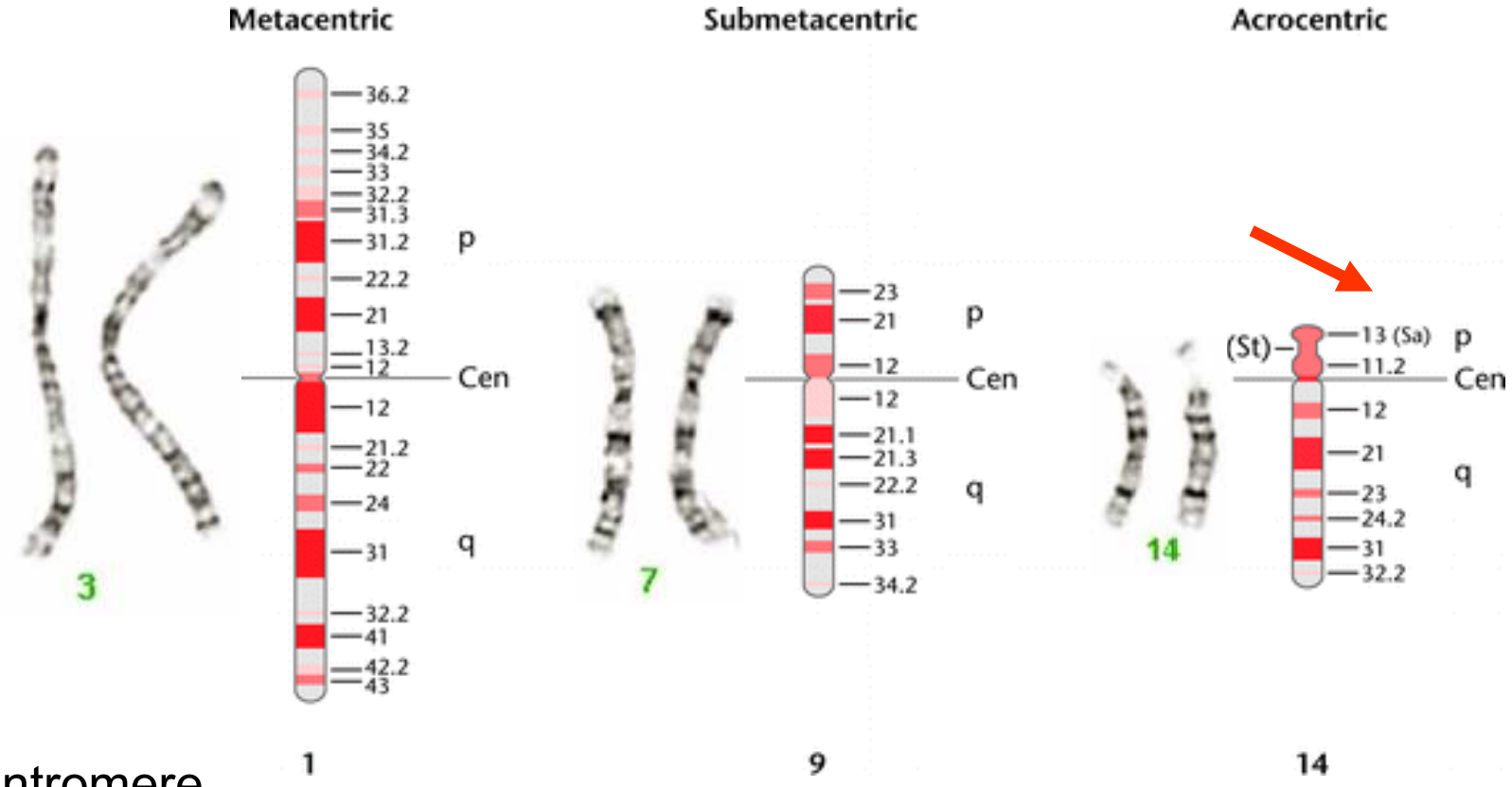
