Cytogenetics
Chromosomal Genetics

Sophie Dahoun

Service de Génétique Médicale, HUG

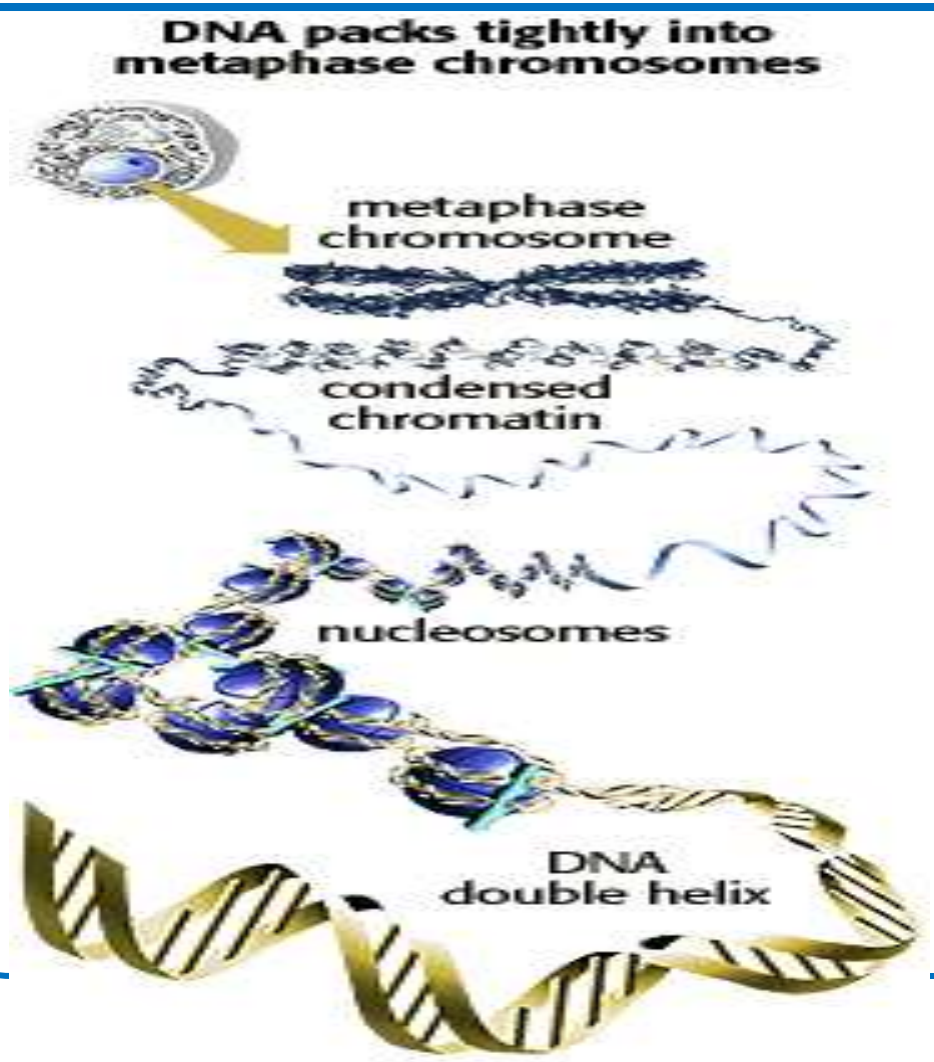
Geneva, Switzerland

sophie.dahoun@hcuge.ch

Training Course in Sexual and Reproductive Health Research

Geneva 2011

Cytogenetics is the branch of genetics that correlates the structure, number, and behaviour of chromosomes with heredity and diseases



Conventional cytogenetics

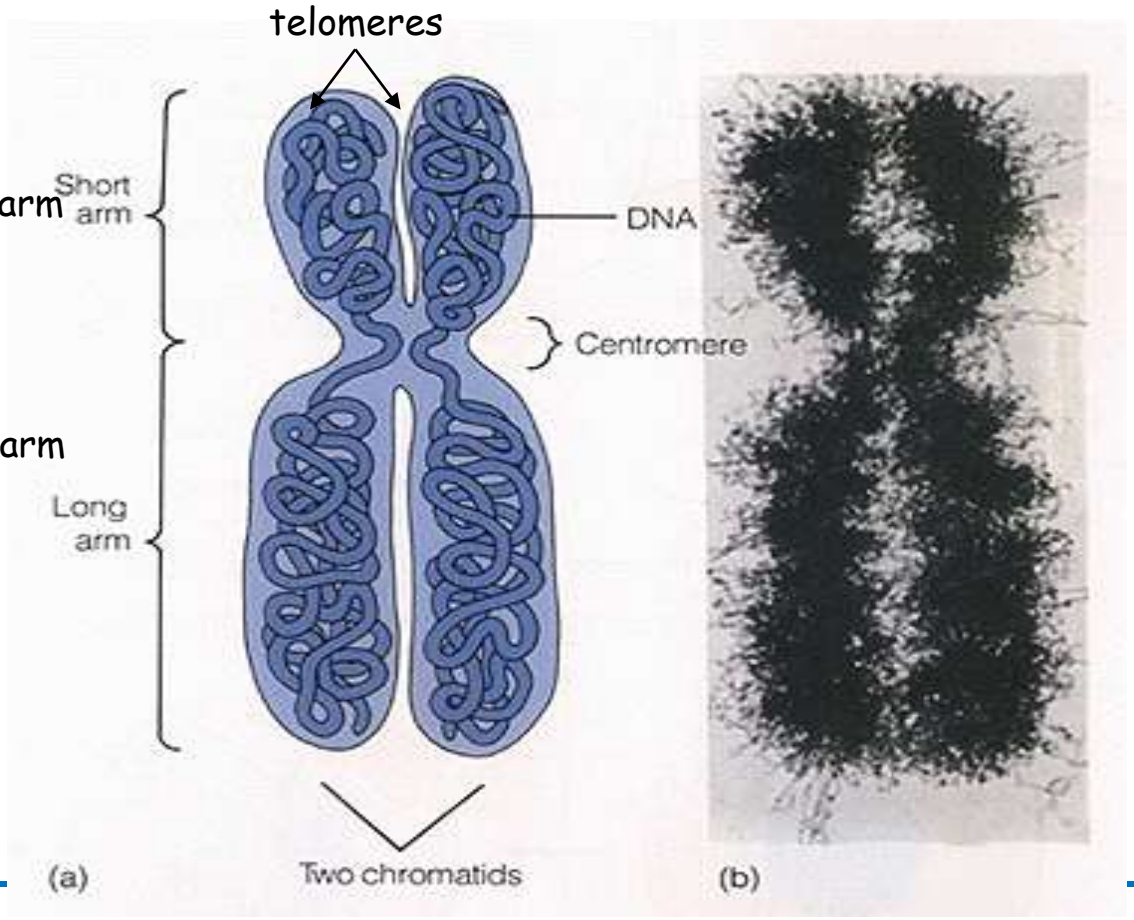
Molecular cytogenetics

Molecular Biology

I. Karyotype

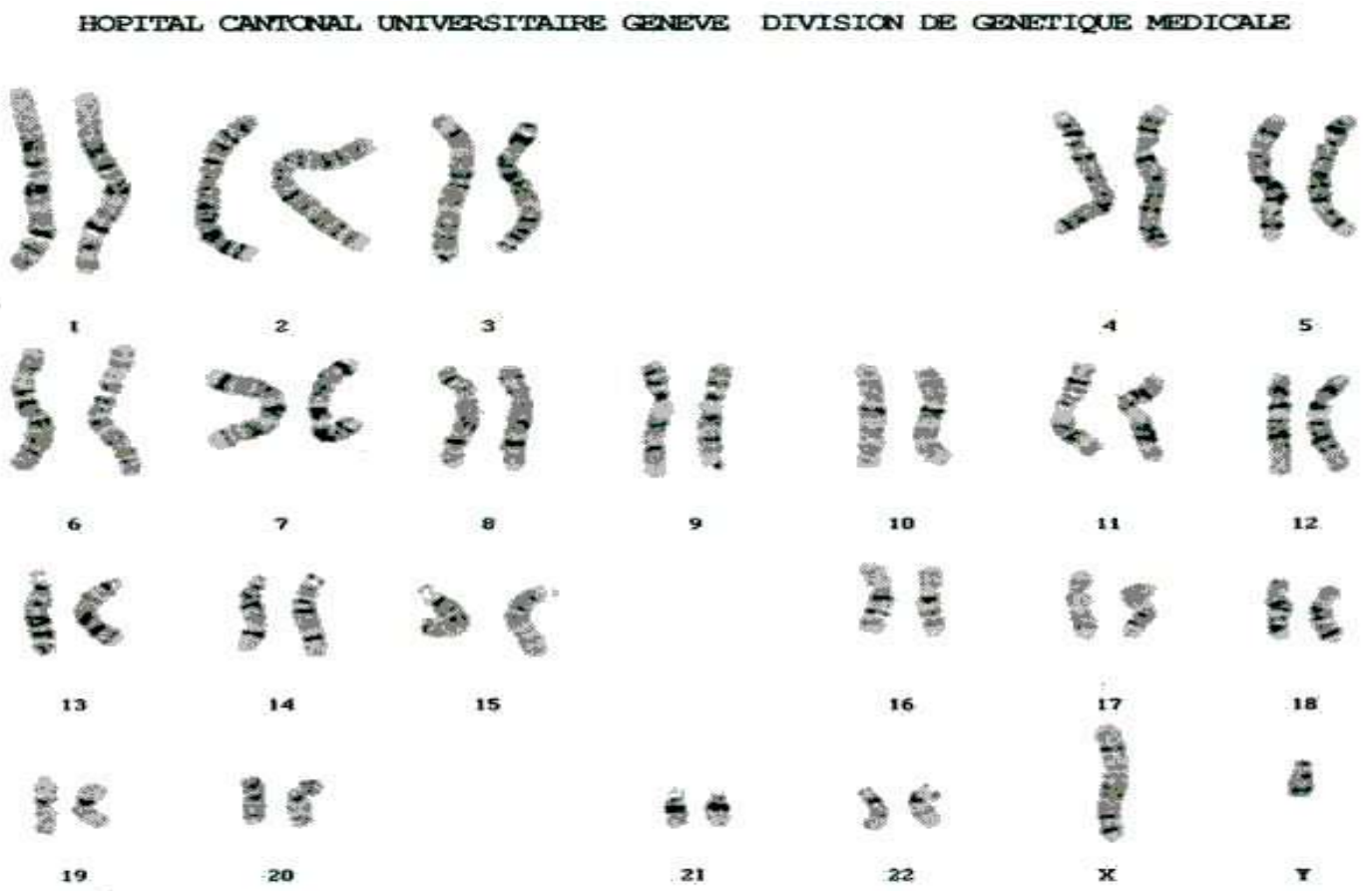
- Definition
- Chromosomal Banding
- Resolution limits
- Nomenclature

