

Cytogenetics Chromosomal Genetics

Sophie Dahoun

Service de Génétique Médicale, HUG

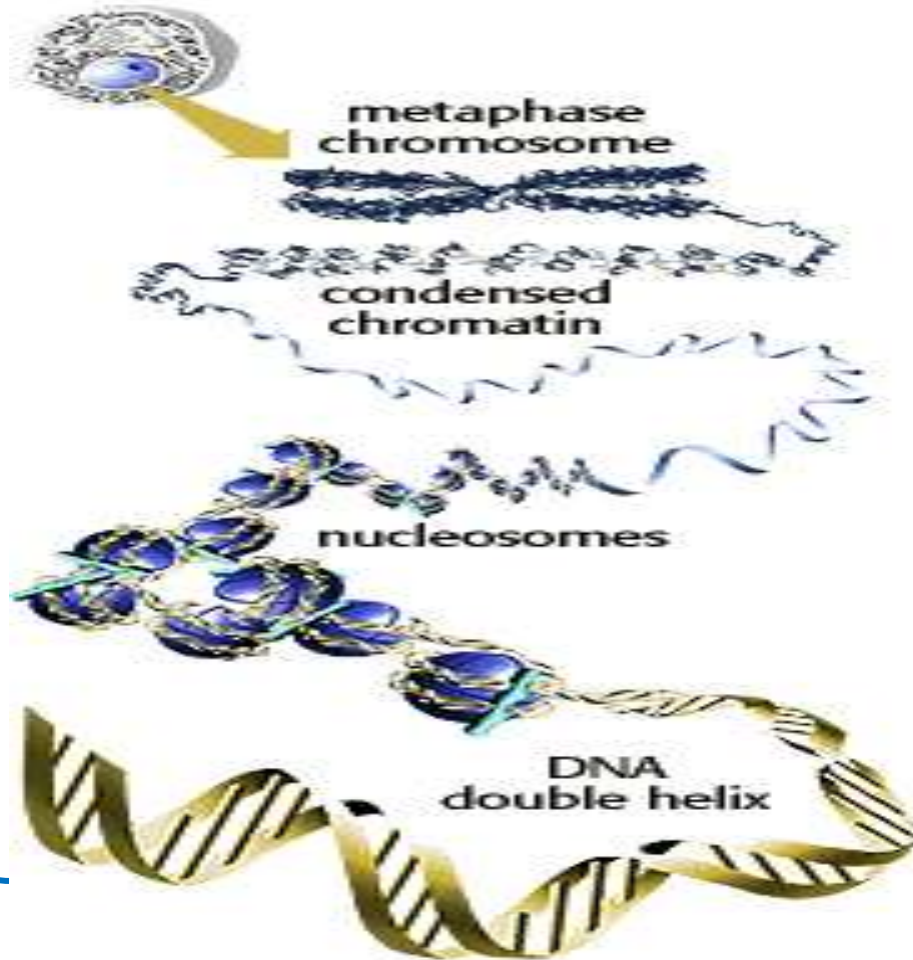
Geneva, Switzerland

sophie.dahoun@hcuge.ch

Training Course in Sexual and Reproductive Health Research
Geneva 2010

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

DNA packs tightly into metaphase chromosomes



Conventional cytogenetics

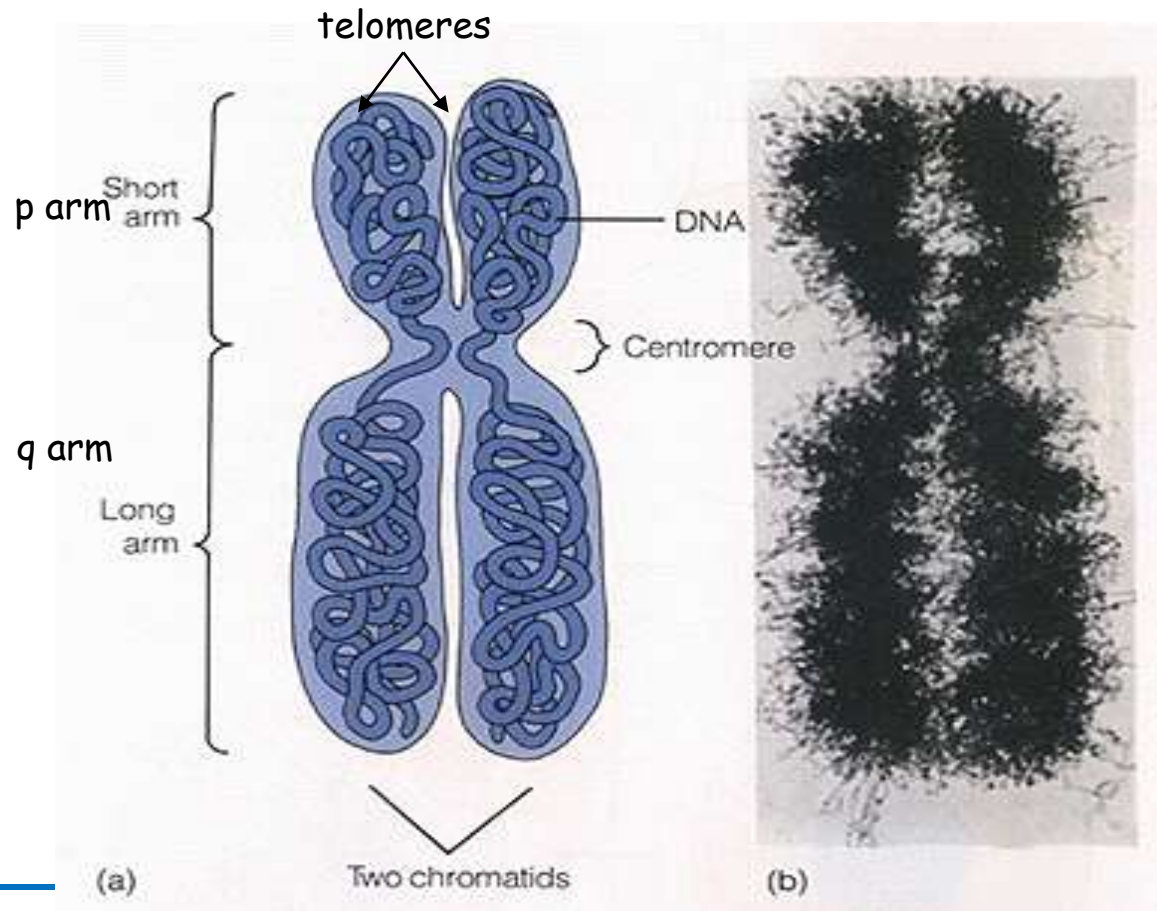
Molecular cytogenetics

Molecular Biology

I. Karyotype

- Definition
- Chromosomal Banding
- Resolution limits
- Nomenclature

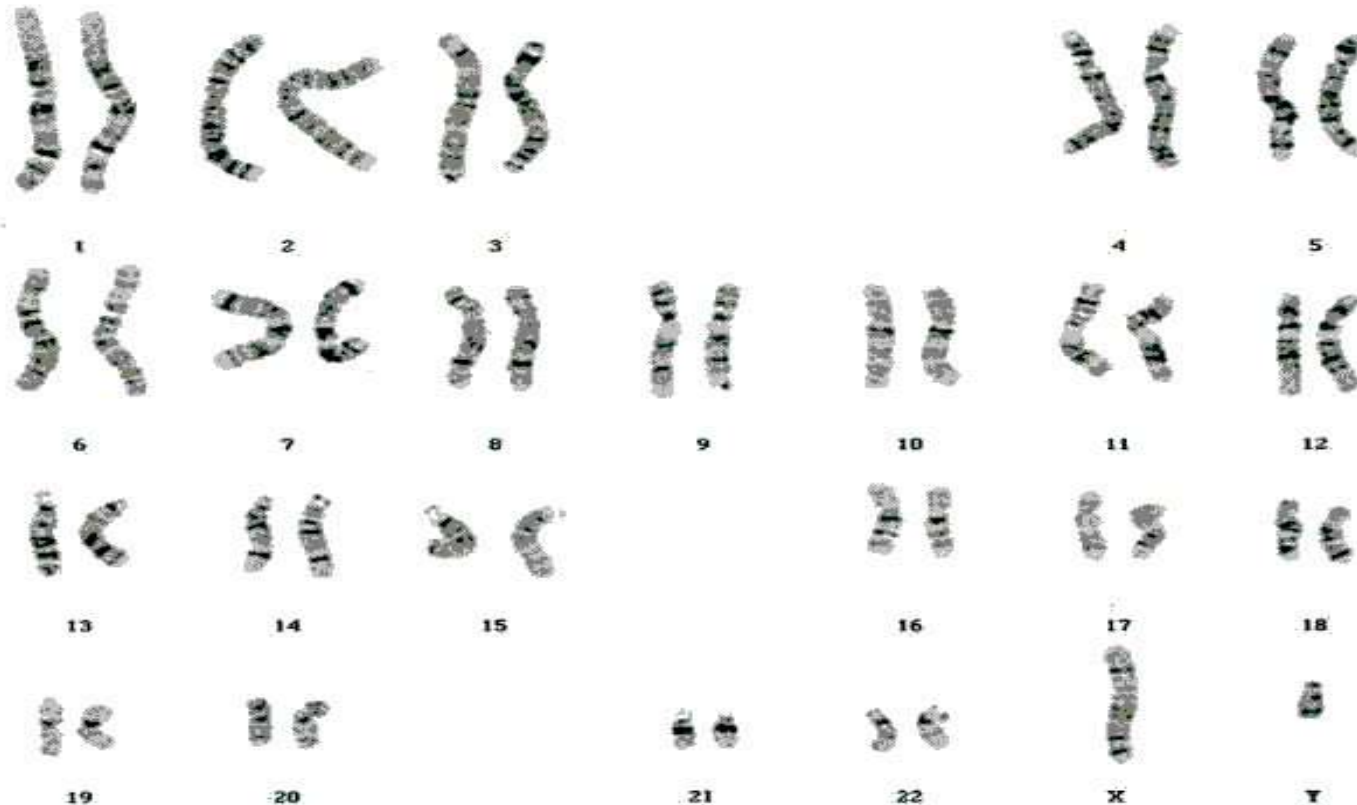
The metaphasic chromosome



G-banded Human Karyotype

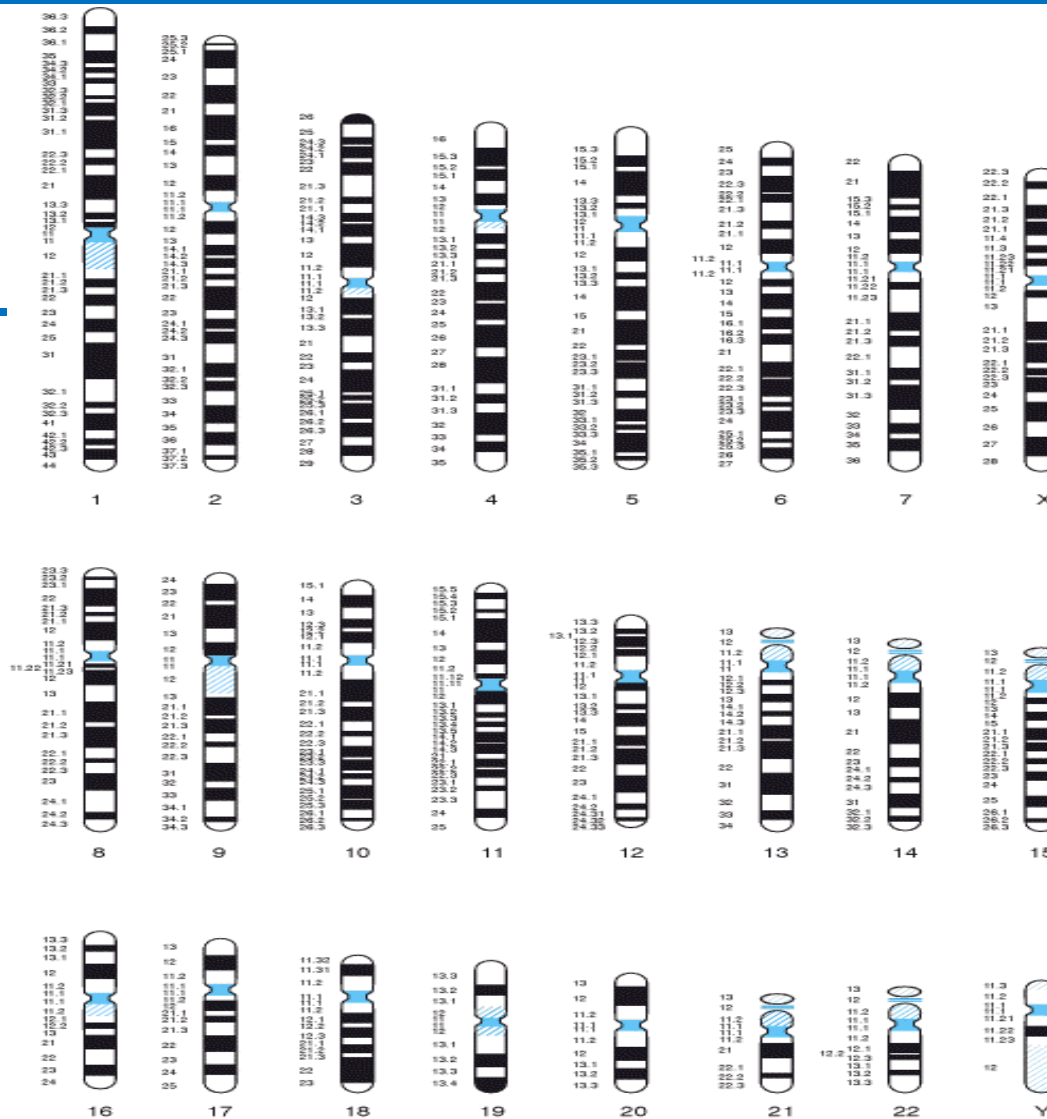
Tjio & Levan 1956

HOPITAL CANTONAL UNIVERSITAIRE GENEVE DIVISION DE GENETIQUE MEDICALE



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.



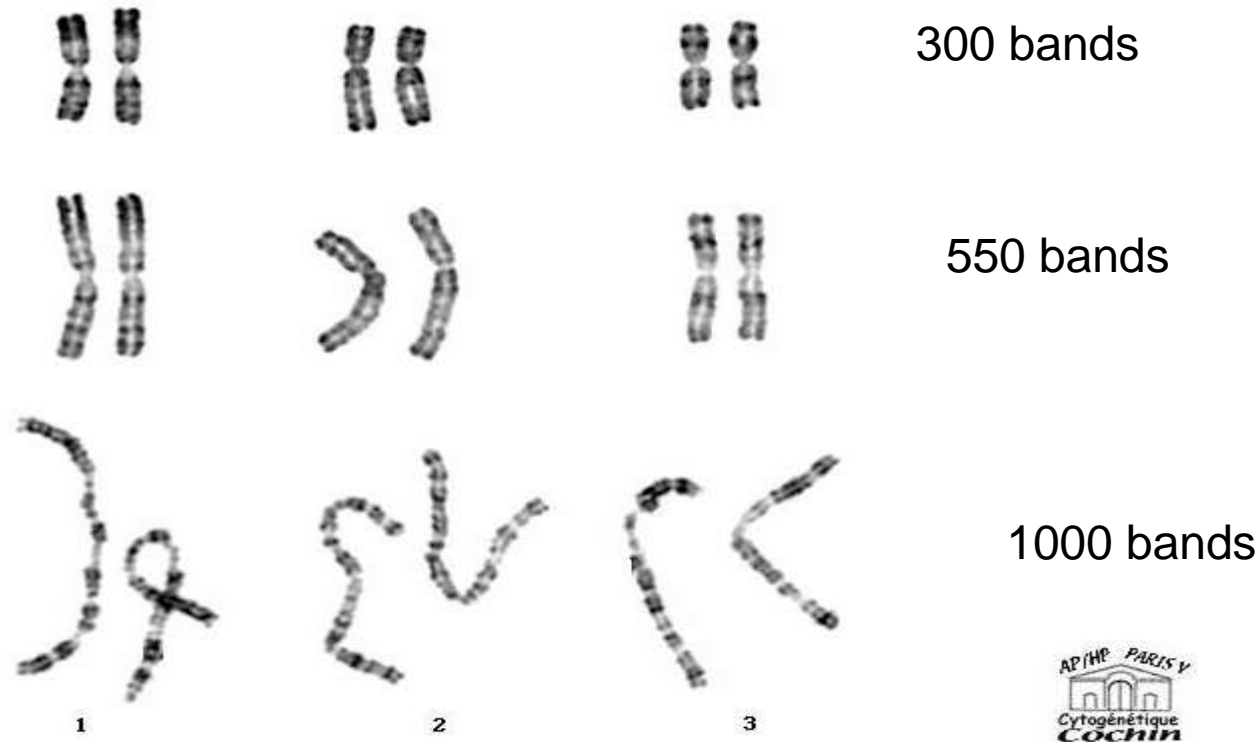
A chromosome banding pattern is comprised of alternating light and dark stripes, or bands, that appear along its length after being stained with a dye. A unique banding pattern is used to identify each chromosome

