Cytogenetics
Chromosomal Genetics

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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

- p arm
- q arm
- telomeres
- DNA
- Centromere
- Two chromatids
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.
Normal male Karyotype 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

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

Cordocentesis
*(Blood from umbilical artery)*

(GW: gestational weeks)
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**

- **Exist at birth**
- **Present in all tissues**
  - Mosaicism in some tissues caused by Postzygotic mitotic abnormality

**Acquired chromosome abnormalities**

- **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 reductive 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

First meiotic division

Trisomy  
Trisomy  
Monosomy  
Monosomy

Second meiotic division

Nondisjunction in meiosis II

Euploid  
Euploid  
Trisomy  
Monosomy

Electrophoresis profiles

offspring after fertilization 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

Maternal age frequency distribution

Maternal Age

Women with MMI error (n=730)  Women with MMII error (n=230)

Allen et al. 2009
Recombination and non disjunction

- Normal
  - 1 chiasma/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

- 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
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

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number of cases</th>
<th>%</th>
<th>Meiotic recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>732</td>
<td>90.7%</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>556</td>
<td>68.9%</td>
<td>Reduced</td>
</tr>
<tr>
<td>MII</td>
<td>176</td>
<td>21.8%</td>
<td>Increased</td>
</tr>
<tr>
<td>Paternal</td>
<td>44</td>
<td>5.5%</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>17</td>
<td>2.1%</td>
<td>Reduced</td>
</tr>
<tr>
<td>MII</td>
<td>27</td>
<td>3.3%</td>
<td></td>
</tr>
<tr>
<td>Mitotic</td>
<td>31</td>
<td>3.8%</td>
<td></td>
</tr>
<tr>
<td>“Maternal”</td>
<td>17</td>
<td>2.1%</td>
<td></td>
</tr>
<tr>
<td>“Paternal”</td>
<td>14</td>
<td>1.7%</td>
<td></td>
</tr>
</tbody>
</table>

*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 nondisjunction in population-based newborn studies

<table>
<thead>
<tr>
<th>Origin</th>
<th>n</th>
<th>Maternal age</th>
<th>Paternal age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>145</td>
<td>30.1</td>
<td>32.0</td>
</tr>
<tr>
<td>MII</td>
<td>50</td>
<td>31.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Paternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI+MII</td>
<td>16</td>
<td><strong>25.6</strong></td>
<td><strong>29.9</strong></td>
</tr>
<tr>
<td>Mitotic</td>
<td>12</td>
<td>28.2</td>
<td>30.5</td>
</tr>
</tbody>
</table>

* *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 depend on the presence or absence of an unbalanced chromosome constitution.

- **Unbalanced Phenotypic consequences**
- **Balanced Normal phenotype**
Chromosomal abnormalities

Numerical

Always unbalanced

Abnormal Phenotype

Structural

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% mosais 47,XXY/46,XY or 47,XXY/46,XX
Turner Syndrome

- Short stature
- Low hairline
- Shield-shaped thorax
- Widely spaced nipples
- Shortened metacarpal IV
- Small finger nails
- Brown spots (nevi)
- Characteristic facial features
- Fold of skin
- Constriction of aorta
- Poor breast development
- Elbow deformity
- Rudimentary ovaries
- Gonadal streak (underdeveloped gonadal structures)
- No menstruation
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

- Abnormal Gametes
- Partial Anomalies

Abnormal zygotes

Balanced

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 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

normal  or  translocation  or  monosomy  or  trisomy
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

1 Chromosome
- Deletion
- Duplication
- Ring

2 Chromosomes
- Translocation
- Insertion

Complex
Ring

1 chromosome
2 breakpoints
Deletion

Terminal deletion
1 chromosome
1 breakpoint

Interstitial deletion
1 chromosome
2 breakpoints

Del(16)(q21)

4
del(4)(q12q21.1)

4
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).