The metaphasic chromosome

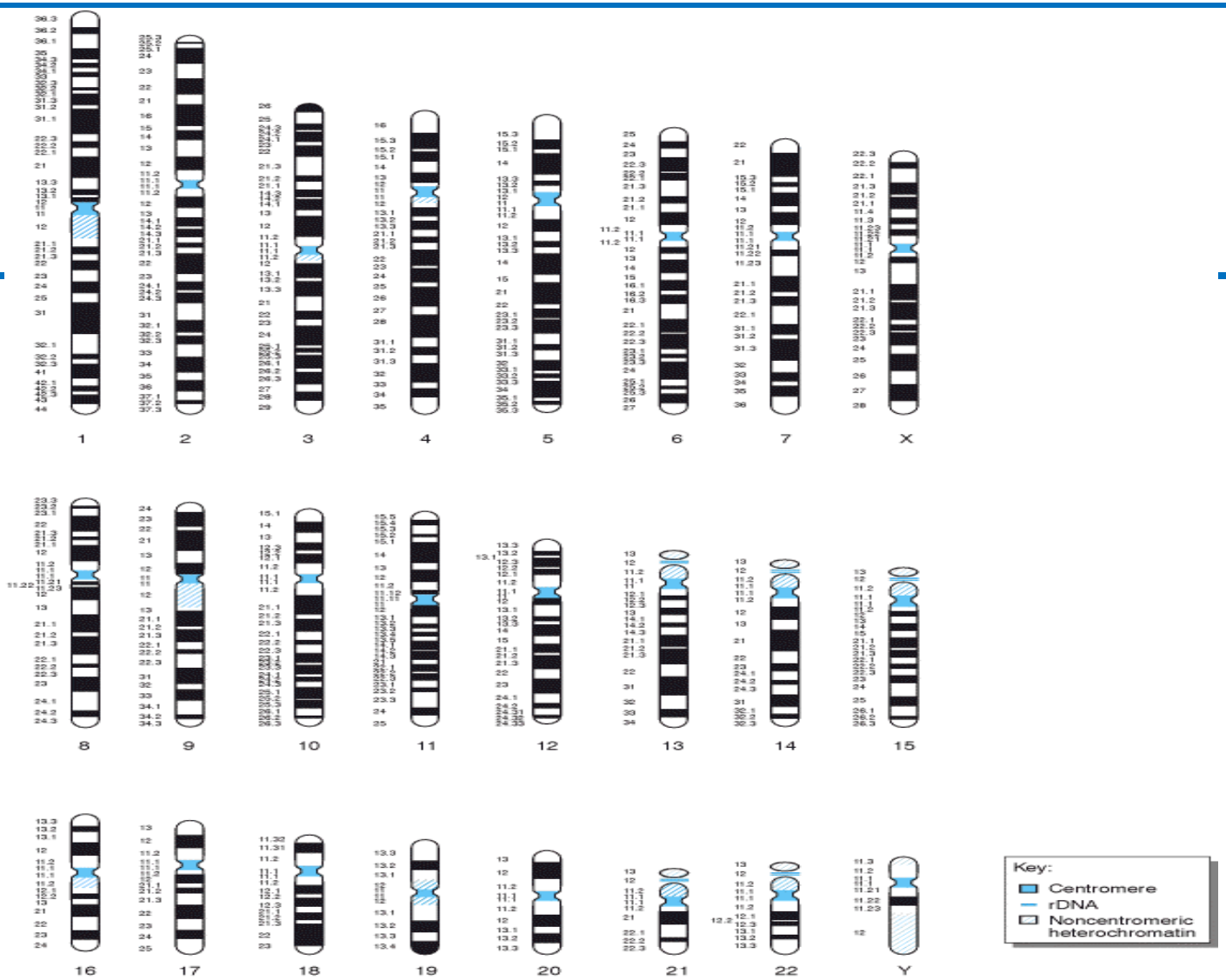


G-banded Human Karyotype

Tjio & Levan 1956



Karyotype: The characterization of the chromosomal complement of an individual's cell, including number, form, and size of the chromosomes.
A photomicrograph of chromosomes arranged according to a standard classification.



A chromosome banding pattern is comprised of alternating light and dark stripes, or bands, that appear along its length after being stained with a dye. A unique banding pattern is used to identify each chromosome

Chromosome banding techniques and staining

- Giemsa has become the most commonly used stain in cytogenetic analysis. Most G-banding techniques require pretreating the chromosomes with a proteolytic enzyme such as trypsin. G-banding preferentially stains the regions of DNA that are rich in adenine and thymine.

- R-banding involves pretreating cells with a hot salt solution that denatures DNA that is rich in adenine and thymine. The chromosomes are then stained with Giemsa.
- C-banding stains areas of heterochromatin, which are tightly packed and contain repetitive DNA.
- NOR-staining, where NOR is an abbreviation for "nucleolar organizing region," refers to a silver staining method that identifies genes for ribosomal RNA.

http://medicine.jrank.org/pages/2026/Chromosomal-Banding-Chromosome-Banding-Techniques.html **Chromosomal Banding - Chromosome Banding Techniques**

Normal male Karyoytype 46,XY

R-banding (right)is the reverse pattern of G bands (left) so that G-positive bands are light with R-banding methods, and vice versa

Limits of resolution

Metaphase Chromosomes at different levels of resolution

300 bands

550 bands

1000 bands

AP/HP PARIS V
Cytogénétique
Cochin

Depending on the length of the chromosomes, the karyotype has a limit of resolution, indicated par the count of bands for a haploid genome

Nomenclature

International System for human Cytogenetic Nomenclature (ISCN) 2009

In designating a particular band,

⇒chromosome number
⇒Arm symbol
⇒Region number
⇒Band number

Description of chromosome abnormalities

⇒Total number of chromosomes including sex chromosomes
⇒Sex chromosome constitution
⇒Numerical abnormalities
⇒For example a female Down syndrome or trisomy 21 is written as 47,XX,+21
⇒Structural changes are designated by letters, for example 'dup' for duplication
Such as 46,XY,dup(1)(q22q25) (duplication of a segment in long arm of chromosome 1, q, in region 2 between bands 22 and 25.

Chromosomes can be studied in any nucleated body cell in an individual

Peripheral blood

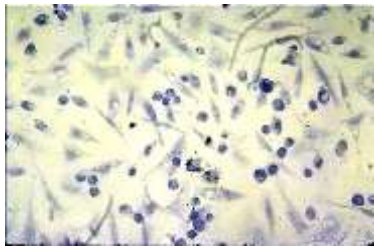
↓

Lymphocyte culture
3 days

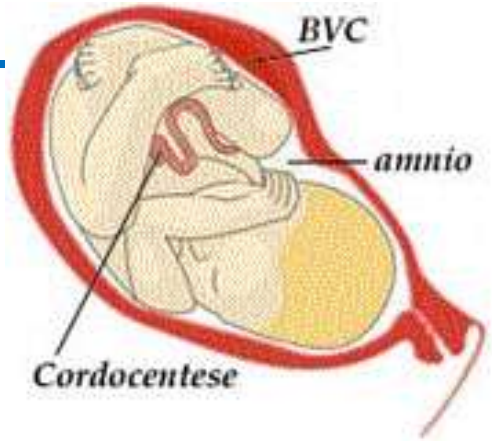
Skin biopsy

↓

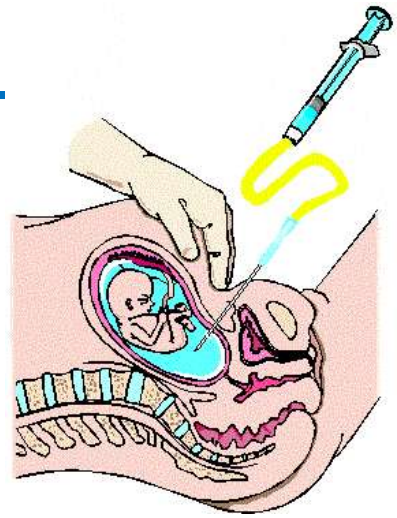
culture of fibroblasts
15 –21 days



Prenatal tests to study fetal chromosomes



Choriocentesis
(Chorion villus biopsy)
Risk of abortion 2-3%



Amniocentesis
Risk of abortion 1%

Choriocentesis ←→

Amniocentesis ←→

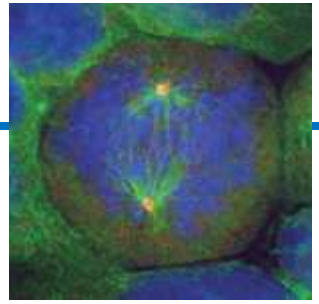
GW 10 11 12 13 14 15 16 17 18 19 20 21 22

Cordocentesis ←→

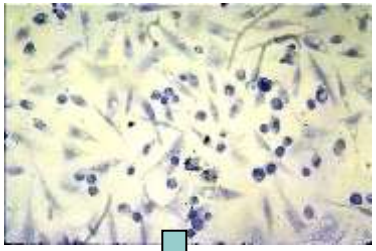
(GW: gestational weeks)

(Blood from umbilical artery)

Chromosome preparation



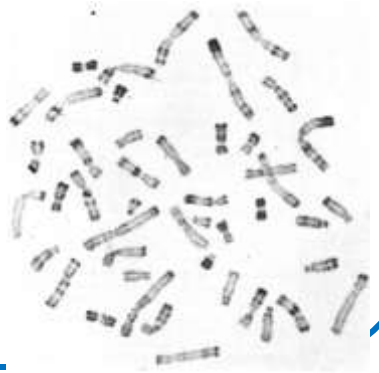
Addition of colchicine inhibits formation of mitotic spindle



Hypotonic solution to disperse chromosomes

Fixation of chromosomes on a slide

Staining of chromosomes

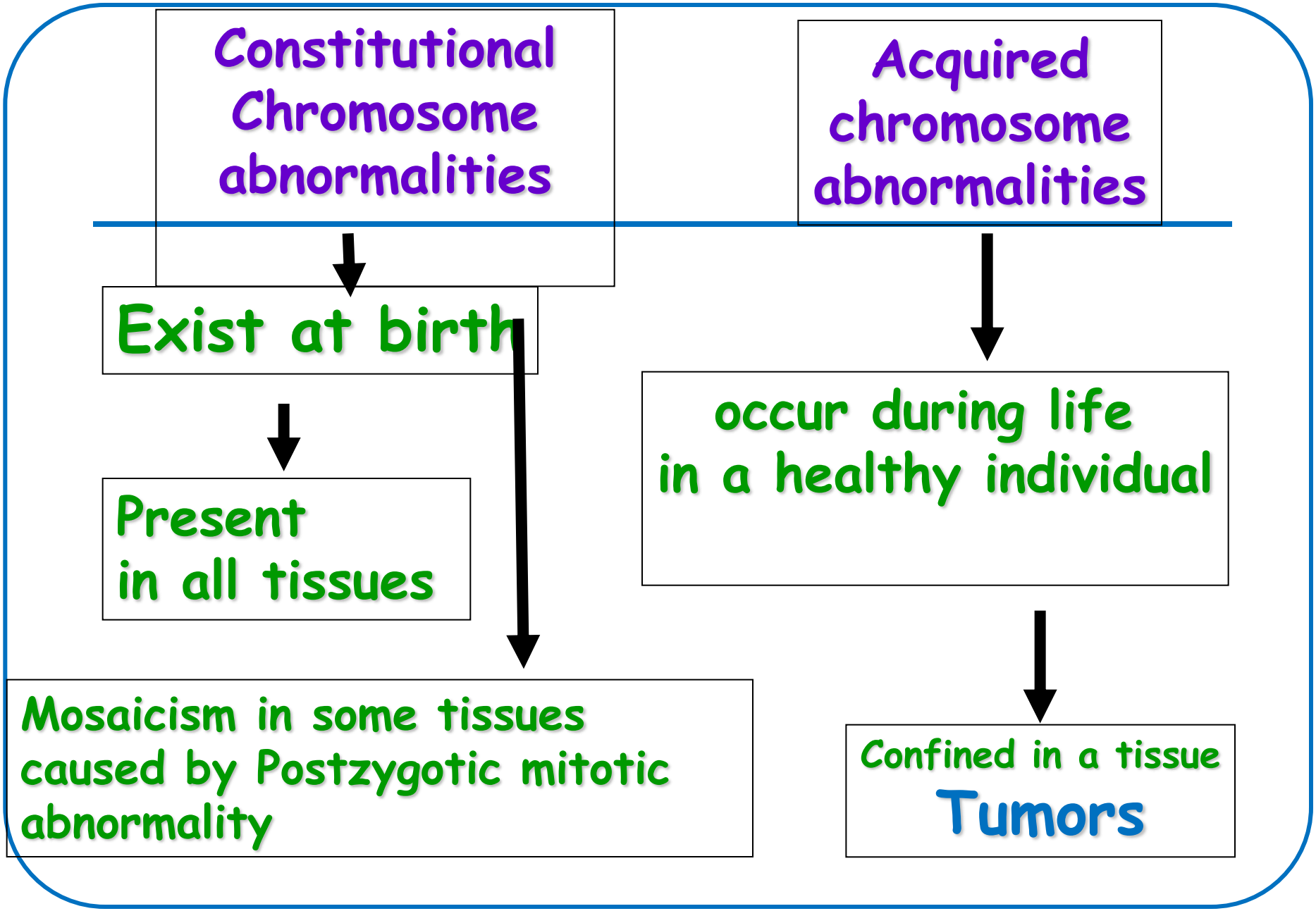


II. Chromosome abnormalities

- Statistics
- Meiosis
  - Description
  - Crossing over, recombination
- Errors of meiosis I
- Errors of meiosis II
- Promoted factors

Chromosome abnormalities

1. Constitutional : exist at birth. These are usually present in all tissues, if present only in some tissues, it is called mosaicism and it means that the abnormality occurred in the mitotic divisions that follow zygote formation
2. Acquired: occur during the life of a healthy individual and are confined to one tissue as sen in tumour cells


