Human Genetics

Thomas Liehr

(1) Institute of Human Genetics, Friedrich Schiller University, Jena; Germany

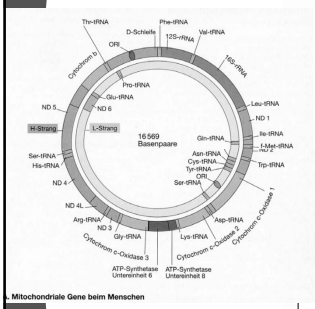
Institut für Humangenetik
Abteilung Molekulare Zytogenetik

Universitätsklinikum Jena

Zytogenetik

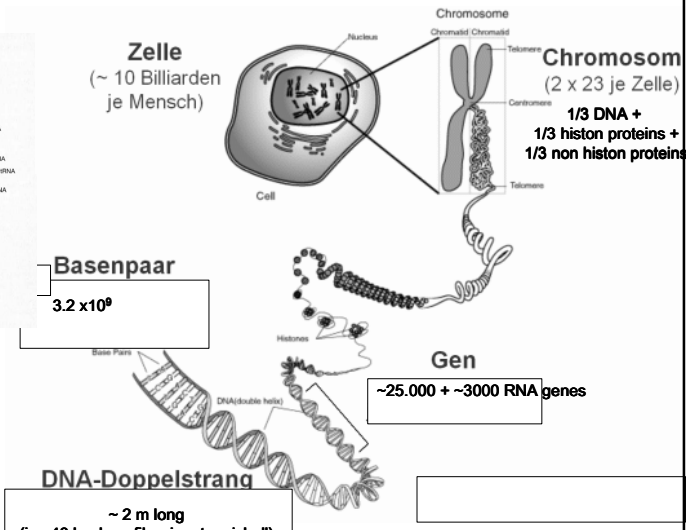
- chromosome structure
- chromosome preparation
- formal cytogenetics
- prenatal cytogenetics
- non-invasive diagnostics
- postnatal cytogenetics
- chromosome anomalies
- tumor(cyto)genetic
- limitations

Cytogenetics
DNA and chromosomes



Mitochondriale Gene beim Menschen

10-500 mitochondria per cell
à 3-6 mtDNA rings



Zelle
(~ 10 Billiarden je Mensch)

Chromosom
(2 x 23 je Zelle)

1/3 DNA +
1/3 histon proteins +
1/3 non histon proteins

Basenpaar

3.2 x10⁹

Gen

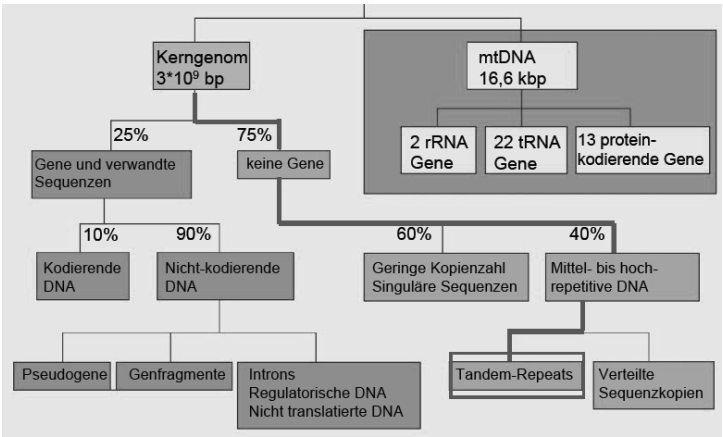
~25.000 + ~3000 RNA genes

DNA-Doppelstrang

~ 2 m long
(i.e. 40 km long fiber in a tennisball)

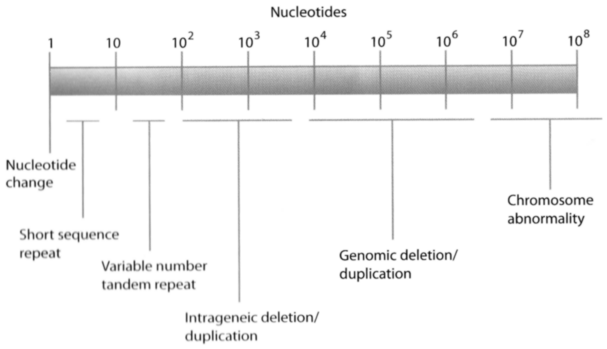
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

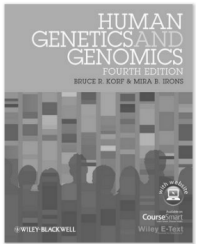
Cytogenetics
DNA and chromosomes



Cytogenetics
DNA and chromosomes

Scale of genetic and genomic variation, from the level of a single nucleotide (far left) to changes in entire chromosomes (right).

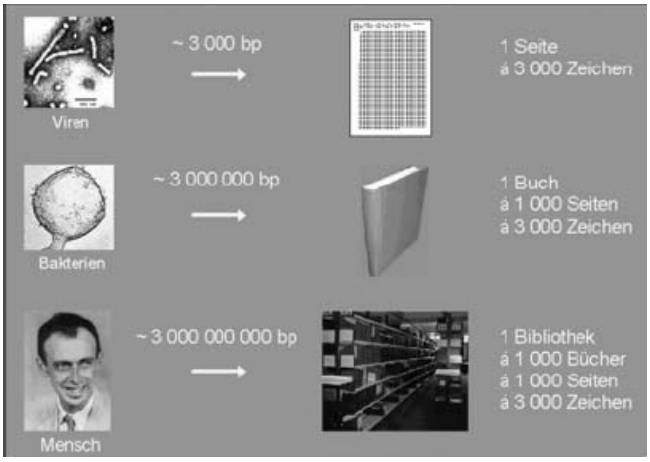




5

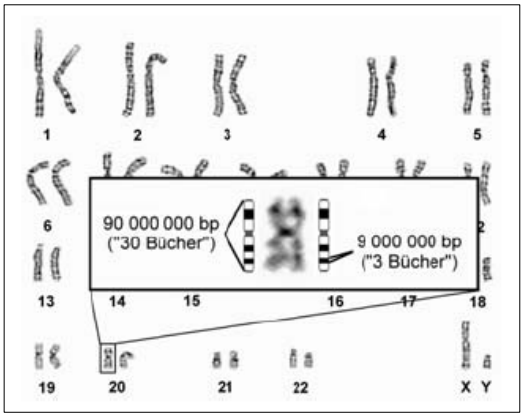
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

Cytogenetics
DNA and chromosomes



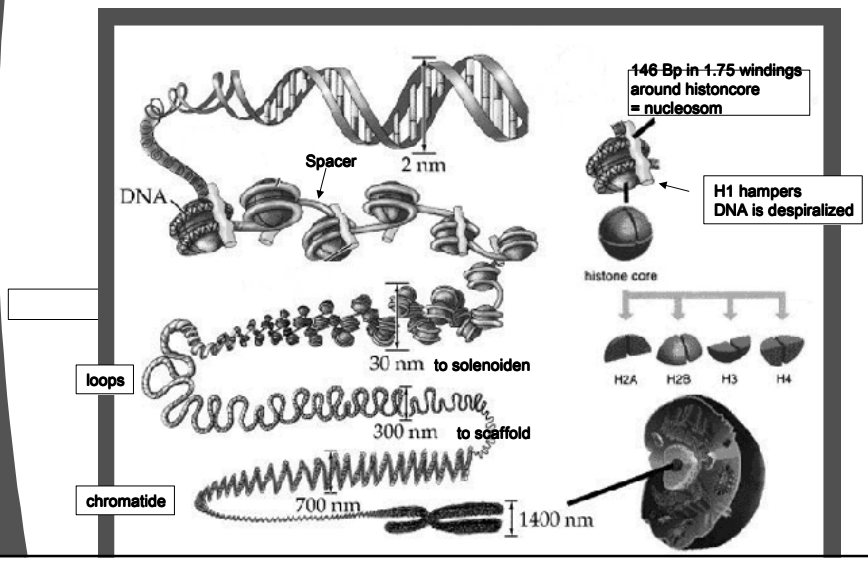
6

Cytogenetics
DNA and chromosomes

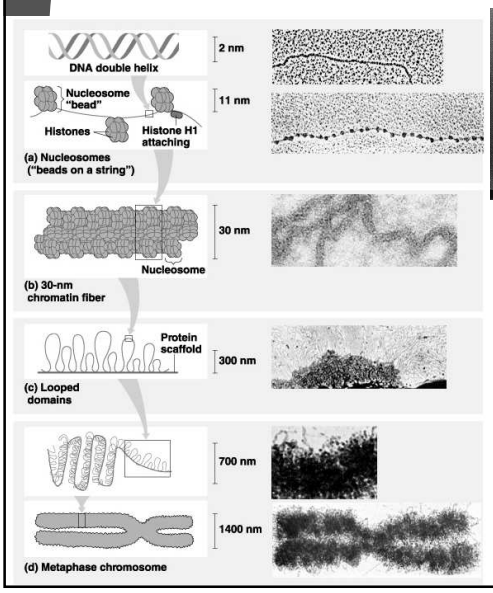


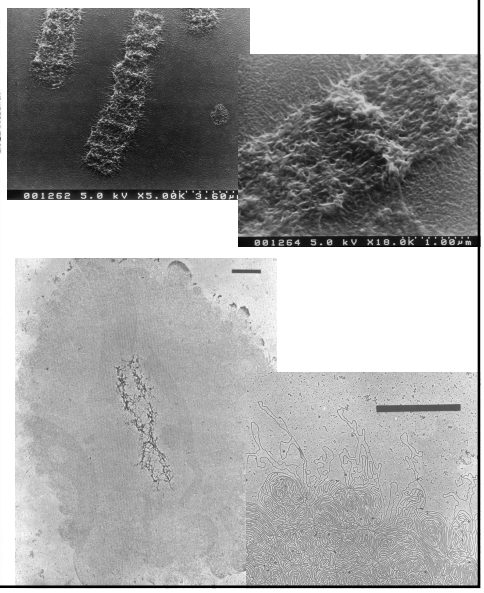
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

Cytogenetics
DNA and chromosomes



Cytogenetics DNA and chromosomes





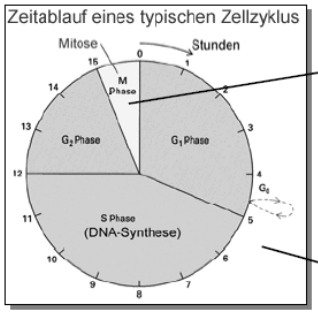
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

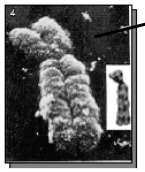
Cytogenetics
chromosome preparation

→ Chromosomes can only be prepared from living and dividing cells

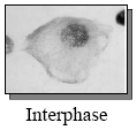
Material used most often:

- blood
- skin fibroblasts
- amnion cells
- chorion
- placenta

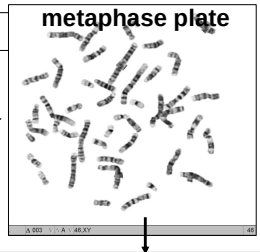


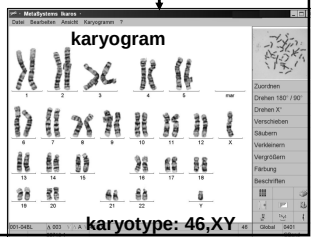


Mitot.Metaphase

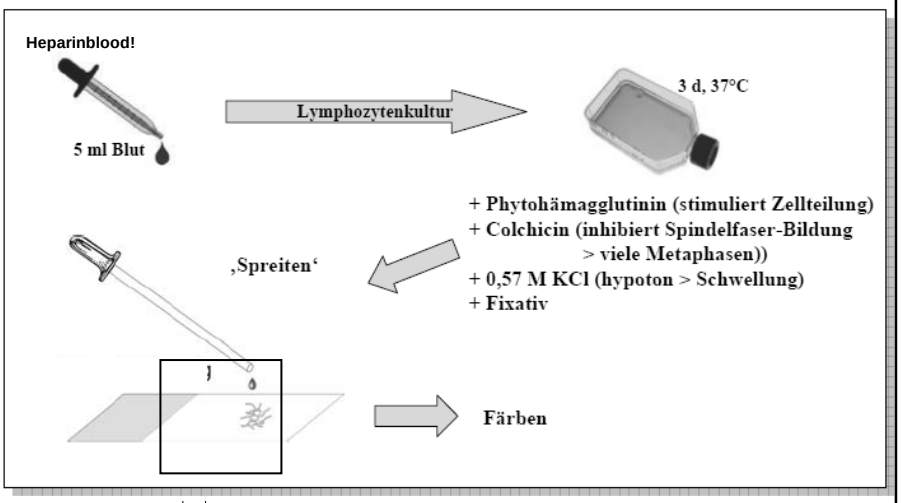


Interphase



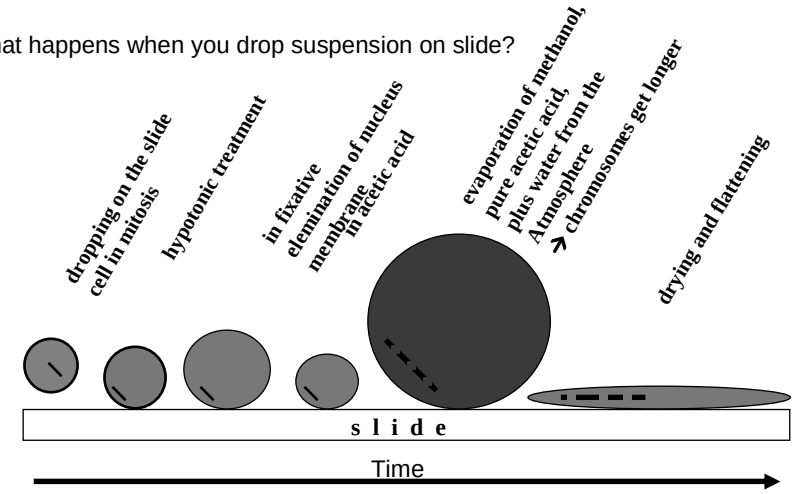


Cytogenetics
chromosome preparation



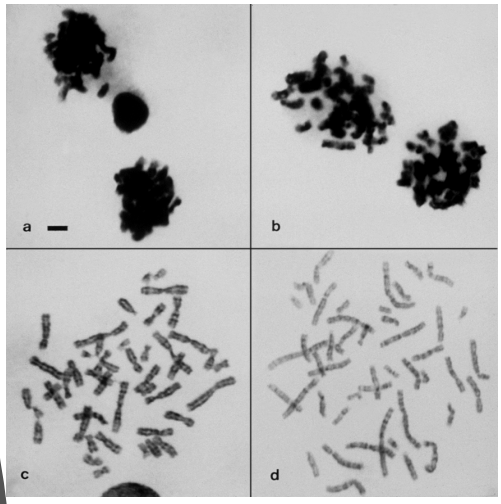
Cytogenetics
chromosome preparation

What happens when you drop suspension on slide?



critical factors: → humidity and temperature (weather)

Cytogenetics
chromosome preparation



Influence of humidity on spriting

At room temp

(**a**) 7% humidity

(**b**) 12% humidity

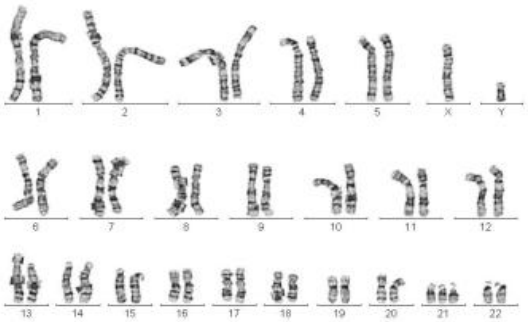
(**c**) 21% humidity

(**d**) 29 % humidity.

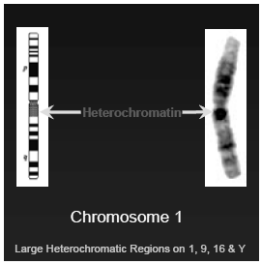
Cytogenetics
formal cytogenetics

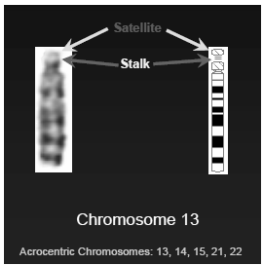
Cytogenetics is about **chromosomes** – numerical or structural changes are identified

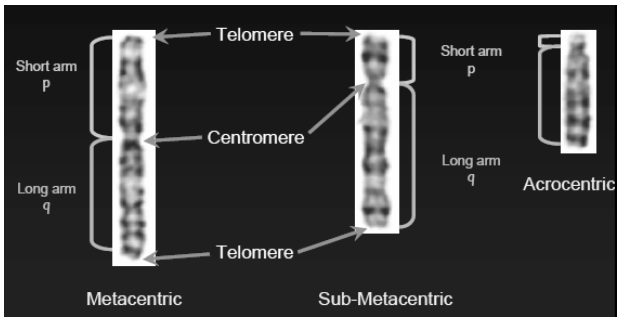
Chromosomes are classified according to : - size
- band pattern
- centromeric position



Cytogenetics
formal cytogenetics







Cytogenetics
formal cytogenetics

centromeres:
- functional: points where spindelapparatus in mitosis is attaching.
- human centromeres have large blocks of repetitive "alphoid“ DNA (Mb size)
- alpha satellites are AT rich - several thousends of such repeats form centr.

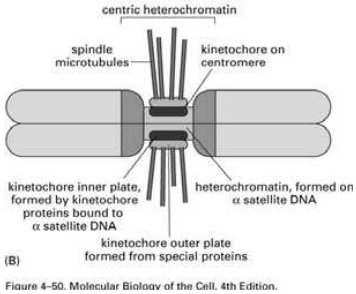
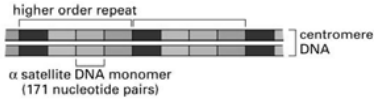


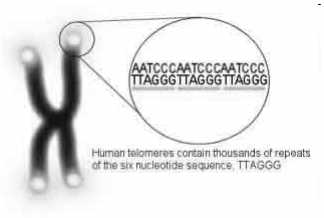
Figure 4–50. Molecular Biology of the Cell, 4th Edition.



Cytogenetics
formal cytogenetics

telomeres:

- stabilizing sequences: hamper fusion with other DNA; enable replication without loss of DNA; mark own cellular DNA and avoid degradation
- high repetitive sequences, motive in human telomeres: 5'TTAGGG3'
- problem at 5´ end, no room for RNA-primer for last Okazaki fragment
- in each division a human cell loses 50-100 telomernucleotides
- telomerase is in many somatic cells (almost) not active
- human fibroblast can divide only 60x in cell culture → senescence
  → **Hayflick Limit**



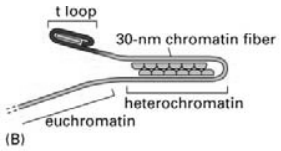
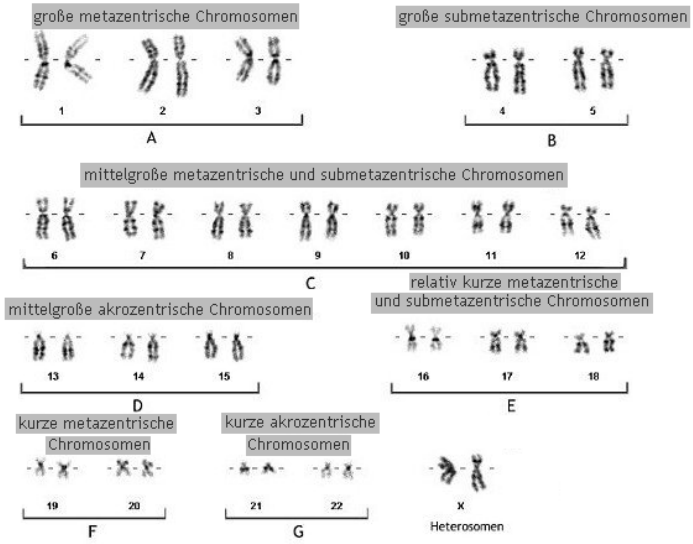


Figure 5–44. Molecular Biology of the Cell, 4th Edition.