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.

Limits of resolution

Metaphase Chromosomes at different levels of resolution



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,

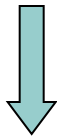
- ⇒ chromosome number
- ⇒ Arm symbol
- ⇒ Region number
- ⇒ Band number

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**

Blood sample is taken



Chromosome



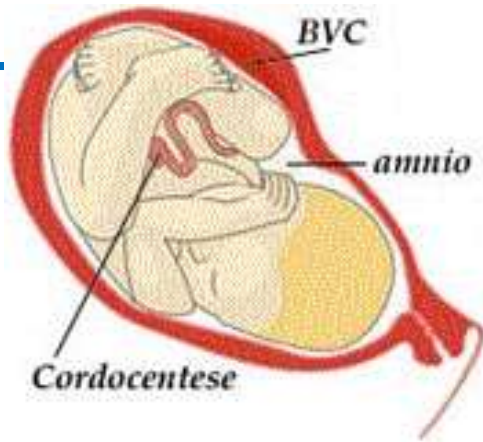
Skin biopsy



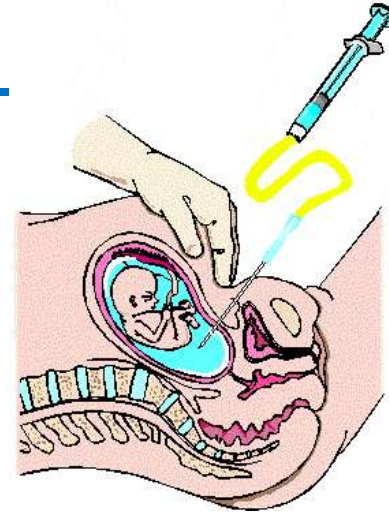
**culture of fibroblasts
15 -21 days**



Prenatal tests to study fetal chromosomes



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



Amniocentesis
Risk of abortion 1%

Choriocentesis

Amniocentesis

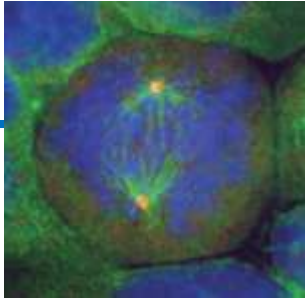
GW 10 11 12 13 14 15 16 17 18 19 20 21 22

Cordocentesis

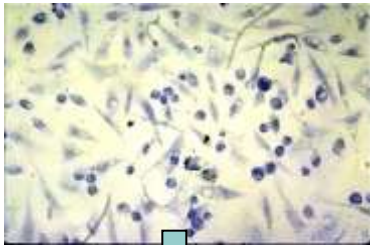
(GW: gestational weeks)