Constitutional Chromosome abnormalities
Acquired chromosome abnormalities
Exist at birth
Present in all tissues
Mosaicism in some tissues caused by Postzygotic mitotic abnormality
occur during life in a healthy individual
Confined in a tissue
Tumors

Frequencies of chromosome abnormalities

- 2% of sperms have Chromosomal abnormalities
- 20% of ova have Chromosomal abnormalities
- So among 100 conceptions, there are 25% chromosome abnormalities

Frequencies of chromosome abnormalities

- In every 100 pregnancies, there occurs 15 spontaneous miscarriages, 50% of which have chromosome abnormalities
- Among 160 births, one baby is born with a chromosome abnormality

2% of sperms have Chromosomal abnormalities
20% of ova have Chromosomal abnormalities

100 conceptions

→ 25 Chromosomal abnormalities

100 Pregnancies

→ 15 miscarriages

→ 50% Chromosomal abnormalities

160 Births

→ 1 child
With a Chromosomal abnormality

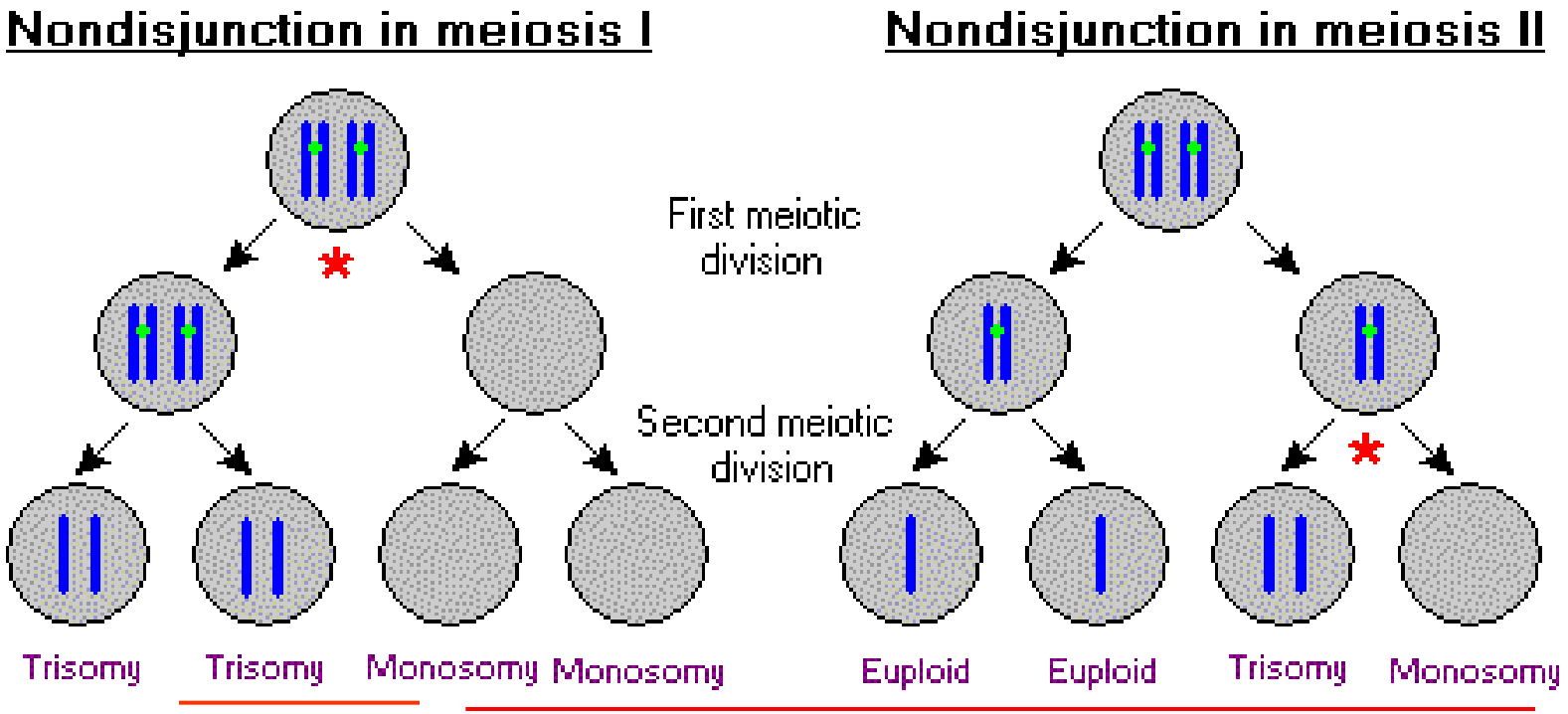
Meiosis

- Is the process of reductional division in which a diploid cell_2N = 46 (2 x sets of chromosomes) is reduced to a haploid cell (N) = 23 (1 set of chromosomes)
- It comprises MI (meiosis I) and MII (meiosis II)
- Meiosis always results in the formation of gametes (ova and sperms)

Non-disjunction in meiosis

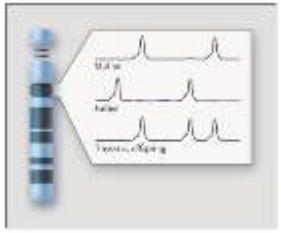
- This is an abnormal division where one daughter cell gets an extra chromosome (24) and the other daughter cell gets one chromosome less than normal (22).
- It can happen in MI or MII.
- Fertilisation with a normal gamete gives either a trisomic zygote (24+23=47) or a monosomic zygote (22+23=45)

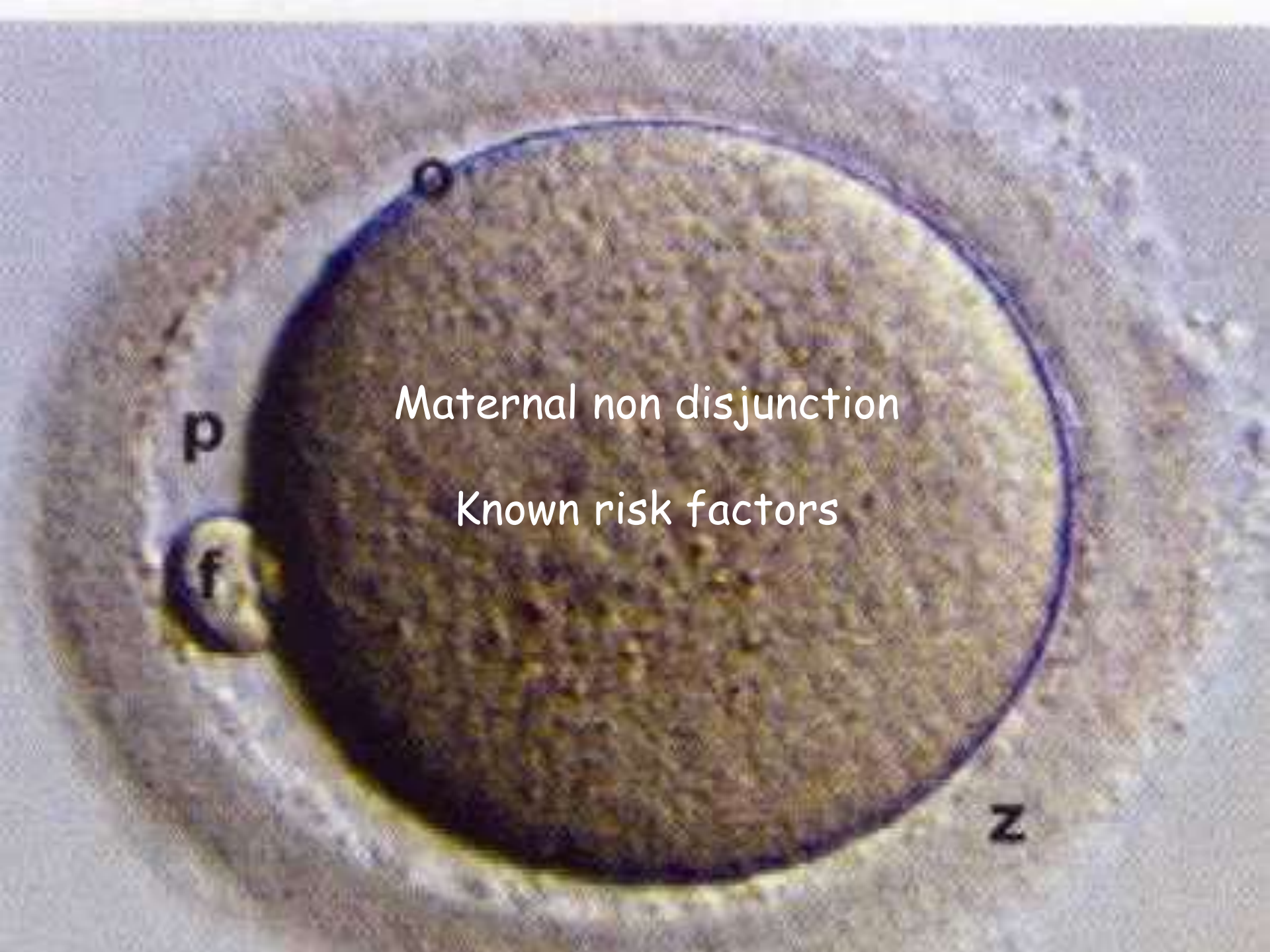
Mechanism. Meiotic nondisjunction



Electrophoresis profiles

offspring after ferilization with another normal gamete



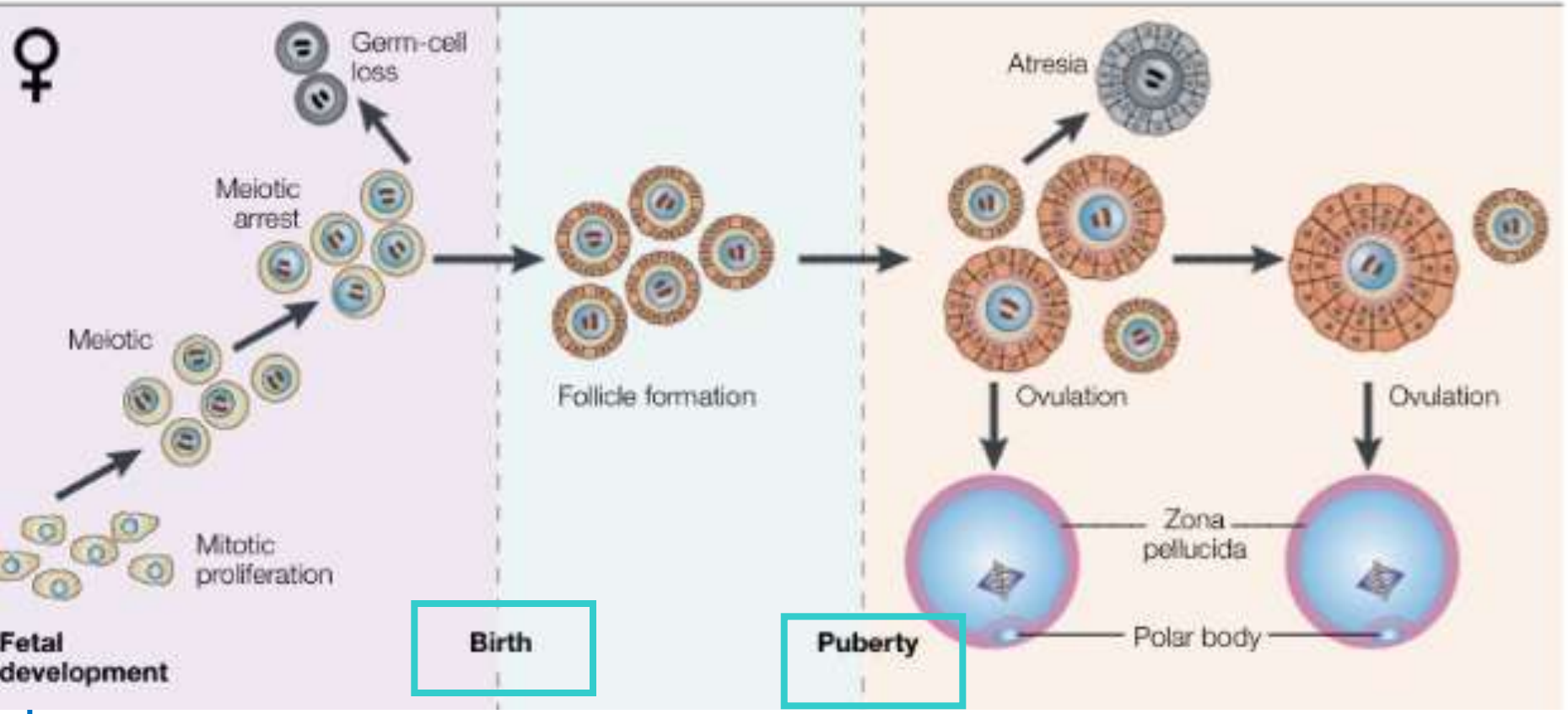


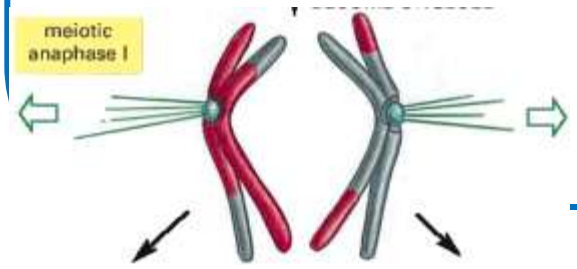
Maternal non disjunction

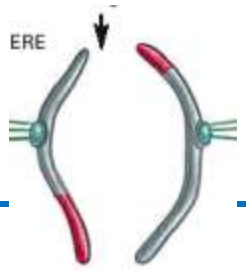
Known risk factors

Period of gametogenesis in the female

meiosis starts at intrauterine life with ovulation starting at puberty. Each month one ovum is produced and 1000 follicles become undergo atresia