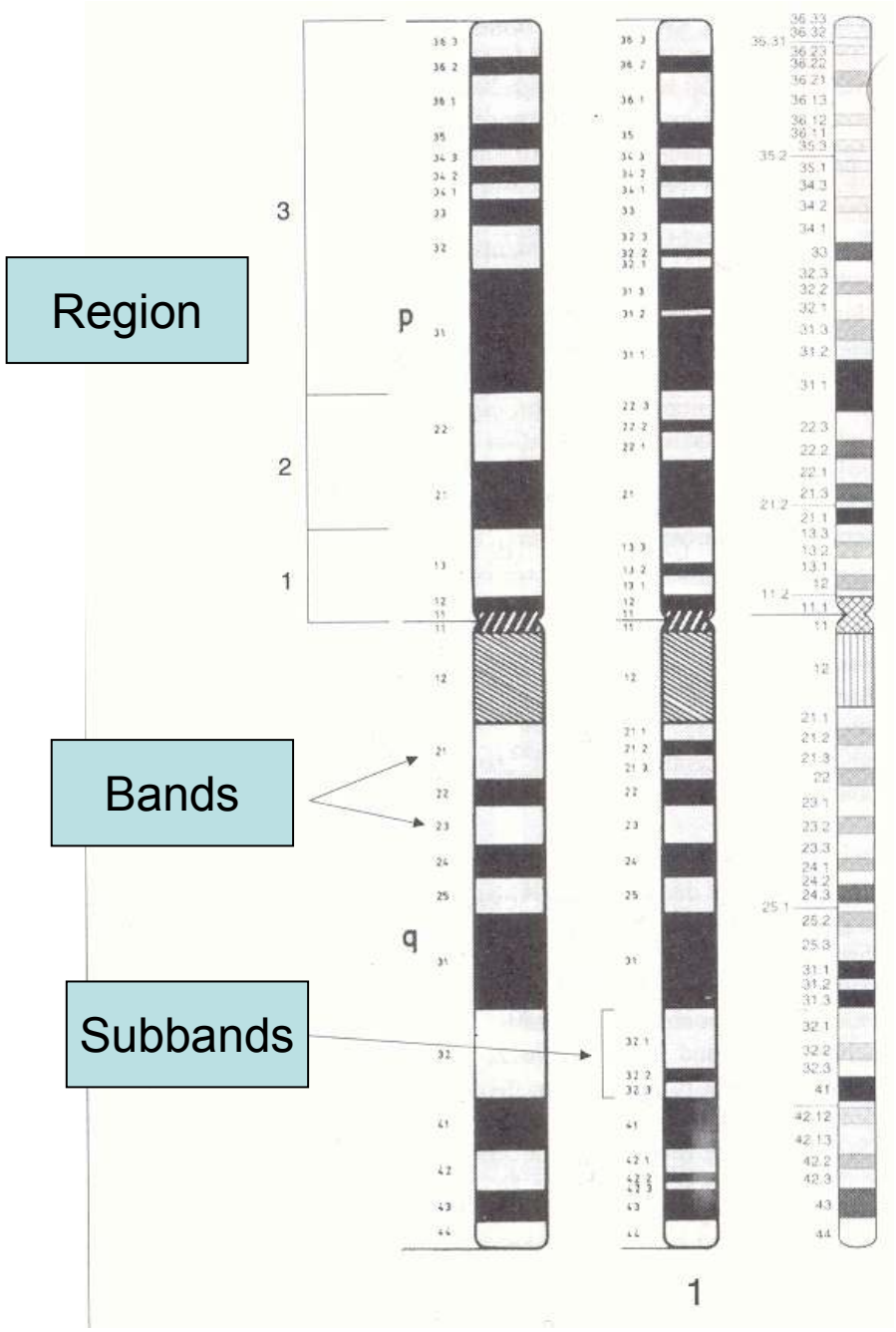
Cytogenetics