17

Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

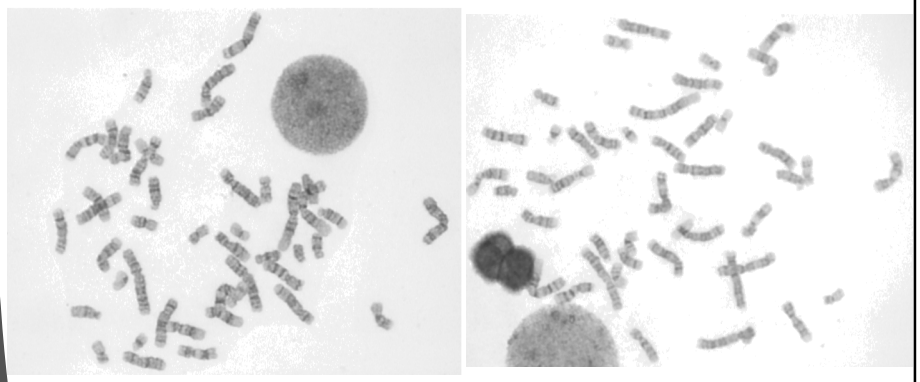
Cytogenetics
formal cytogenetics



18

Cytogenetics
karyotyping

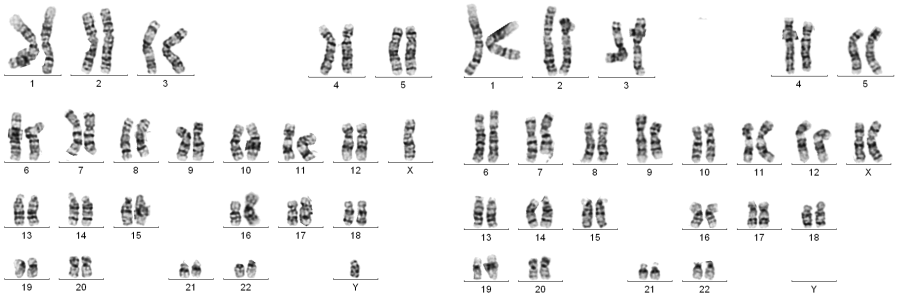
A **B**



Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

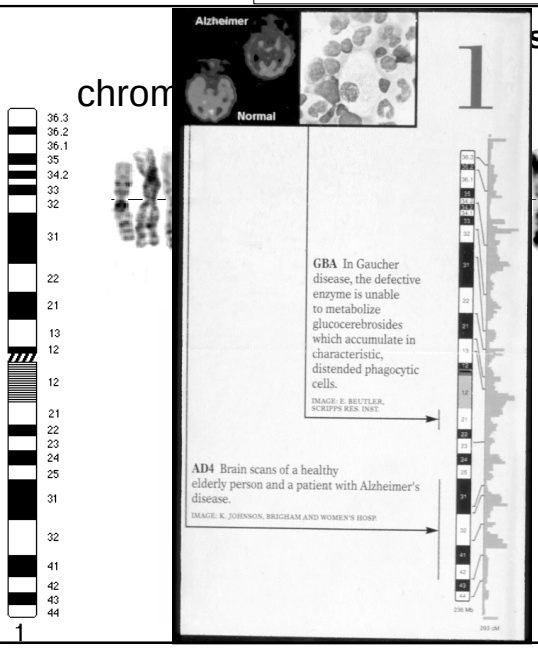
Cytogenetics
karyotyping

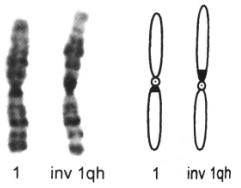
A **B**



karyotype: 46,XY 46,XX

chrom s



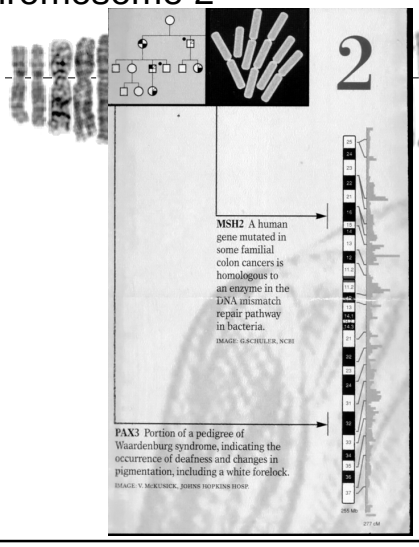


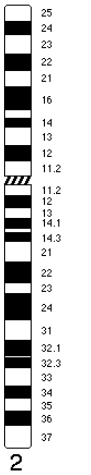
features
- largest chromosome
- metacentric
- dark centromeric region goes in q-arm
- distaler part in p-Arm is light

21

Cytogenetics
karyotyping

chromosome 2





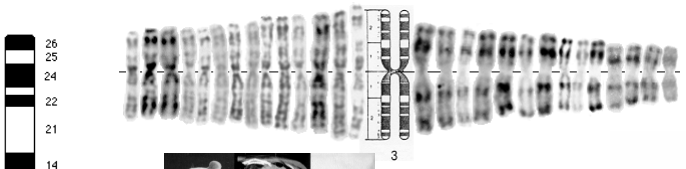
~ 0.6%

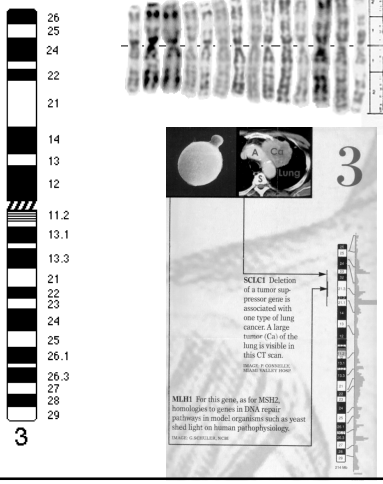
features
- second largest chromosome
- submetacentric
- relatively evenly banded

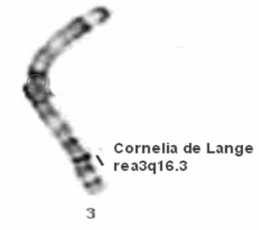
22

Cytogenetics
karyotyping

chromosome 3





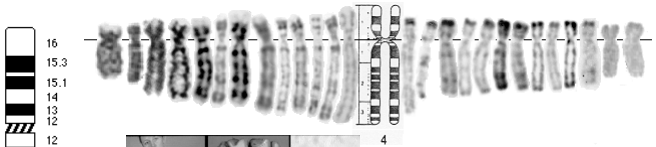


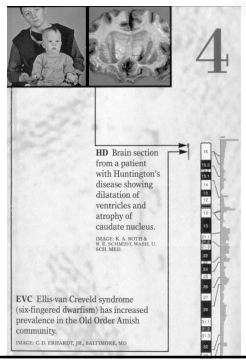
features
- similar but smaller than chromosome 1
- metacentric
- p-arm starts with dark band

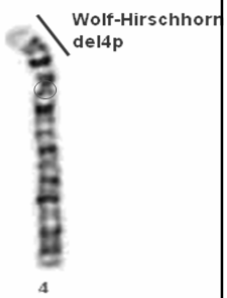
23

Cytogenetics
karyotyping

chromosome 4





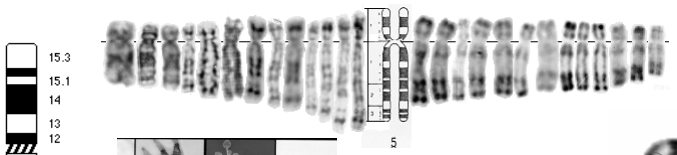


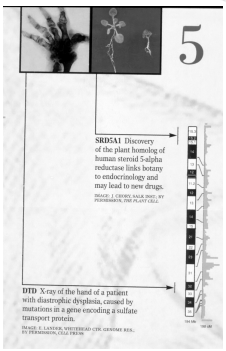
features
- large submetacentric chromosome
- dark band in 'shoulder'
- p-arm with 2 dark bands
- q-arm evenly banded

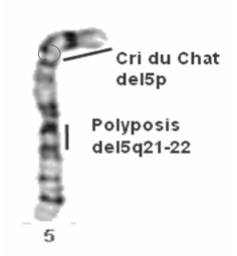
24

Cytogenetics
karyotyping

chromosome 5





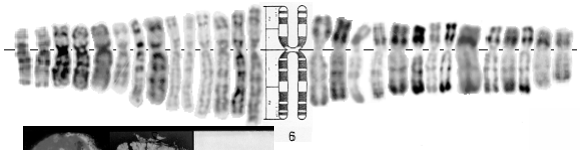


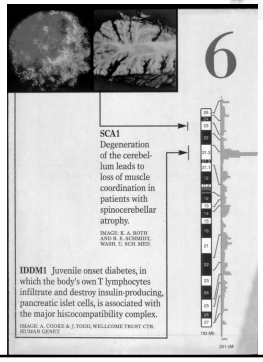
features
- large submetacentric chromosome
- q-arm central with 3 dark bands

25

Cytogenetics
karyotyping

chromosome 6



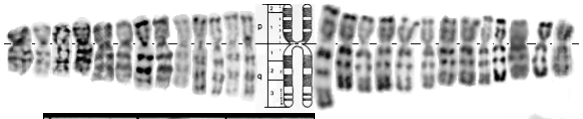


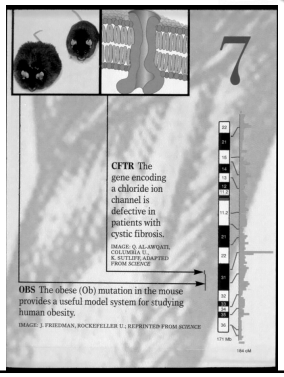
features
- similar sized as chromosomes 4 and 5 but longer p-arm
- submetacentric
- p-Arm rel. light

26

Cytogenetics
karyotyping

chromosome 7

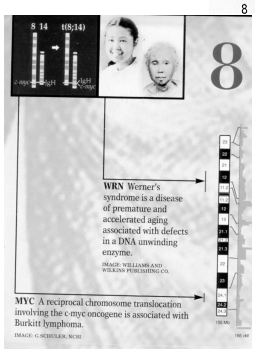


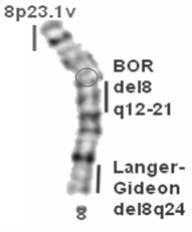


features
- long p-arm resembling a rabbit ear)
- submetacentric
- may be mixed up with #10
  if you look ad q-arm

Cytogenetics
karyotyping

chromosome 8

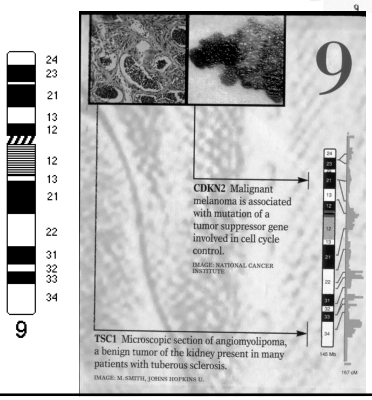




features
- may be mixed up with X-chr
- submetacentric
- p-arm with 2 dark bands
- q-arm light with one distal dark band

Cytogenetics
karyotyping

chromosome 9

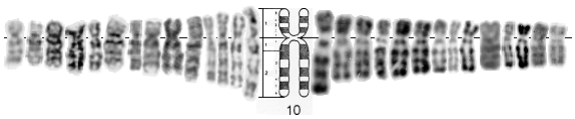


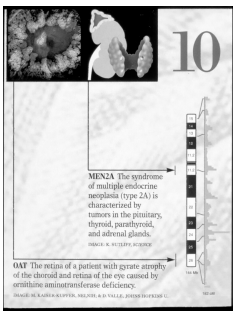
~ 0.6% of pop.

features
- dark centromeric region goes in q-arm
- submetazentrisch
- p-arm with two dark bands and 'heart-shape'
- q-arm with two distal dark bands

Cytogenetics
karyotyping

chromosome 10

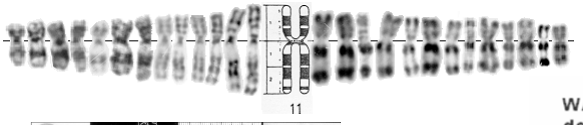


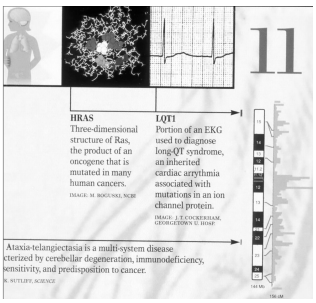


features
-submetacentric
- q-arm with one proximal dark shoulder band

Cytogenetics
karyotyping

chromosome 11



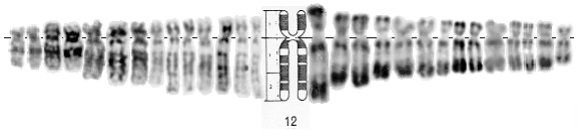


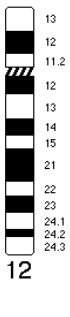
features
- may be mixed up with chromosome 12
- more compact than #12
- submetacentric
- p-arm with two dark bands