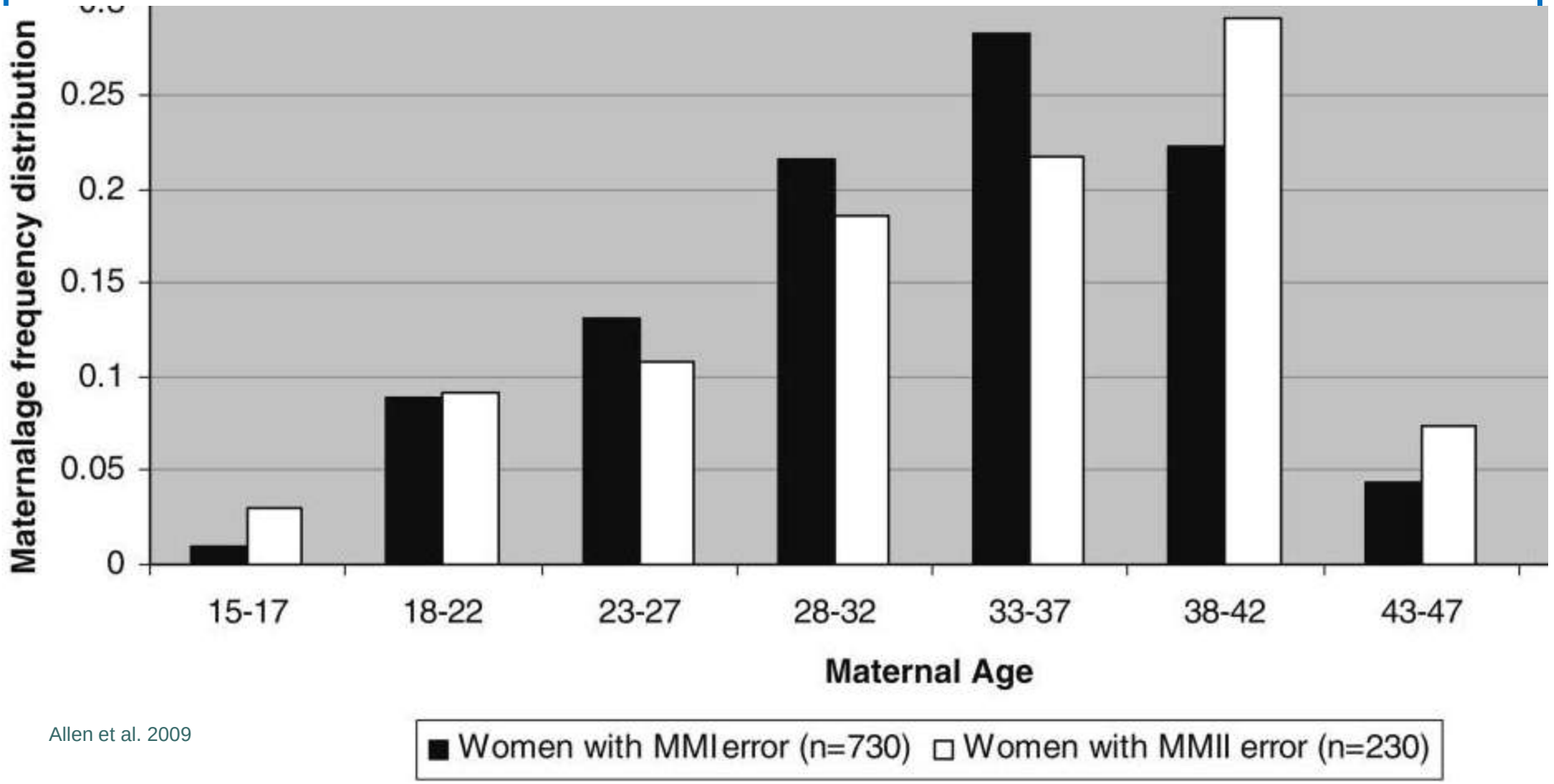




Known predisposing causes for non-disjunction in the female

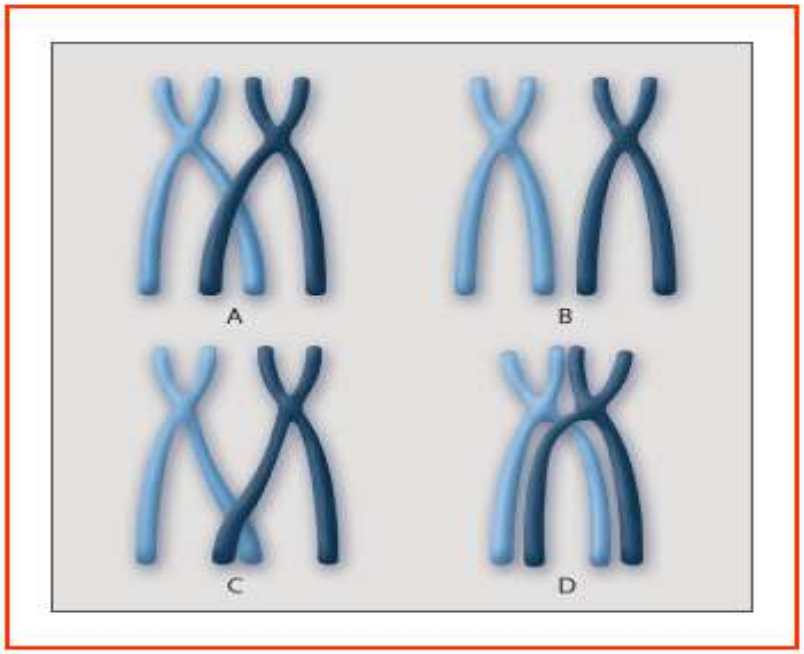
- Advanced maternal age
- Sites and rate of meiotic recombination (crossing over or chiasma formation)
- Genetic factors
- Mosaicism with trisomic cells in ovaries

Advanced Maternal Age



Allen et al. 2009

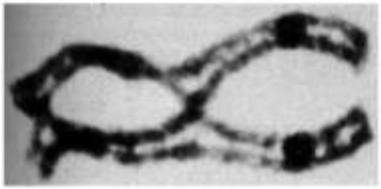
Recombination and non disjunction



- Normal
  - 1chiasma/chromosome A
- Trisomy 21 MMI,
  - 45% achiasma B
  - 41% 1 telomeric chiasma C
- Trisomy 21 MMII
  - Pericentromeric Chiasma D