(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 seen in tumour cells

**Constitutional
Chromosome
abnormalities**

**Acquired
chromosome
abnormalities**

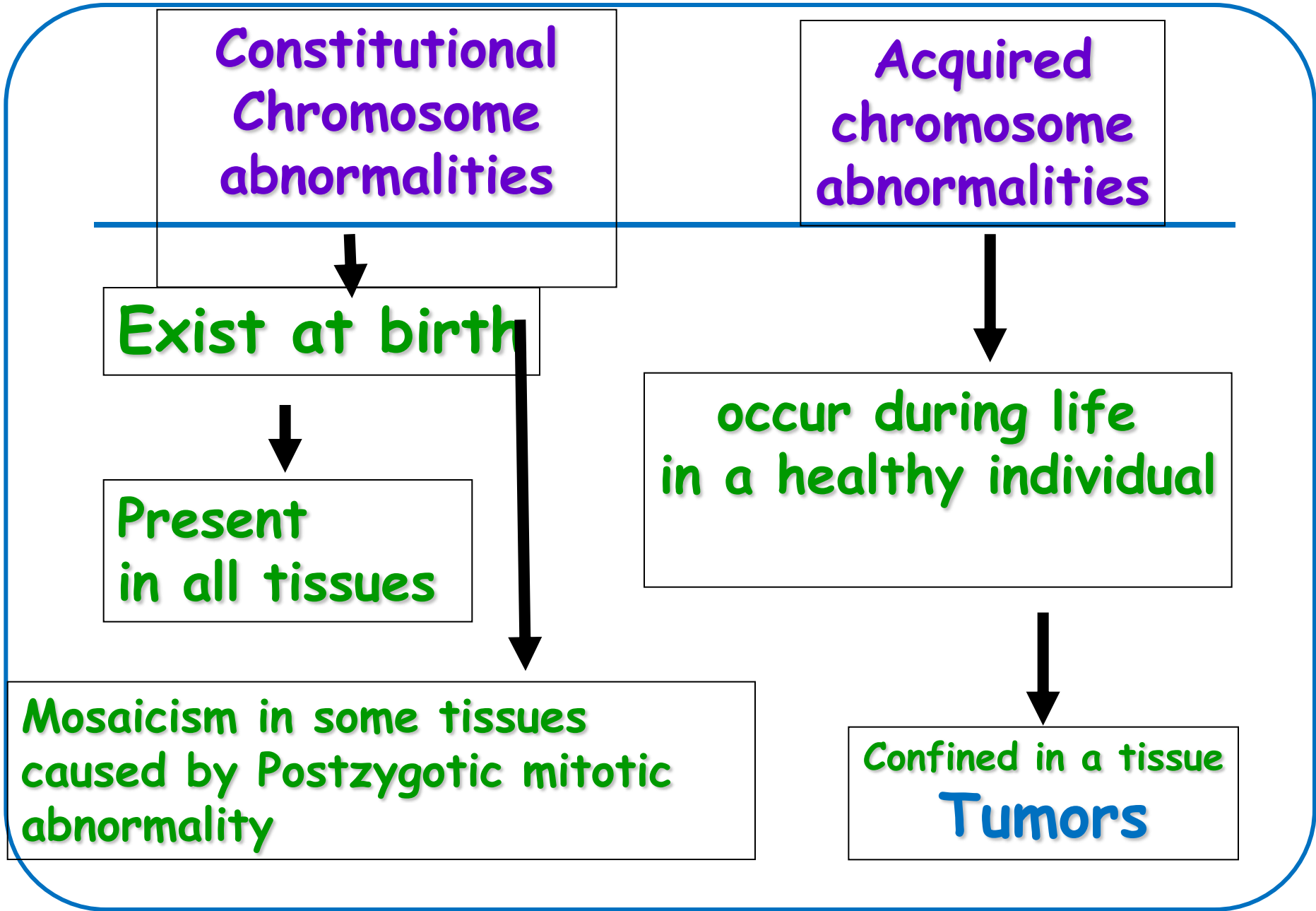
Exist at birth

**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

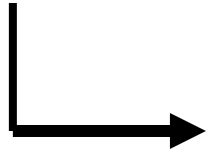
- 2% of sperms have Chromosomal abnormalities
- 20% of ova have Chromosomal abnormalities
- So among 100 conceptions, there are 25% chromosome abnormalities

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

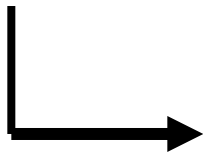
2% of sperms have Chromosomal abnormalities
20% of ova have Chromosomal abnormalities

100 conceptions



25 Chromosomal abnormalities

100 Pregnancies

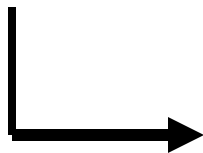


15 miscarriages



50% Chromosomal abnormalities

160 Births



1 child
With a Chromosomal abnormality

Meiosis

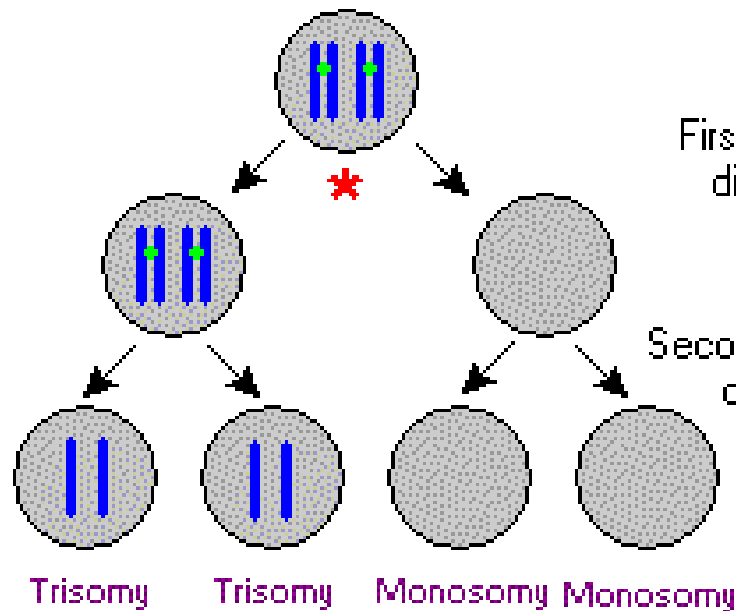
- Is the process of reductional division in which a diploid cell $2N = 46$ (2 x sets of chromosomes) is reduced to a haploid cell $(N) = 23$ (1 set of chromosomes)
- It comprises MI (meiosis I) and MII (meiosis II)
- Meiosis always results in the formation of gametes (ova and sperms)

Non-disjunction in meiosis

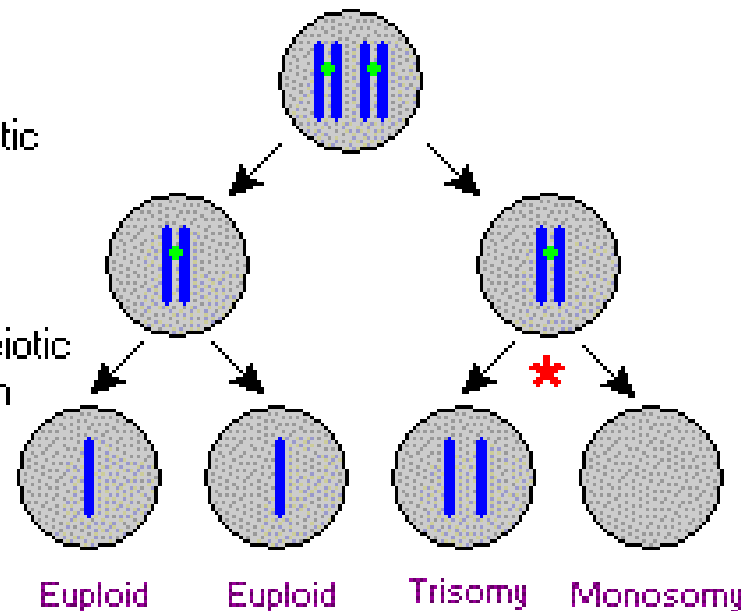
- This is an abnormal division where one daughter cell gets an extra chromosome (24) and the other daughter cell gets one chromosome less than normal (22).
- It can happen in MI or MII.
- Fertilisation with a normal gamete gives either a trisomic zygote ($24+23=47$) or a monosomic zygote ($22+23=45$)

Mechanism. Meiotic nondisjunction

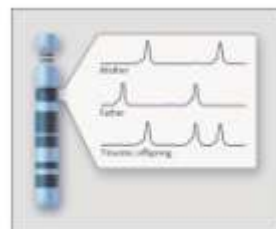
Nondisjunction in meiosis I



Nondisjunction in meiosis II



Electrophoresis profiles



offspring after fertilization with another normal gamete



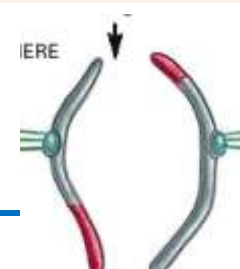
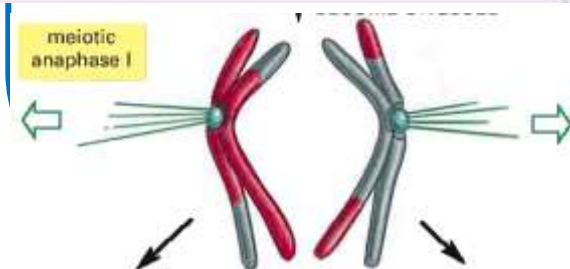
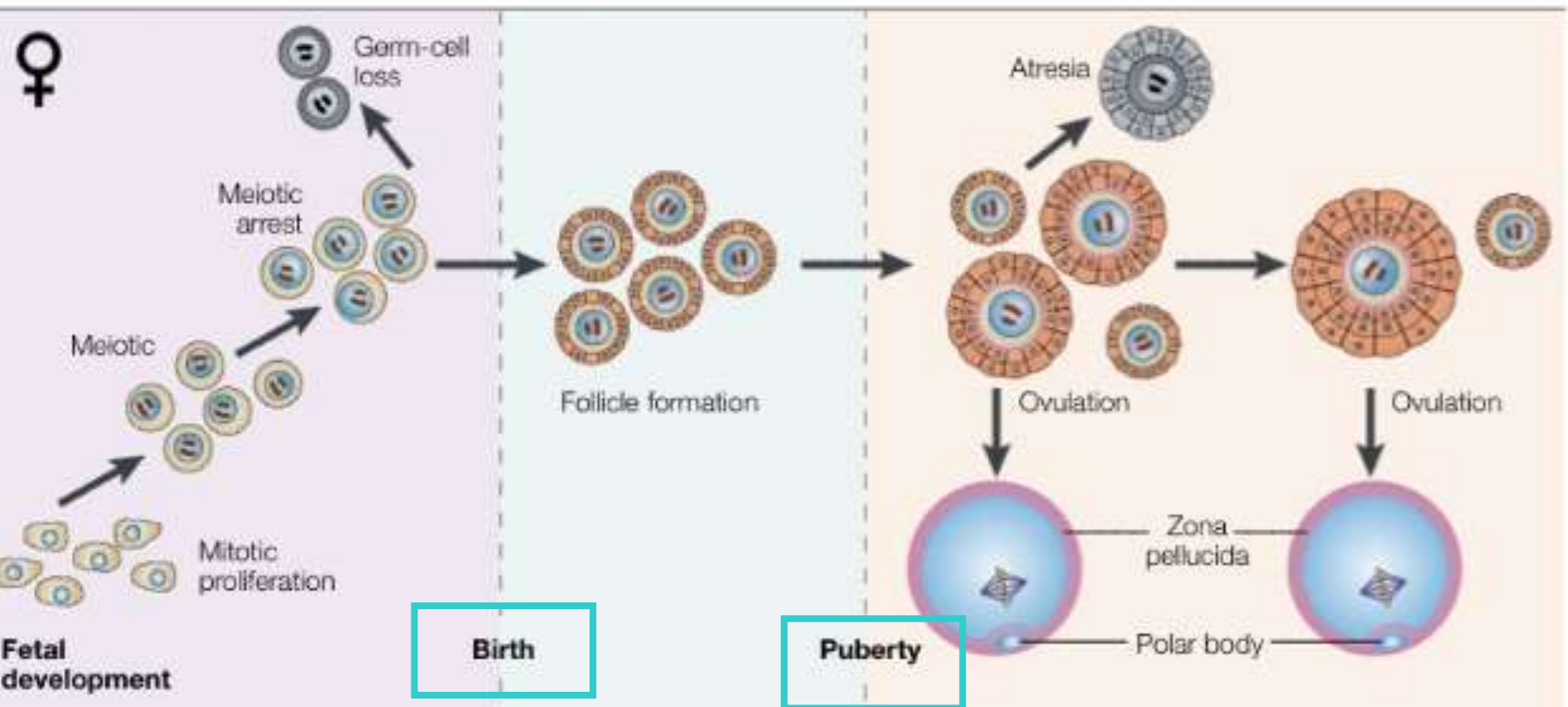
A microscopic image of a cell, likely a zygote or early embryo, showing a large, dark, circular nucleus. The cell is surrounded by a lighter, granular cytoplasm. Three labels are present: 'p' is located on the left side of the nucleus, 'f' is located just below 'p' and appears to be inside a small, lighter-colored structure, and 'z' is located at the bottom right of the nucleus. The text 'Maternal non disjunction' and 'Known risk factors' is overlaid in the center of the image.

