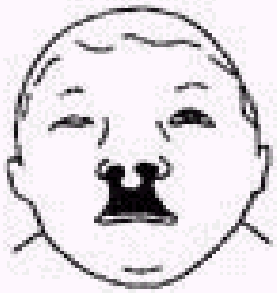




Trisomy 13 syndrome
= Patau 症候群

脳奇形
口唇／口蓋裂
1/5000～6000

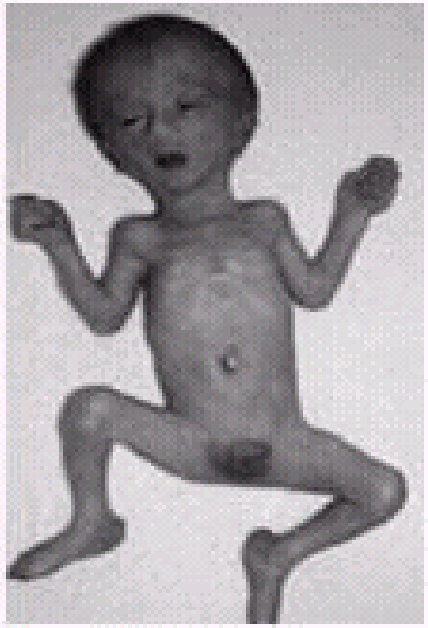




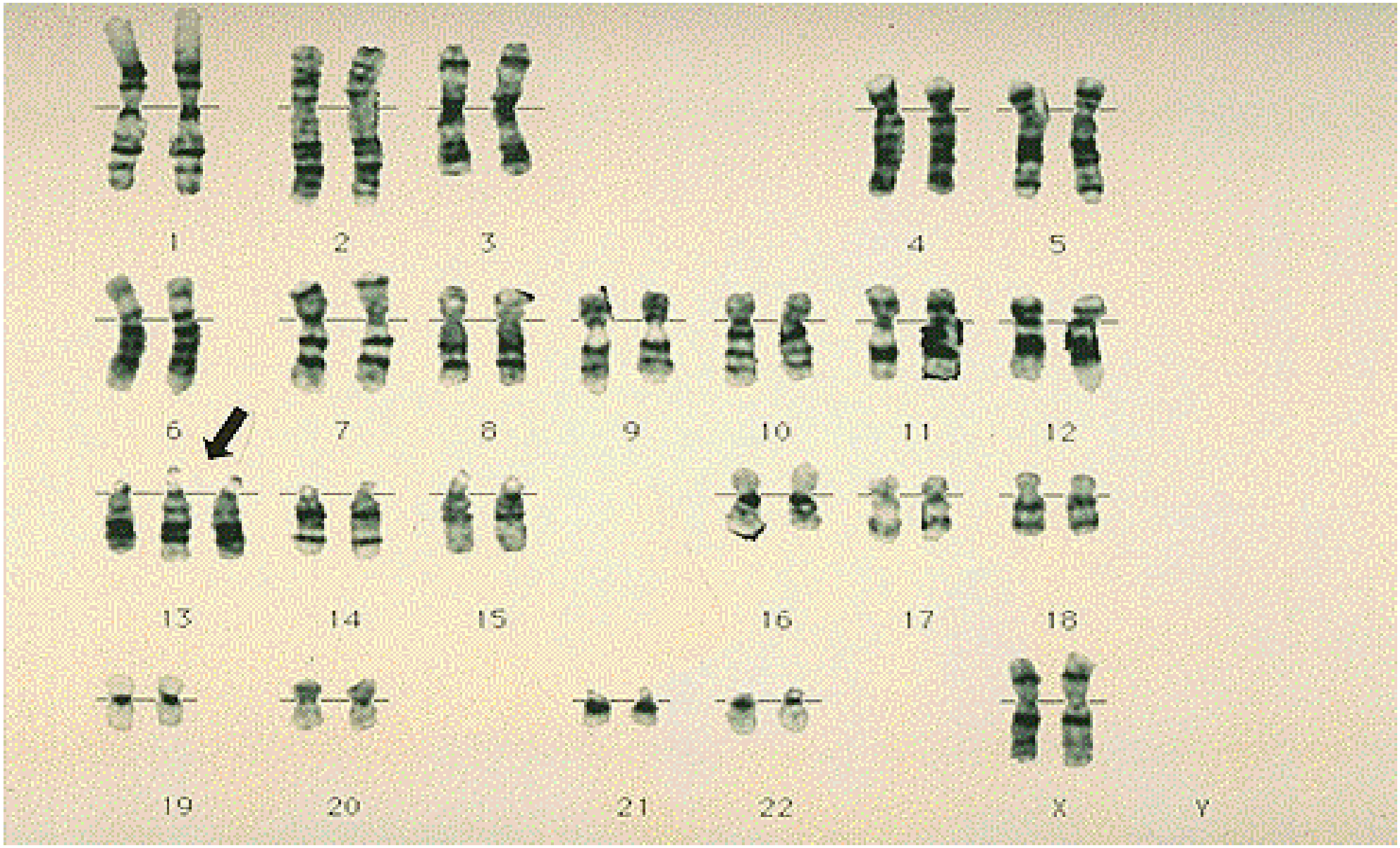
小・無眼球症，虹彩欠損，
小頭症，無嗅脳症，
耳介低位，両眼隔離，
多指，心室中隔欠損，
心房中隔欠損，嚢胞腎，
重複尿管，臍ヘルニア，
停留睾丸，発育不全，
精神発達遅滞，
白血球核付属物，
好中球過分葉

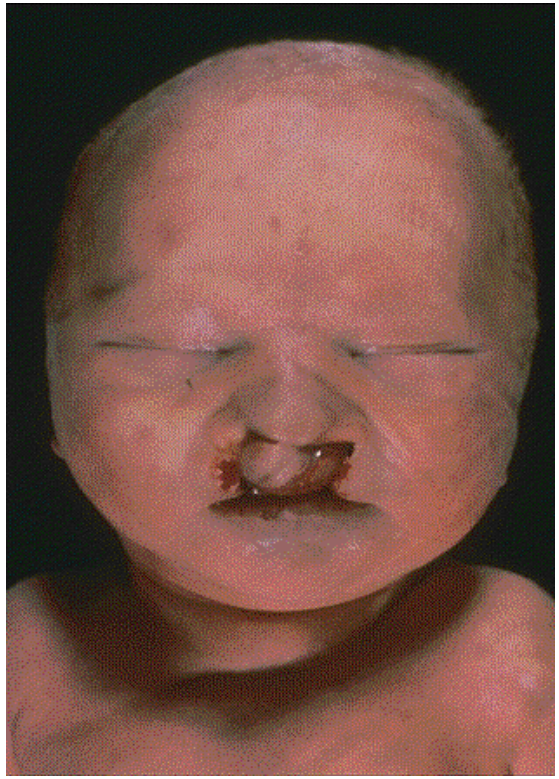
Trisomy 18 syndrome
= Edward 症候群

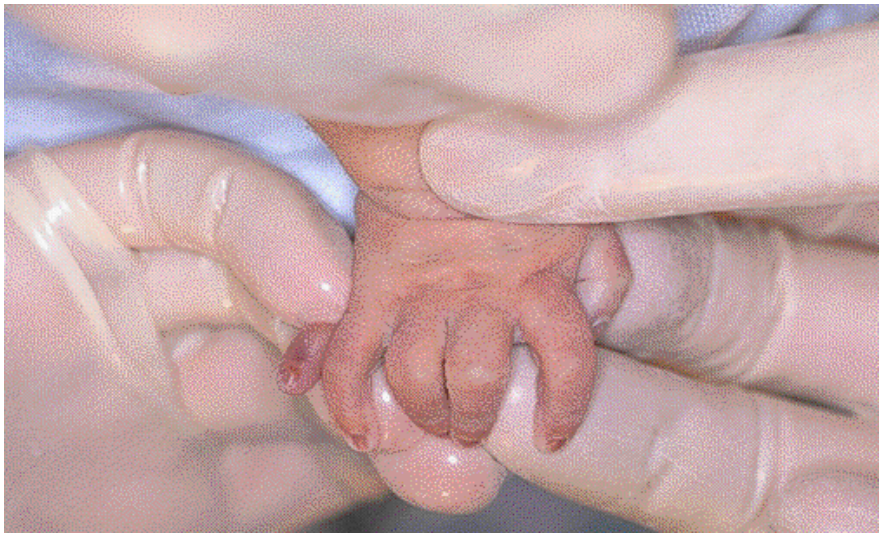
手指の重なり
女：男＝3：1
1/3000～6000

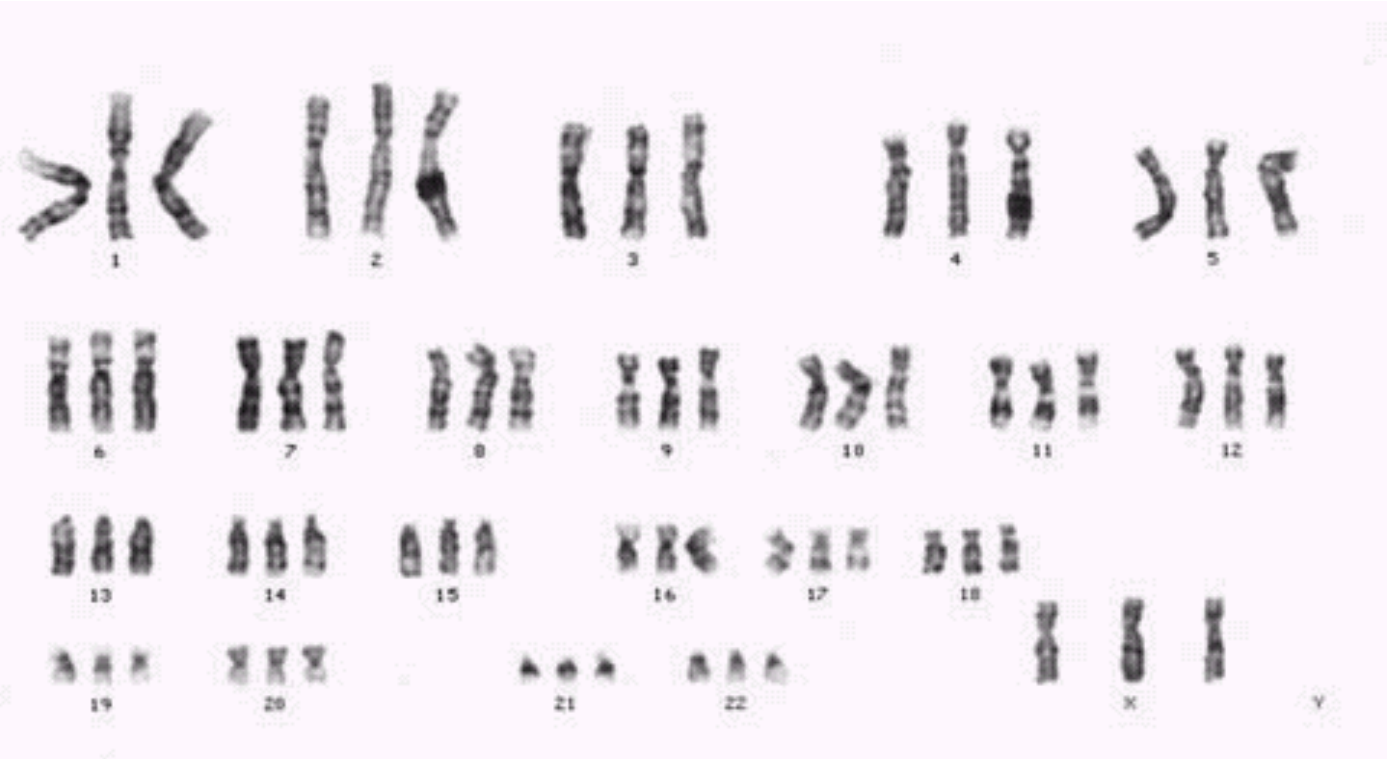


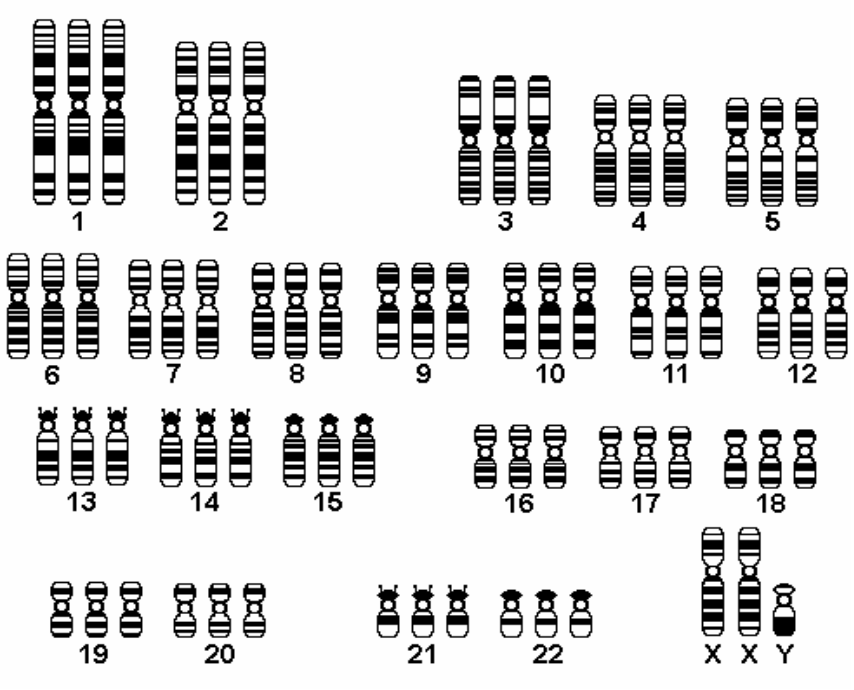
弓状眉，両眼隔離，
大泉門開大，後頭部突出，
耳介変形，耳介低位，
小顎，翼状頸，猿線，
指の屈曲拘縮，胸骨短小，
乳頭間開離，動脈管開存，
心室中隔欠損，馬蹄腎，
重複尿管，Meckel 憩室，
狭骨盤，停留睾丸，
巨大陰核，筋緊張亢進，
踵の後方突出，
握り椅子状足，
短小背屈第Ⅰ趾，
精神発達遅滞