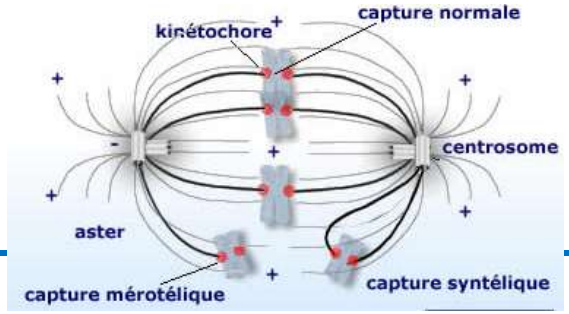
Two-hit model of non disjunction

- Establishment of "susceptible " exchange in the fetal oocyte



Diplotene

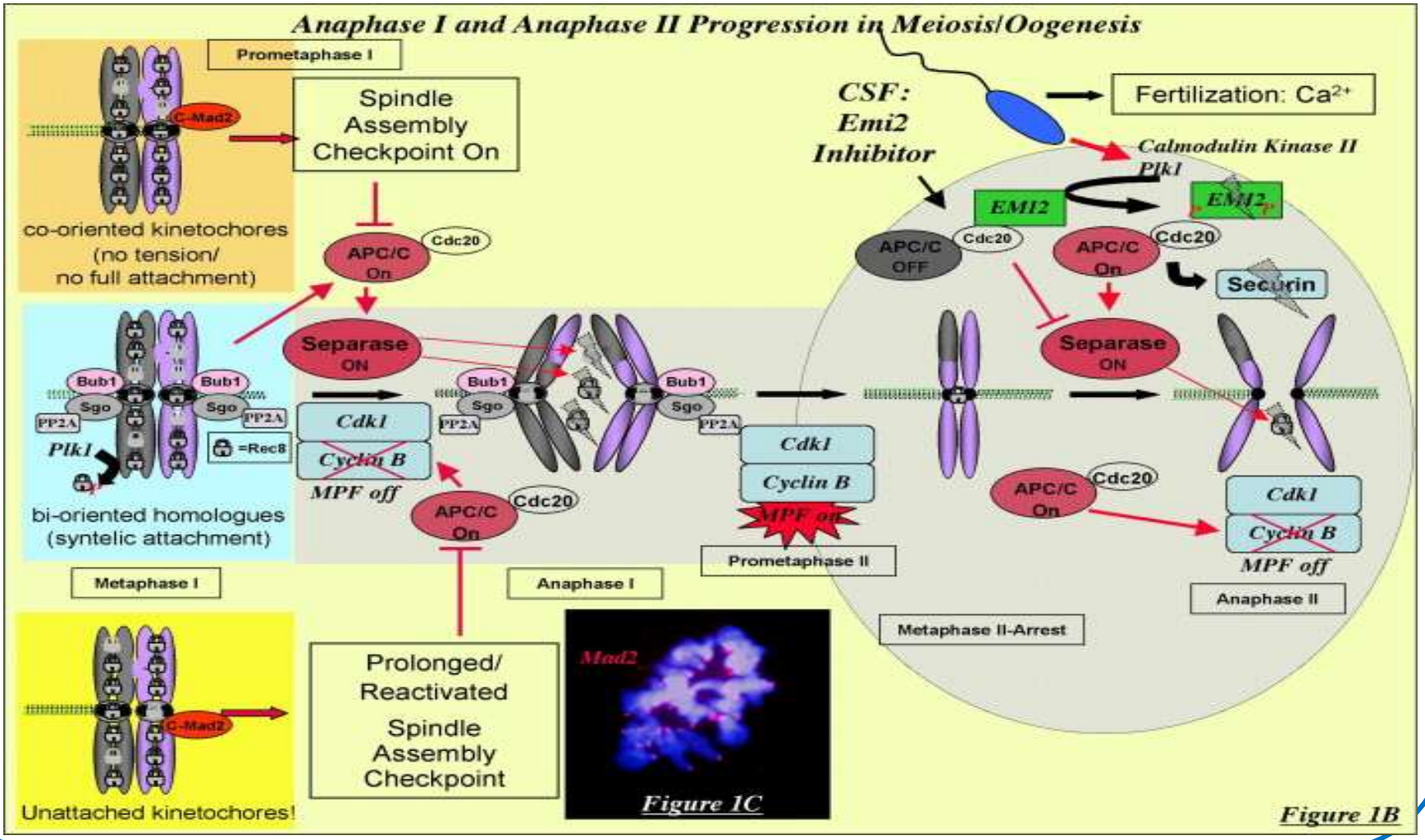
- Age dependant abnormal processing



Genetic factors

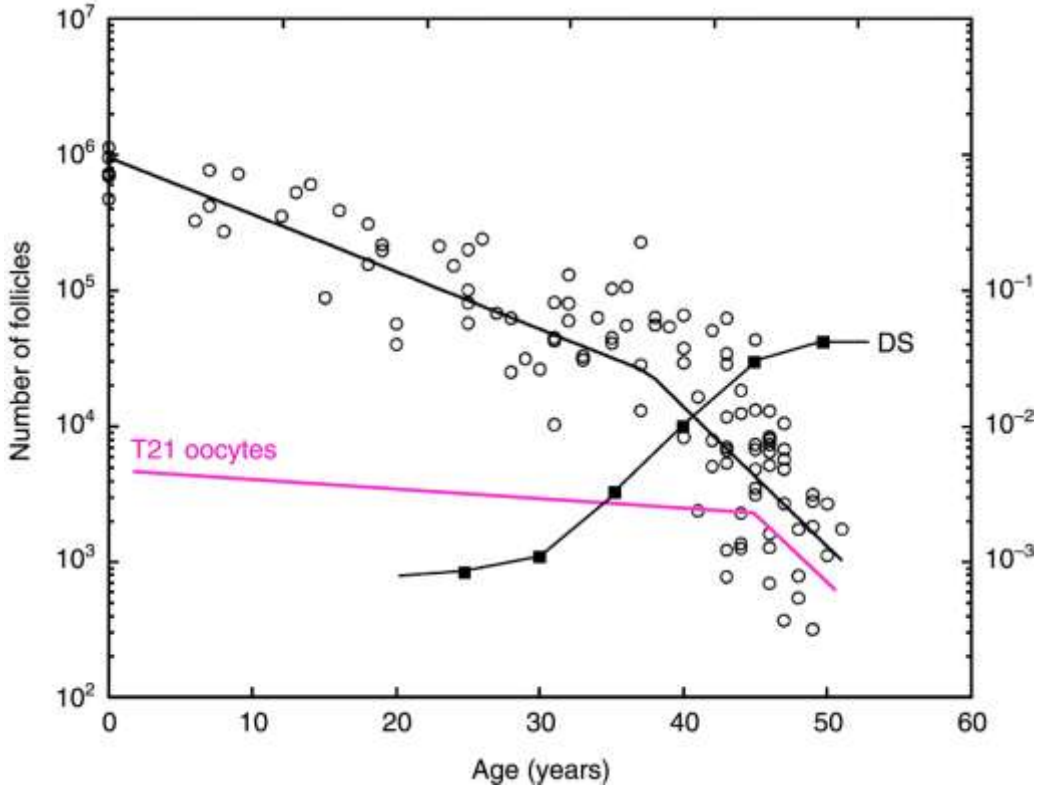
- Homologous chromosomes pairing
- Assembly of the synaptonemal complex
- Chiasmata formation
- Sister chromosome cohesion
- Spindle formation
- etc…

Mutations in the genes that function during meiosis may play a role in causing non-disjunction



Vogt 2008

Germinal mosaicism: the gonads have some cells with trisomy 21 and so some gametes are trisomic



0.54% mosaicism observed by Hultén *et al*. (2008).

accumulation of trisomy 21 oocytes in the ovarian reserve of older women

Paternal non disjunction

datas

Where did non disjunction causing trisomic Down syndrome occur?

- Maternal MI 69%
- Maternal MII 21.5%
- Paternal MI 2%
- Paternal MII 3.5%
- Post zygotic 4%

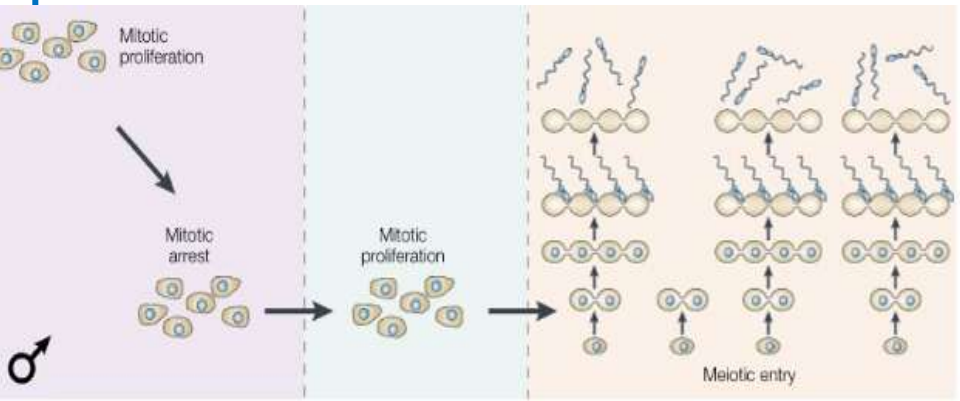
Table 1. Origin of nondisjunction in human trisomy 21 by DNA polymorphism analysis

Origin[a]	Number of cases	%	Meiotic recombination
Maternal	732	90.7%	
MI	556	68.9%	Reduced
MII	176	21.8%	Increased
Paternal	44	5.5%	
MI	17	2.1%	Reduced
MII	27	3.3%	
Mitotic	31	3.8%	
"Maternal"	17	2.1%	
"Paternal"	14	1.7%	

[a] MI = meiosis I, MII = meiosis II, "Maternal" and "Paternal" refer to the parental origin of the chromosome that was duplicated by postzygotic nondisjunction. Data from Antonarakis et al. (1993), Lamb et al. (1996), Savage et al. (1998).

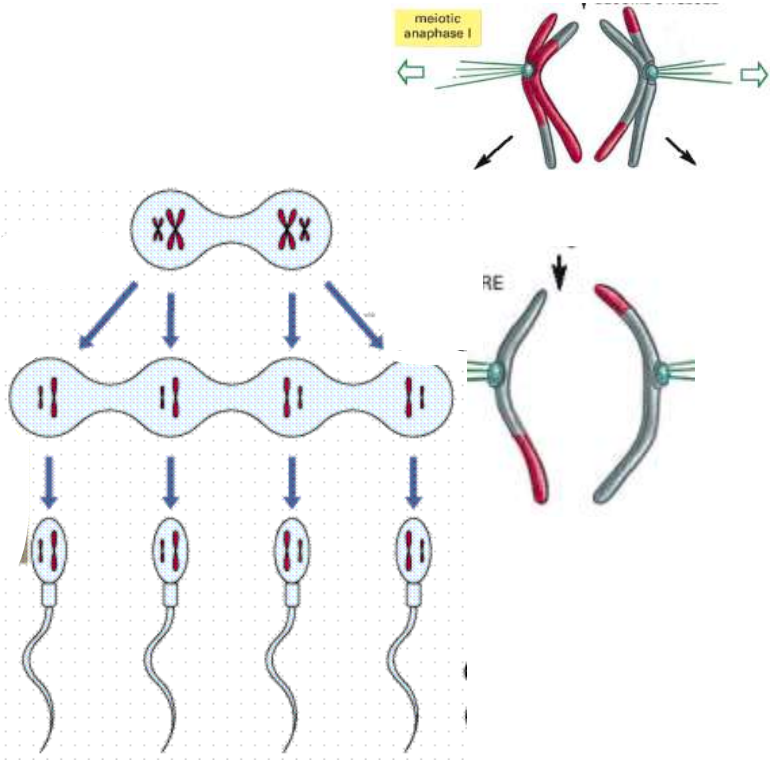
M.B. Petersen and M. Mikkelsen 2000

Period of gametogenesis in the male
Meiosis starts at puberty



birth

puberty



Paternal age

Table 2. Mean parental ages by origin of non-disjunction in population-based newborn studies

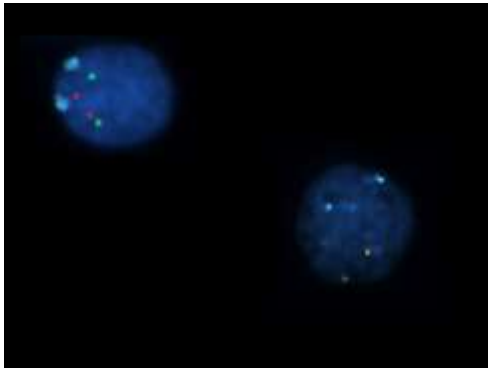
Origin[a]	n	Maternal age	Paternal age
Maternal			
MI	145	30.1	32.0
MII	50	31.2	33.3
Paternal			
MI+MII	16	25.6	29.9
Mitotic	12	28.2	30.5

[a] MI = meiosis I, MII = meiosis II. Data from Mikkelsen et al. (1995) and Yoon et al. (1996).

M.B. Petersen and M. Mikkelsen 2000

Germinal mosaicism

- FISH to determine testicular T21 mosaicism in four male fetuses showed that male 21 trisomy germinal mosaicism is very low compared to female ovarian T21 mosaicism



Hultén MA et al;2010

Chromosomal abnormalities

- Numerical
  - Unbalanced
  - Autosomal
  - Sex chromosomes
- Structural
  - Unbalanced vs balanced
  - Transmission

Consequences of chromosomal abnormalities

Depends on presence or absence of unbalanced chromosome constitution

Unbalanced
Phenotypic consequences

Balanced
Normal phenotype

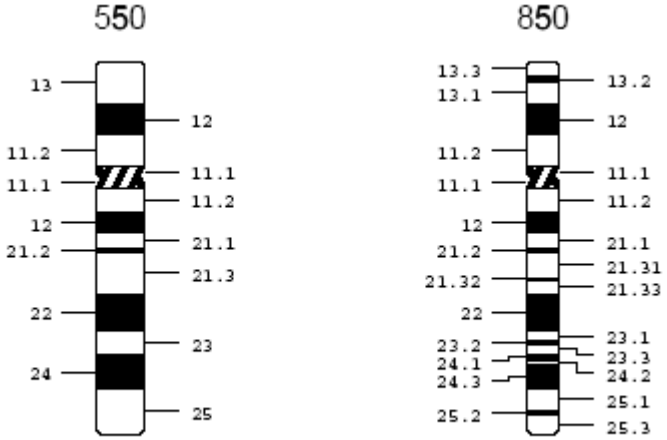
Chromosomal abnormalities

Numerical

21

Always unbalanced

Structural



Unbalanced or Balanced

Abnormal Phenotype

Normal phenotype

Numerical Anomalies (Aneuploidies)

Extra Chromosomes

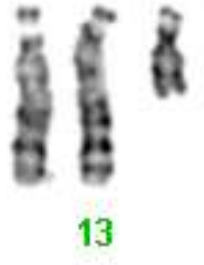
+1 Trisomy 47
+2 Tetrasomy 48
+3 Pentasomy 49
+23 Triploidy 69
+46 Tetraploidy 92

Deficient Chromosome

-1 Monosomy 45

Chromosome's Segment

Partial Trisomy



Viable aneuploidies

Autosomes

extra or deficient chromosome material

- Mental Retardation
- Dysmorphy
- +/- Internal Malformations
- +/- Growth Retardation

Chromosome syndromes

Down's syndrome
Trisomy 21

Edward's syndrome
Trisomy 18

Patau's syndrome
Trisomy 13

Malformations (examples)

- Congenital heart defects
- Renal abnormalities
- Brain abnormalities

Down syndrome

Frequency: 1/800 livebirths

In newborn: hypotonia and dysmorphic features

Frequently associated malformations :

- Cardiovascular in 50% of cases
- Digestive: duodenal atresia or stenosis

Mental retardation :

- IQ around 50 at 5 years of age.