Maternal non disjunction

Known risk factors

Period of gametogenesis in the female

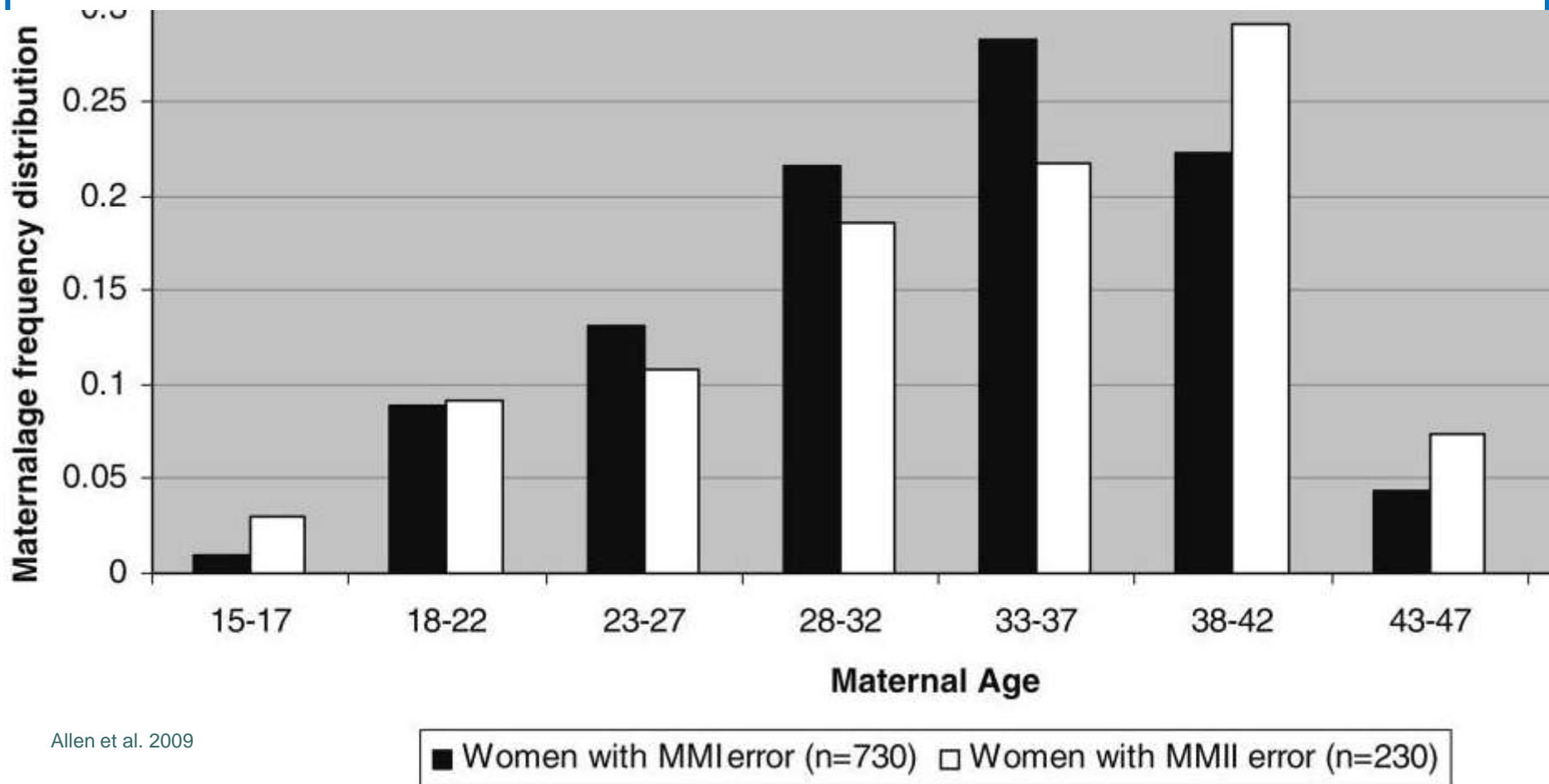
meiosis starts at intrauterine life with ovulation starting at puberty. Each month one ovum is produced and 1000 follicles become undergo atresia



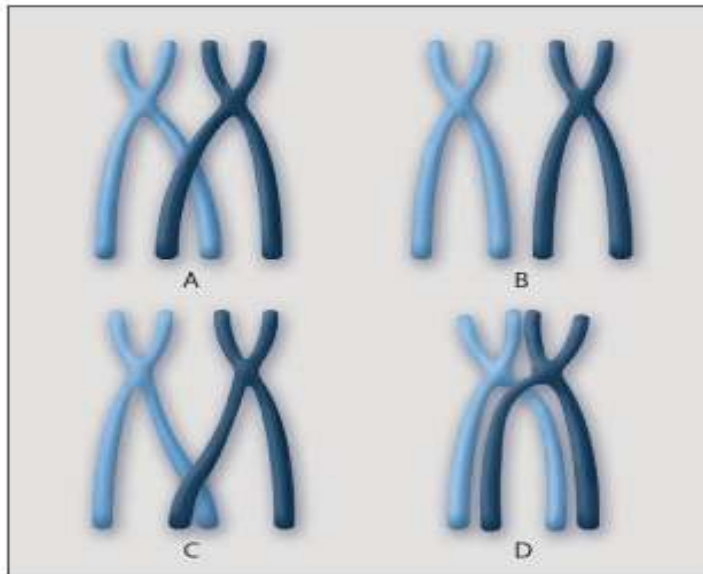
Known predisposing causes for non-disjunction in the female