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



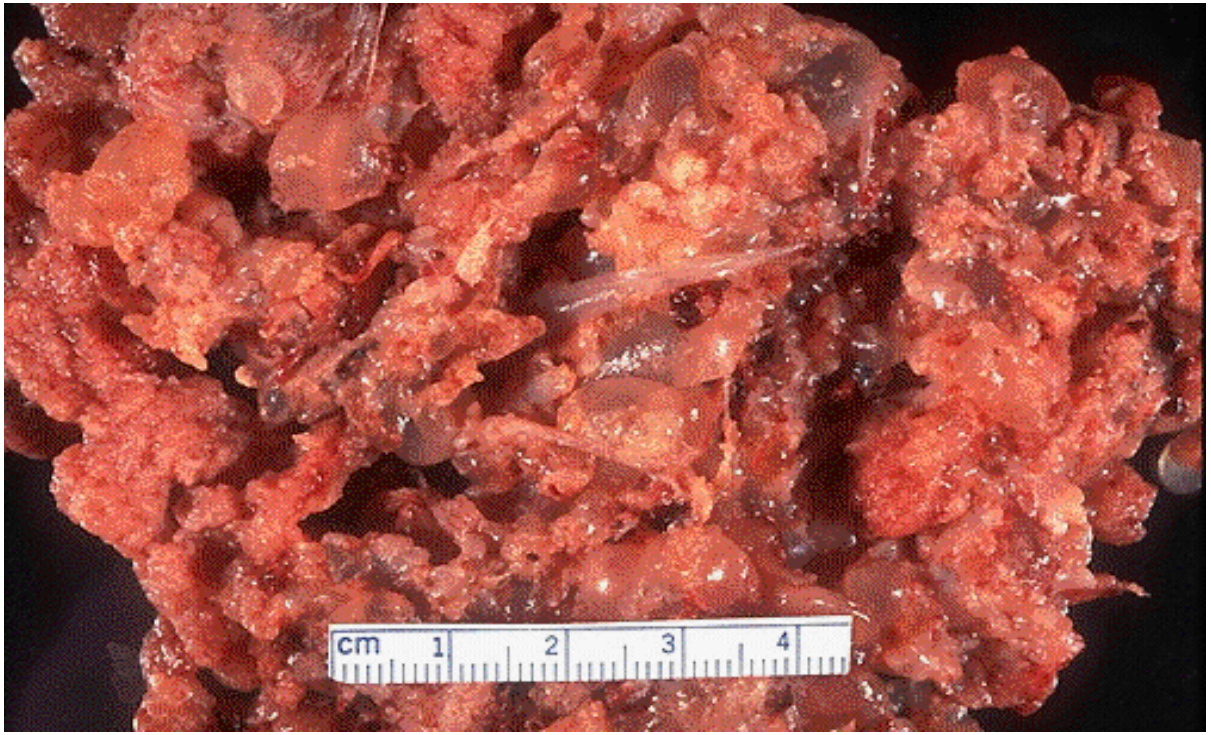


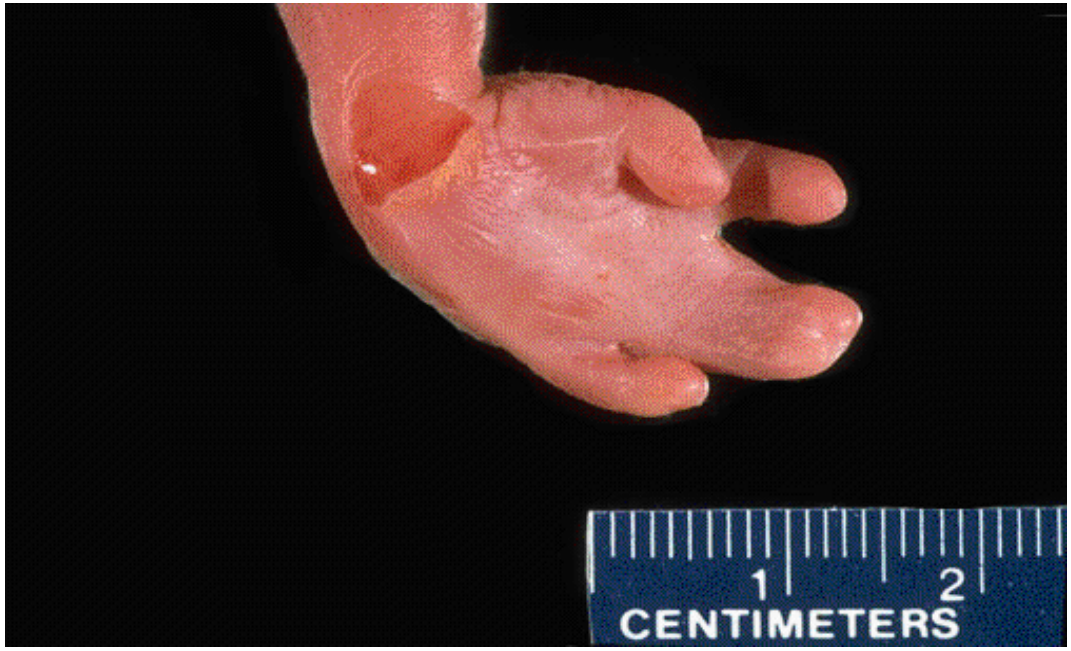

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y



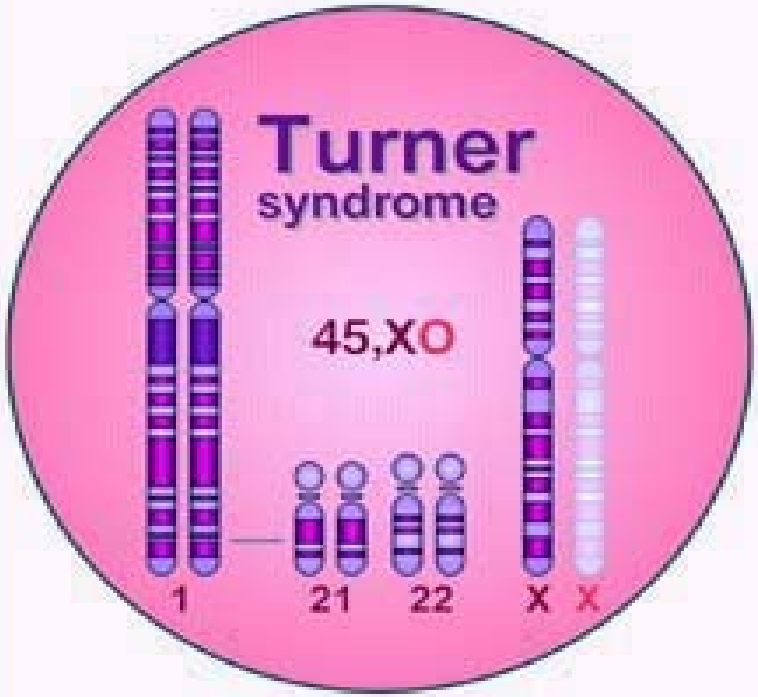
This is triploidy, which occurs when there is double fertilization of an ovum (dispermy). The result may be 69, XXX or 69, XXY or 69, XYY. The extra set of paternal chromosomes predisposes to formation of a partial mole, features of which may or may not be grossly or microscopically apparent.

Scattered grape-like villi are present in this placenta, consistent with a partial hydatidiform mole.

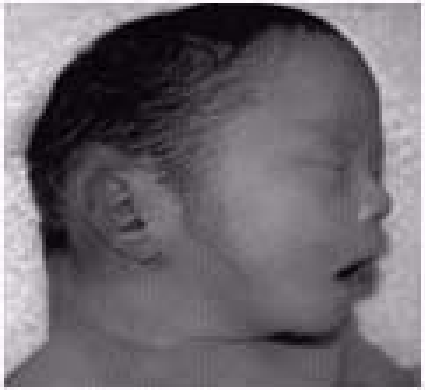




A characteristic fetal finding with triploidy is syndactyly involving the third and fourth digits of one or both hands or feet.



著しい低身長
性腺機能低下
翼状頸
外反肘
楯状胸



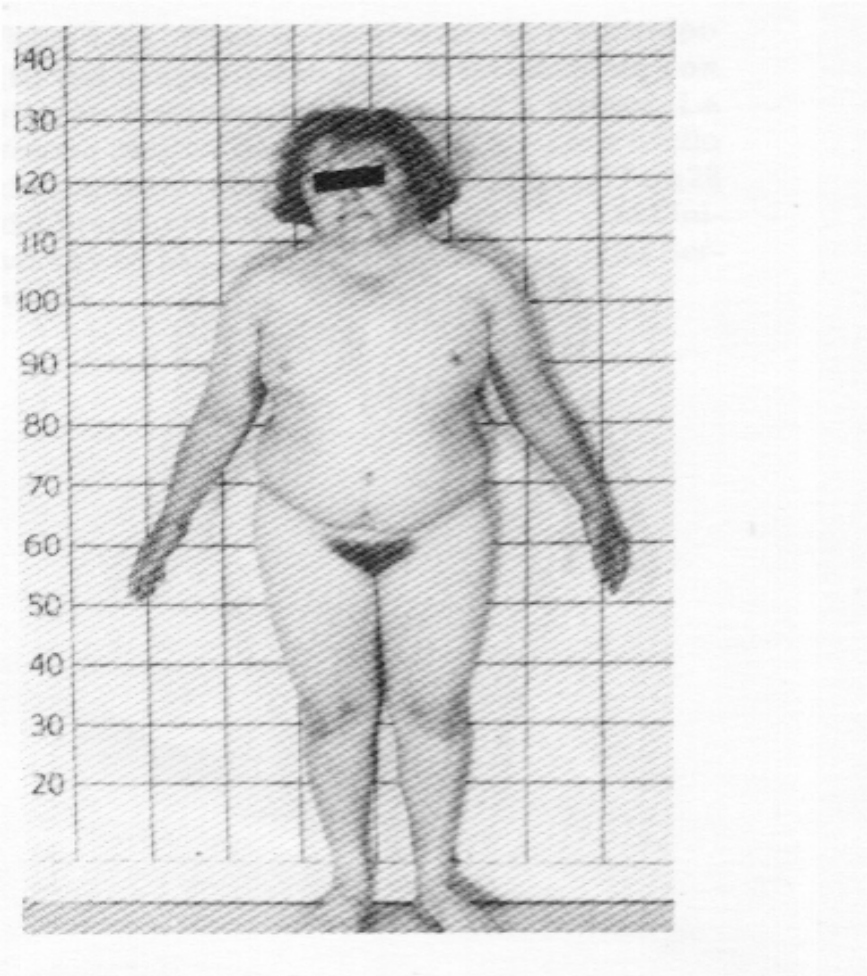
小人症，スフィンクス様顔貌，
口角下垂，大動脈縮窄症，
乳頭間開離，爪形成不全，

線維組織のみからなる性腺，
無月経，第二次性徴の欠如，

多発性色素性母斑，
手背・足背のリンパ性浮腫，

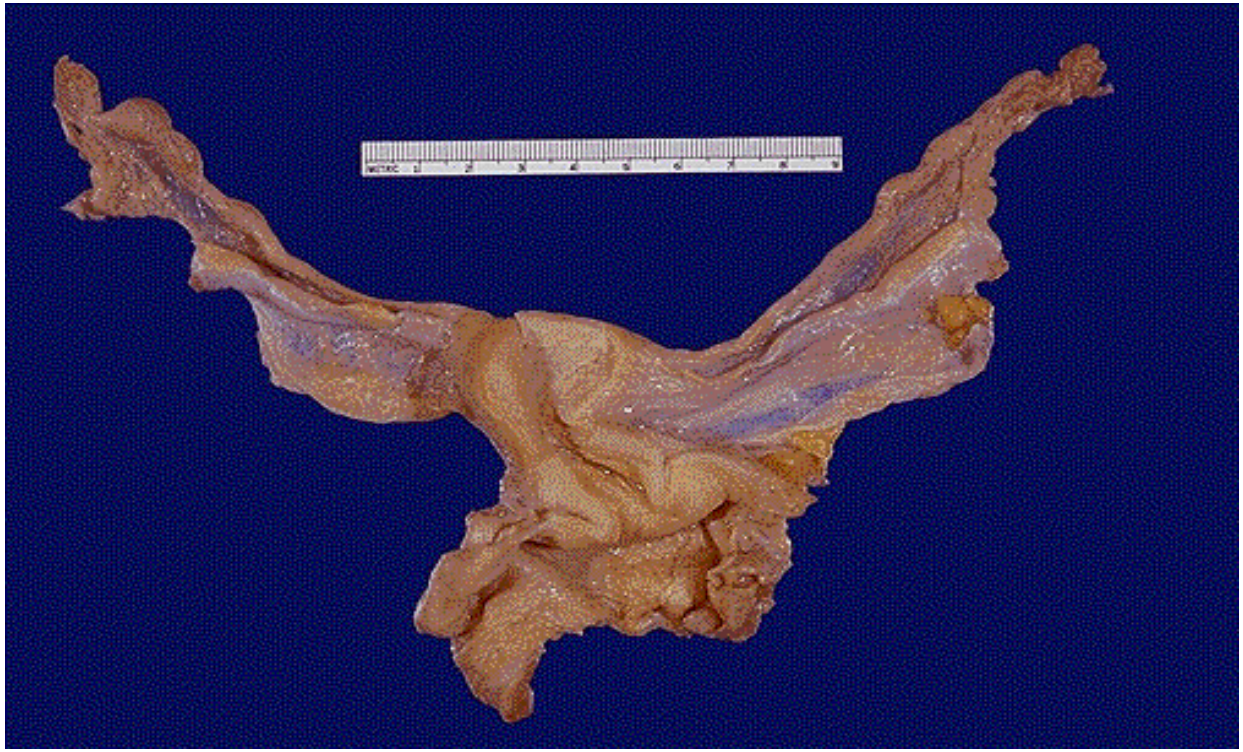
尿中17ケトステロイド↓
尿中ゴナドトロピン↑，
尿中エストロゲン↓

Sindrome di Turner.

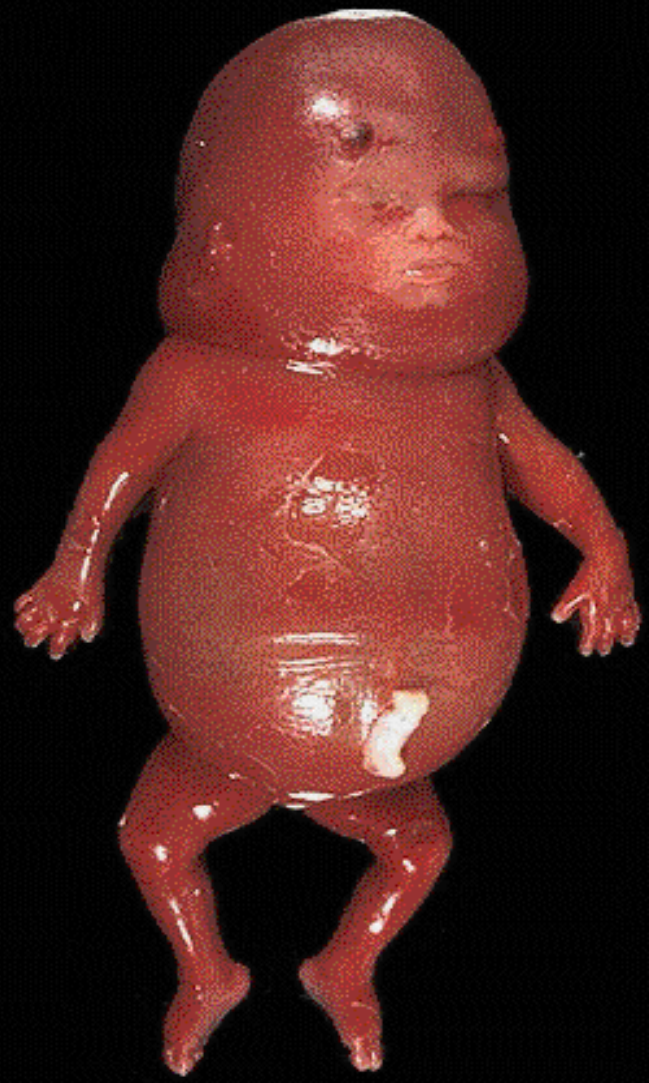


- bassa statura
- pterigio del collo
- torace a scudo
- gomito valgo
- mamelle iposviluppate

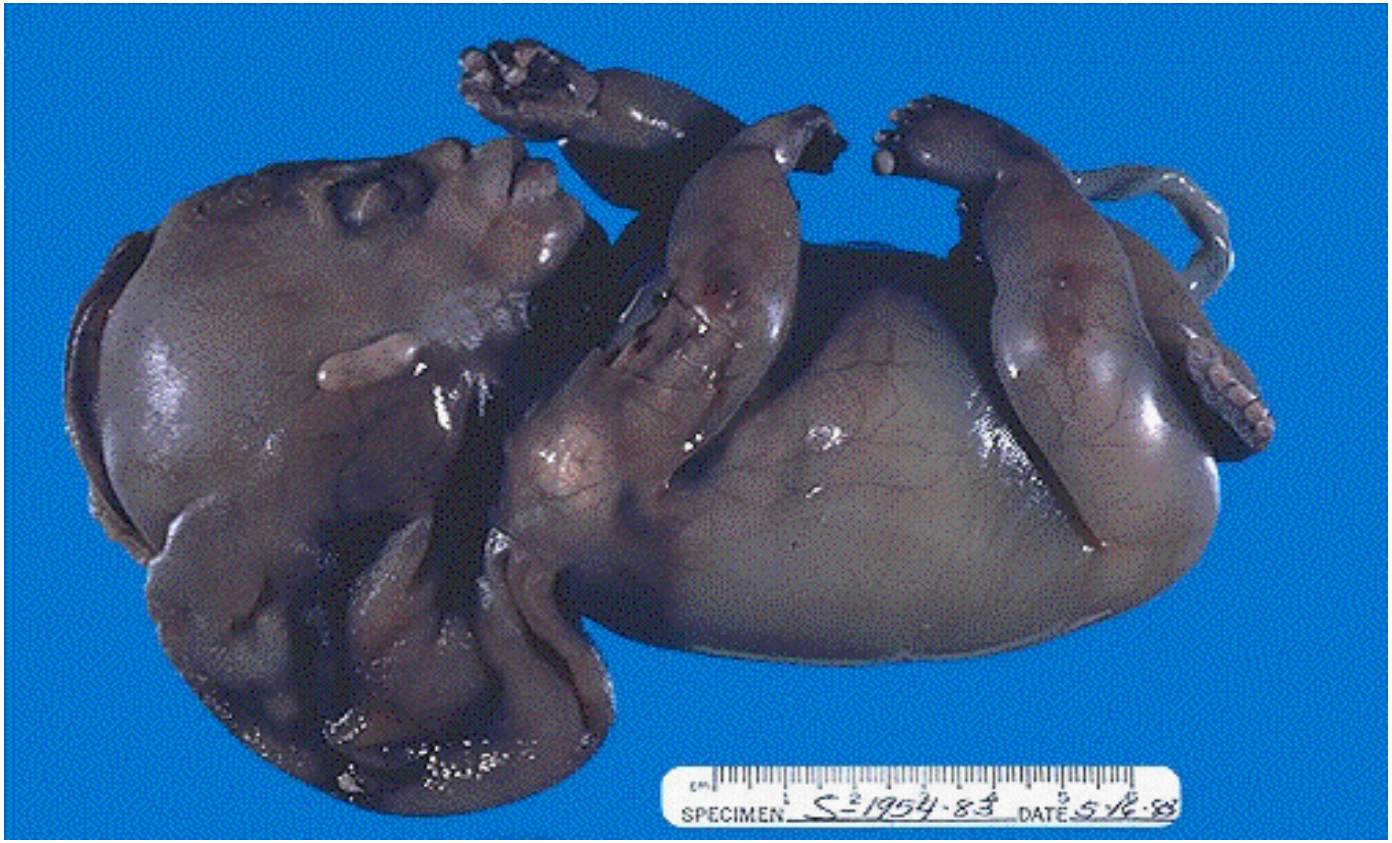
After puberty, the ovaries should develop into plump 3 to 5 cm ovoid organs, but these "streak" ovaries are typical for Turner's syndrome.