Chromosome abnormalities in Down syndrome

- 95% trisomy 21
- 2.5% translocation of chromosome 21 and another acrocentric chromosome
- 2.5% mosaicism

Aneuploidies of sex chromosomes

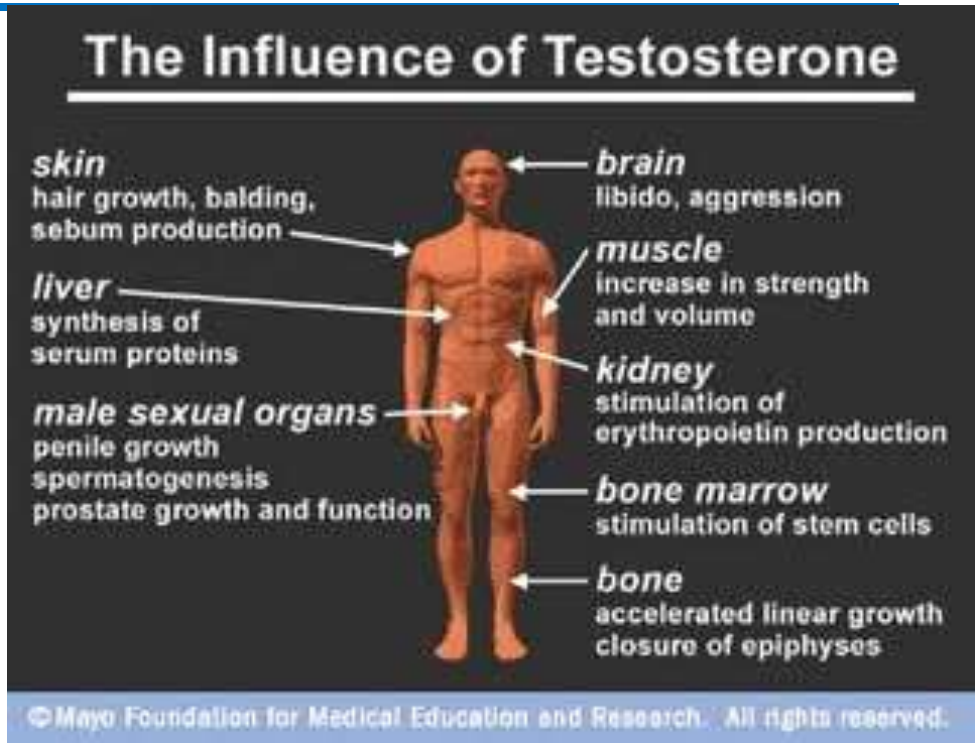
Mildly or not dysmorphic
Mild or no mental retardation

+/- height

Fertility problems

Klinefelter syndrome

- No frontal baldness
- Poor beard growth
- Breast development
- Female type pubic hair pattern
- Small testicles
- Long legs

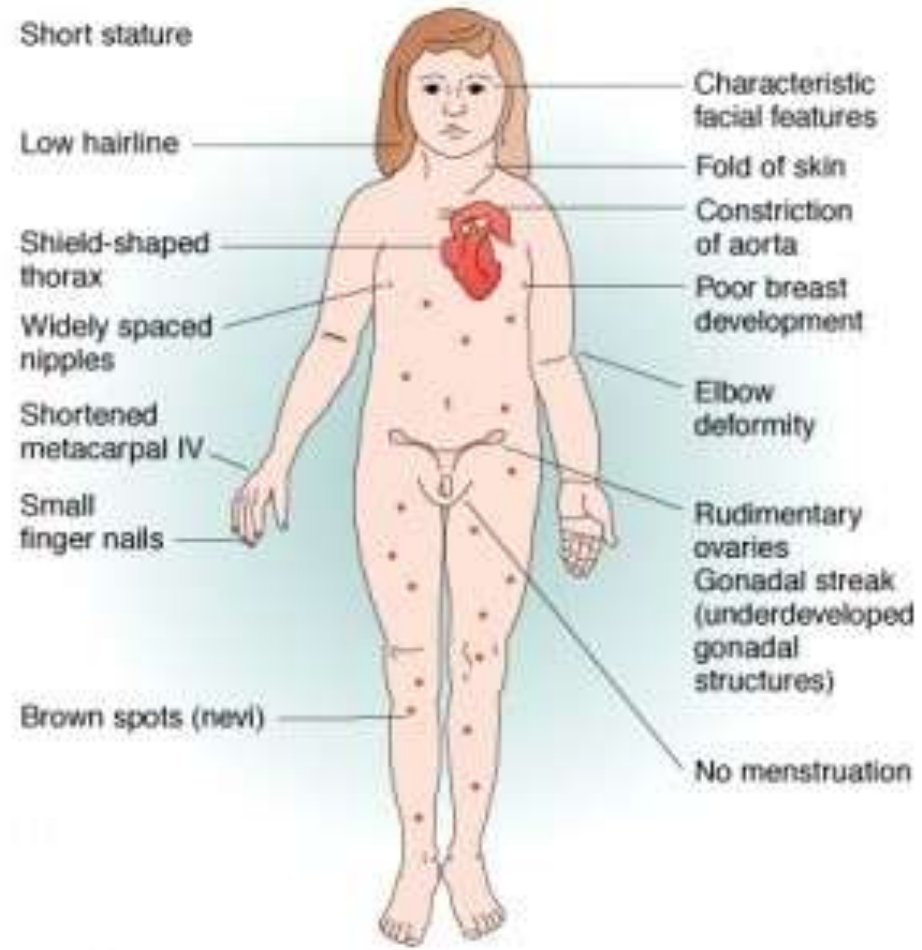


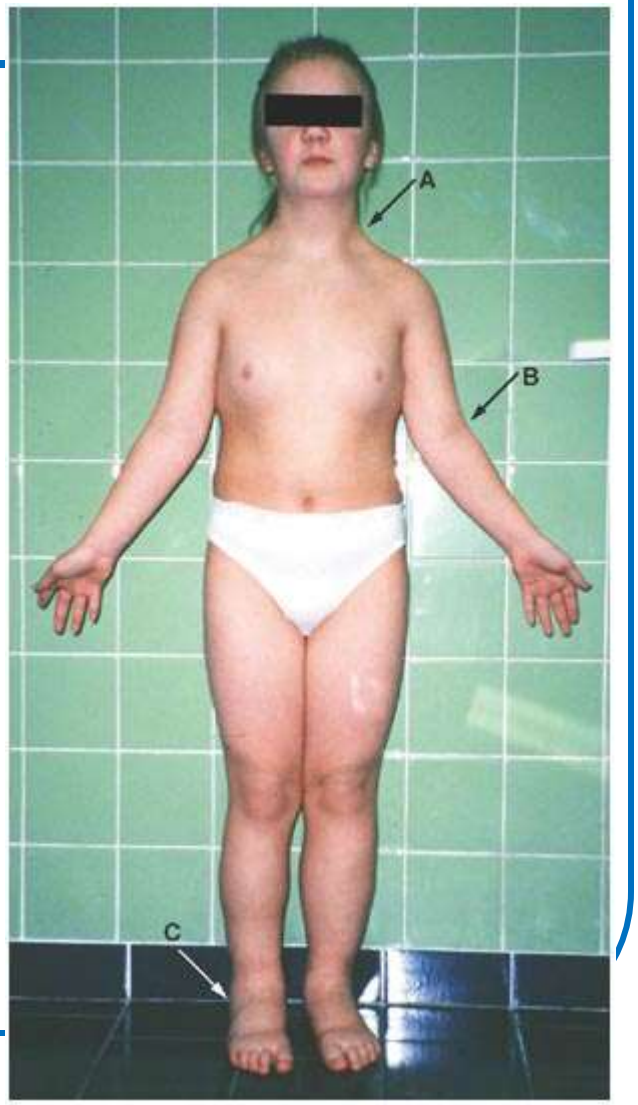
Cytogenetics :

85 % 47,XXY in all the studied cells

15% ***mosaics*** **47,XXY/46,XY or 47,XXY/46,XX**

Turner Syndrome





Cytogenetics Turner syndrome

45,X in 50% of cases, the X chromosome is of maternal origin in 76 % of the cases

45 % of the remaining cases are either numerical variation or structural variation

mosaic : 46,XX/45,X

structural anomalies (could be mosaic) :

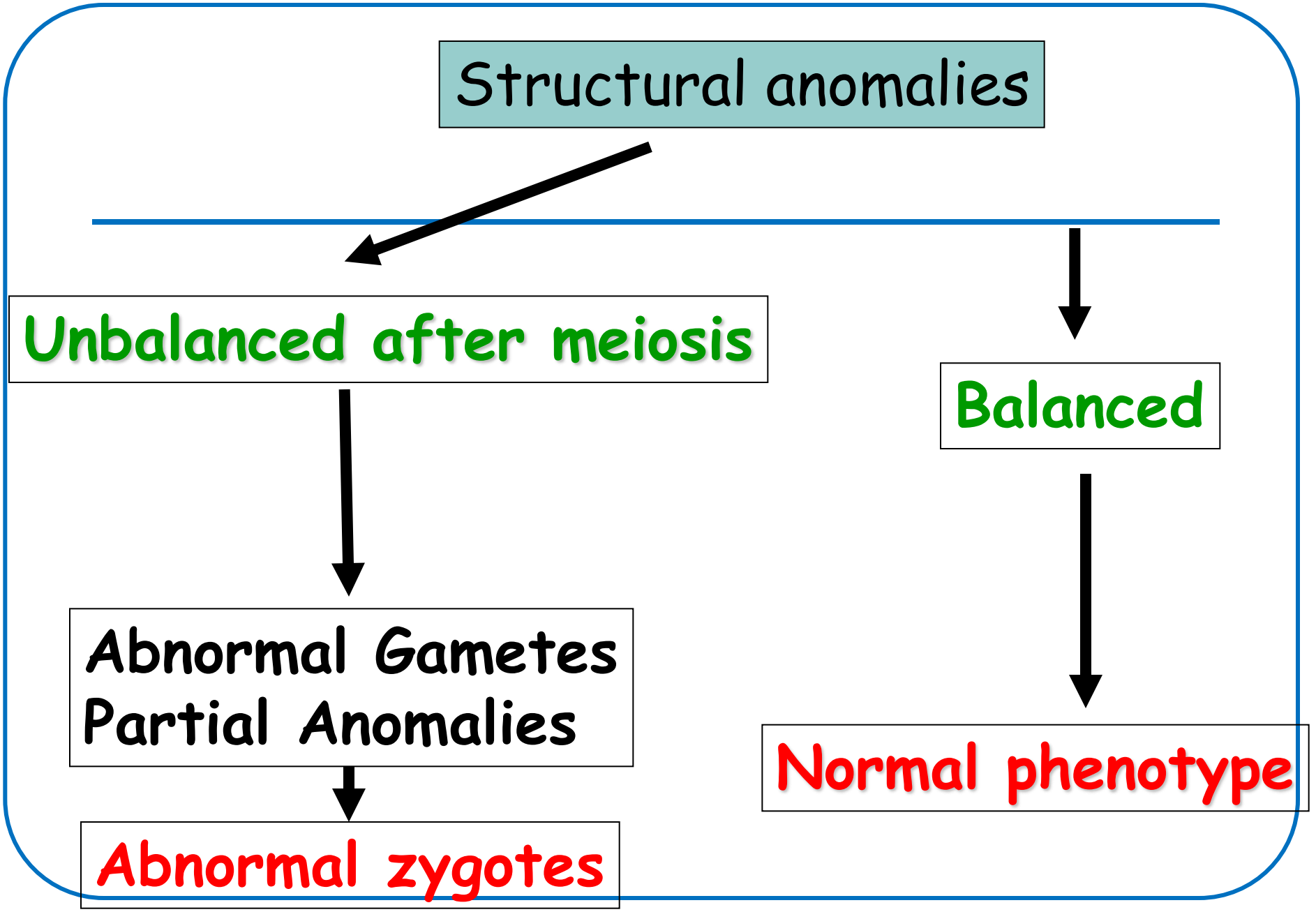
ring X : 46,X,r(X)

deletions : del Xp,del(Xq)

isochromosome X : 46,X,i(Xq)