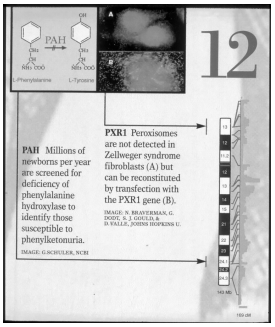
31

Cytogenetics
karyotyping

chromosome 12





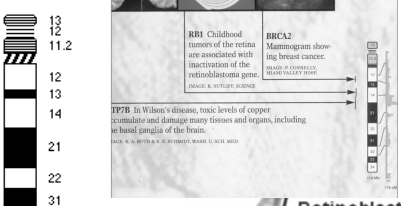


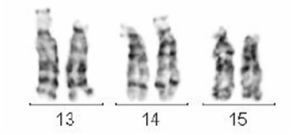
features
- may be mixed up with chromosome 11
- submetacentric
- p-arm one dark band and shorter than #11

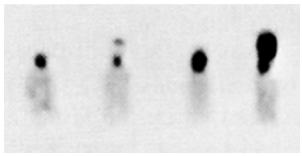
32

Cytogenetics
karyotyping

chromosome 13



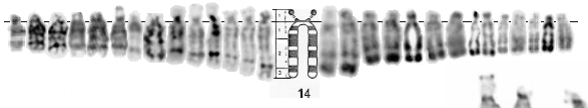


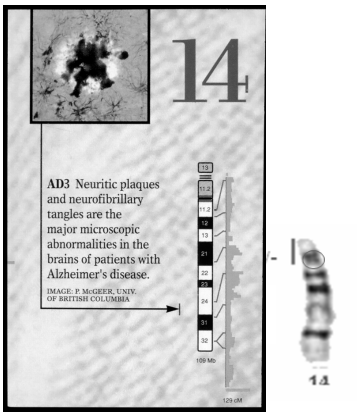


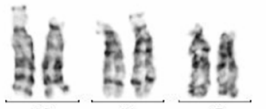
features
- hanging light shoulders
- acrocentric
- p-arm variable - satellite DNA
- q-arm distal dark

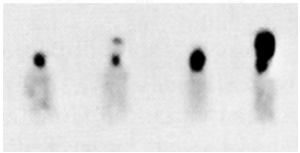
Cytogenetics
karyotyping

chromosome 14





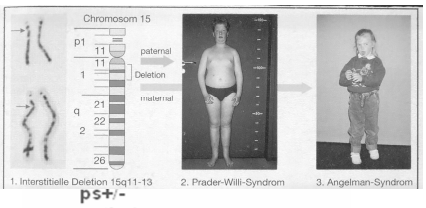


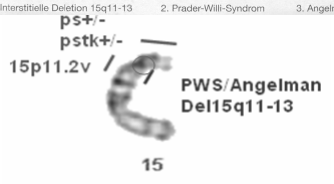


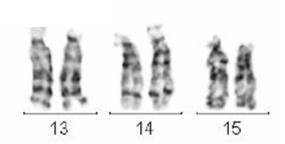
features
- bracket dark shoulders
- acrocentric
- p-arm variable - satellite DNA
- q-arm proximal and distal one dark band each

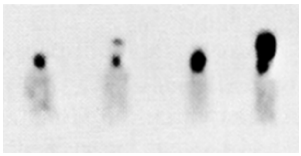
Cytogenetics
karyotyping

chromosome 15







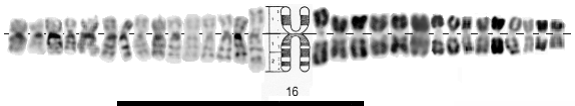


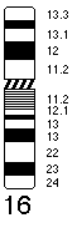
features
- hanging light shoulders
- acrocentric
- p-arm variable - satellite DNA
- q-arm proximal dark

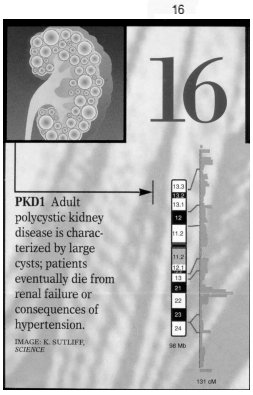
35

Cytogenetics
karyotyping

chromosome 16





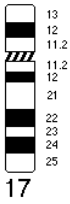


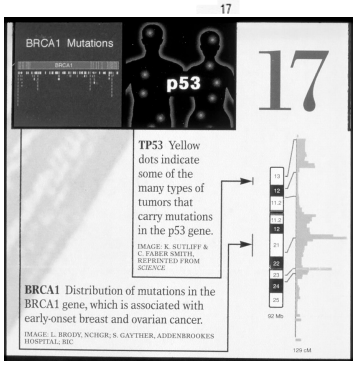
features
- dark centromeric region goes in q-arm
- submetacentric
- p-arm very light
- q-arm two dark bands

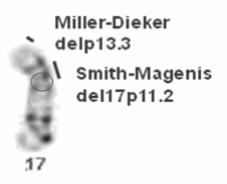
36

Cytogenetics
karyotyping

chromosome 17



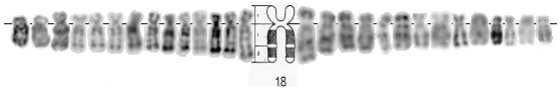


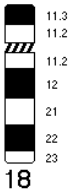


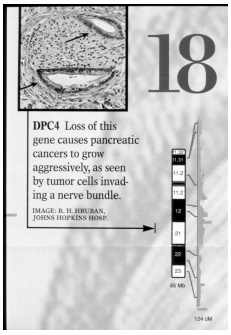
features
- looks like a torpedo
- submetacentric
- q-arm with two distal dark bands

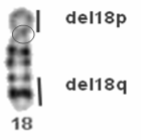
Cytogenetics
karyotyping

chromosome 18





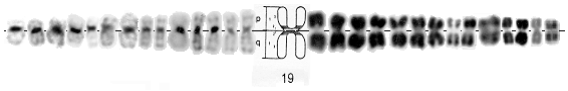


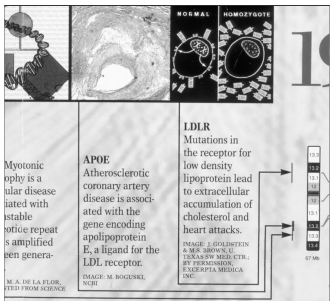


features
- submetacentric
-light p-arm hell
- q-arm one distal and one proximal dark band

Cytogenetics
karyotyping

chromosome 19



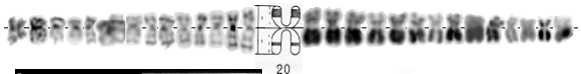


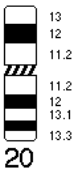
features
- lightest chromosome (gene rich)
- metacentric
- very dark centromere

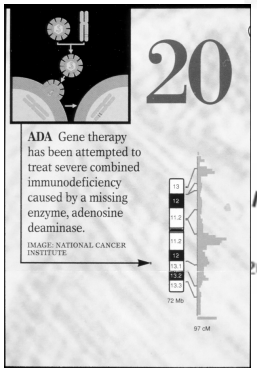
39

Cytogenetics
karyotyping

chromosome 20







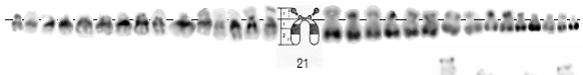
Alagille
del20p1.2

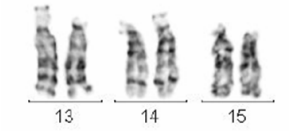
features
- metacentric
- p-arm dark central band

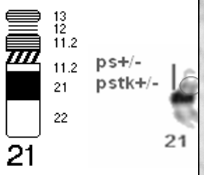
40

Cytogenetics
karyotyping

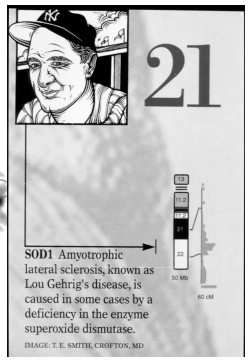
chromosome 21

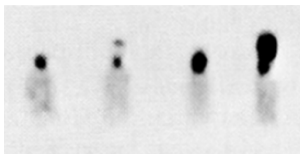






ps+/-
pstk+/-

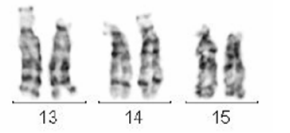


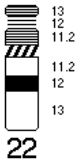


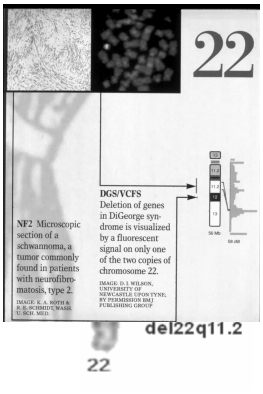
features
- acrocentric
- smallest chromosome
- p-arm variable – satellite DNA
- q-arm with dark shoulder band

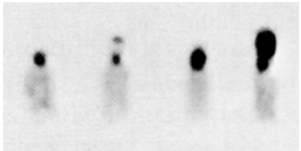
Cytogenetics
karyotyping

chromosome 22





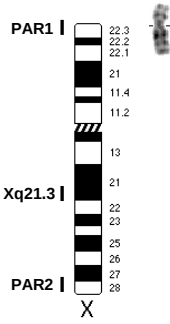


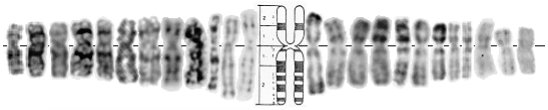


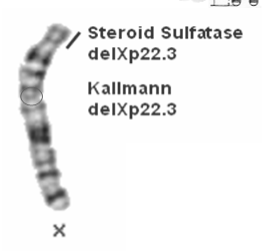
features
- acrocentric
- very light
- p-arm variable – satellite DNA

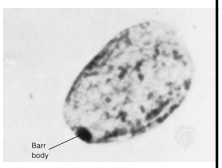
Cytogenetics
karyotyping

X-chromosome









features
- submetacentric
- similar size like chromosome 7
- p-arm longer than other C-chromosomes
- central dark band

PAR1 = pseudoautosomal region - 2.7 Mb – also called p-PAR. - obligatory crossing-over in male with Y-chr.

PAR2 - 0.33 Mb – also called q-PAR - non obligatory crossing-over in male with Y-chr.

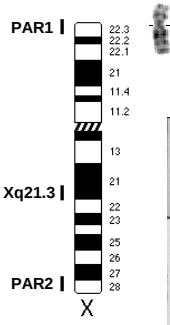
In both PAR ~ 30 genes, which escape **X-inactivation.**

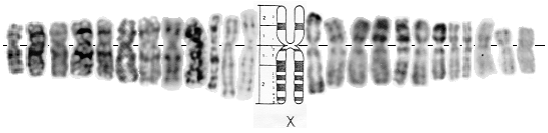
besides PARs another homologous region: Xq21.3 and Yp11.1.