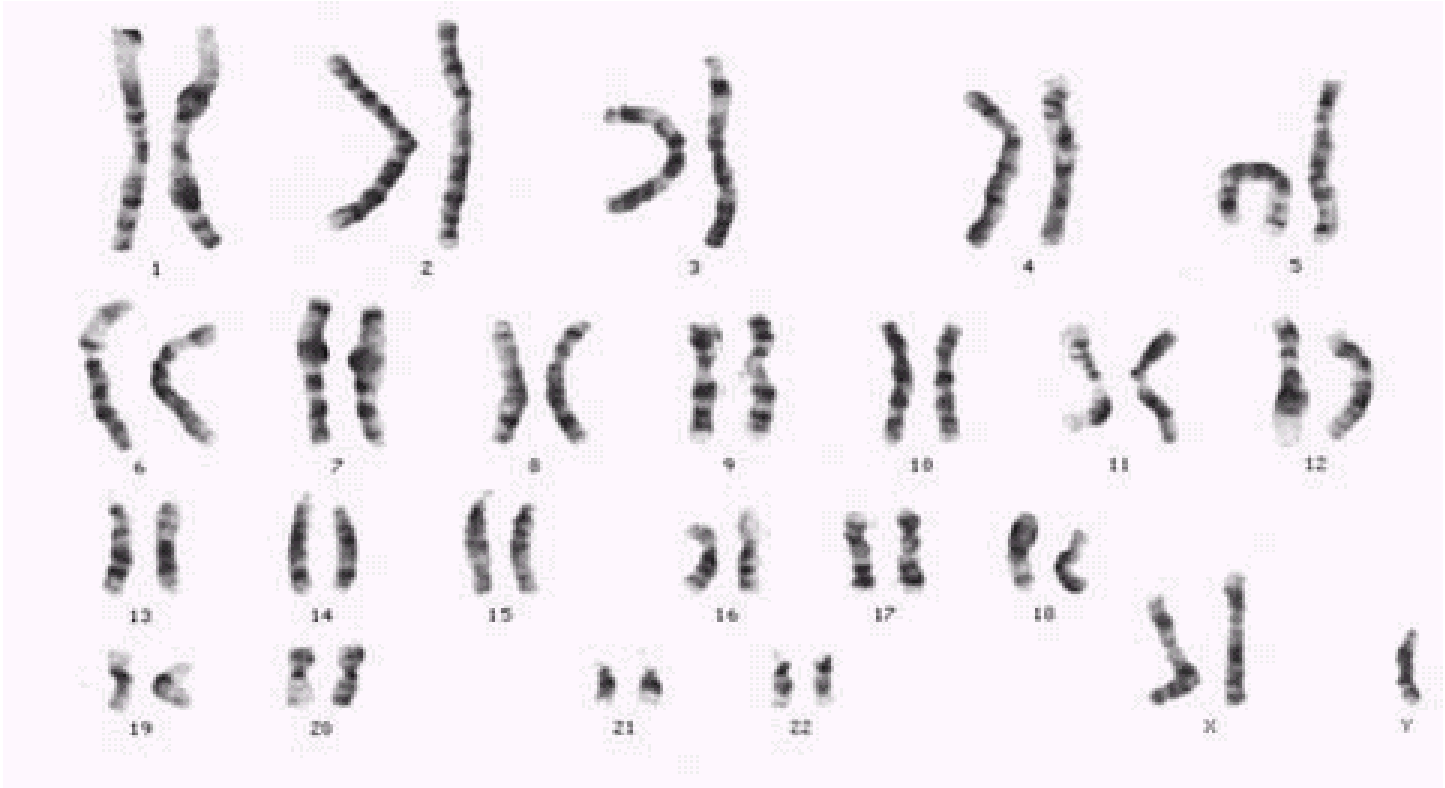


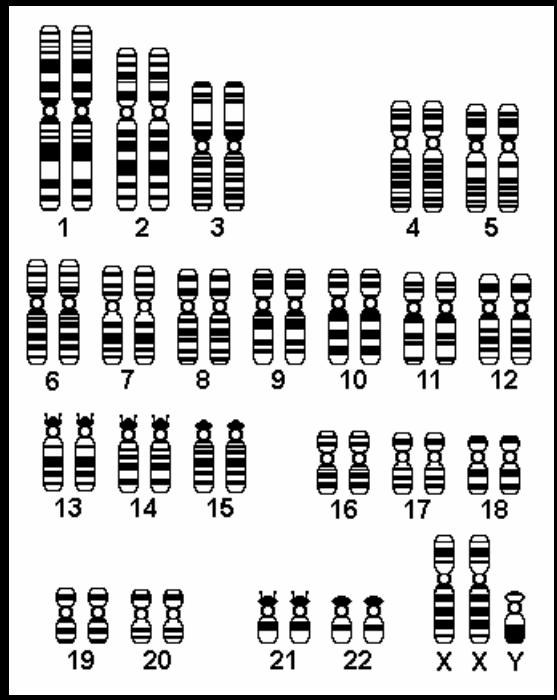
Fetal hydrops indicates a poor prognosis, regardless of the cause, and in about a third of stillbirths, the causse for hydrops is not found. However, chromosomal abnormalities should be considered, and foremost among them should be Turner's syndrome



Bilateral cystic hygroma of the neck, caused by a developmental abnormality of vascular channels, is characteristic (but not specific) for Turner's syndrome.




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
X
Y

Sindrome di Klinefelter.

- ginecomastia
- sproporzione degli arti
- ipogonadismo
- problemi di comportamento

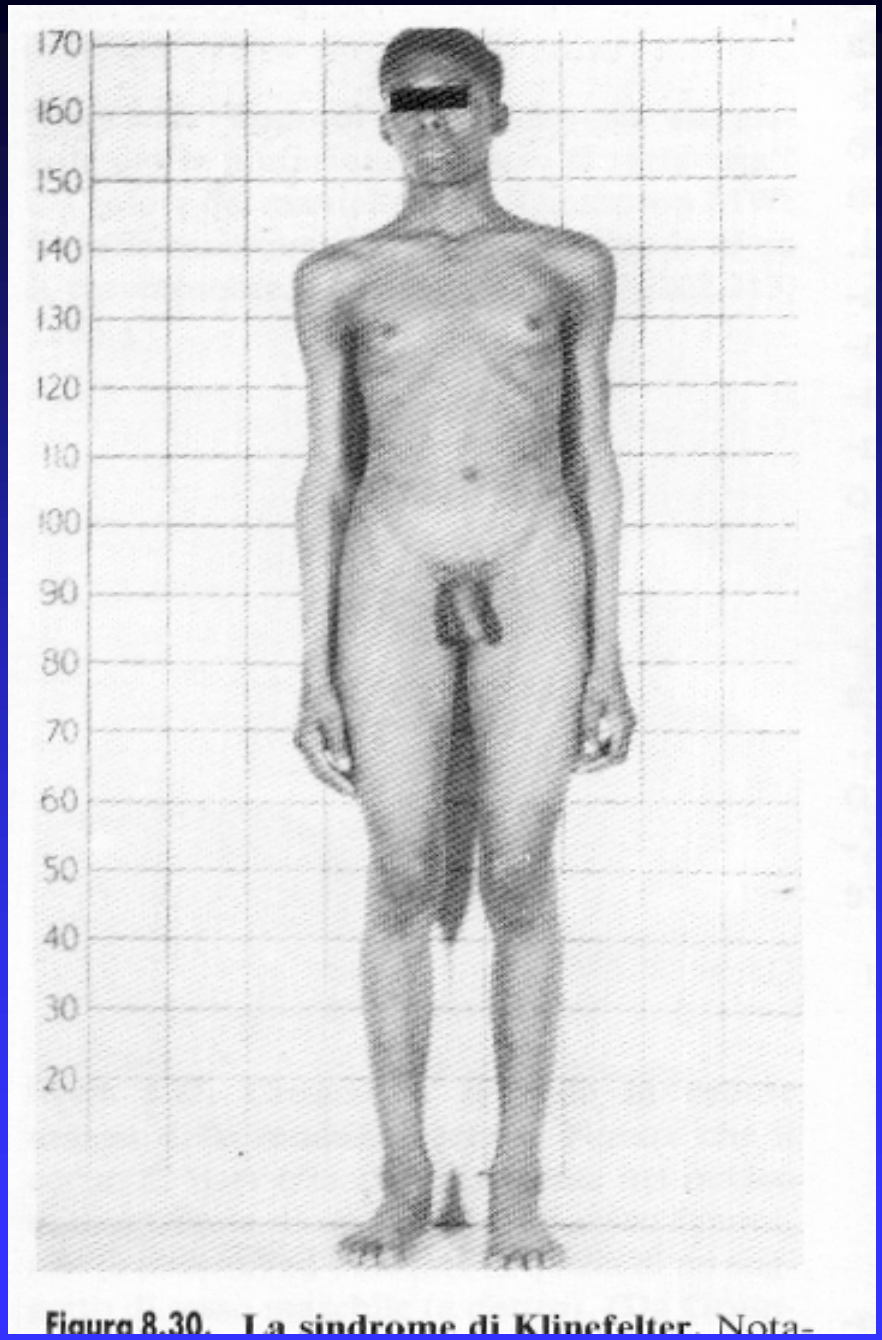
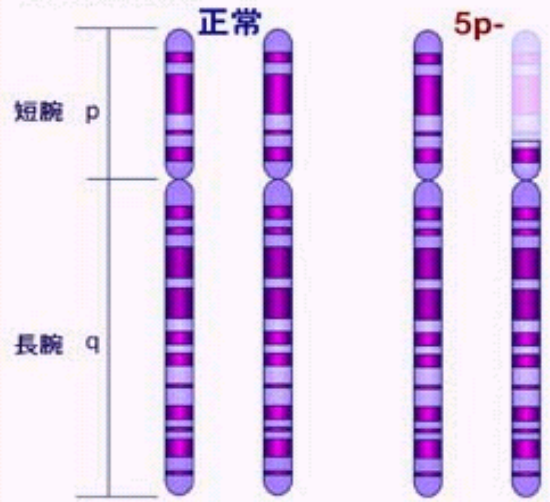


Figura 8.30. La sindrome di Klinefelter. Nota-

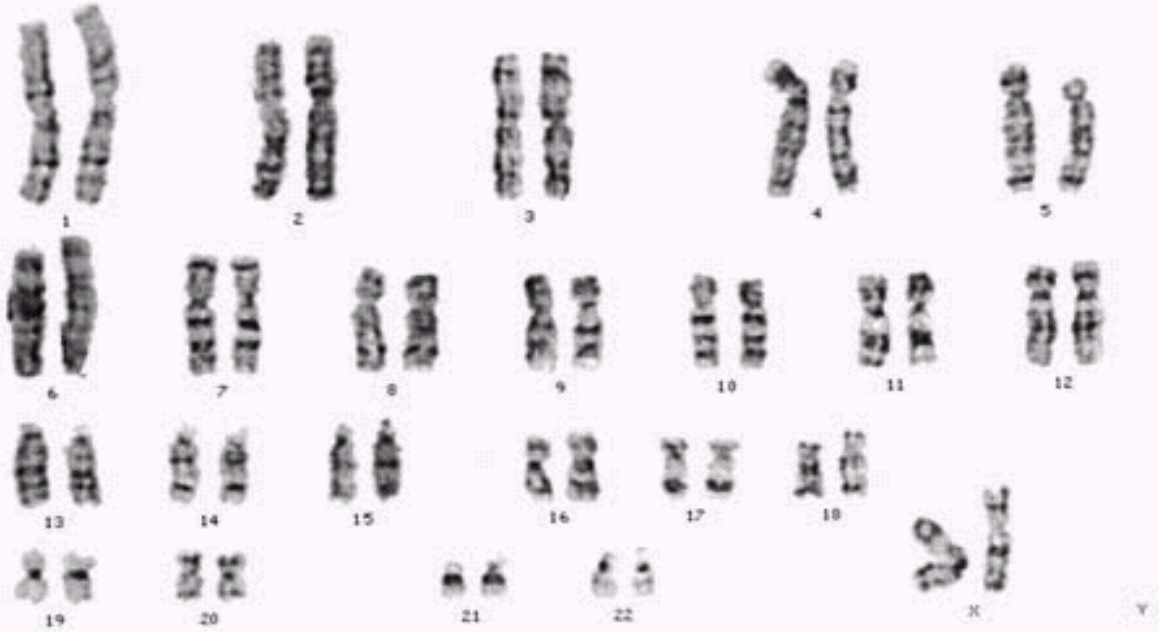
5p- syndrome = cri du chat syndrome

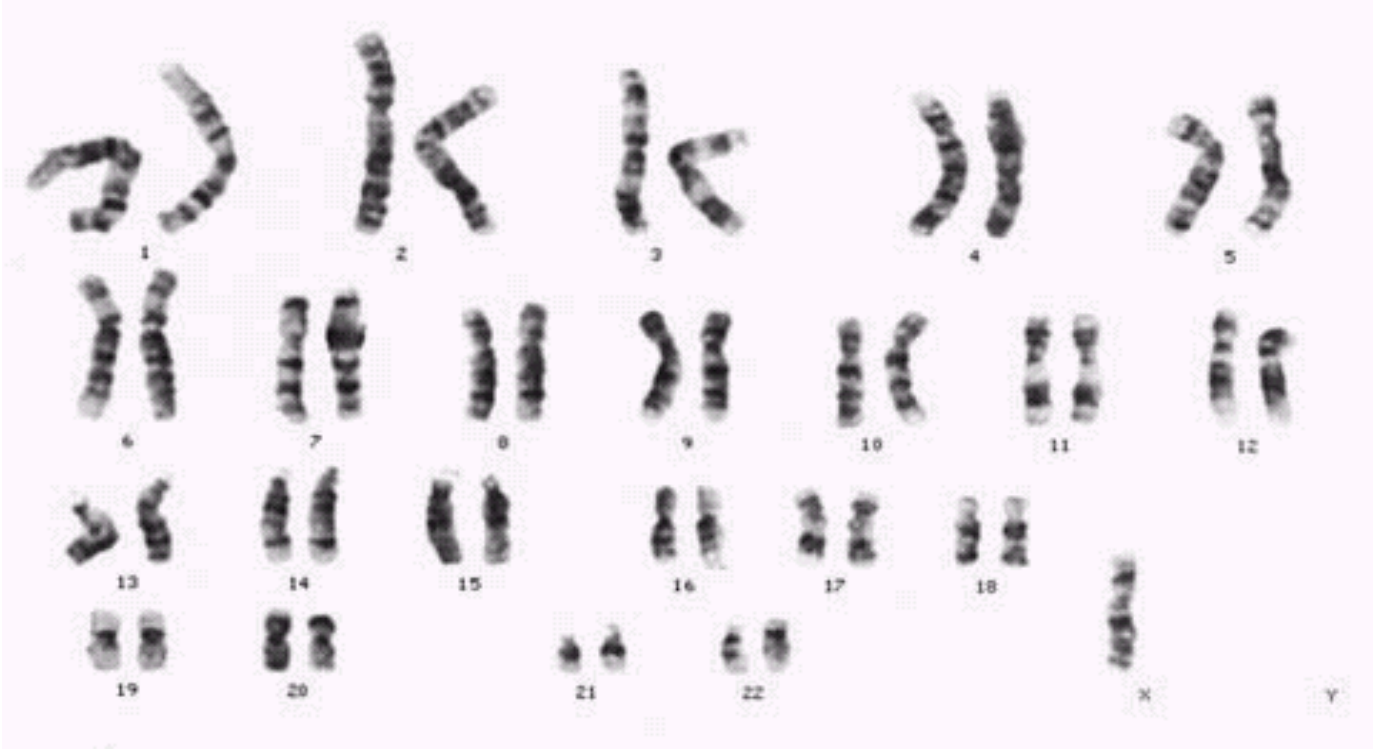
= ネコなき症候群



子猫の啼き声（新生児期に一過性）
円形顔貌
女児に圧倒的に多い
精神発達遅滞
1/10000
5 番染色体短腕欠損

小頭，瞼裂斜下，両眼隔離，
小顎，耳介前円柱，一部に猿線，
先天性心疾患




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

Chromosome 18 Registry

- There are five major syndromes that occur when there are abnormalities of chromosome 18.
- Within each syndrome there are a variety of characteristics and a wide range in severity.
- Some individuals are mosaic or have translocations involving another chromosome and so do not fit exactly into one of these syndromes.
- The most frequent abnormalities of chromosome 18 are 18q-, 18p-, ring 18, trisomy 18, and tetrasomy 18p.