- 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



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

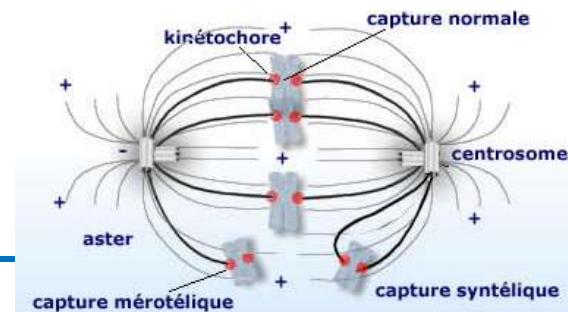
Two-hit model of non disjunction

- Establishment of "susceptible " exchange in the fetal oocyte



NinIntana

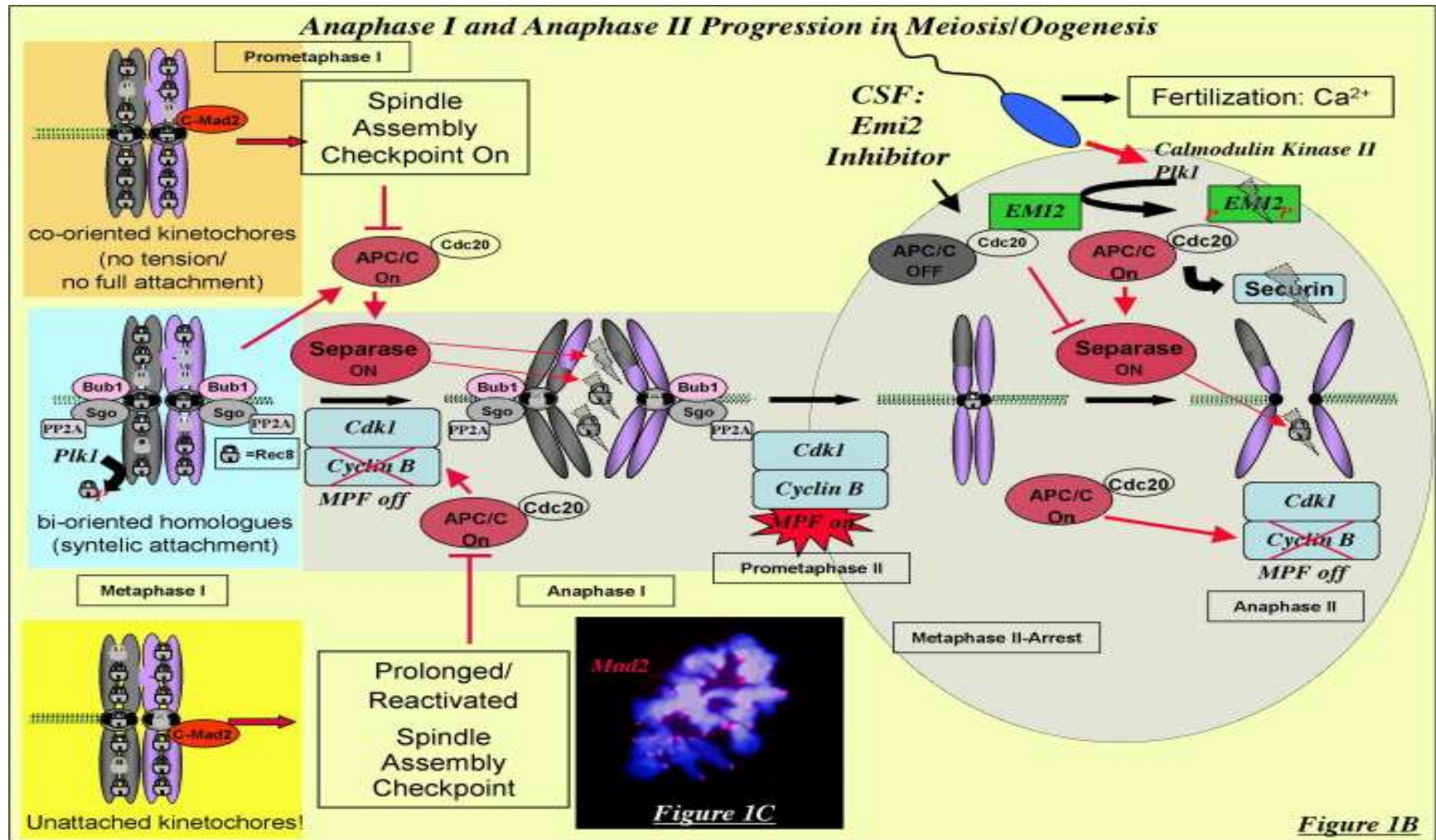
- Age dependant abnormal processing



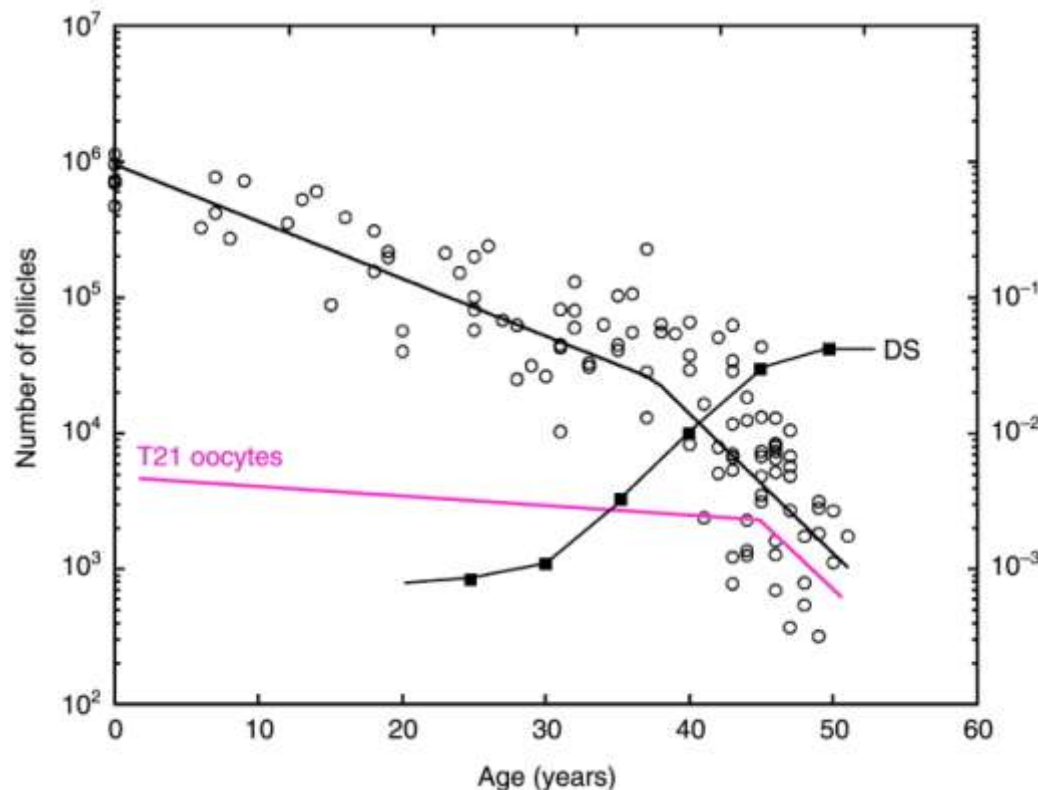
Genetic factors

- 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



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

A microscopic image showing numerous sperm cells. Each cell consists of a small, oval-shaped head and a long, thin, wavy tail (flagellum). The cells are distributed across the field of view, with some appearing more clearly than others. The background is a light, grainy texture.

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%

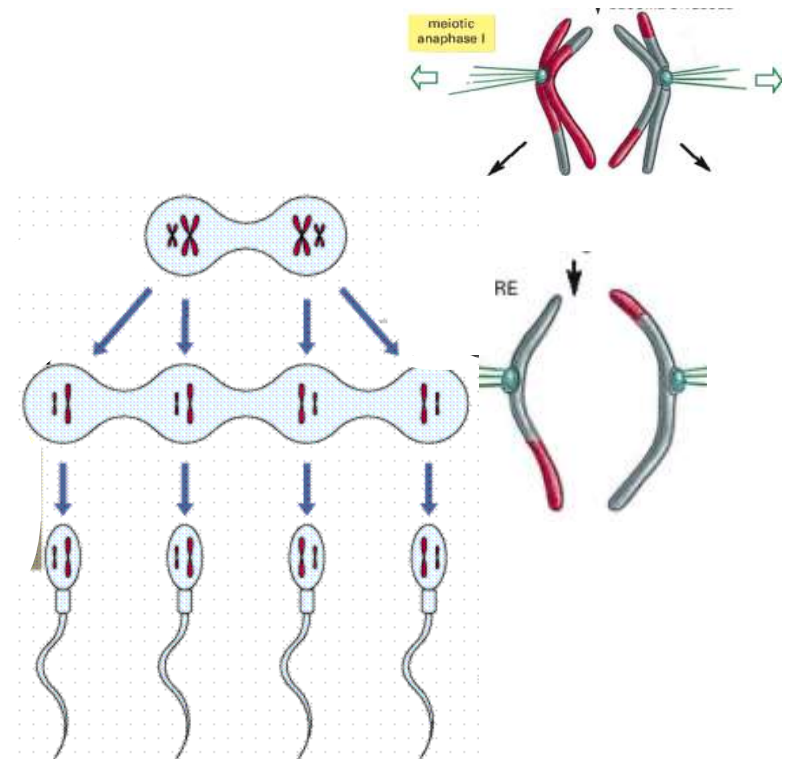
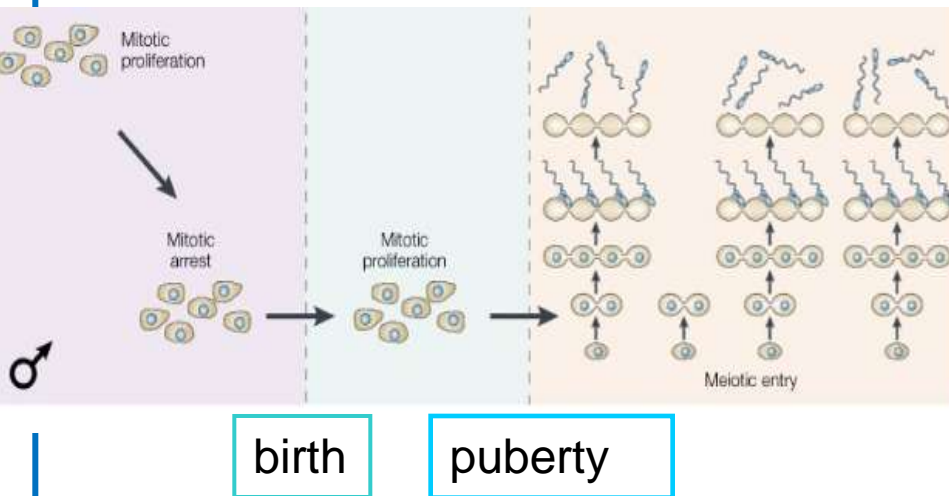
Table 1. Origin of nondisjunction in human trisomy 21 by DNA polymorphism analysis

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).

Period of gametogenesis in the male

Meiosis starts at puberty



Paternal age

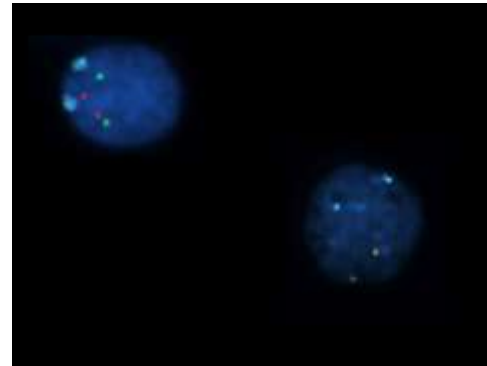
Table 2. Mean parental ages by origin of non-disjunction in population-based newborn studies

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).