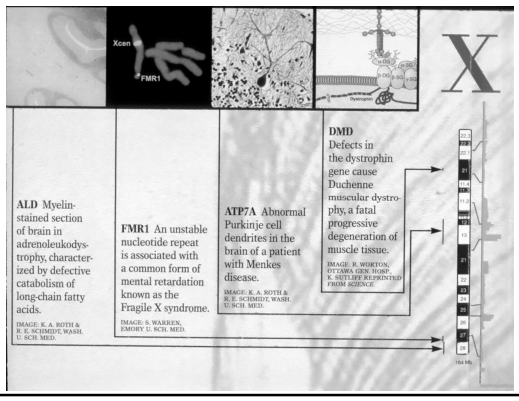
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

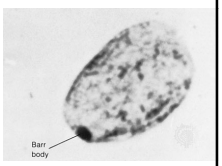
Cytogenetics
karyotyping

X-chromosome



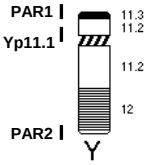


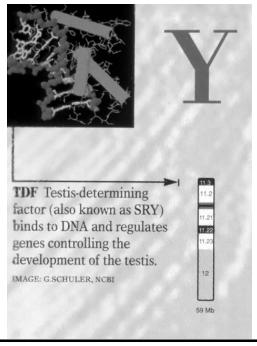




Cytogenetics
karyotyping

Y-chromosome

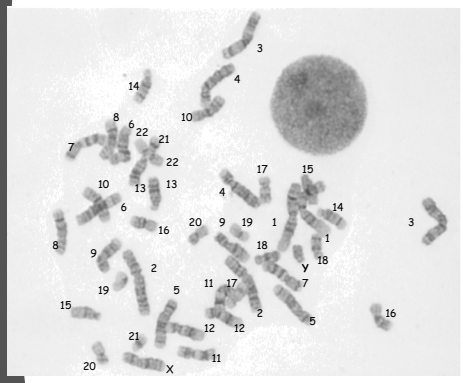




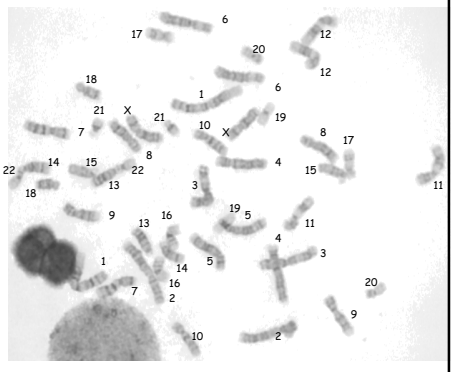
features
- submetacentric
- dreived from X-chromosome
- very small p-arm
- very dark distal q-arm - size variable heterochromatin

Cytogenetics
karyotyping

A



B



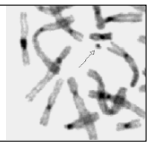
Cytogenetics
formal cytogenetics

GTG-banding
(**G**-bands by **t**rypsin using **G**iemsa) ➥ black and white pattern

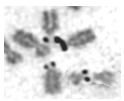
R-banding
(**R**everse) ➥ inverted GTG-banding

CBG-staining
(**C**-bands by **B**arium hydroxide using **G**iemsa)

➥ heterochromatin (centromeres and others)

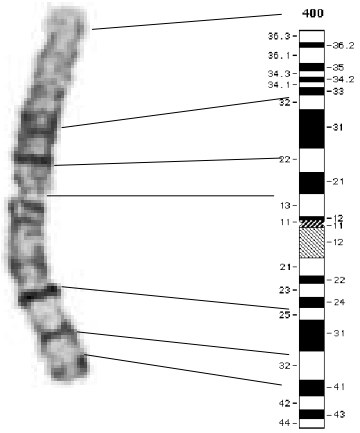


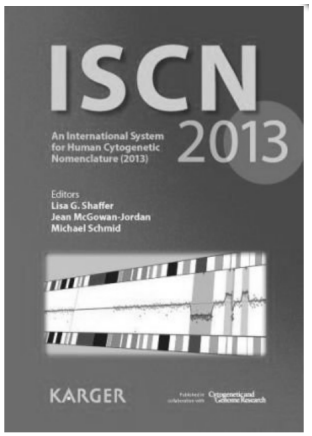
NOR-staining
(**n**ucleolus **o**rganizing **r**egion) ➥ active NORs



Cytogenetics
formal cytogenetics

Ideogram is a schematic drawing of each chromosome.



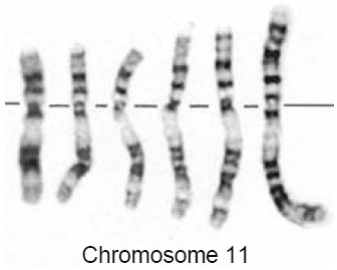


Cytogenetics
formal cytogenetics

Banding resolution

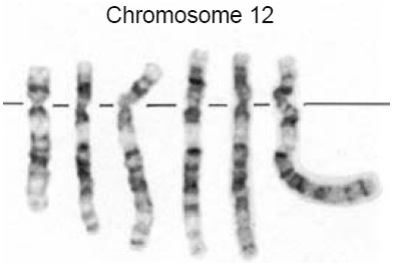
Number of band in haploide chromosome set (22 autosomes with X and Y)

300 400 550 700 850



Chromosome 11

300 400 550 700 850

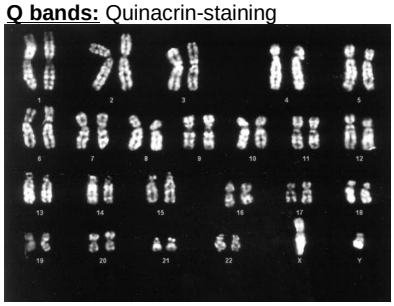


Chromosome 12

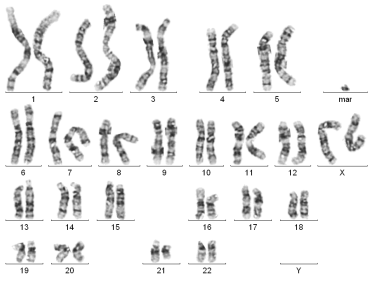
Cytogenetics
formal cytogenetics

Band: part of a chromosome which can be distinguished from its neighborhood, may be dark or light

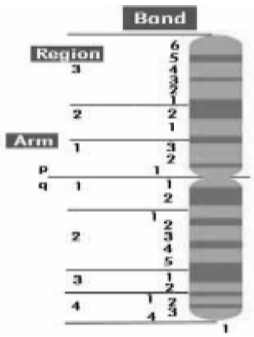
Q bands: Quinacrin-staining



G bands:



Cytogenetics
formal cytogenetics

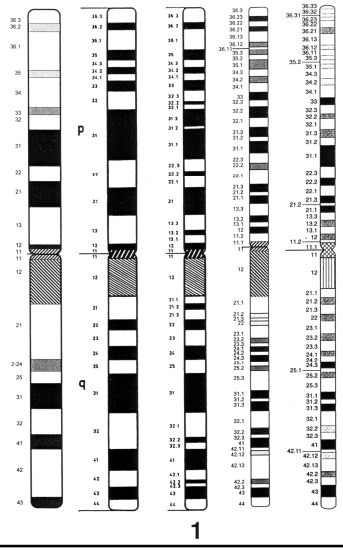


band: part of a chromosome which can be distinguished from its neighborhood, may be dark or light

landmark: bands on which a normal chromosome can be easily recognized

region: sector between two landmarks

Cytogenetics
formal cytogenetics



- there are five different grey values for band intensities.

-Band numbering from centromere to telomere centromere is 10.

- Subbands reflect only nomenclature not biology of band splitting

- One band ~ 5-10 Mb

- G-positive: AT-rich, late replicating, gene poor

- G-negative: GC-rich, early replicating, gene rich

- C-positive: repetitive satellite DNA

- NOR-positive: 18S and 28S rRNA

Cytogenetics
formal cytogenetics

Often used symbols and abbreviations

add	additional material of unknown origin
arrow (->)	from - to, in detailed system
brackets, square ([])	surround the number of cells
cen	centromere
colon, single (:)	break, in detailed system
colon, double (::)	break and reunion, in detailed system
comma (,)	separates chromosome numbers, sex chromosomes, and chromosome abnormalities
decimal point (.)	denotes sub-bands
del	deletion
de novo	designates a chromosome abnormality which has not been inherited
der	derivative chromosome
dic	dicentric
dup	duplication
fra	fragile site
h	heterochromatin, constitutive
hsr	homogeneously staining region
i	isochromosome
ins	insertion
inv	inversion
mar	marker chromosome
mat	maternal origin
minus sign (-)	loss
p	short arm of chromosome
parentheses	surround structurally altered chromosome and breakpoints
pat	paternal origin
plus sign (+)	gain
q	long arm of chromosome
question mark (?)	questionable identification of a chromosome or chromosome structure
r	ring chromosome
rec	recombinant chromosome
s	satellite
sce	sister chromatid exchange
semicolon (;)	separates altered chromosomes and breakpoints in structural chromosome rearrangements involving more than one
slant line (/)	separates clones
t	translocation
ter	terminal (end of chromsome)
upd	uniparental disomy

Cytogenetics
formal cytogenetics

some principles:

1. First is given number of chromosomes, comma, sex-chromosomes, (comma, abnormal autosomes):

   46,XX (normal female) **47,XX,+21** (female, trisomy 21)
   46,XY (normal male)

2. anomalies of sex-chromosomes to mention before aberrant autosomes,
   autosomes are mention in numerical order irrespective of aberration – aberrations to divide by comma:

   47,X,t(X;13)(q27;q12),inv(10)(p13q22),+21 (female, trisomy 21, translocation X and 13 pericentric inversion chromosome 10)

3. After mentioning kind of rearrangement is mentioned the chromosome in question in round brackets:

   inv(2) **del(4)** **r(18)**

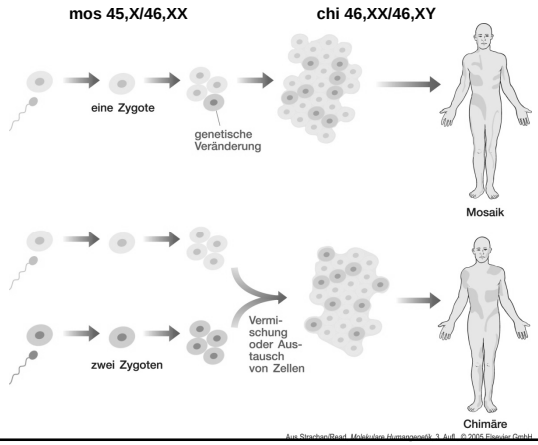
4. Square brackets give clone size - mosaics

   45,X[10]/46,XX[5]

Cytogenetics
formal cytogenetics

some principles:

5. One can distinguish mosaics (**mos**; cells come from one zygote) and chimera (**chi**; cells come from different zygotes)



Cytogenetics
formal cytogenetics

some principles:

Karyotype formulas may be short

46,XX (normal female)
46,XY (normal male)

47,XX,+21 (female, trisomy 21)

long

47,X,t(X;13)(q27;q12),inv(10)(p13q22),+21 (female, trisomy 21, translocation X and 13 pericentric inversion chromosome 10)

Very, very long

78,XX,-Y,der(1)t(1;9)(p35;?),+2,del(2)(p21)x2,+3,der(3)t(3;12)(p11;?)x2, +5,+7,del(7)(q22)x2,der(8)t(8;16)(p11;?),+del(9)(p13),del(9)(p13),der(9;10)(q10;q10)x2,10,der(10)t(10;16)(q11;?)x2,+11,+12,del(12)(q15)x2,+14,+15,+17,der(17)t(8;17)(?;p11)x2,-18,+19,+20,-21,+22[cp25].