- Smith-Magenis Syndrome (SMS) is a syndrome in which there is a small, missing section (deletion) of chromosome 17.
- The exact incidence is not known, but it is estimated that SMS occurs in 1 out of 25,000 births.
- An individual with SMS may have just a few or many of the features, including short stature, characteristic facial appearance with flattened mid-face and down-turned mouth, speech delay and articulation problems, developmental delay and learning disabilities, and hyperactivity.
- They may never show significant behavioral problems, but some degree of self-injury and sleep disturbance occurs in most individuals with SMS. http://www.smithmagenis.org

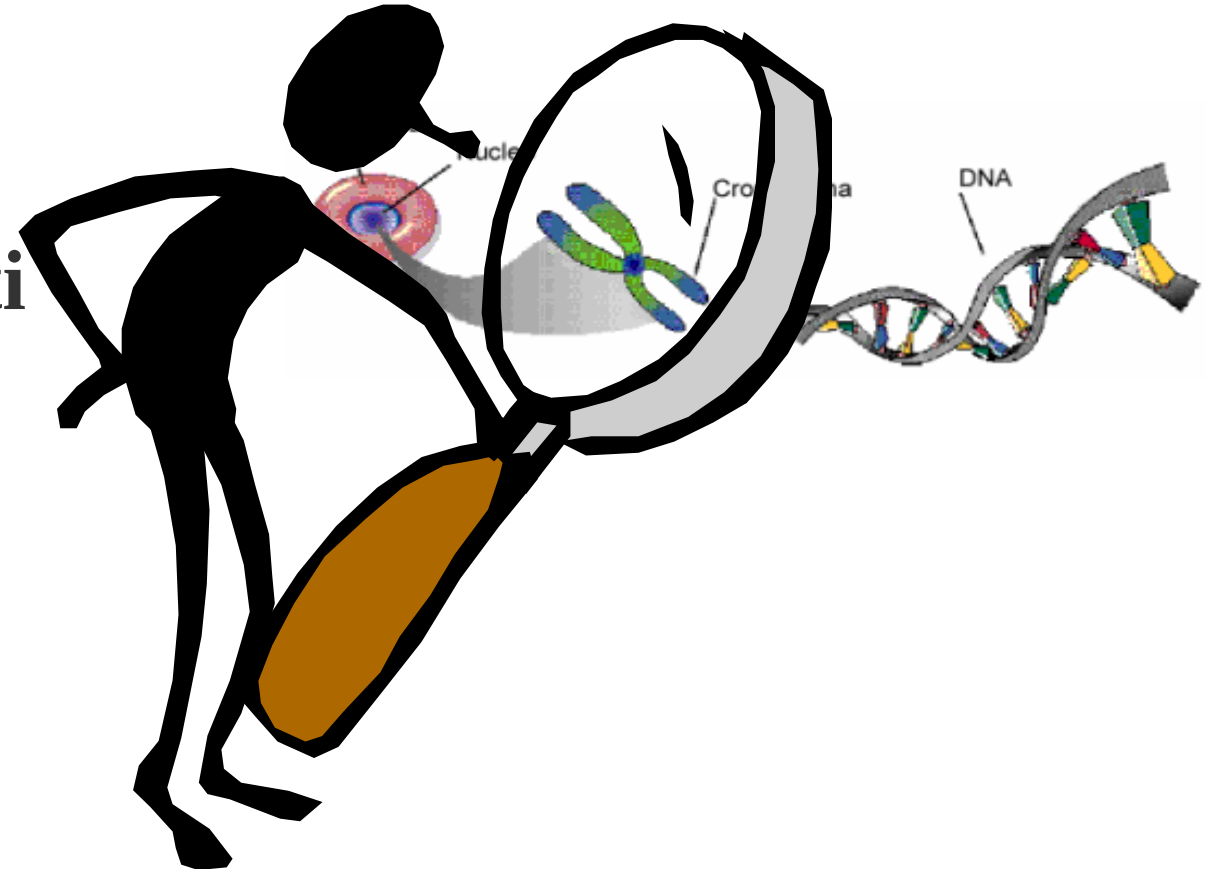
LO STUDIO DEI CROMOSOMI: CITOGENETICA

- alcuni concetti di citogenetica
- classificazione
- la frequenza delle malattie cromosomiche ed alcune patologie più frequenti
- quando la citogenetica tradizionale non ce la fa...

Quando il cariotipo non ce la fa

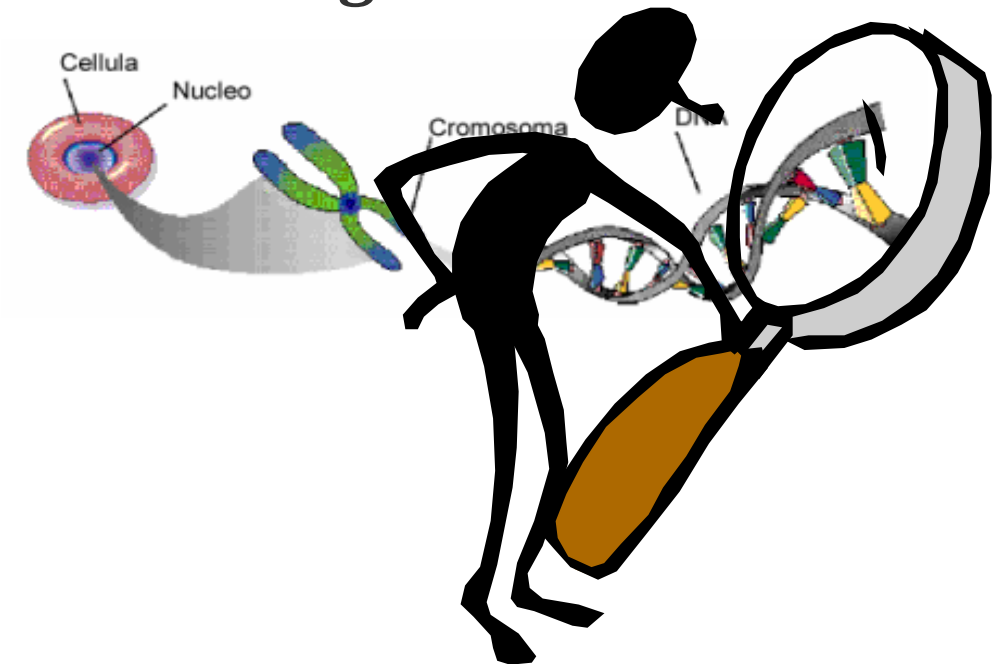
La Citogenetica Classica

- **Permette di identificare riarrangiamenti cromosomici coinvolgenti non meno di 5 Mb.**



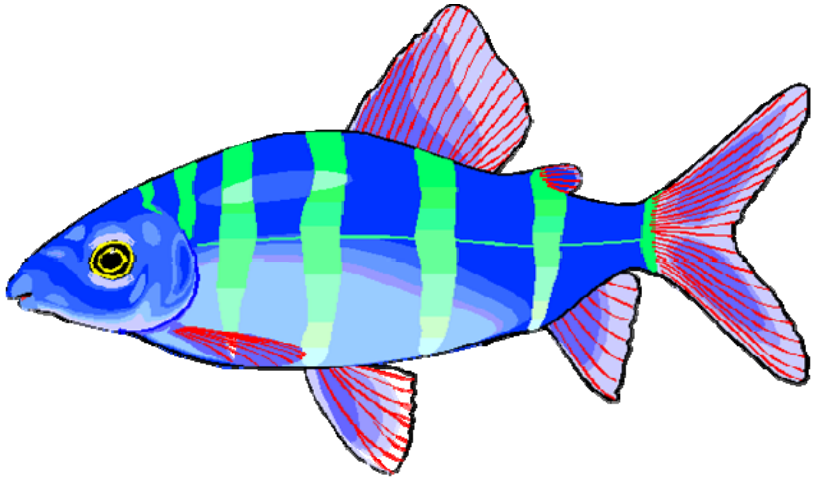
La Citogenetica Molecolare

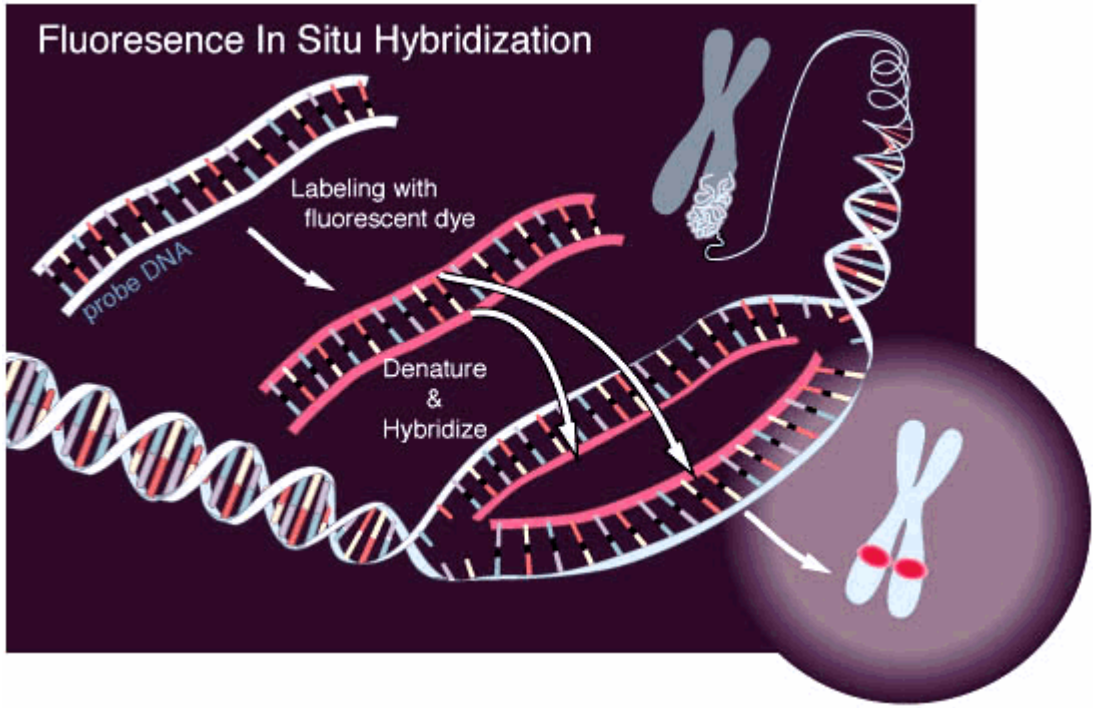
Abbina la possibilità di un'analisi del DNA, propria delle tecniche di biologia molecolare, con la struttura cromosomica il cui studio è oggetto della citogenetica classica.



La Citogenetica Molecolare

- **Permette un'analisi mirata di una regione cromosomica consentendo di mettere in evidenza riarrangiamenti di alcune centinaia di chilobasi.**



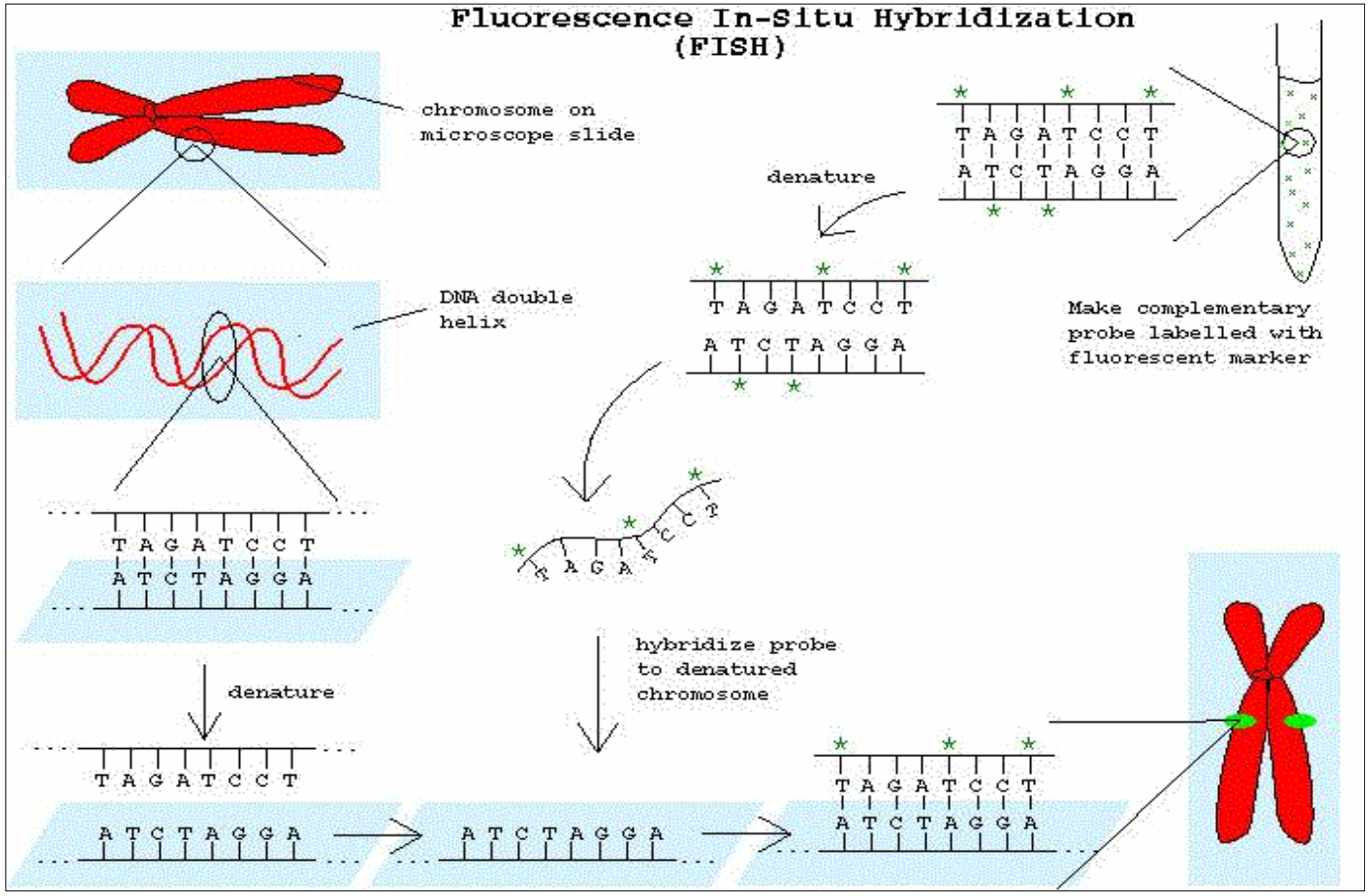


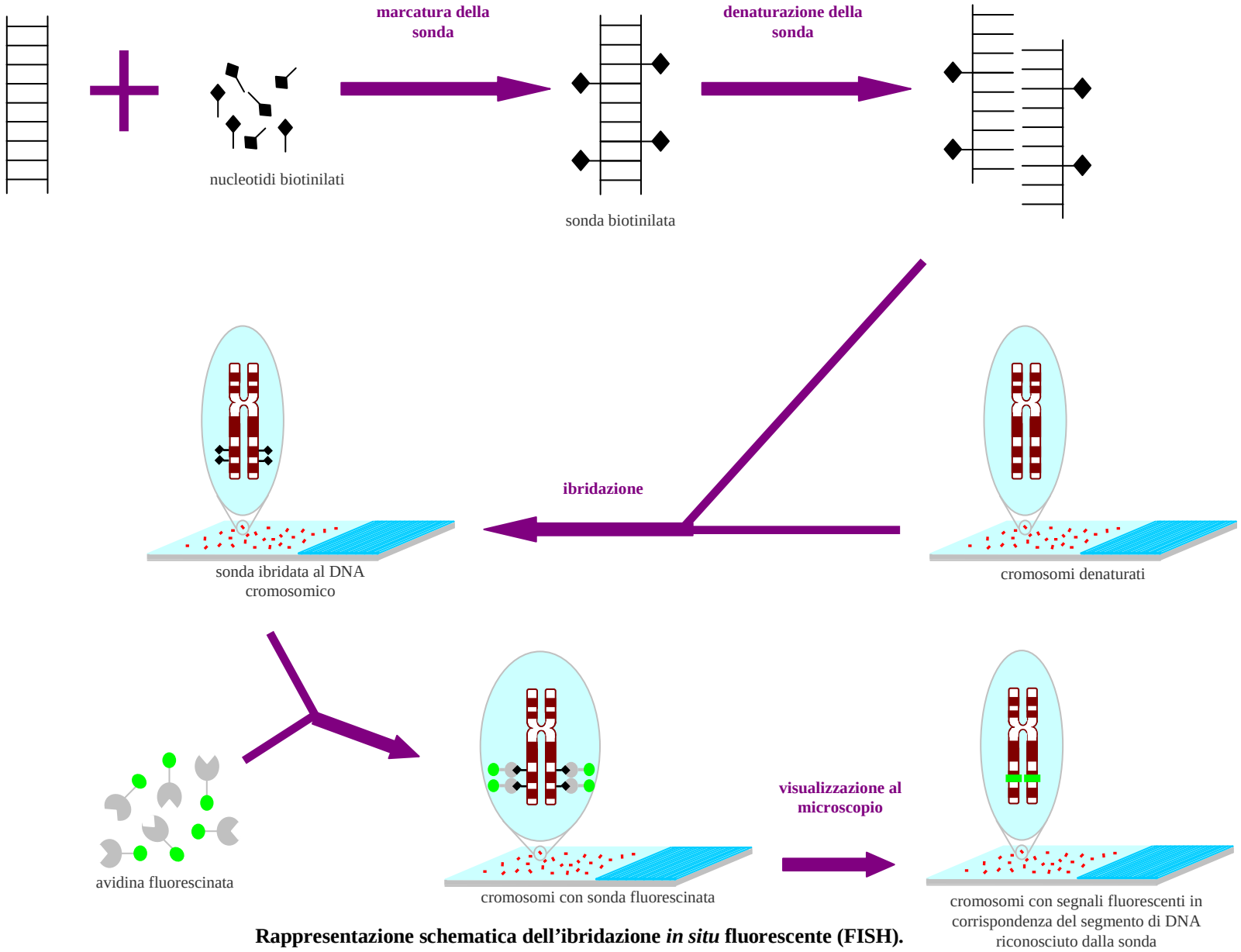
Tecniche di citogenetica molecolare

- **FISH** (fluorescence in situ hybridization)
- **PRINS** (primed in situ labeling)
- **PCR *in situ*** (polymerase chain reaction *in situ*)
- **CGH** (comparative genomic hybridization)
- ecc.