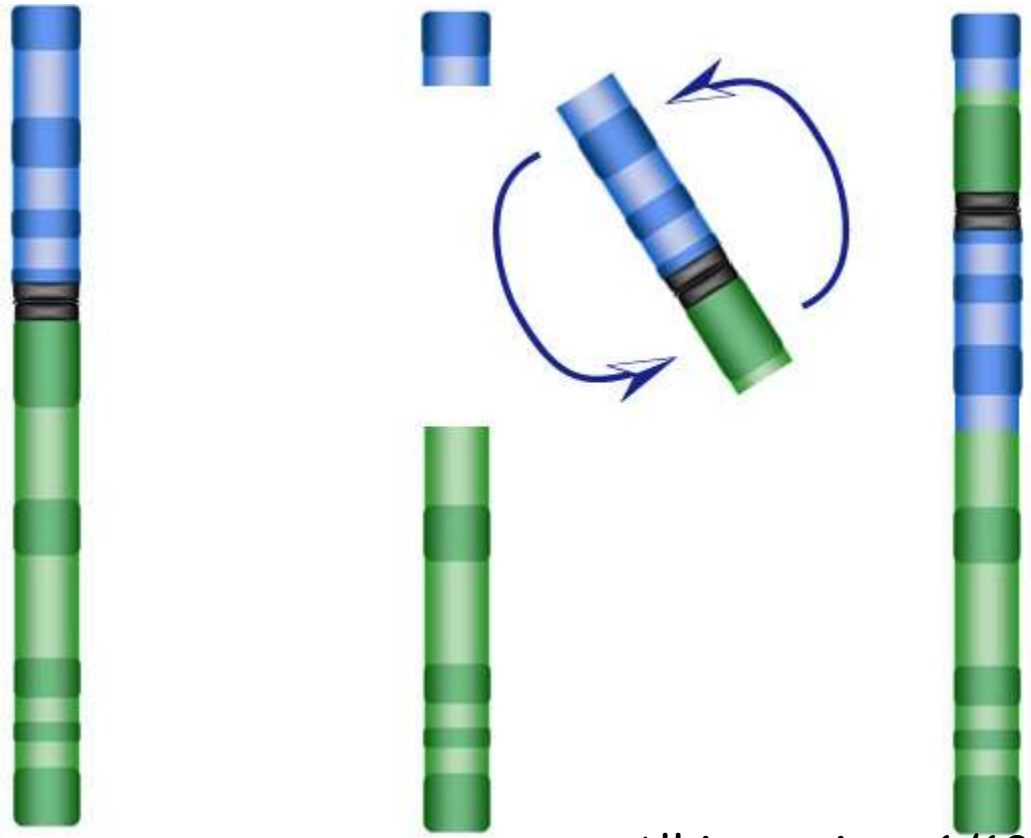
Structural Balanced Anomalies

- 1 Chromosome
  - Inversion
    - pericentric
    - paracentric
- 2 Chromosomes
  - Translocation
    - reciprocal
    - Robertsonian
  - Insertion
- Complex


Structural anomalies
Unbalanced after meiosis
Balanced
Abnormal Gametes
Partial Anomalies
Normal phenotype
Abnormal zygotes

Pericentric Inversion

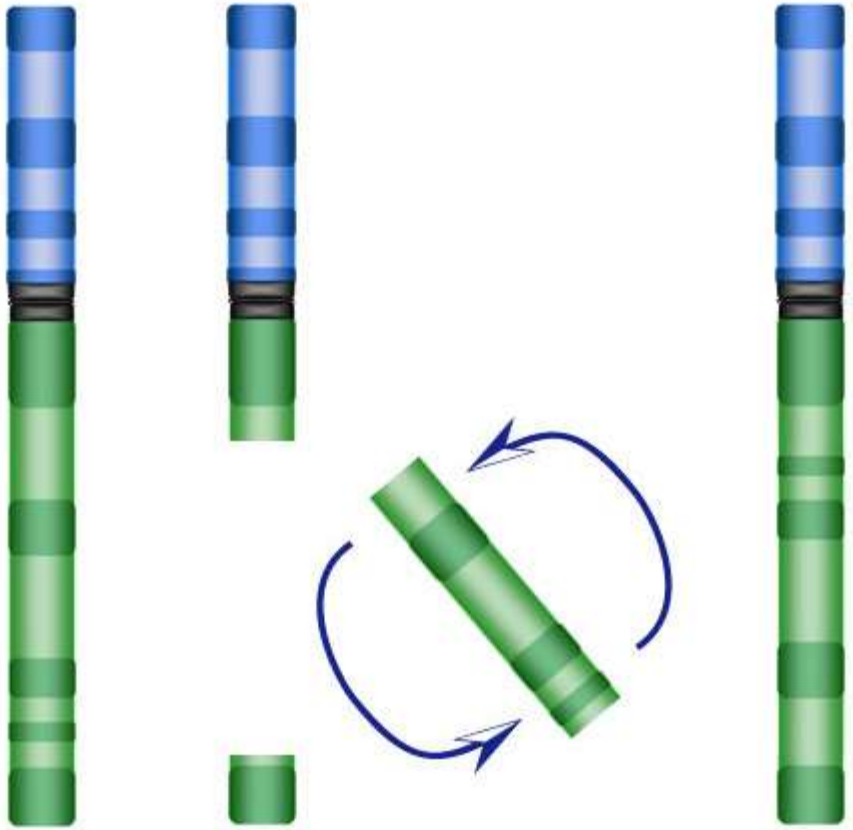
1 chromosome
2 breakpoints



All inversions 1/1000 newborns

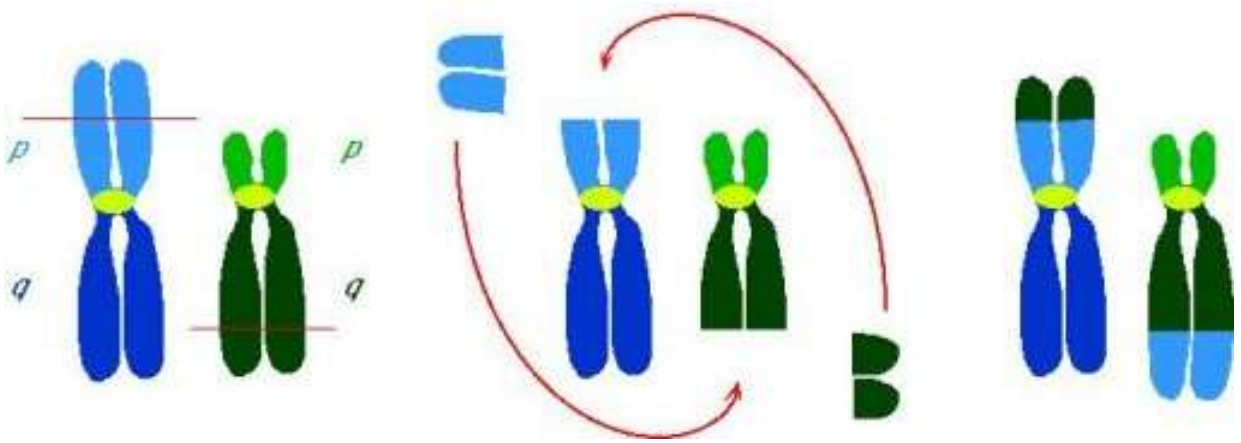
Paracentric inversion

1 chromosome
2 breakpoints



Reciprocal translocation

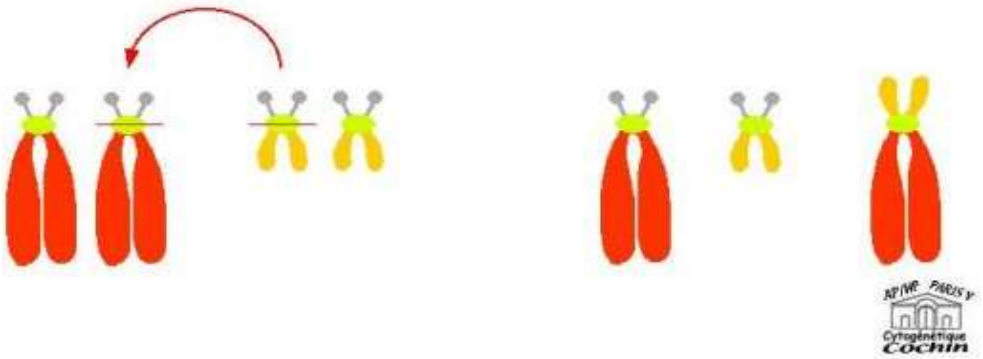
2 chromosomes
2 breakpoints



all translocations 1/500 newborns

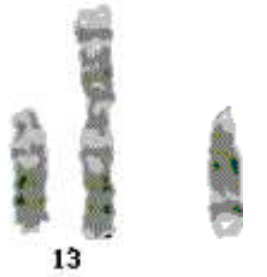
Example: translocation between q arm of a choromosome 11 and q arm of a chromosome 22

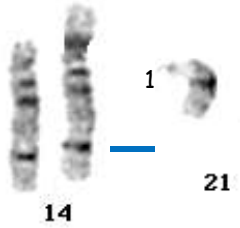
Robertsonian Translocation
ACROCENTRICS



Robertsonian translocations 1/833 newborns
Evans et al.1978

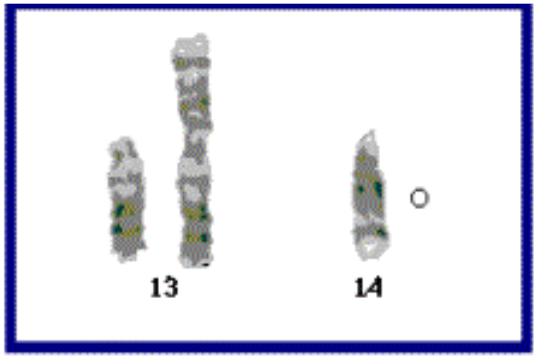
45,der(13;14)(q10;q10) => 73%
45,der(14;21)(q10;q10) => 10%