Cytogenetics
formal cytogenetics

some principles:

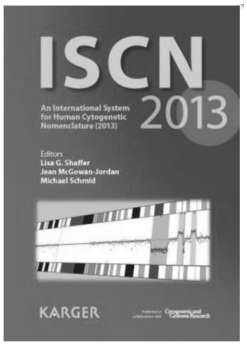
6. If an aberration is inherited from one parent it can be marked by **mat** or **pat**:

   46,XX,t(5;6)(q34;q23)mat,inv(14)(q12q31)pat

   If no-parental origin de novo (**dn**):

   46,XX,t(5;6)(q34;q23)mat,inv(14)(q12q31)dn

→ There are many more rules



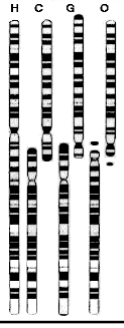
Cytogenetics
formal cytogenetics

There is homology of chromosomes between closely related species.

Chromosomenumber is species specific

	(2n)
Zea mays (corn)	20
Oryza sativa (rise)	42
Ascaris megacephalus (round worm)	2
Stylonychia mytilus (see animal)	ca. 300
Gallus domesticus (chicken)	78
Drosophila melanogaster (fruit fly)	8
Bombyx mori (silkmoth)	56
Lysandra atlantica (buttefly)	446
Felis catus (cat)	38
***Homo sapiens* (Human)**	**46**

	Chromosomenzahl (2n)
Gorilla gorilla	48
Pan troglodytes	48
Pongo pygmaeus	48
Homo sapiens	**46**

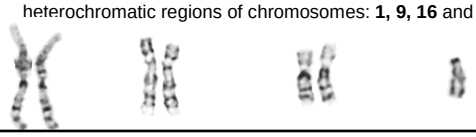


Cytogenetics
Structural variants

→ Are only found if you study a lot of chromosomes in a population.

1. Heteromorphism

heterochromatic regions of chromosomes: **1, 9, 16** and **Y**

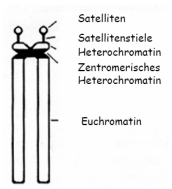


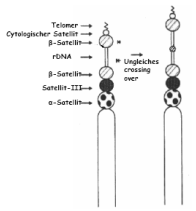
short arms of acrocentric chromosomes: **13, 14, 15, 21** and **22**

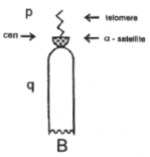
centromeric regions of all chr.

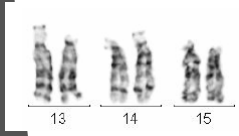
inversions of chromosomes **2** and **9**

Cytogenetics
Structural variants
1. Heteromorphism
Acrocentric chromosomes

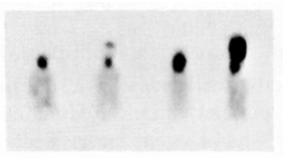








GTG

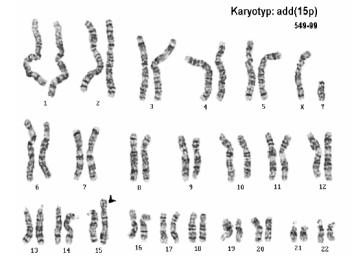


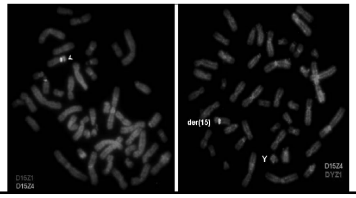
CBG

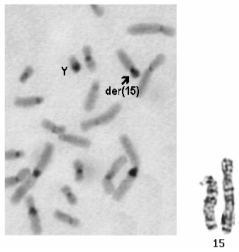
NOR

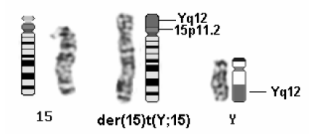
Cytogenetics
Structural variants
1. Heteromorphism
Acrocentric chromosomes

Why to be interested in such variants?





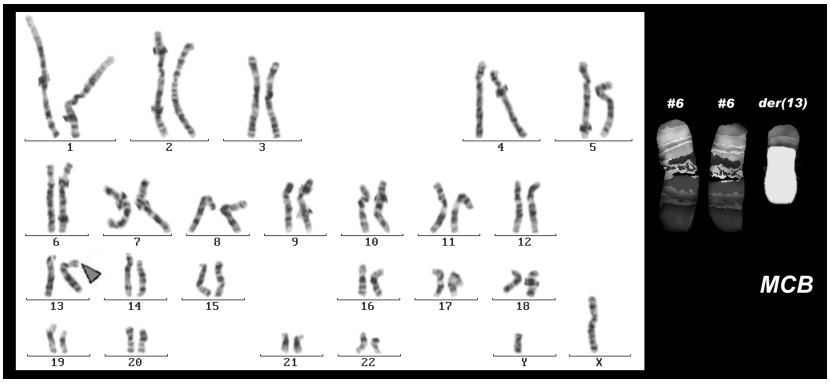




Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

Cytogenetics
Structural variants
1. Heteromorphism
Acrocentric chromosomes

Why to be interested in such variants?



Cytogenetics
Structural variants
1. Heteromorphism
Acrocentric chromosomes

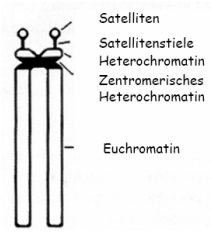
ISCN 2009:

Variations in length of heterochromatic segments (**h**), centromeres (**cen**), cen stalks (**stk**) or satellites (**s**) can be described.

Examples: 16qh+ 12cenh+pat 9phqh+ 1q41h+
Yqh- 10cenh+mat 9ph+

Acrocentric chromosomes

21ps+
22pstk+
15pss
13pstkstk
15cenh+mat,15ps+pat
14cenh+pstk+ps+
17ps
Yqs



Cytogenetics
Structural variants
1. Heteromorphism
Acrocentric chromosomes
Satellite associations

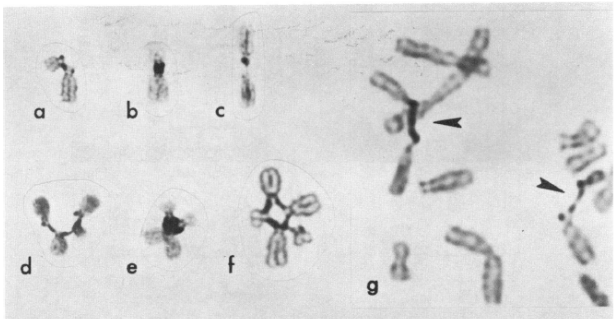


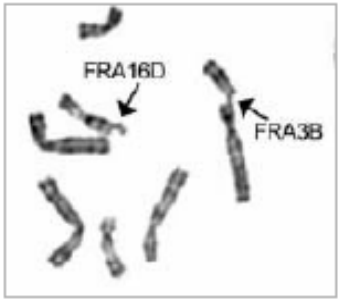
FIG. 3.—Human acrocentric chromosomes in association, showing variation in Ag-staining: *a*, short Ag-stained connective between single chromatids; *b*, increased silver deposit between two chromosomes in association; *c*, association between one chromosome with Ag-stained material and one without; *d*, chain of four chromosomes; *e*, tight ring of four chromosomes; *f*, open ring of four chromosomes (with connectives between adjacent chromosomes only) plus a single chromatid association with a fifth chromosome; *g*, long Ag-stained connectives.

Cytogenetics
Structural variants

→ Are only found if you study a lot of chromosomes in a population.

2. Fragile sites

… are cytogenetically visible gaps or breaks, which appear under specific cultural circumstances and may span several hundred kb.



65

Cytogenetics
chromosome breakage syndromes

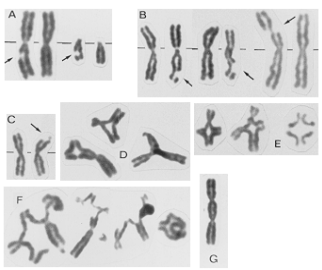
… sind Krankheiten, denen ein Defekt im **DNA Reperaturmechanismus** zugrunde liegt. Alternativ werden sie auch als **Mutagenhypersensitivitäts Syndrome** bezeichnet.

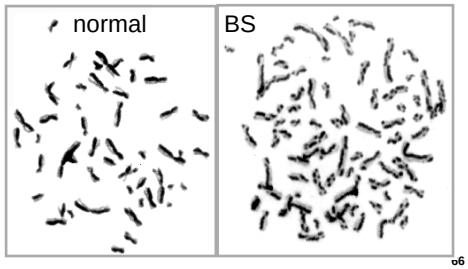
Klassische CBS: - **Fanconi-Anämie**
- **Bloom-S.**
- **Ataxia-Telangiektasia**

Seltene CBS: - **Nijmegen-Breakeage-S.**
- **Immundef.-Cen. inst-Facial ano (ICF)-S.**

→ werden autosomal **rezessiv vererbt**

FA



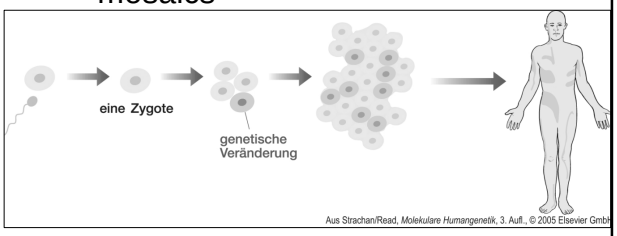


66

Cytogenetics
mosaics

mosaic: at least two different cell lines in one probe / sample.

mos 46,XX[15]/47,XX,+21[5]



causes: - preparation induced or cultural artifacts = pseudomosaics
- clonal cell lines (in independent cultures) = real mosaics

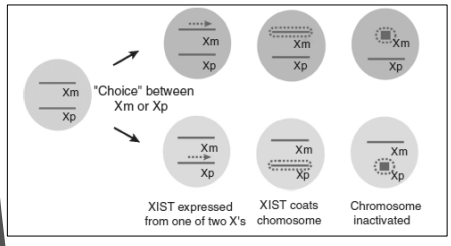
properties: - develop postzygotic
- percentage of cell population can be different in variant tissues
- thus, if mosaic in one tissue → **may be** phenotype less severe
- often cells with aberrant karyotype is lost in quick dividing tissues during life
- incidence: 2:1000 life births

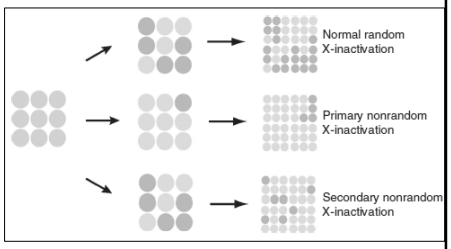
Cytogenetics
mosaics – special forms

Each woman: - with resp. to activity of X-chromosomes (**Lyon-hypothesis**)

→ thus, also women may show symptoms of X-chromosomal disorder!