FISH (Fluorescence *in situ* hybridization)

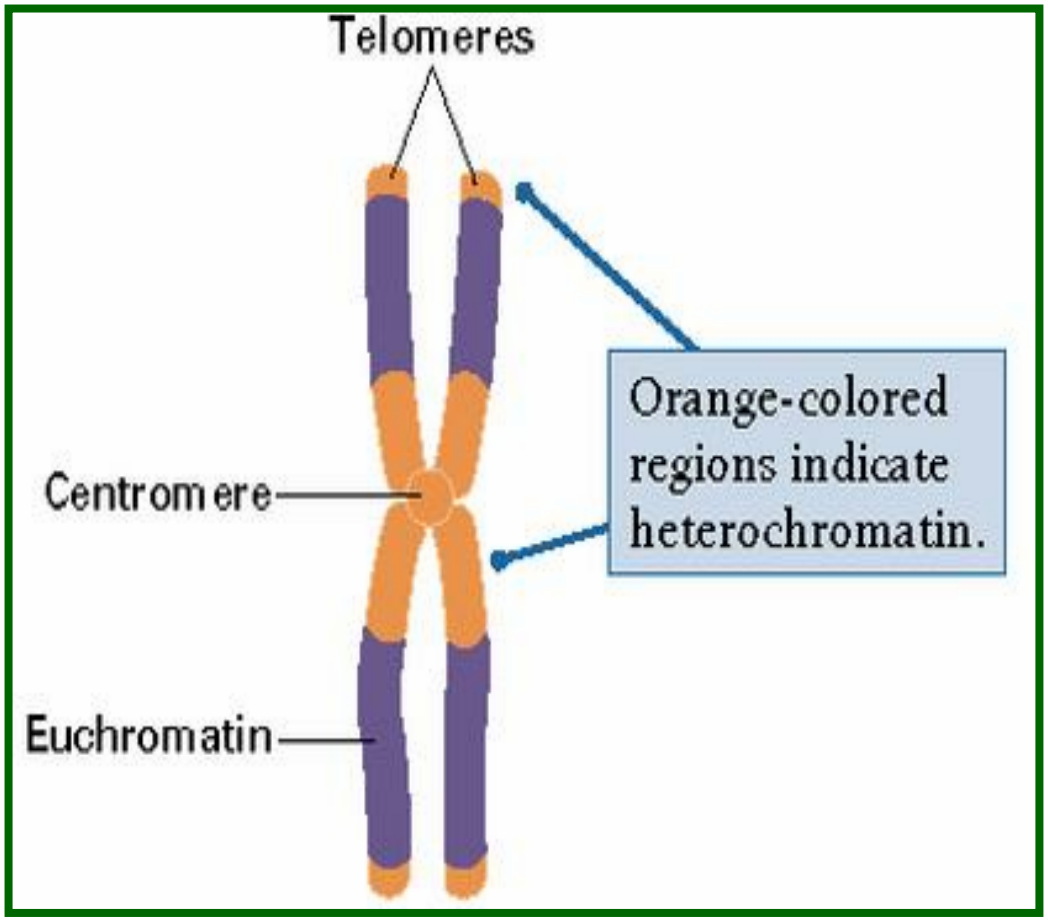
- E’ una tecnica di ibridazione che permette, dopo fissazione di metafasi e nuclei in interfase su vetrino, di identificare sequenze specifiche negli acidi nucleici.
- Tale identificazione avviene mediante sonde marcate in maniera non isotopica, impiegando fluorocromi che emettono a diverse lunghezze d’onda.





Rappresentazione schematica dell'ibridazione *in situ* fluorescente (FISH).

Nel cromosoma si distinguono:

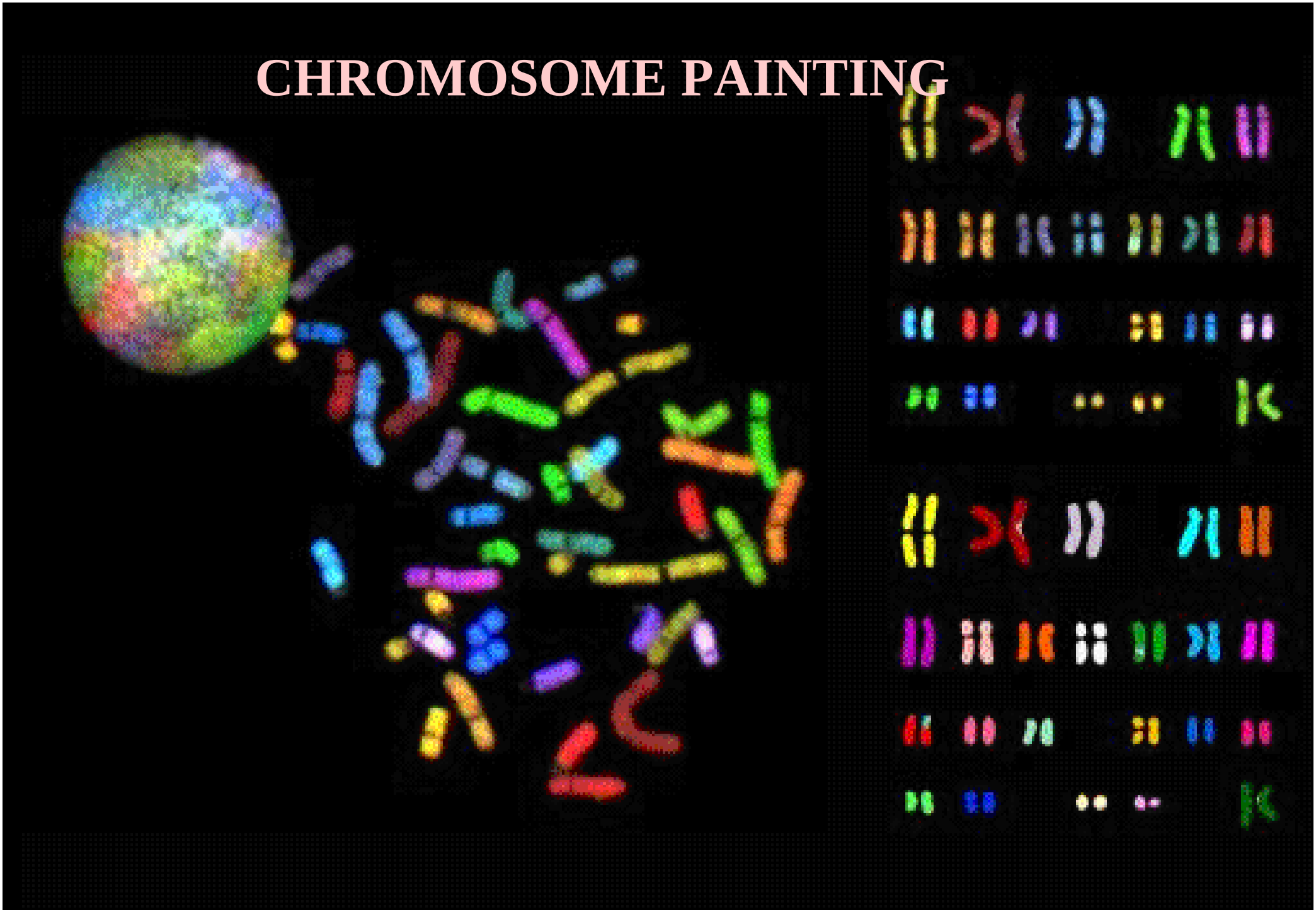


TIPI DI SONDE

- **Sequenze ripetute:**
  - **alfa satellite**
  - **beta satellite**
  - **satelliti classici**
  - **telomeriche**
- **Sequenze uniche:**
  - **DiGeorge**
  - **Prader Willi**
  - **Williams**
  - **ecc.**
- **Painting**

TIPI DI SONDE	ABERRAZIONI CROMOSOMICHE IDENTIFICABILI	MATERIALE IMPIEGATO
Sequenze ripetute	- Trisomie - Monosomie	Nuclei in interfase
Painting	- Riarrangiamenti cromosomici - Identificazione di cromosomi marcatori	Metafasi
Sequenze uniche	- Microdelezioni e duplicazioni - Riarrangiamenti cromosomici	Metafasi e nuclei in interfase

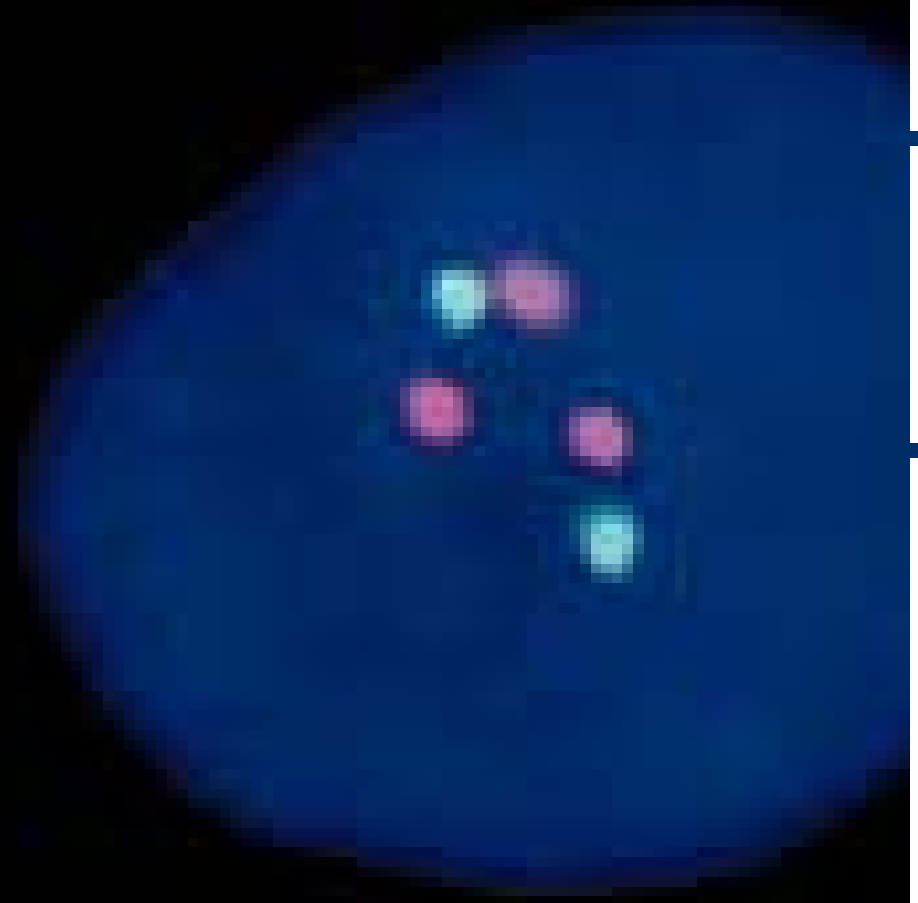
CHROMOSOME PAINTING

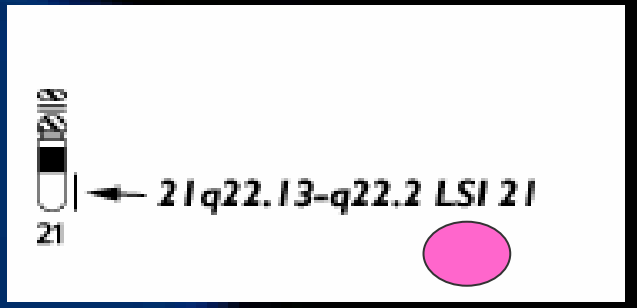


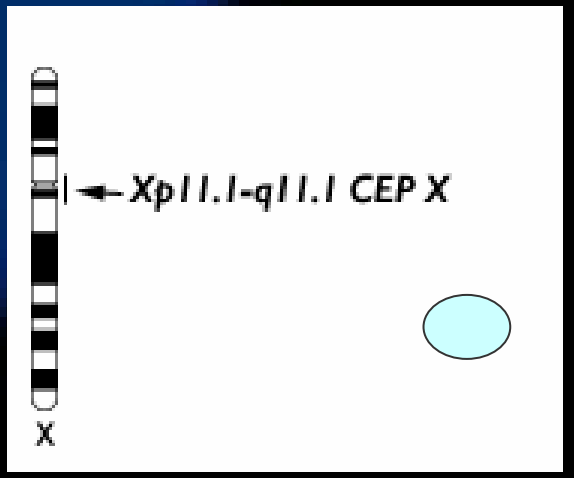
PRINCIPALI SINDROMI DA MICRODELEZIONE

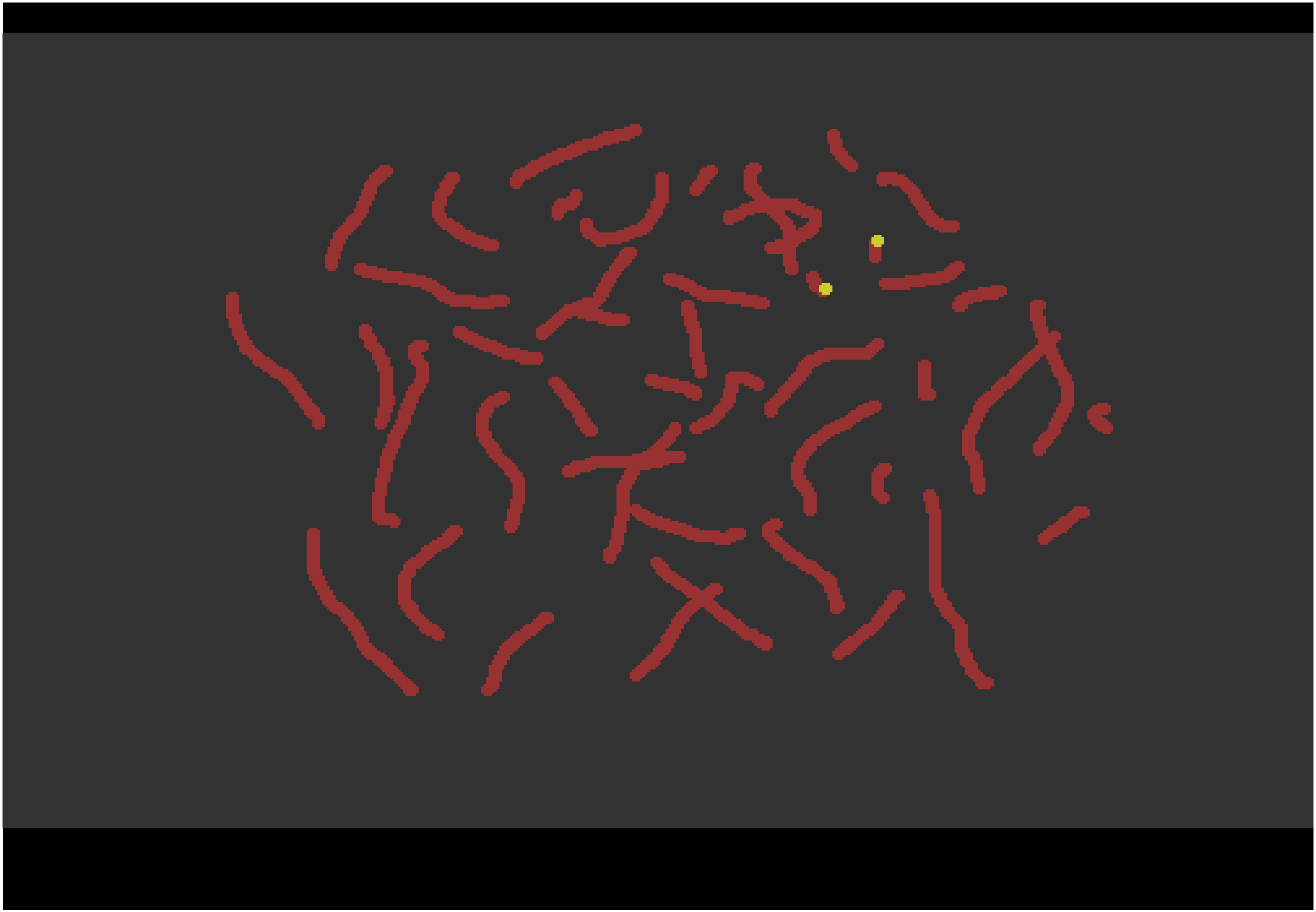
SINDROME	LOCALIZZAZIONE CROMOSOMICA	SOGGETTI CON MICRODELEZIONE
Prader Willi/Angelman	15q11.13	70%
Williams	7q11.23	90%
DiGeorge/Velocardiofacciale	22q11.2	75%
Smith-Magenis	17p11.2	95%
Miller-Dieker	17p13.3	90%