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

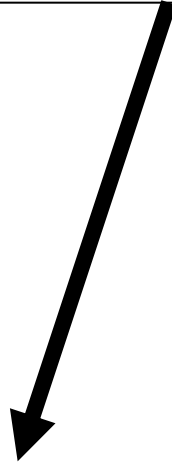
- Numerical
 - Unbalanced
 - Autosomal
 - Sex chromosomes
- Structural
 - Unbalanced vs balanced
 - Transmission

Consequences of chromosomal abnormalities

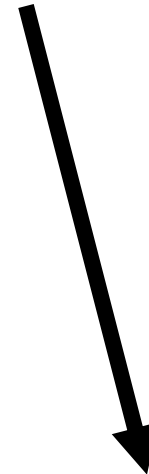
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graph TD; A[Consequences of chromosomal abnormalities] --> B[Depends on presence or absence of unbalanced chromosome constitution]; B --> C[Unbalanced Phenotypic consequences]; B --> D[Balanced Normal phenotype];
```



Depends on presence or absence of unbalanced chromosome constitution



**Unbalanced
Phenotypic consequences**



**Balanced
Normal phenotype**

Chromosomal abnormalities

Numerical



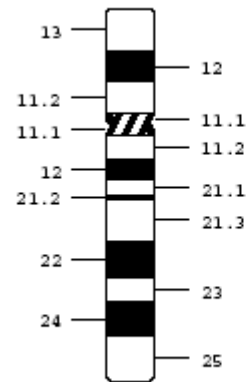
21

Always unbalanced

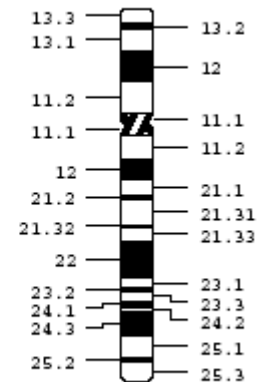
Abnormal Phenotype

Structural

550



850



Unbalanced or Balanced

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
- Dysmorphism
- +/- 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

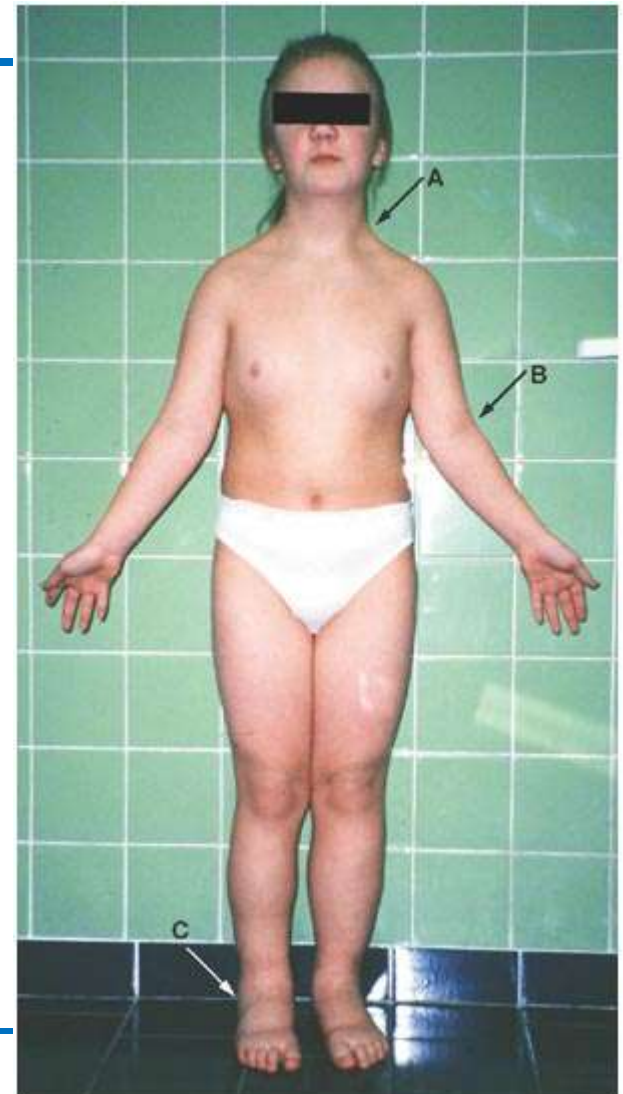
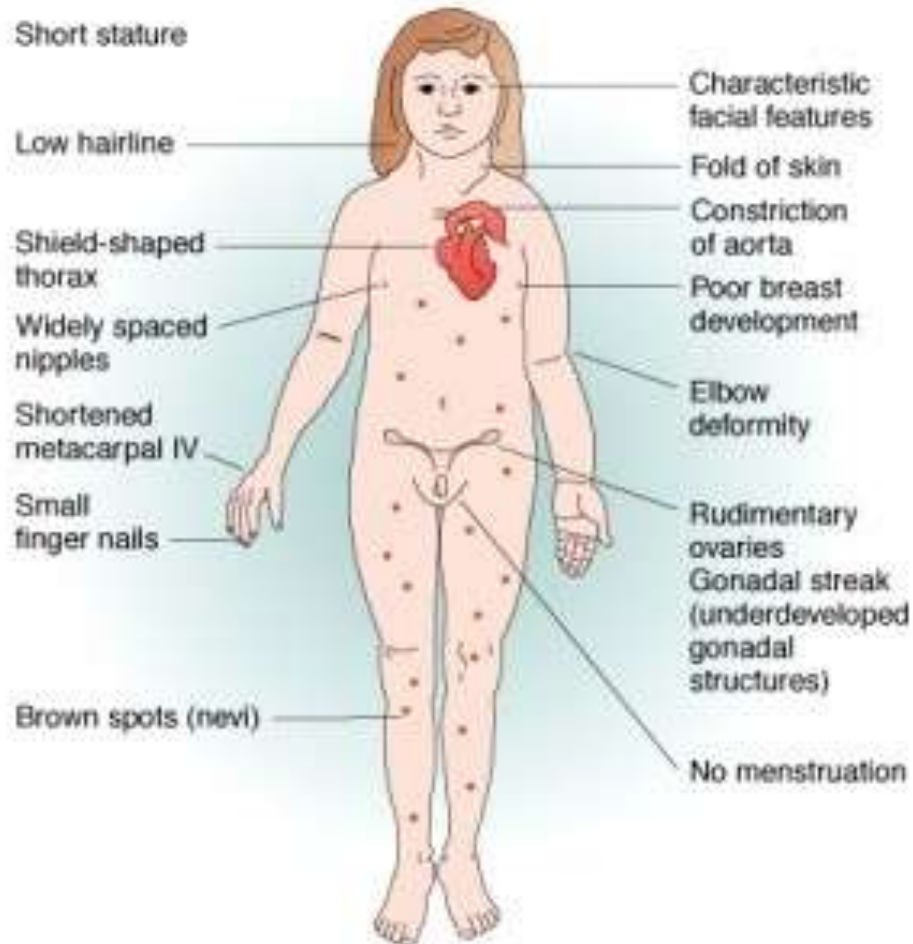


Cytogenetics :

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

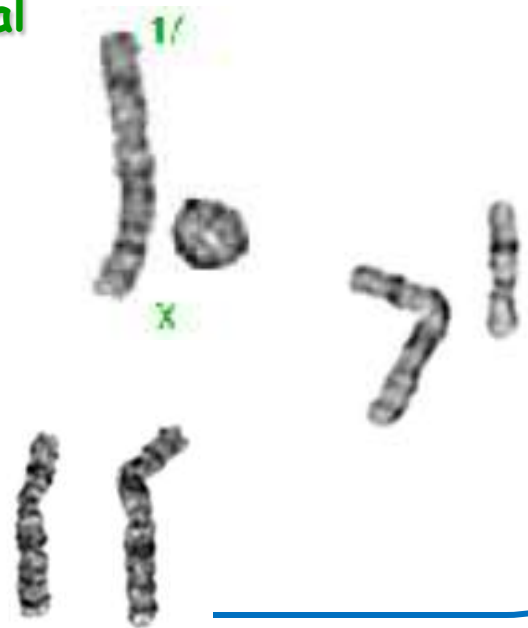
mosaic : 46,XX/45,X

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



```
graph TD; A[Structural Balanced Anomalies] --> B[1 Chromosome]; A --> C[2 Chromosomes]; A --> D[Complex]; B --> E["Inversion<br/>pericentric<br/>paracentric"]; C --> F["Translocation<br/>reciprocal<br/>Robertsonian<br/>Insertion"];
```

1 Chromosome

Inversion
pericentric
paracentric

2 Chromosomes

Translocation
reciprocal
Robertsonian
Insertion

Complex

Structural anomalies