→ In case of defect X-chromosome the 50:50 distribution may be relocated towards the 'healthy X-chromosome' = skewed X inactivation





facultative heterochromatin

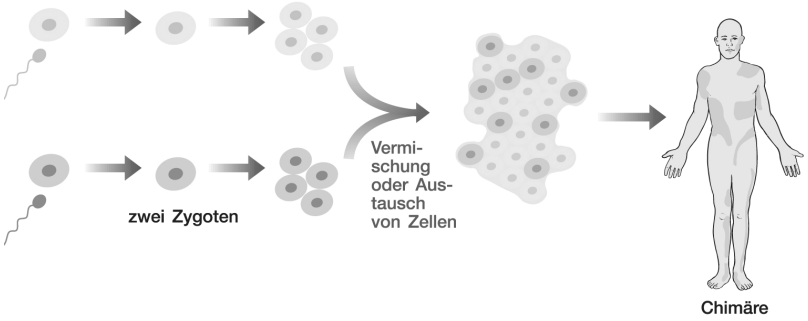
Cytogenetics
mosaics – special forms

chimera: may be due to

→ twin fusion in utero to one individual

→ individual after transplantation e.g. bone marrow

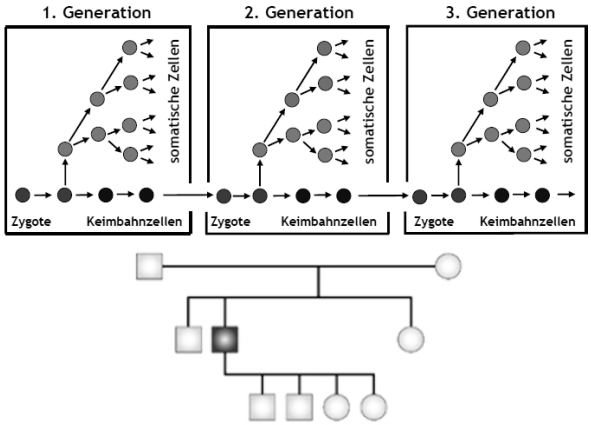
→ every woman who was already pregnant (residual embryonic cells)



Aus Strachan/Read, *Molekulare Humangenetik*, 3. Aufl., © 2005 Elsevier GmbH

Cytogenetics
mosaics – special forms

somatic mosaics : develop postzygotic and have no influence on germ line cells (e.g. tumor)

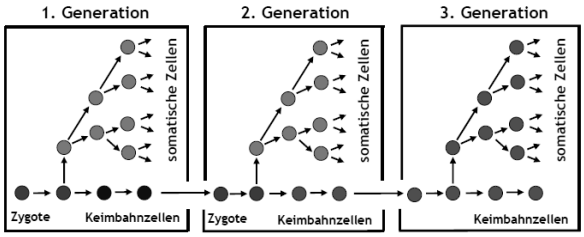


Cytogenetics
mosaics – special forms

germ line mosaics: - mutation happens in germ line
→ then there are two different germ cell populations

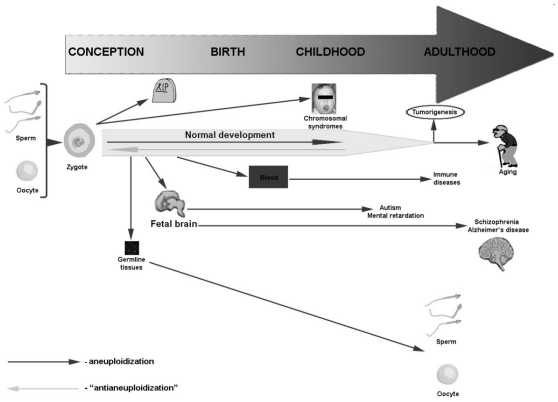
- mutations not detectable in parental somatic cells

- higher risk for diseased offspring than in new-mutations!



Cytogenetics
mosaics – summary

- mosaics develop postzygotic
- most chromosome aberrations appear already in one of both **meiotic divisions**.



Cytogenetics
prenatal diagnostics

invasive prenatal diagnostics

→ There is a risk

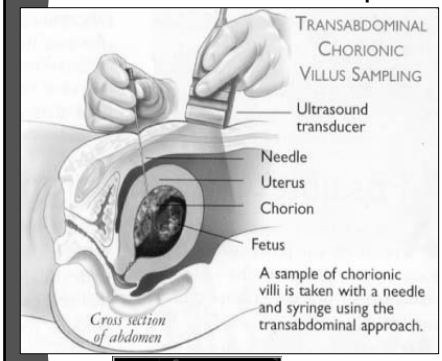
- **chorionic villi biopsy** (placenta)
- **amniocentesis** (amnion cells)
- **chordocentesis** (umbilical chord blood)

If to do invasive diagnostics or not depends on

question, age and wish of pregnant woman.

chorionic villi biopsy

Cytogenetics
prenatal diagnostics

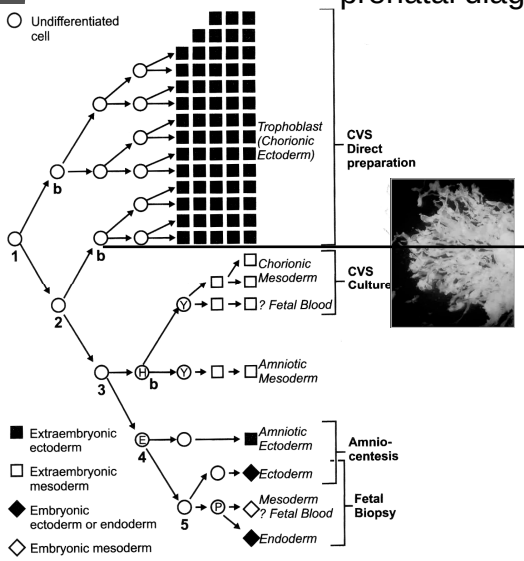


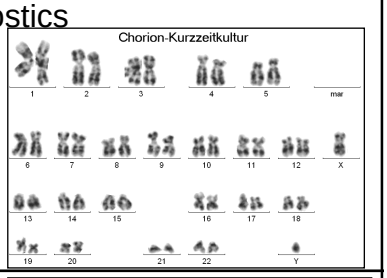
- from **9.-12. week of gestation**
- biopsy under sonographic control (take ~ 15 mg)
- N.B. embryo develops from one cell of 16-32 cell stage - all other cells become amnion and trophoblast → mosaics possible!
- Chromosomes by **direct preparation** (1 day)
- also chromosome after **long term culture** (2-3 weeks)
- **risk** (transabdominal) **0.3-0.5%** (dependent on MD doing punctuation)

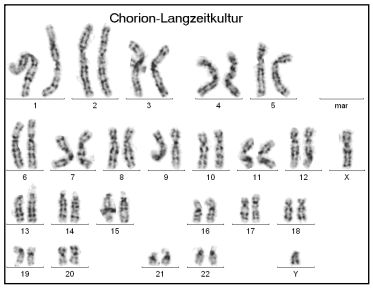
Institut für Humangenetik
Abteilung Molekulare Zytogenetik

Universitätsklinikum Jena

chorionic villi biopsy — Cytogenetics prenatal diagnostics





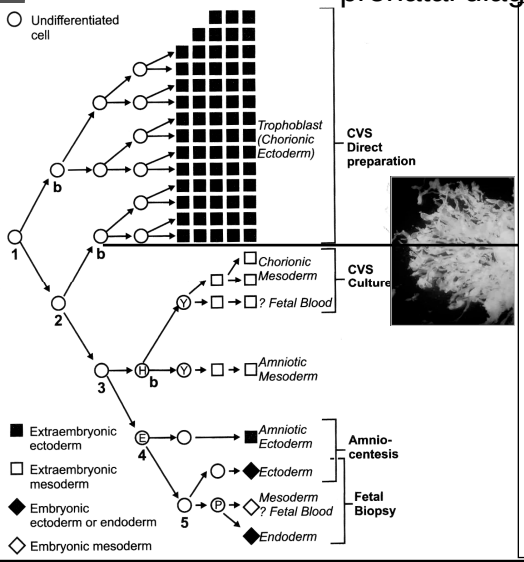


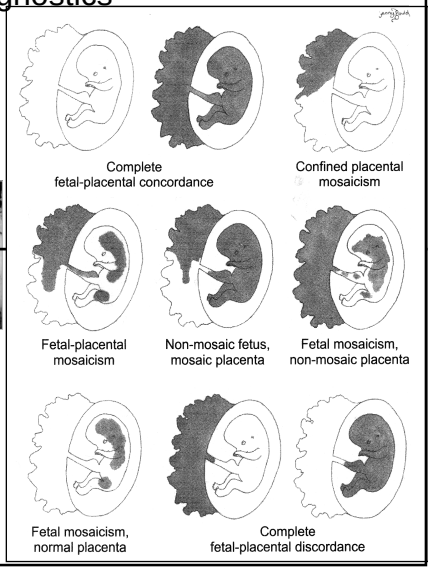
75

Institut für Humangenetik
Abteilung Molekulare Zytogenetik

Universitätsklinikum Jena

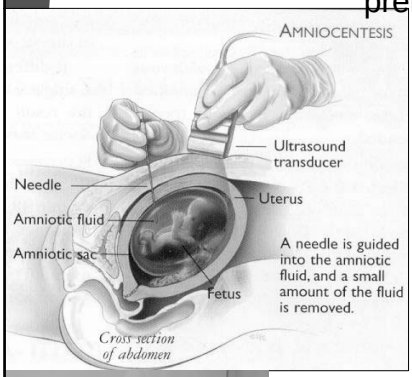
chorionic villi biopsy — Cytogenetics prenatal diagnostics

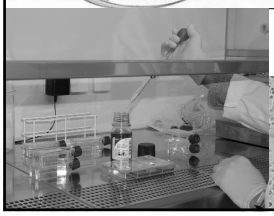


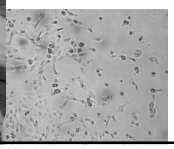


Cytogenetics
prenatal diagnostics

amniocentesis







- between **14.-20. week of gestation**
- fluid under sonographic control (take ~ 17 ml)
- mainly cells are **skinfibroblasts** and also cells from **stomach** and **bladder**
- chromosome from **cell culture** (2 weeks)
- **add. diagnostics**: FISH or STR **quicktest** for aneuploidies of chromosomes 13,18,21,X,Y ; **molecular genetic** and **biochemical** diagnostics (AFP-(Alpha-Feto-Protein) – neural tube defects)
- **risk 0.3-0.5%** (dependent on MD doing punctuation)

http://www.youtube.com/watch?v=K9itd1Ot-kg&feature=related Pres. 4-2

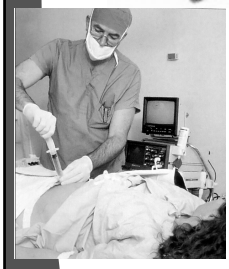
77

Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

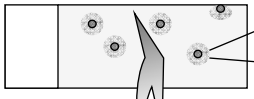
Cytogenetics
prenatal diagnostics

amniocentesis

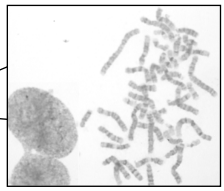
in situ culture



slide with cell clones

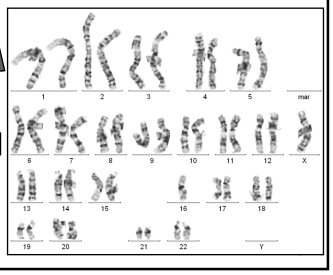


metaphase from periphery



cultural artifact?
mosaic?