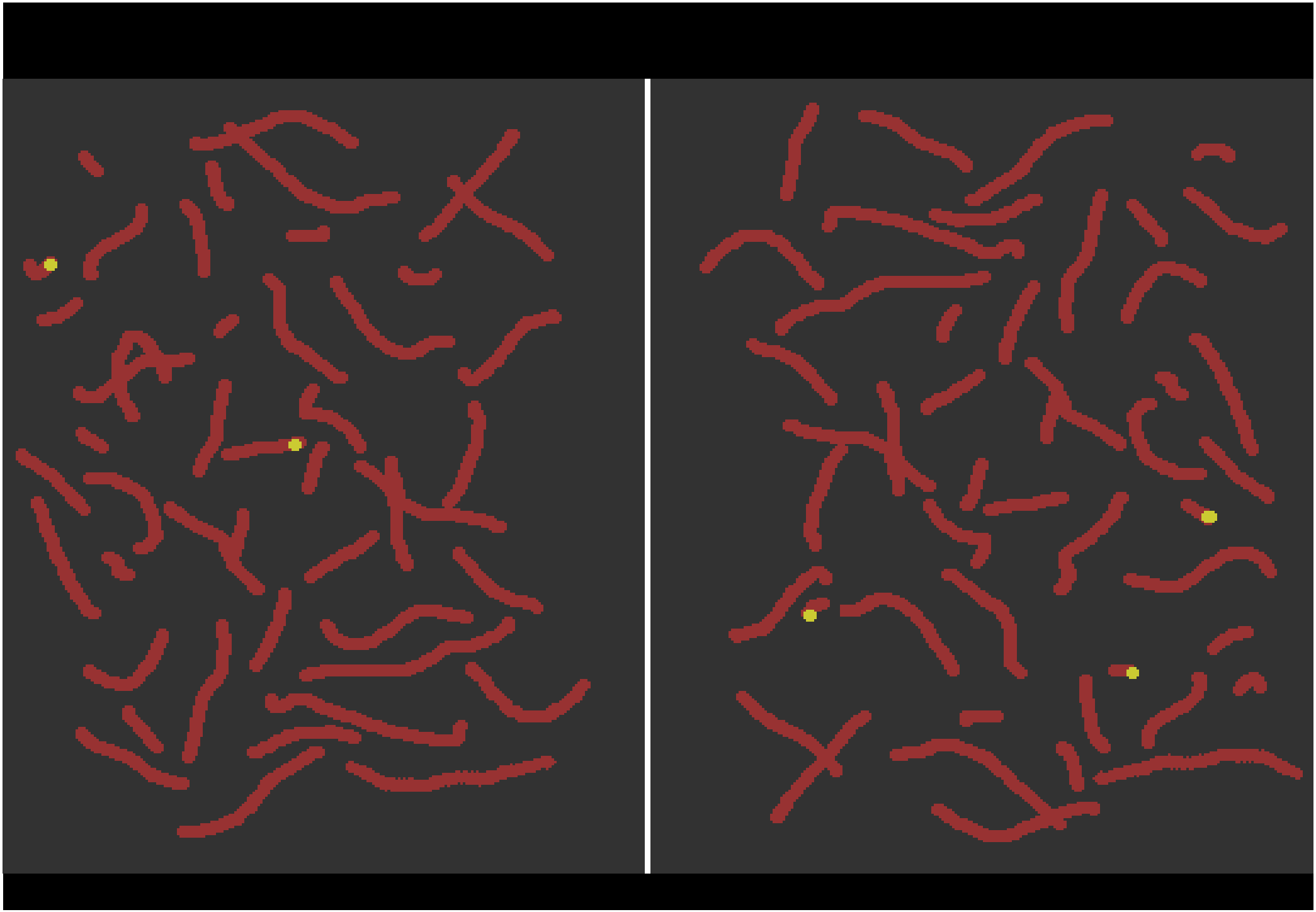
DIAGNOSI DI ANEUPLOIDIE



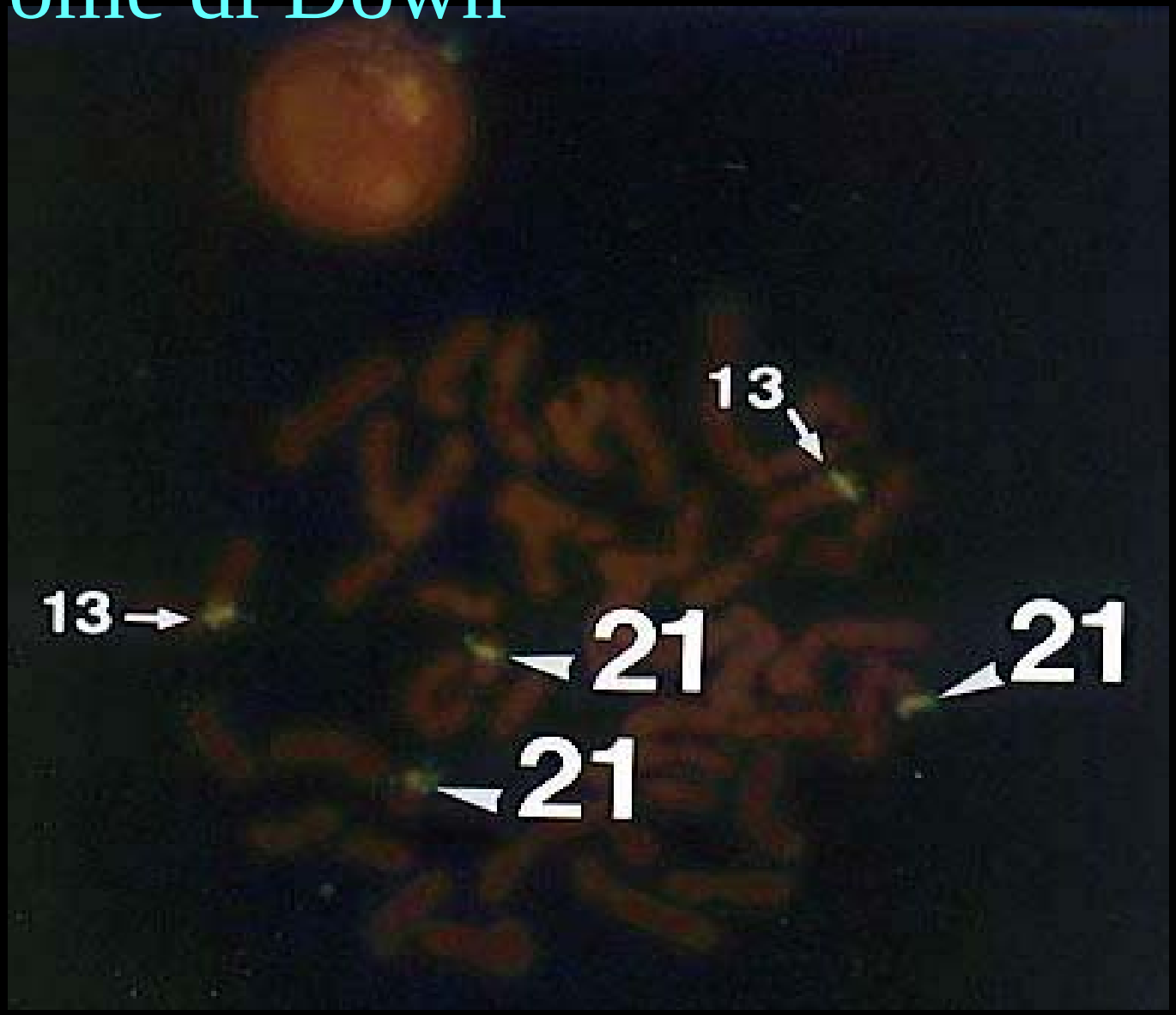






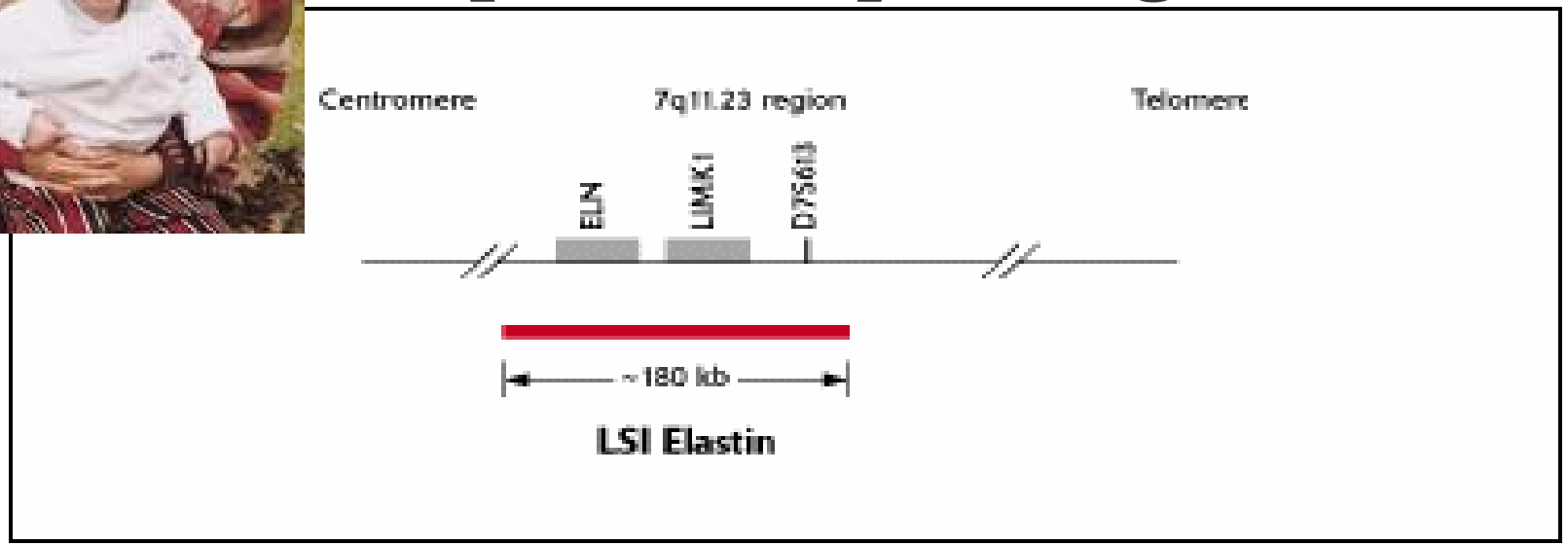


Sindrome di Down

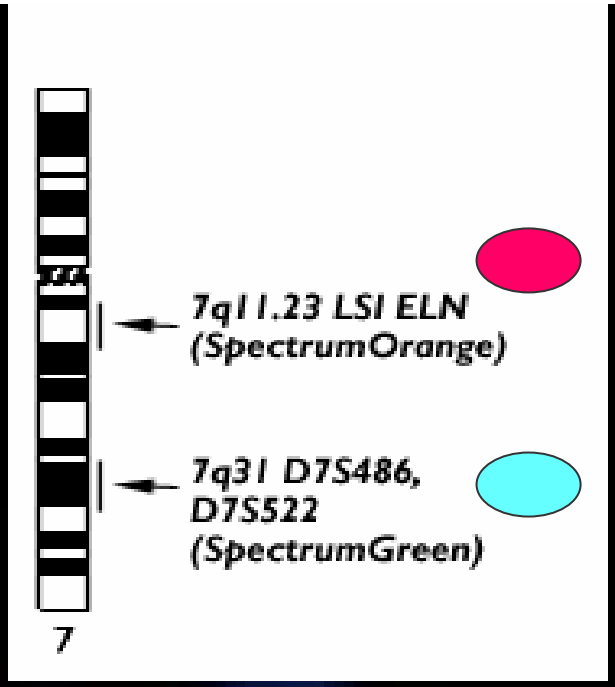


SINDROME DI WILLIAMS

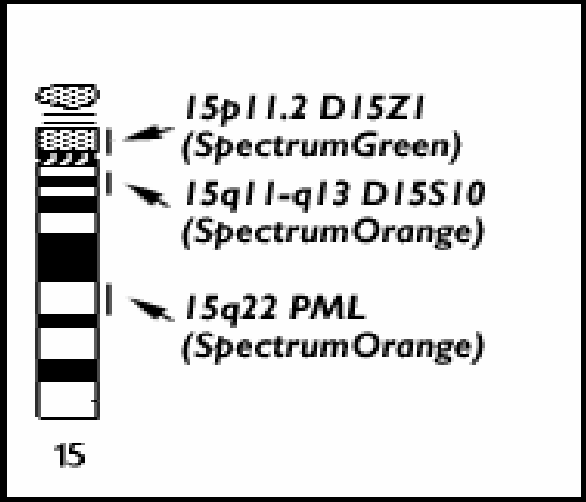
- faccia caratteristica
- ritardo di crescita
- problemi cardiaci
- problemi psicologici