```
graph TD; A[Structural anomalies] --> B[Unbalanced after meiosis]; A --> C[Balanced]; B --> D[Abnormal Gametes<br/>Partial Anomalies]; D --> E[Abnormal zygotes]; C --> F[Normal phenotype]
```

Unbalanced after meiosis

Balanced

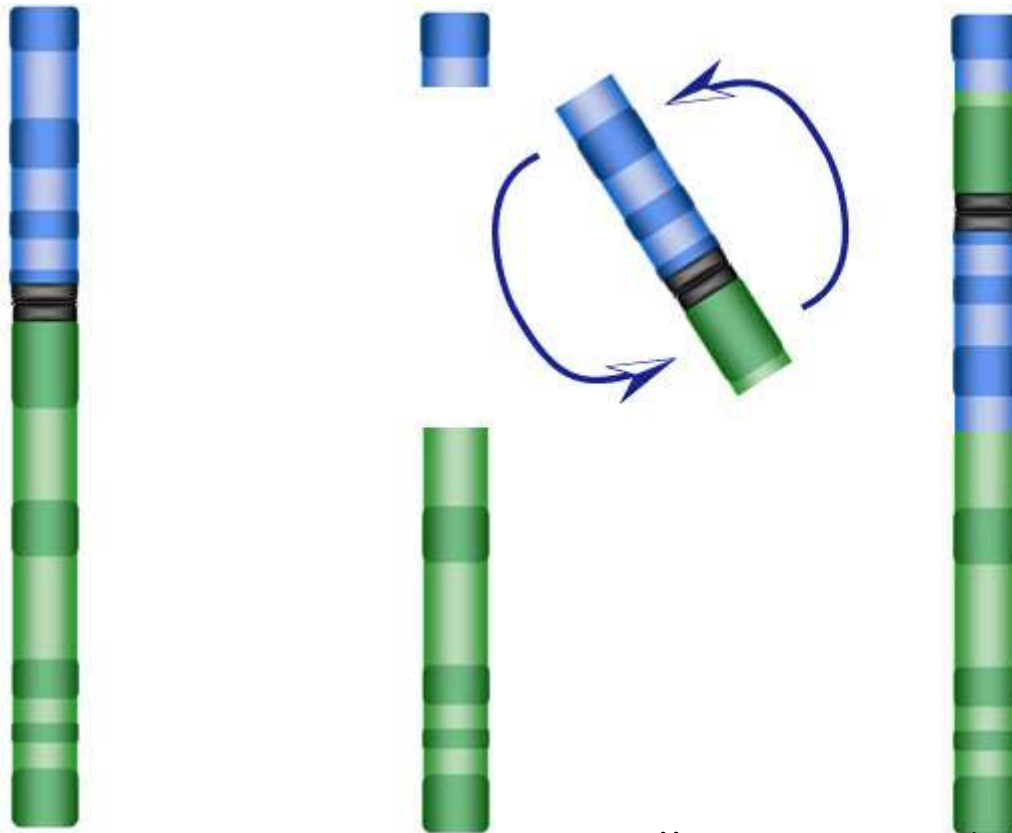
Abnormal Gametes
Partial Anomalies

Abnormal zygotes

Normal phenotype

Pericentric Inversion

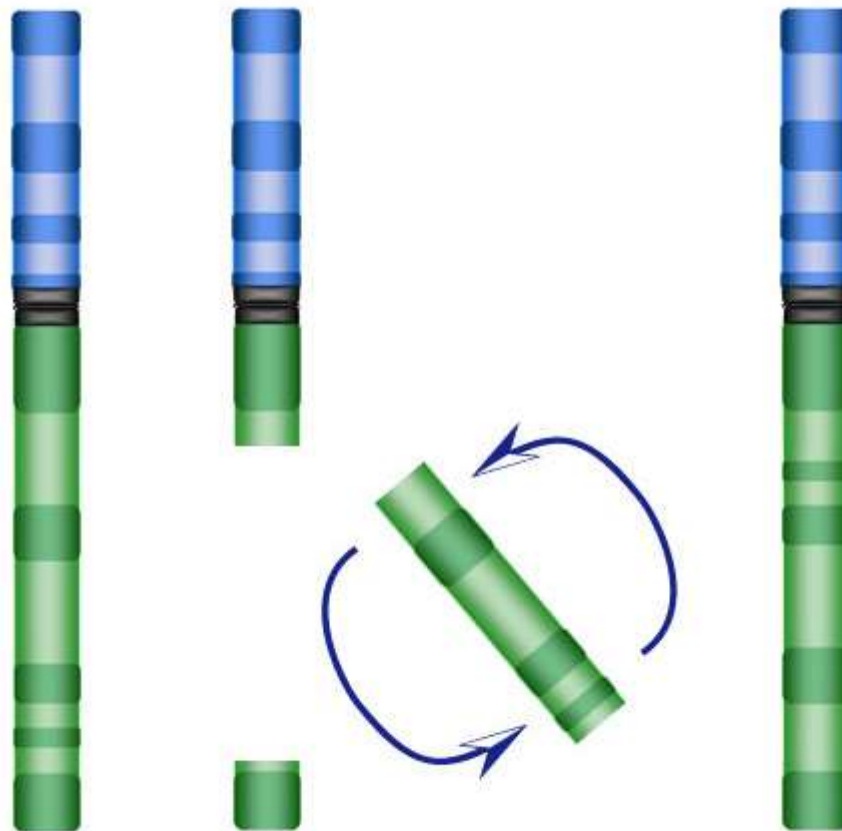
1 chromosome
2 breakpoints



All inversions 1/1000 newborns

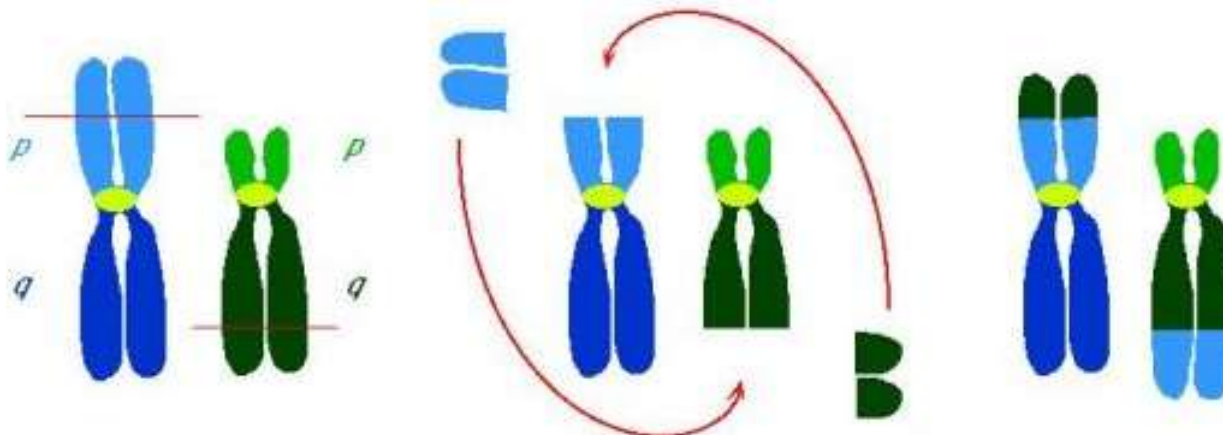
Paracentric inversion

1 chromosome
2 breakpoints



Reciprocal translocation

2 chromosomes
2 breakpoints

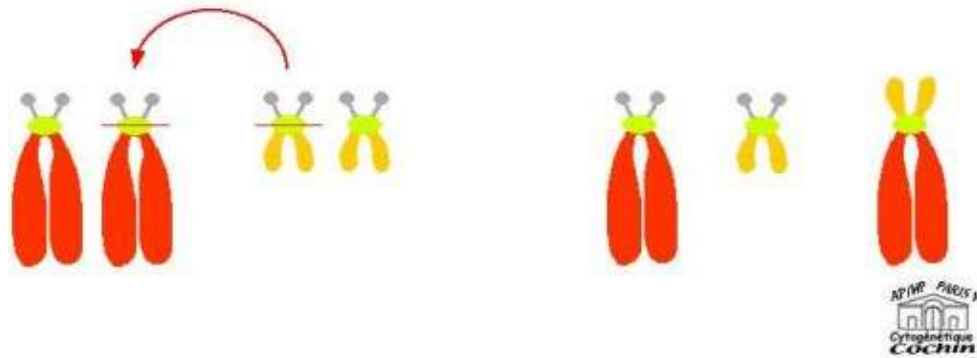


all translocations 1/500 newborns

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



Robertsonian Translocation ACROCENTRICS

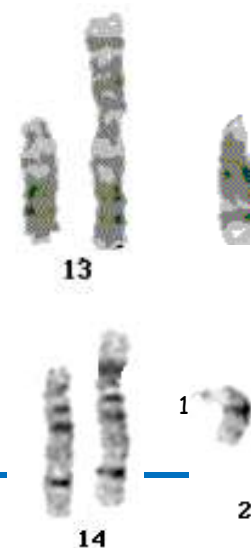


Robertsonian translocations 1/833 newborns

Evans et al.1978

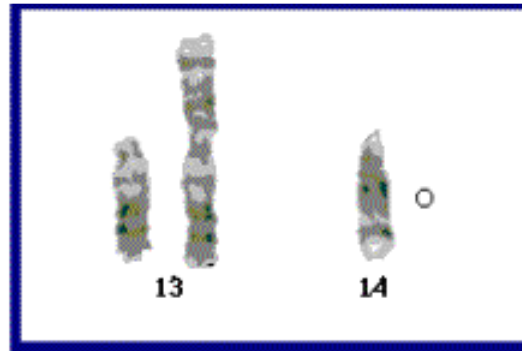
45,der(13;14)(q10;q10) => 73%

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

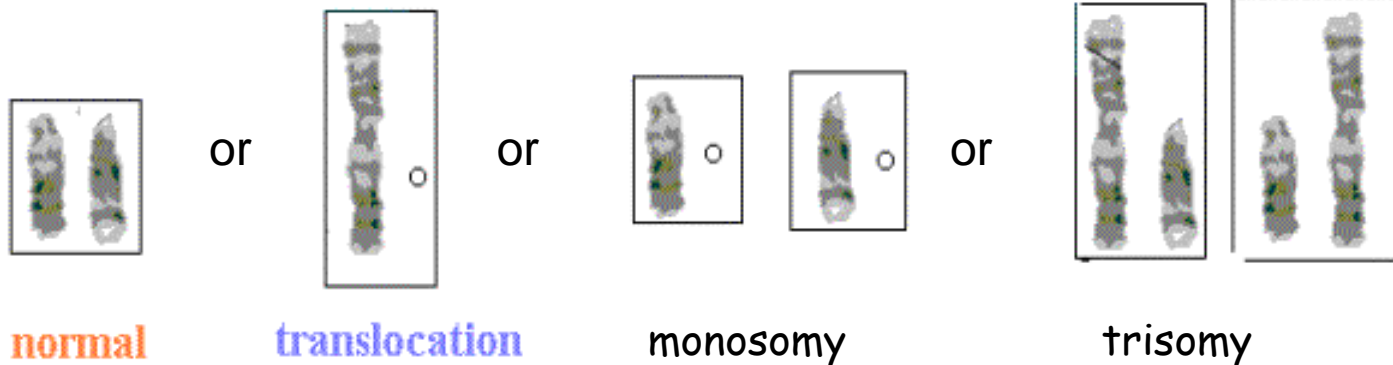


Meiosis chromosomal segregation of a t(13;14) translocation

Constitutional karyotype

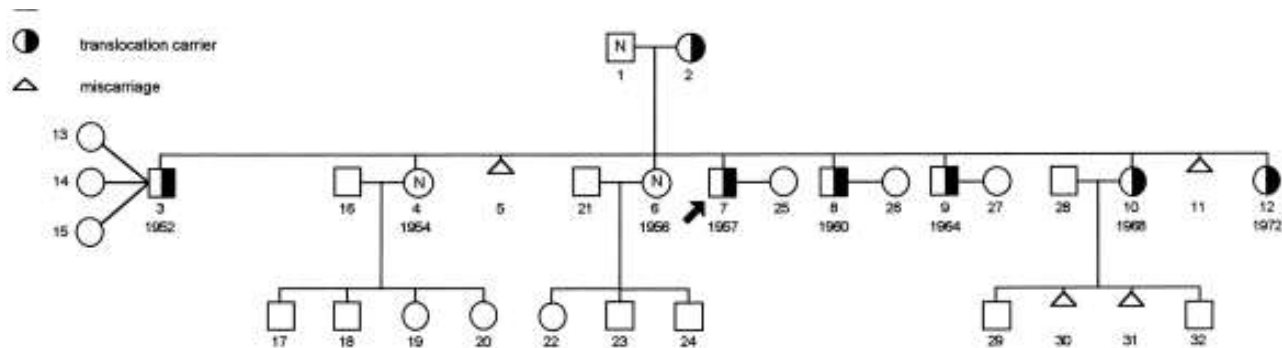


Gametes



Clinical Consequences of a Translocation

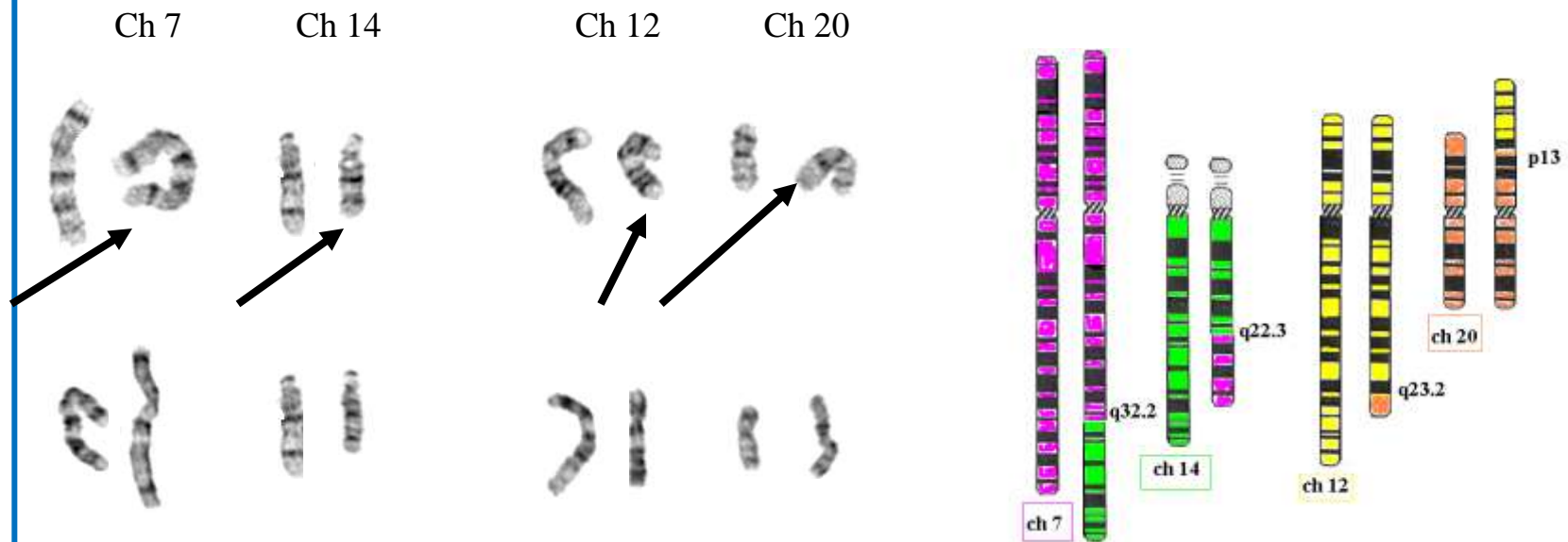
- Infertility
- Miscarriages



- **Trisomy** by transmission of unbalanced translocation



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