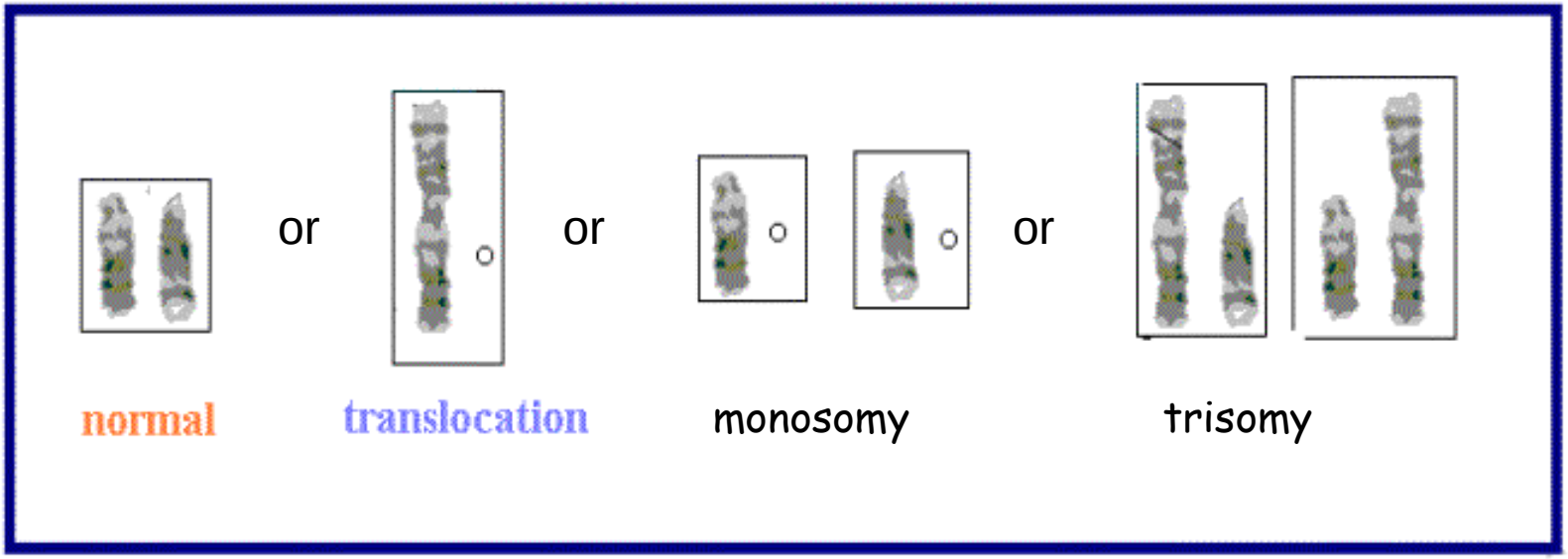


Meiosis chromosomal segregation of a t(13;14)translocation

Constitutional caryotype

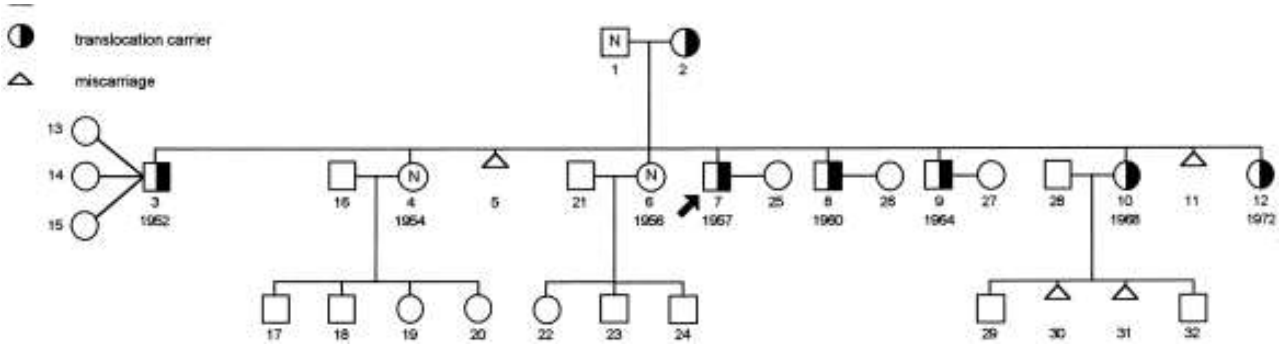


Gametes



Clinical Consequences of a Translocation

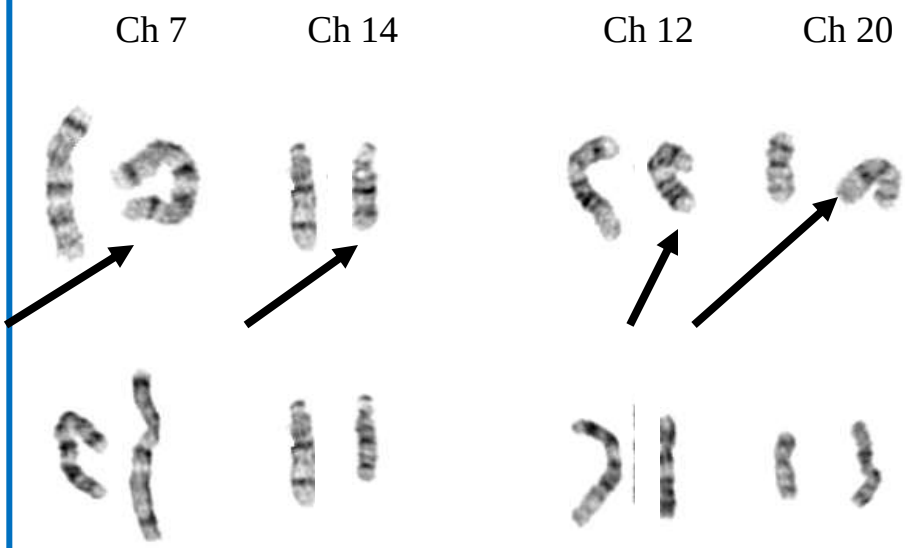
- **Infertility**
- **Miscarriages**

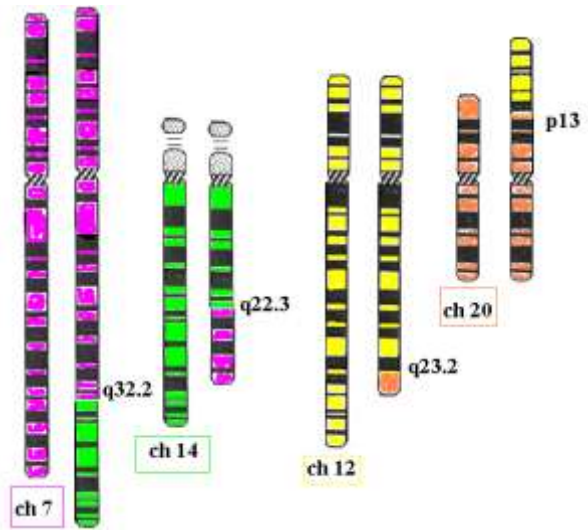


- **Trisomy** by transmission of unbalanced translocation

Ogur *et al.* 2006, Berend *et al.* 2000, 2002

Partial Karyotype (GTG banding) of the double translocation t(7;14)(q32.2;q22.3),t(12;20)(q23.2;p13)





Exemple of complex karyotype with 2 familial translocations

Unbalanced Structural Anomalies

Complex

1 Chromosome

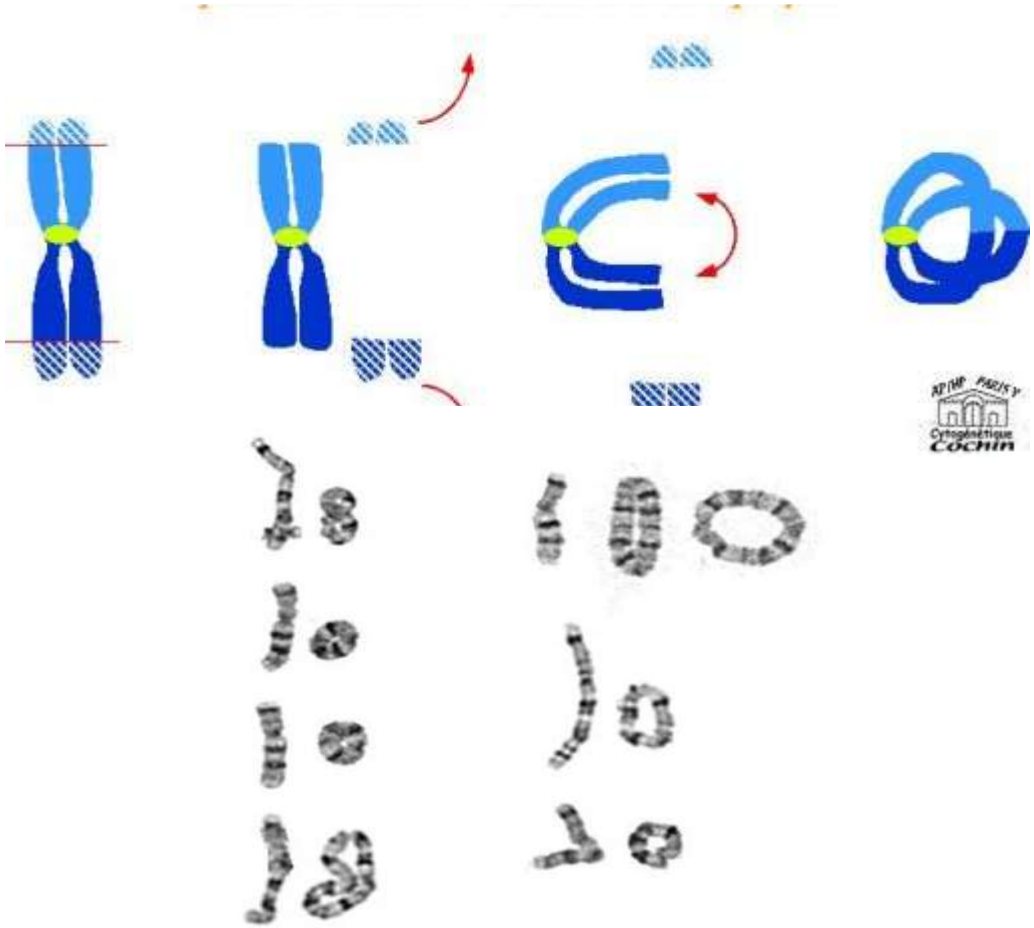
Deletion
Duplication
Ring

2 Chromosomes

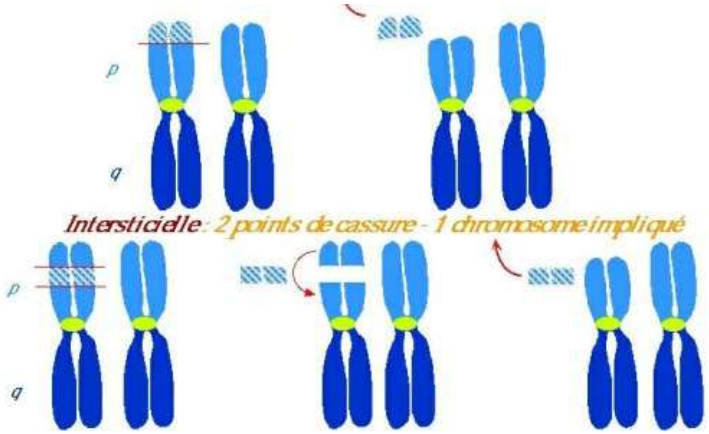
Translocation
Insertion

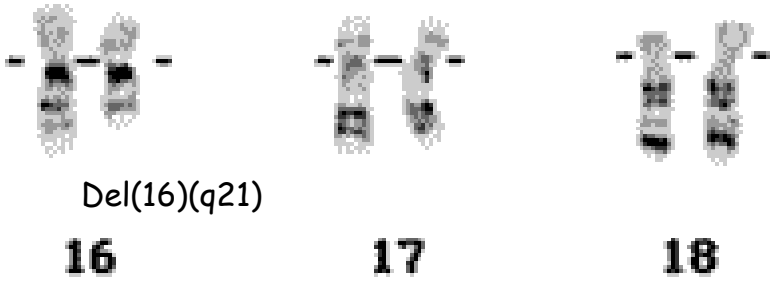
Ring

1 chromosome
2 breakpoints

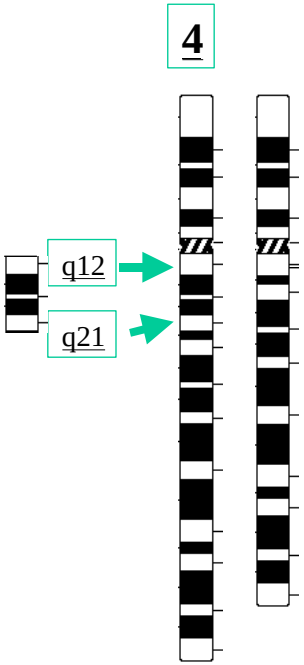


Deletion





Terminal deletion
1 chromosome
1 breakpoint

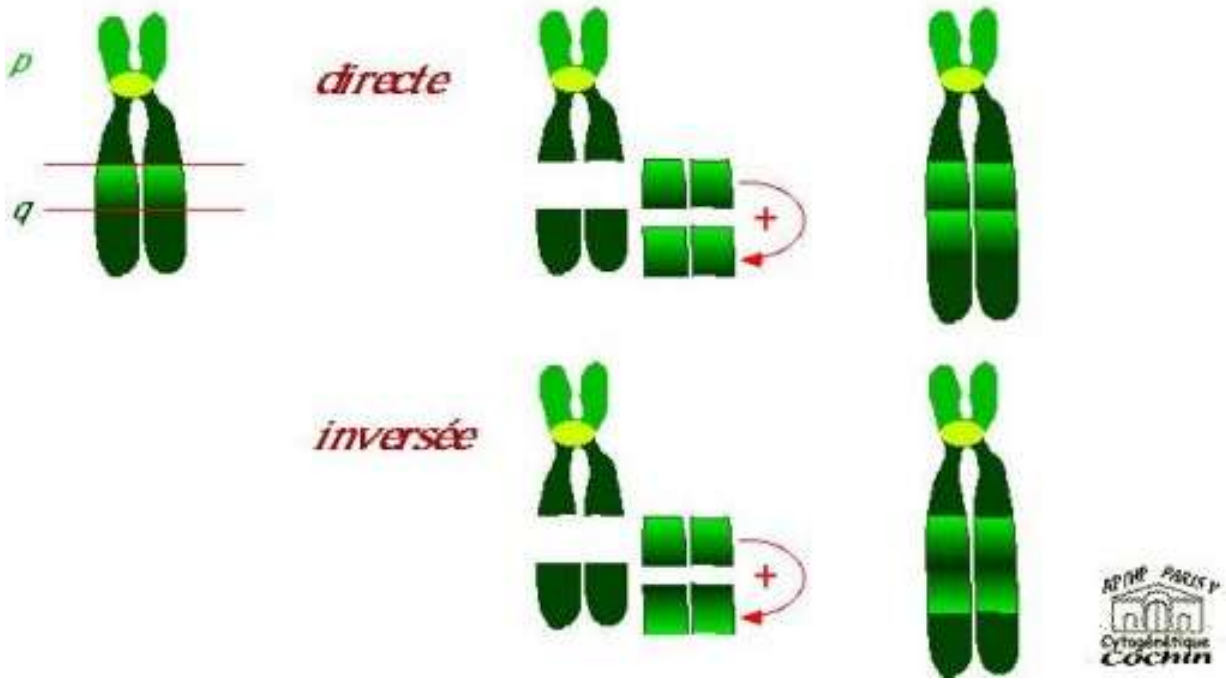


del(4)(q12q21.1)

Interstitial deletion
1 chromosome
2 breakpoints

Duplication

1 chromosome
2 breakpoints



Conclusions

- Chromosomes can be studied in any nucleated cell postnatally as well as prenatally from chorion villus samples and amniocytes
- 1/160 newborns has a chromosome abnormality
- The most common syndromes are Down syndrome (trisomy 21) and Klinefelter syndrome (47,XXY)