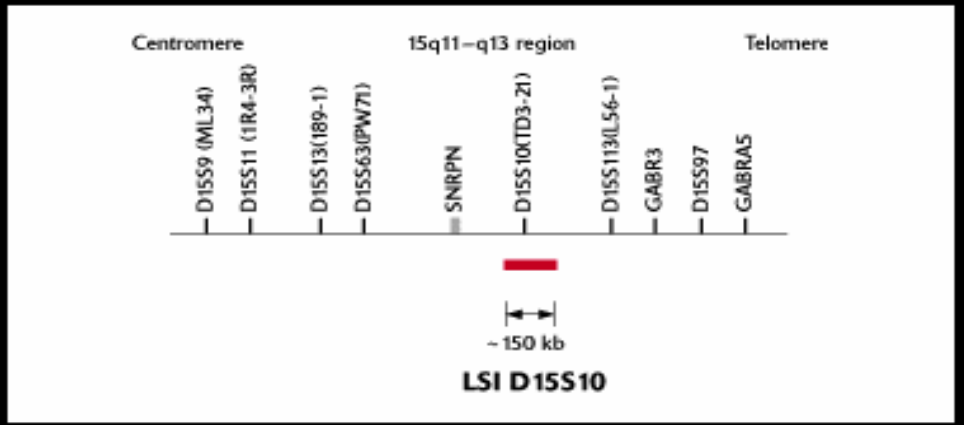


SINDROME DI WILLIAMS

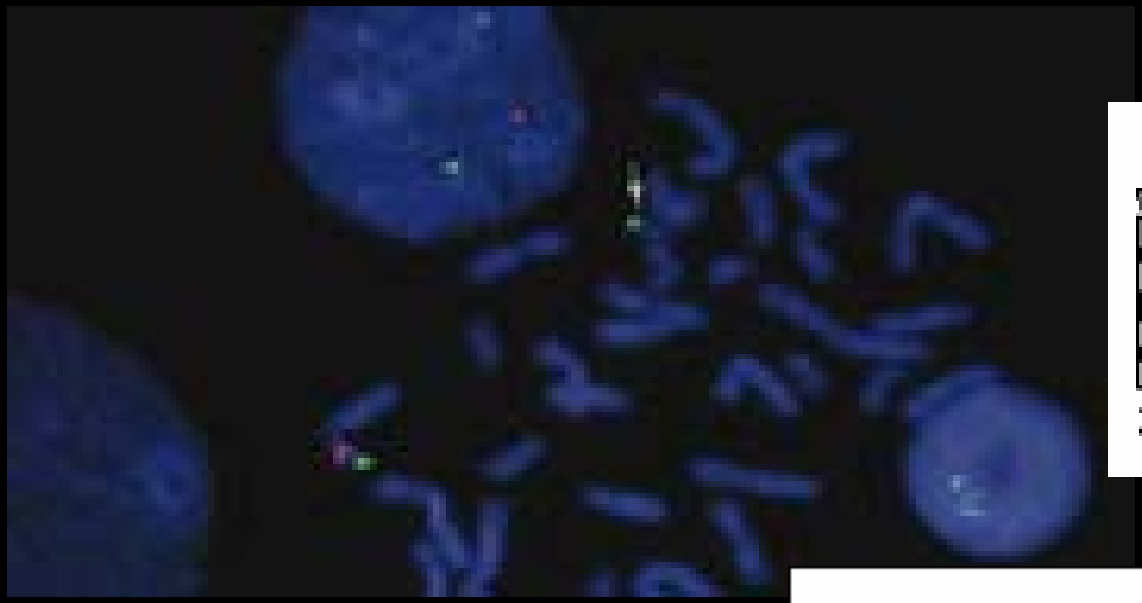


SINDROME DI PRADER-WILLI





SINDROME DI DI-GEORGE



FISH

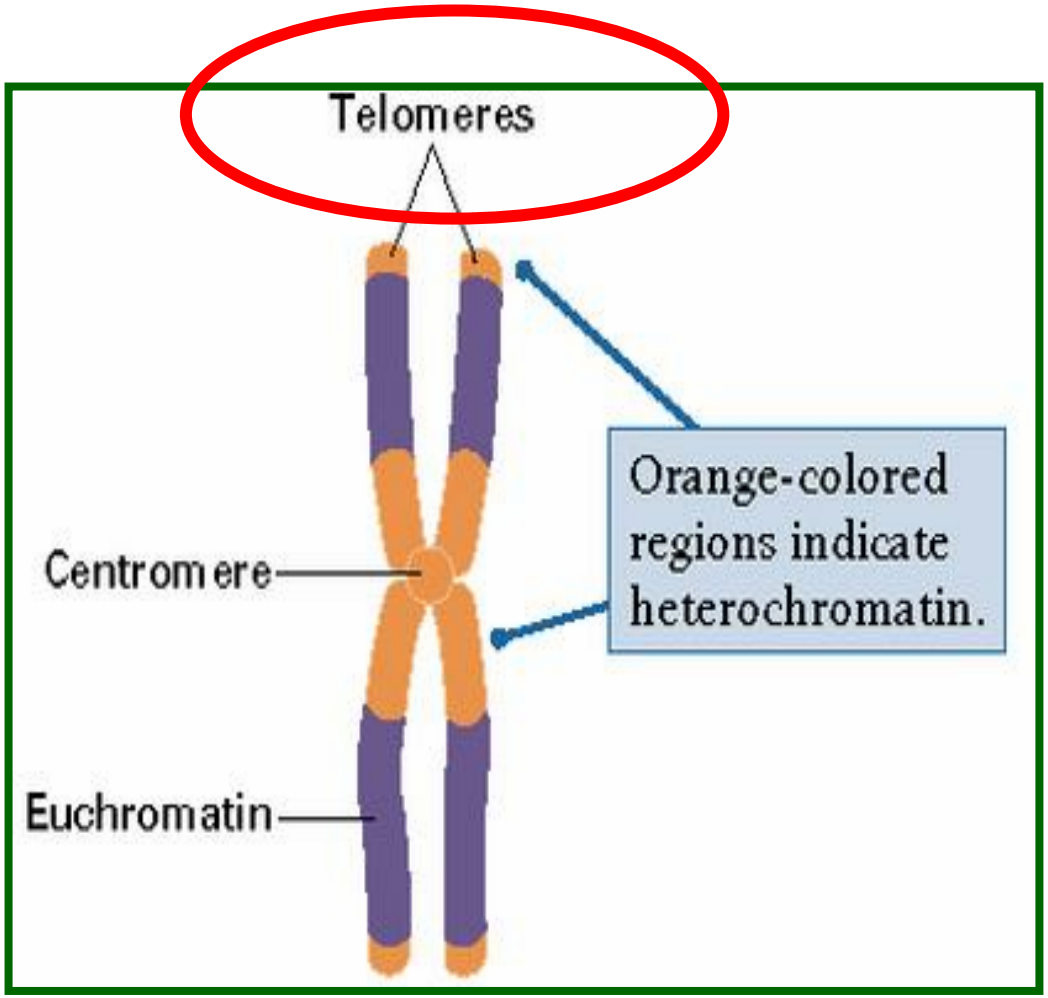
VANTAGGI

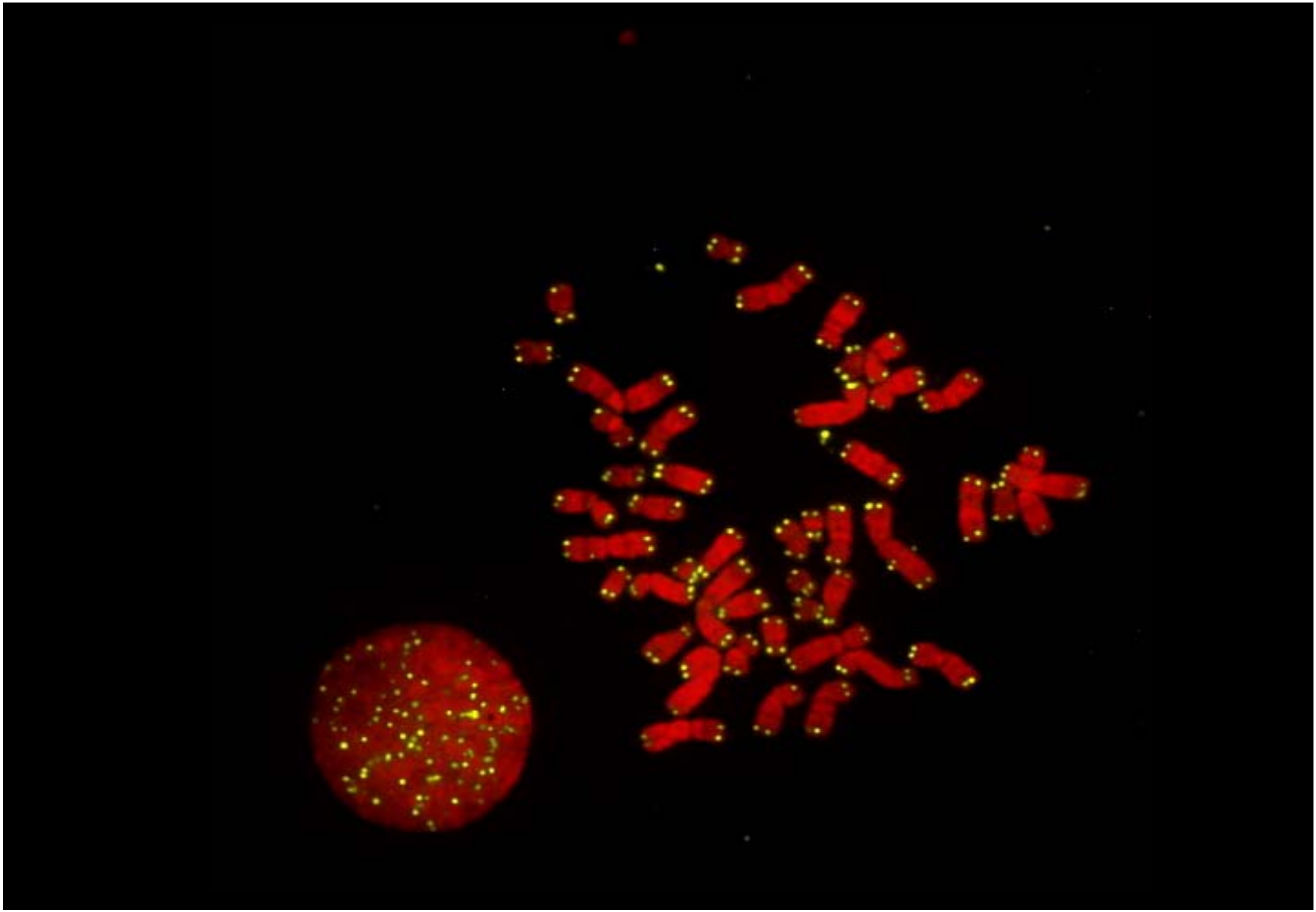
- rapidità
- identificazione di microdelezioni e riarrangiamenti complessi
- diagnosi su nucleo

SVANTAGGI

- costi elevati
- diagnosi non completa

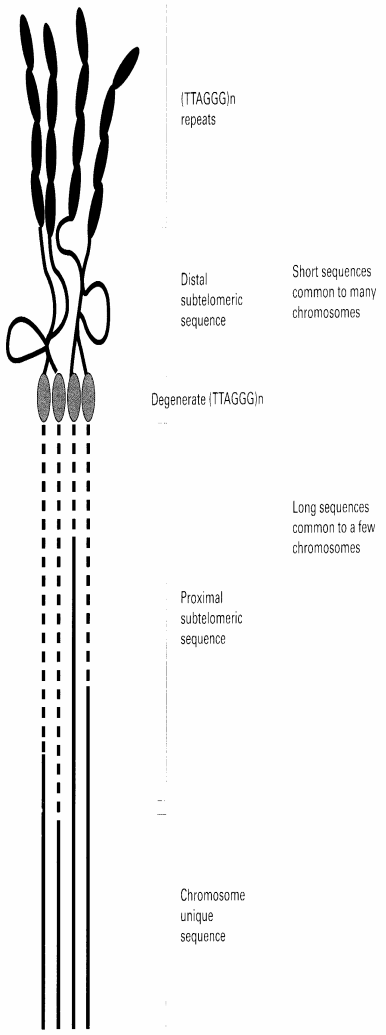
Nel cromosoma si distinguono:





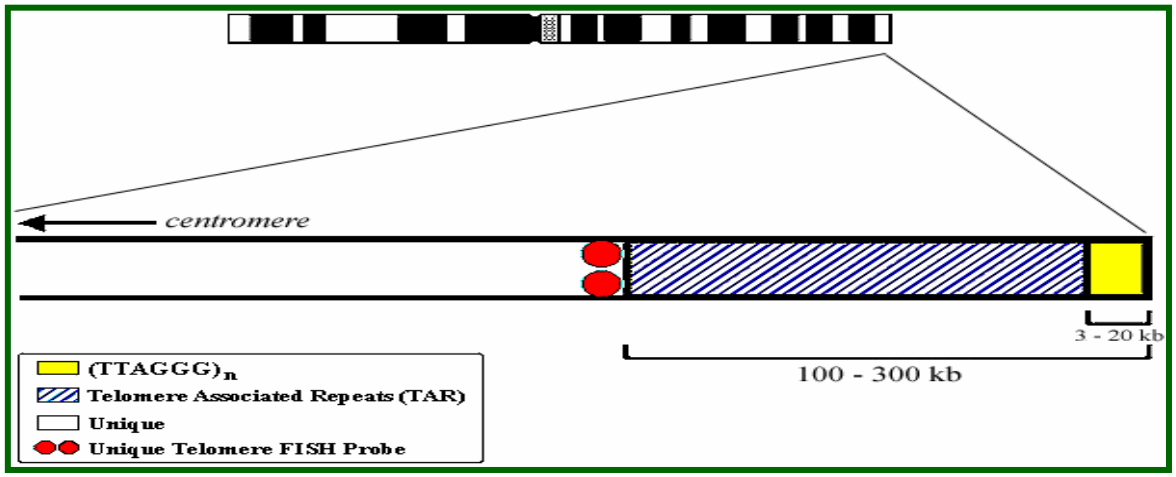
Ricordiamo che le estremità di ciascun cromosoma sono costituite da:

- Sequenze **comuni**
- Famiglie **complesse**
- Sequenze **uniche**

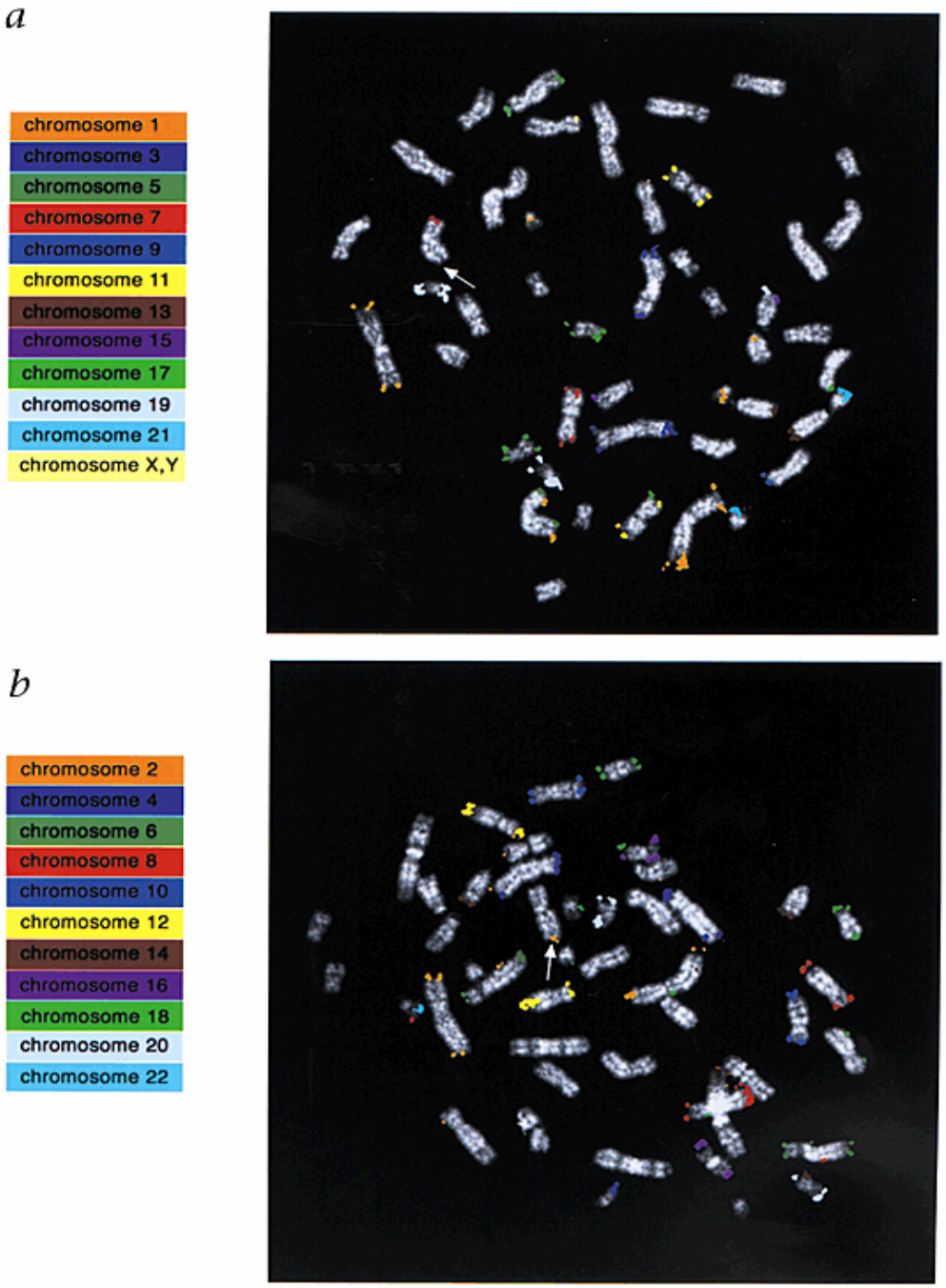


Sequenze uniche

- Le regioni subtelomeriche sono particolarmente ricche di CpG islands e geni.
- Si pensa abbiano la più alta densità genica dell'intero genoma.
- Data l'alta densità genica anomalie cromosomiche coinvolgenti queste aree possono essere associate ad anomalie fenotipiche e ritardo mentale.