```
graph TD; A[Unbalanced Structural Anomalies] --> B[1 Chromosome]; A --> C[2 Chromosomes]; A --> D[Complex]; B --> E[Deletion<br/>Duplication<br/>Ring]; C --> F[Translocation<br/>Insertion];
```

1 Chromosome

Deletion
Duplication
Ring

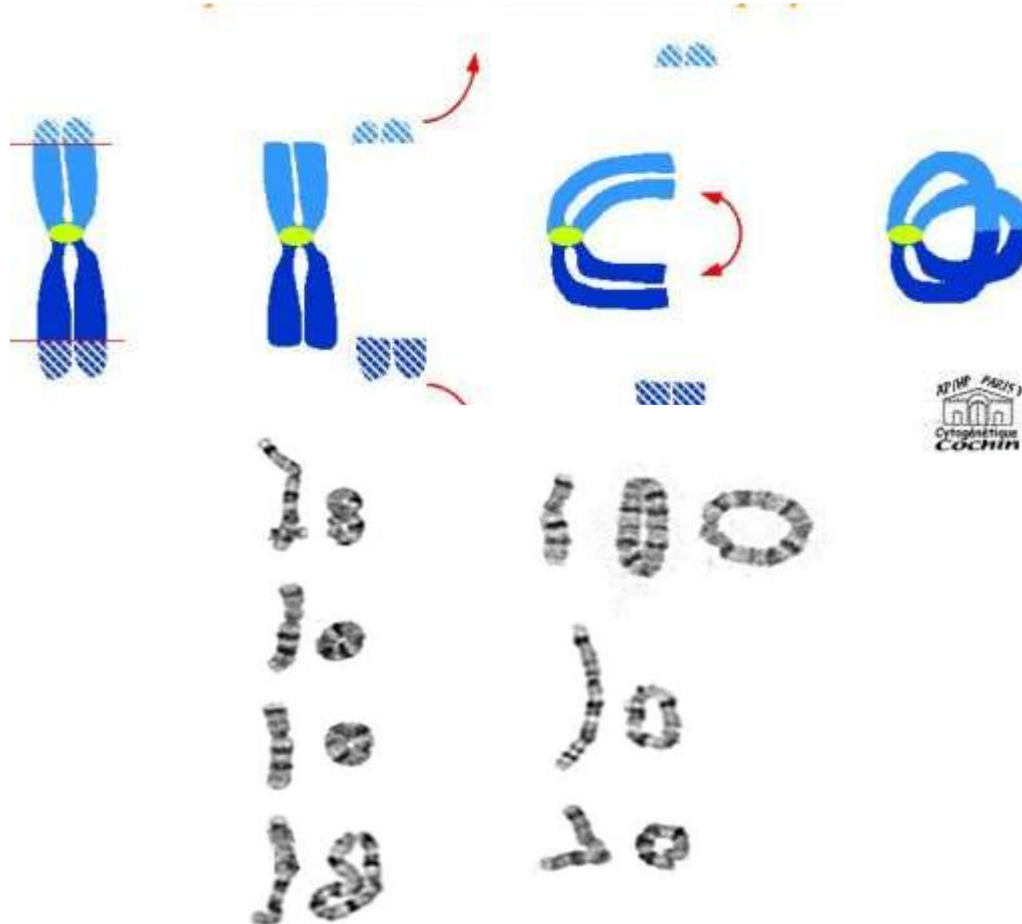
2 Chromosomes

Translocation
Insertion

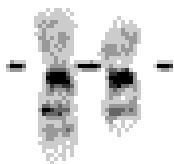
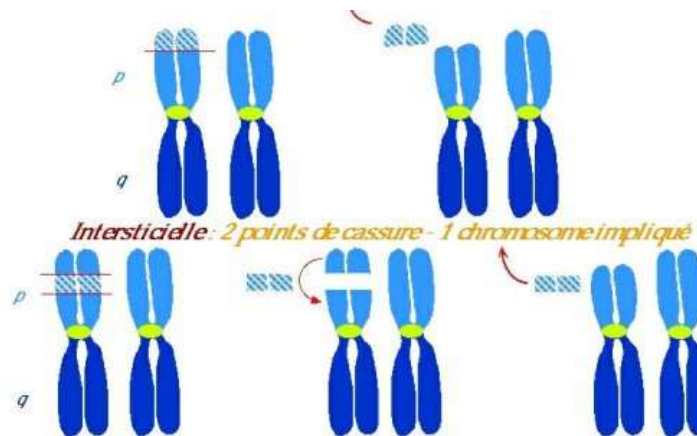
Complex

Ring

1 chromosome
2 breakpoints

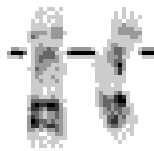


Deletion

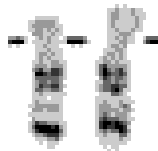


Del(16)(q21)

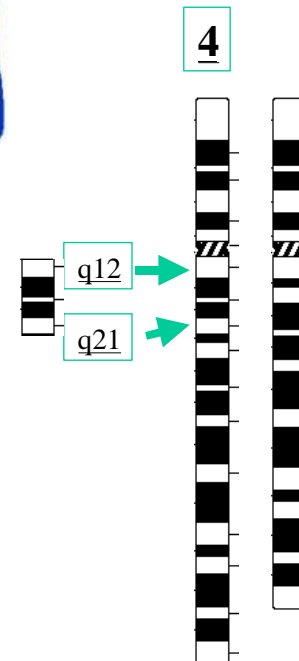
16



17



18



del(4)(q12q21.1)

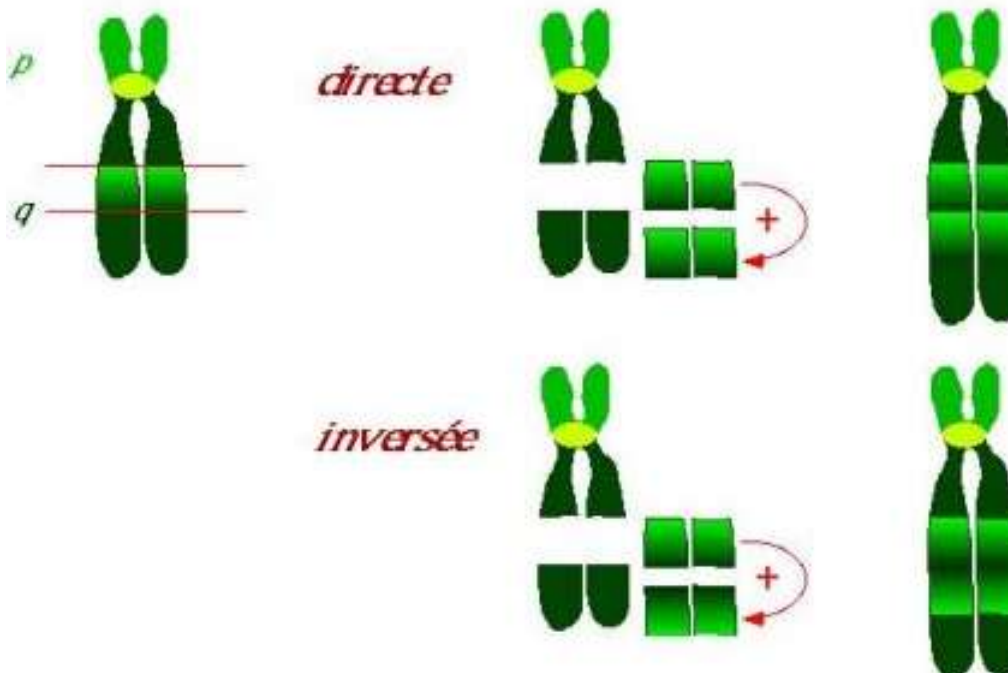


Terminal deletion
1 chromosome
1 breakpoint

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)