Karyogram: 45,X,-X,-16

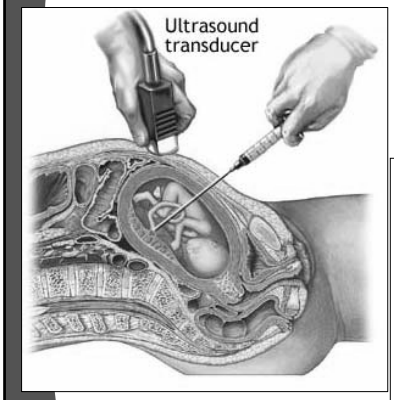


Preliminary result by phone:
2 clones: numerically normal ♀/♂
≥3 clones: normal ♀/♂

final written report :
>10 clones: normal ♀/♂

Cytogenetics

chordocentesis prenatal diagnostics

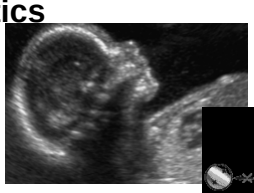


- from **20. week of gestation**
- **fetal blood cells** under sonographic control (take ~2ml)
- chromosomes from **lymphocyte culture** (3 days)
- **add. diagnostics possible**
- **risk** (transabdominal) **0.3-1.0%**
  (dependent on MD doing punctuation)

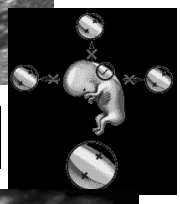
79

Cytogenetics

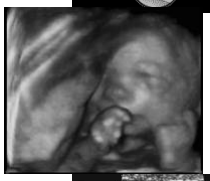
sonography prenatal diagnostics



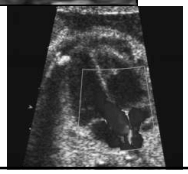
3 basic-**sonography - methods**
for phenotyping and measuring



Doppler-sonography (measuring bloodstreams in fetus), **high resolution sonography** and **3D sonography** from 19. week of gestation



Add. **first-trimester-screening** (11.-14. w.o.g.) → individual risk for birth of a child with trisomy 21
(mat. age, sonography, hormones of woman (ß-HCG, PAPP-A) – detection level >95%

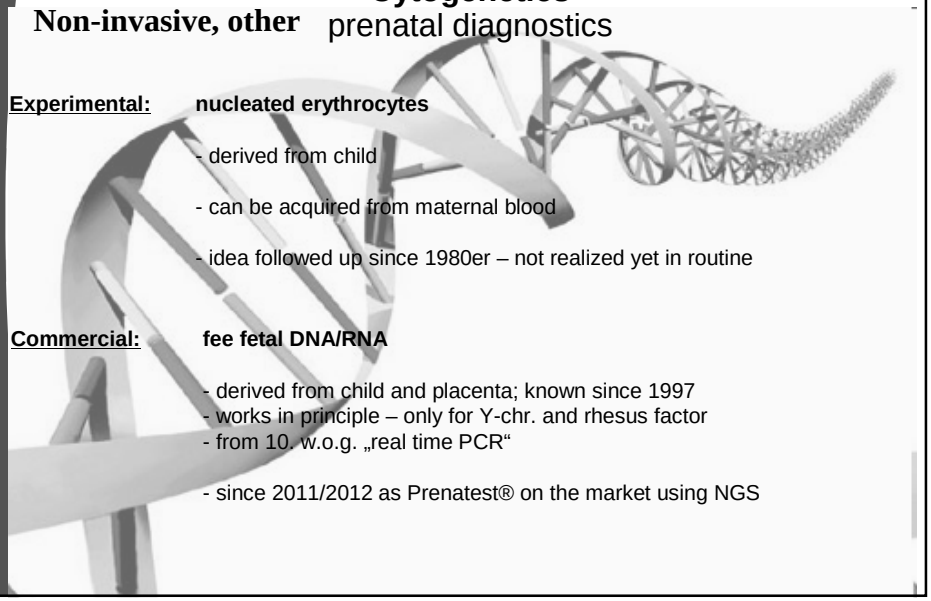


also poss. **triple-test**: risk estimation from:
age of mother, hormones of woman (ß-HCG, PAPP-A)
and AFP (alpha-feto-protein)

No risk - BUT – there is no 100% security !

Cytogenetics

Non-invasive, other prenatal diagnostics



Experimental: **nucleated erythrocytes**

- derived from child
- can be acquired from maternal blood
- idea followed up since 1980er – not realized yet in routine

Commercial: **fee fetal DNA/RNA**

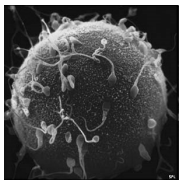
- derived from child and placenta; known since 1997
- works in principle – only for Y-chr. and rhesus factor
- from 10. w.o.g. „real time PCR“
- since 2011/2012 as Prenatest® on the market using NGS

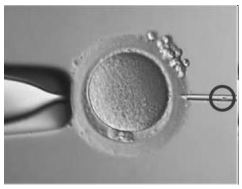
Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

Cytogenetics

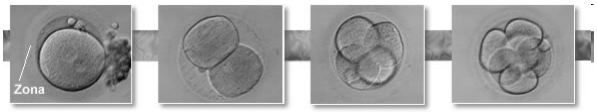
IVF and PID prenatal diagnostics

after (IVF) and intracytoplasmatic sperminjection (ICSI)

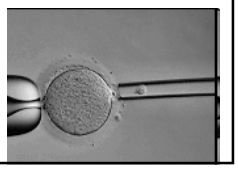




→ Take 1 morulacell (8-cellstadion, day 3) for diagnostics



http://www.youtube.com/watch?v=LYQVxd1MHuk Pres. 4-3



"Embryonenschutzgesetzes" not allowed in Germany (at least under discussion)
→ **polar body diagnostics**!

Cytogenetics
prenatal diagnostics

Indications prenatal cyto.

– advanced maternal age (>35 y)
– aberrant first-trimester-screening
– sonographic abnormalities
– abnormal tripletest
– psychological reasons
– previous pregnancy with chromosomal aberration
– pos. family anamnesis (like Down syndrome)
– others

§218

– TOP without giving reason until 12. w.o.g.

– TOP until 21./22. w.o.g. to „avoid risk of a severe impact on somatic or psychological health of pregnant ”

Cytogenetics
diagnostics
“**Gen Diagnostik Gesetz”**

since 1.2.2010:

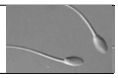
- no genetic diagnostics (GDG) without informed written consent of patient – has to be present in lab !
- only responsible MD is allowed to give result to patient
- material has to be destroyed after diagnostics is finished
- records have to be stored 10 years and then destroyed
- no prenatal diagnostics for late manifesting diseases

Cytogenetics
prenatal diagnostics

Frequency of aneuploidies

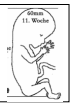
oocyte 20%

sperm 10%

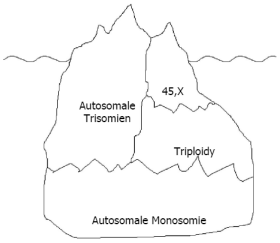


conception 30%

1. Trimenon embryo 10%



life born 0.6%



85

Institut für Humangenetik
Abteilung Molekulare Zytogenetik
Universitätsklinikum Jena

Cytogenetics
prenatal diagnostics

abortions/ miscarriages

- **abortions are most frequent adverse event** in human reproduction
  (15-20% of all pregnancies, 80% in early embryonic phase)
- diagnostics should be offered after 2. abortion
- **reasons**: chromosomal, endocrinolog., autoimmunolog., exogen
- 2-5% of abortions are repeated, after 3 abortions = **habitual abortions**
  risk of repetition after
  1 abortion ➔ 15%
  2 abortions ➔ 25%
  3 abortions ➔ 30-45%

In such cases one finds chrom. aberrations in one of parent in 3-8%
(= 6x over general population)

86

Cytogenetics
prenatal diagnostics
abortions/ miscarriages

Bezeichnung	Definition
Fehlgeburt	= Abort
Frühabort	Weniger als 12+0 SSW*
Spätabort	12+0 SSW* oder später und weniger als 500 g Geburtsgewicht
Totgeborenes	500 g oder mehr Geburtsgewicht und Kind ohne Lebenszeichen
Frühgeborenes	Weniger als 37+0 SSW* mit Lebenszeichen oder Totgeborenes mit 500 g Geburtsgewicht oder mehr
Reifgeborenes	37+0 SSW* oder älter, unabhängig von Gewicht oder Zustand

Legende:
*Die Schwangerschaftswochen (SSW) werden immer ab dem 1. Tag der letzten Regel gezählt (auf lateinisch p.m = post menstruationem): z. B. 17+3 SSW = 17 Wochen und 3 Tage nach dem Beginn der letzten Regel
** In der Literatur wird teilweise die Grenze erst bei 16+0 SSW gezogen, Saling empfiehlt aus klinischen Gesichtspunkten heraus (s. auch Tabelle 2) die frühere Grenze bei 12+0 SSW.

Possible reasons
- **genetic**
- **immunologic**
- **nicotine/ alcohol/ caffeine** or pollutants (rare)
- impaired **blood clotting** (rare)
- **infection of mother Mutter**: rubella, measles, toxoplasmosis
- bad regulated **diabetes mellitus** or **pregnancy associated diabetes**
- other risk factors → **prehistory**
- **fever** (prostaglandin), **diarrhea** (peristaltic)

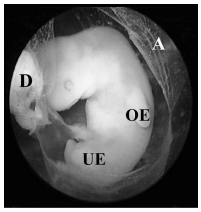
often reason remains unclear.

Institut für Humangenetik
Abteilung Molekulare Zytogenetik

Universitätsklinikum Jena

Cytogenetics
prenatal diagnostics
abortions/ miscarriages

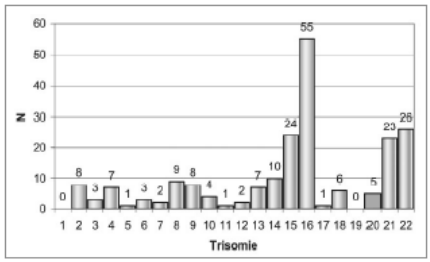
diagnostics
- **anamnesis** (**genetic problems** in family?, other diseases, **drink and tobacco use** or exposure to pollutants)
- **infections in vagina and uterine orifice?**
- vaginal **sonography**
- **karyotyping** of both partners
- **hormone status** (TSH, LH, prolactine, androgens, progesterone)
- others



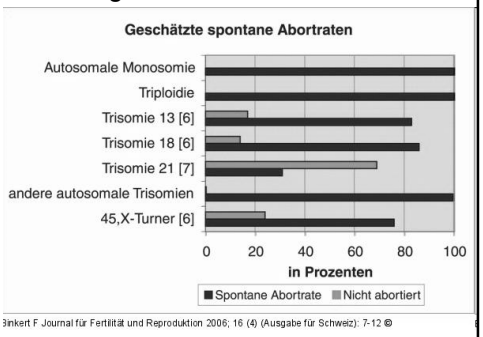
Cytogenetics
prenatal diagnostics

abortions/ miscarriages

most frequent trisomies in abortions



12: Häufigkeit unterschiedlicher Trisomien, die unter 439 erfolgreich karyotypisierten Aborten gefunden wurden. (Zytogenetische Untersuchungen: Zytogenetisches Labor, SMZ-Ost, Donauspital, Abteilung für Pathologie, Vorstand Prof. Reiner.)



Hinkert F Journal für Fertilität und Reproduktion 2006; 16 (4) (Ausgabe für Schweiz): 7-12 ©

What is aborted and what survives?