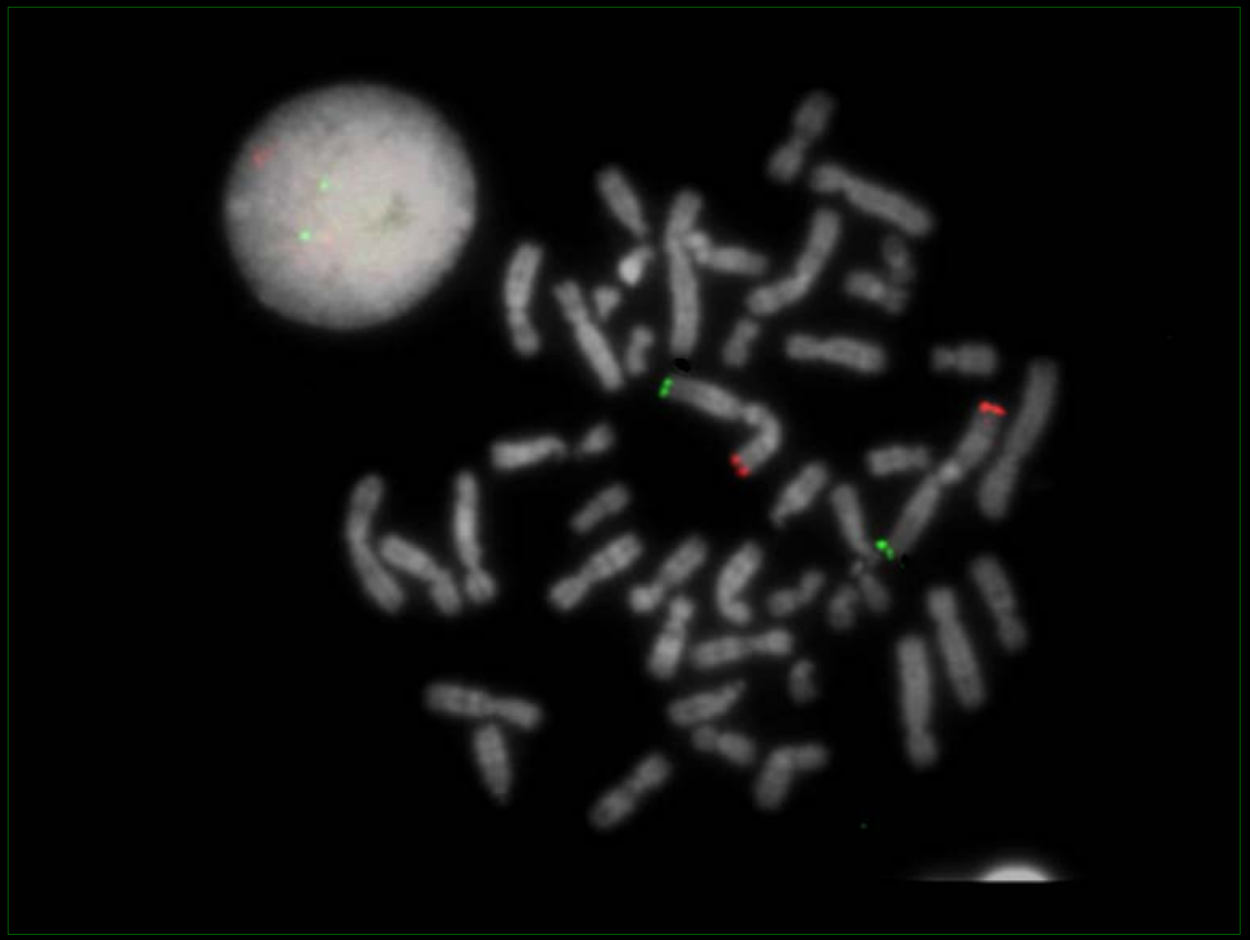
E’ stato recentemente dimostrato che il ritardo mentale può essere causato da riarrangiamenti cromosomici subtelomerici non evidenziabili mediante tecniche di citogenetica classica ma mediante tecniche di citogenetica molecolare a causa delle loro ridotte dimensioni.



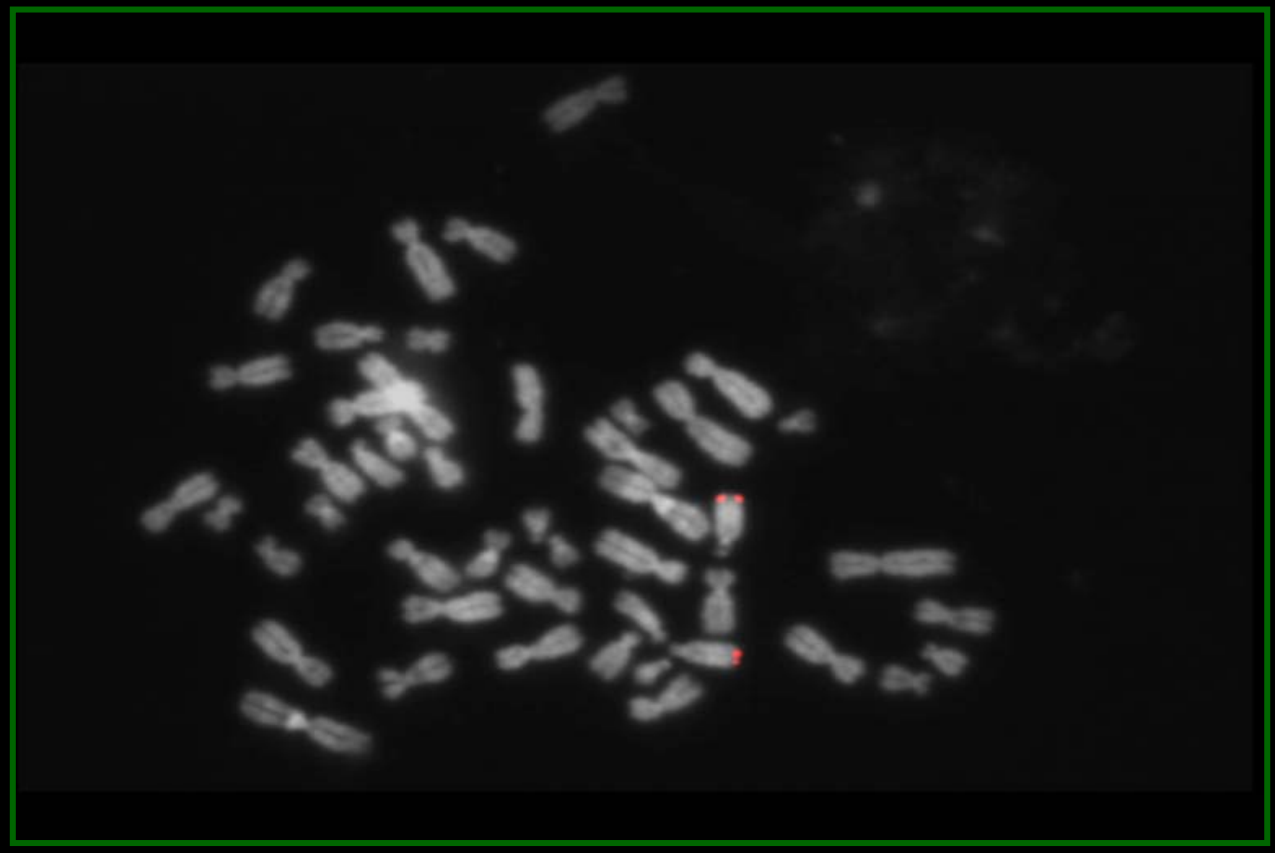
Color classification (goldFISH) analysis of the telomeric signals in metaphases from patient 3.

***a*, The M-TEL1 probes identified a deletion of 7q on one chromosome 7 homolog (arrow). *b*, The M-TEL2 probes identified an additional chromosome 2 signal (orange) on the derivative chromosome 7 (arrow). One further FISH experiment with 2p and 2q subtelomeric probes was sufficient to confirm an unbalanced translocation resulting in monosomy for 7q and trisomy for 2q in this patient. The respective pseudocolors are given on the left. In both *a* and *b*, there are some apparent misclassifications, for example 5p and q, 11p, 16q, 19q. However, in all cases the misclassification involved only one out of a possible two sister chromatids on one chromosome arm. Note also that the 9q and XqYq PACs were not included in this early version of the M-TEL assay, because of cross-hybridization with several other telomeres[18]. Instead, the first-generation cosmids were applied separately in a dual-color hybridization. However, the newer BAC probes for 9q and XqYq do not cross-hybridize and were applied in the more recent M-TEL 1 hybridizations (see Table 1 and Fig. 4).**

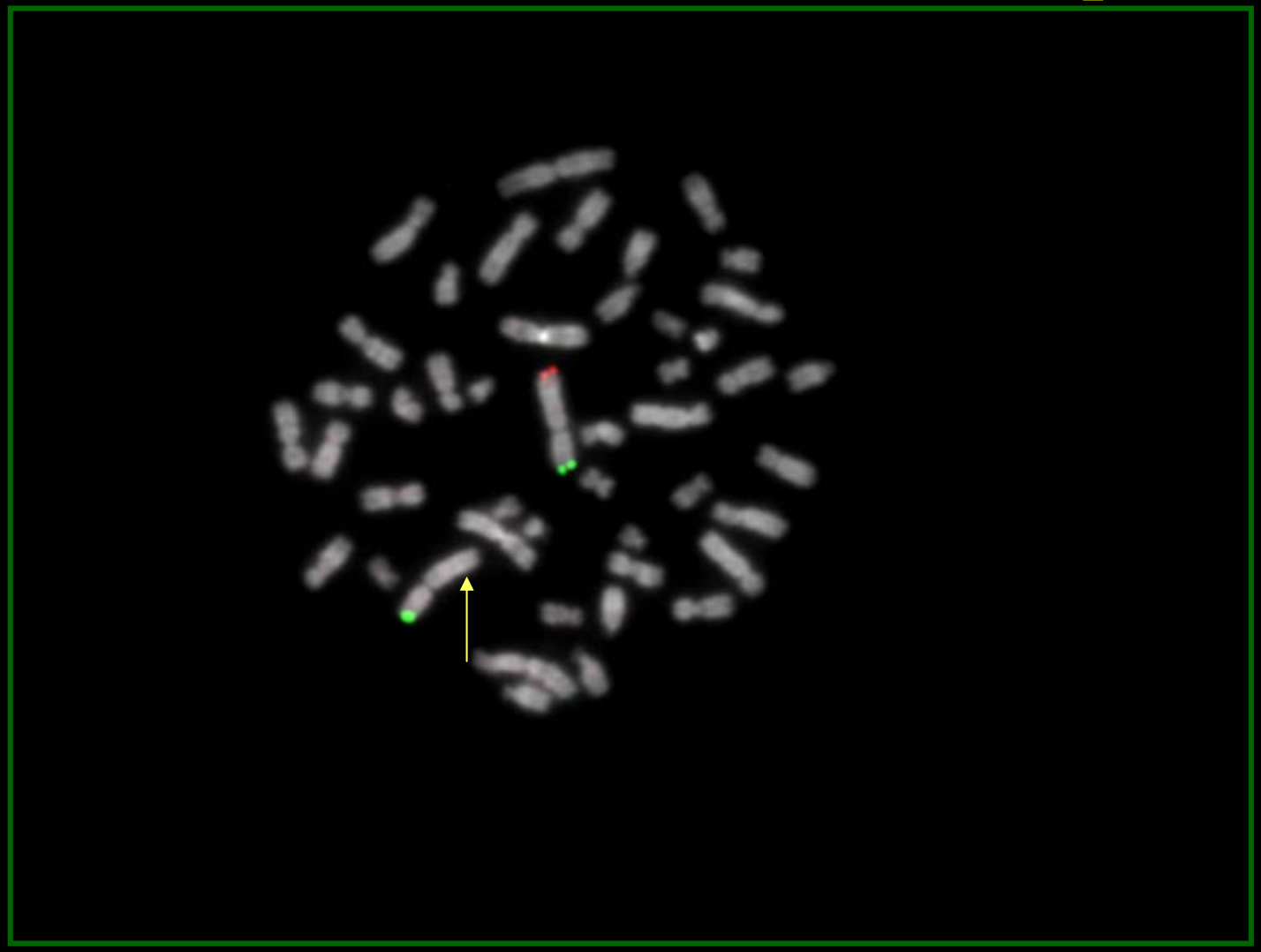
Visualizzazione delle sequenze subtelomeriche del cromosoma 1



Visualizzazione delle sequenze subtelomeriche del cromosoma 13



Delezione subtelomerica 2q



Cause genetiche del ritardo mentale

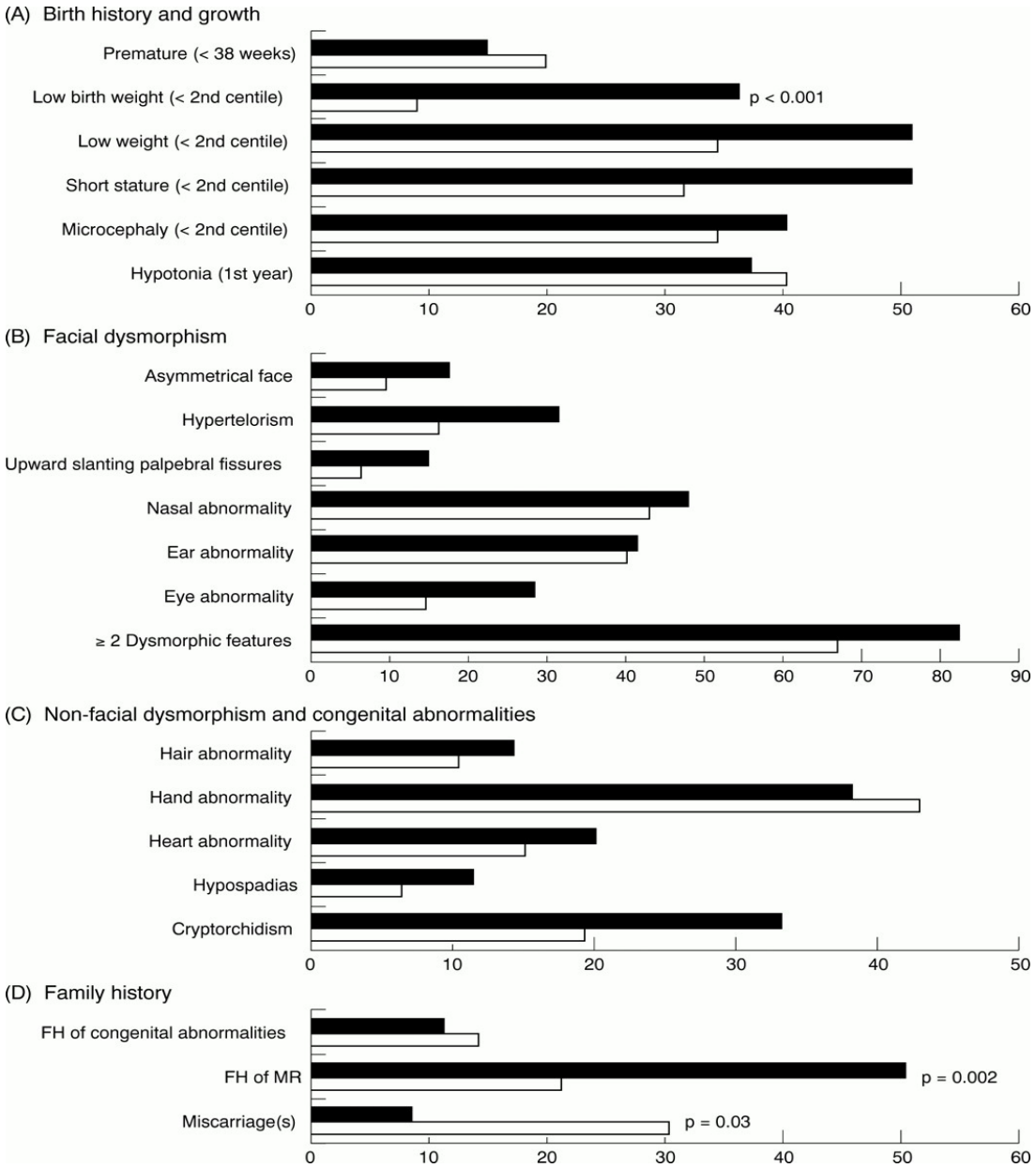
Più di 500 malattie genetiche sono associate a ritardo mentale:

- **Cromosomiche** (Sindrome di Down, ecc.)
- **Monofattoriali** (S. FraX, errori congeniti del metabolismo, ecc.)
- **Multifattoriali** (craniostenosi, ecc.)
- **Riarrangiamenti criptici subtelomerici**, si possono considerare alla base di circa il 7.4% dei casi di ritardo mentale idiopatico da moderato a severo e dello 0.5% dei casi di ritardo mentale lieve.

Knight S. et al. Lancet 354: 1676-1681; 1999

Ritardo mentale

- Può essere considerato una patologia eterogenea ad eziologia mista costituita da un numero molto elevato di entità nosologiche differenti, le cui cause sono solo in parte note.
- E’ stato stimato che la prevalenza del ritardo mentale nella popolazione generale si aggira attorno al 2-3%; la grande maggioranza rientra in ritardi di modesta entità, mentre lo 0.3-0.4% della popolazione generale presenta un ritardo severo.



The frequencies of features in children with subtelomeric abnormalities (black, n=29) compared to controls (white, n=110) concerning (A) birth history and growth, (B) facial dysmorphism, (C) non-facial dysmorphism and congenital abnormalities, and (D) family history. Only the features with frequencies above 10% are shown.