Ideogram of representative G-banded chromosomes



Cen - centromere
St - stalks
Sa - satellites

Human Chromosomes.pdf


Region
Bands
Subbands
3
2
1
p
q
1

Cytogenetic polymorphism

Chromosome polymorphism:

1qh+

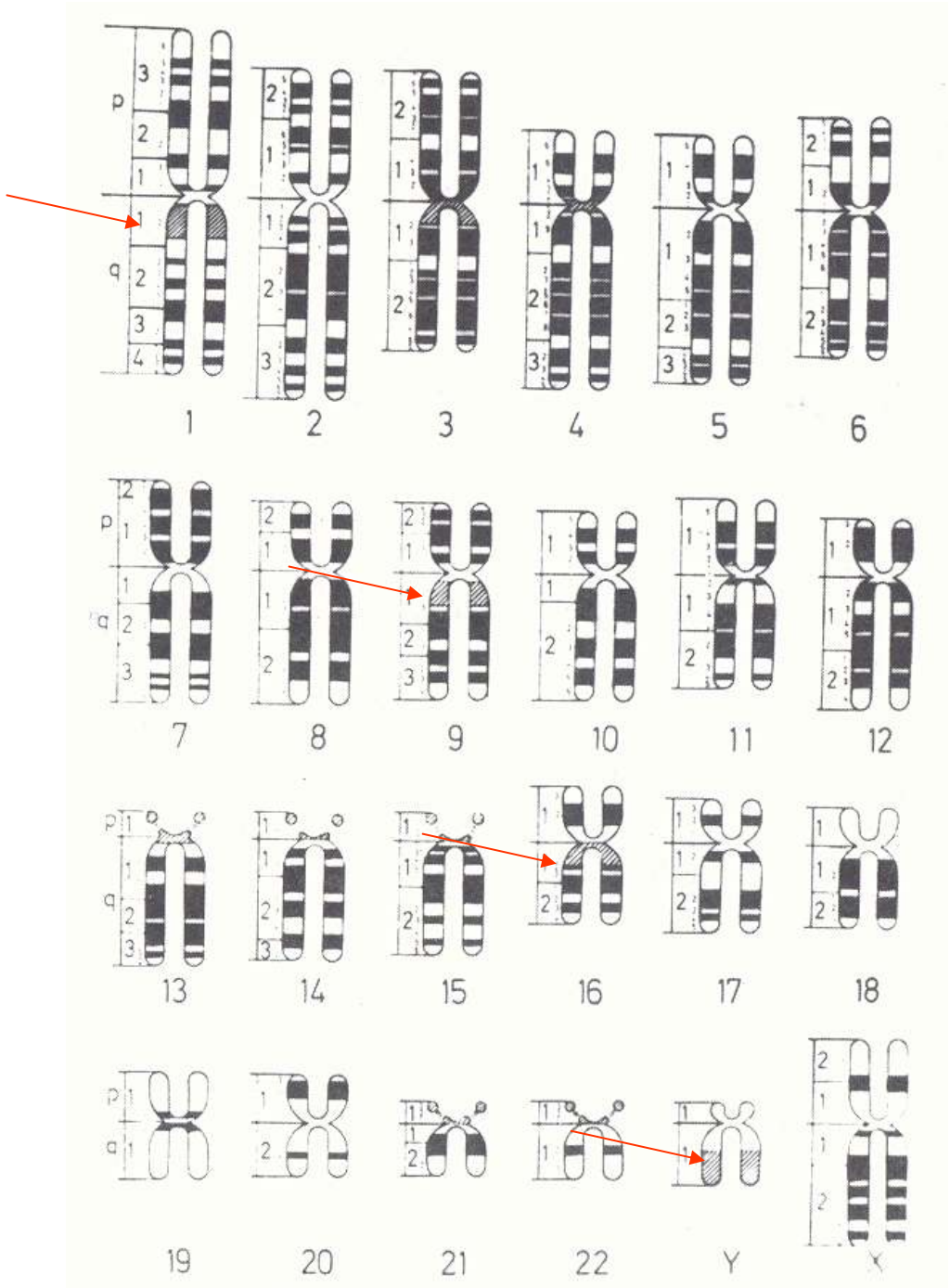
9qh+, 9qh- (increase/decrease of ht. in q-arm), 9ph (ht. in p-arm only), 9phqh (ht. in p and q)

16qh+

Yqh+

Example:

46, XY, 1qh+, 9qh+, 16qh+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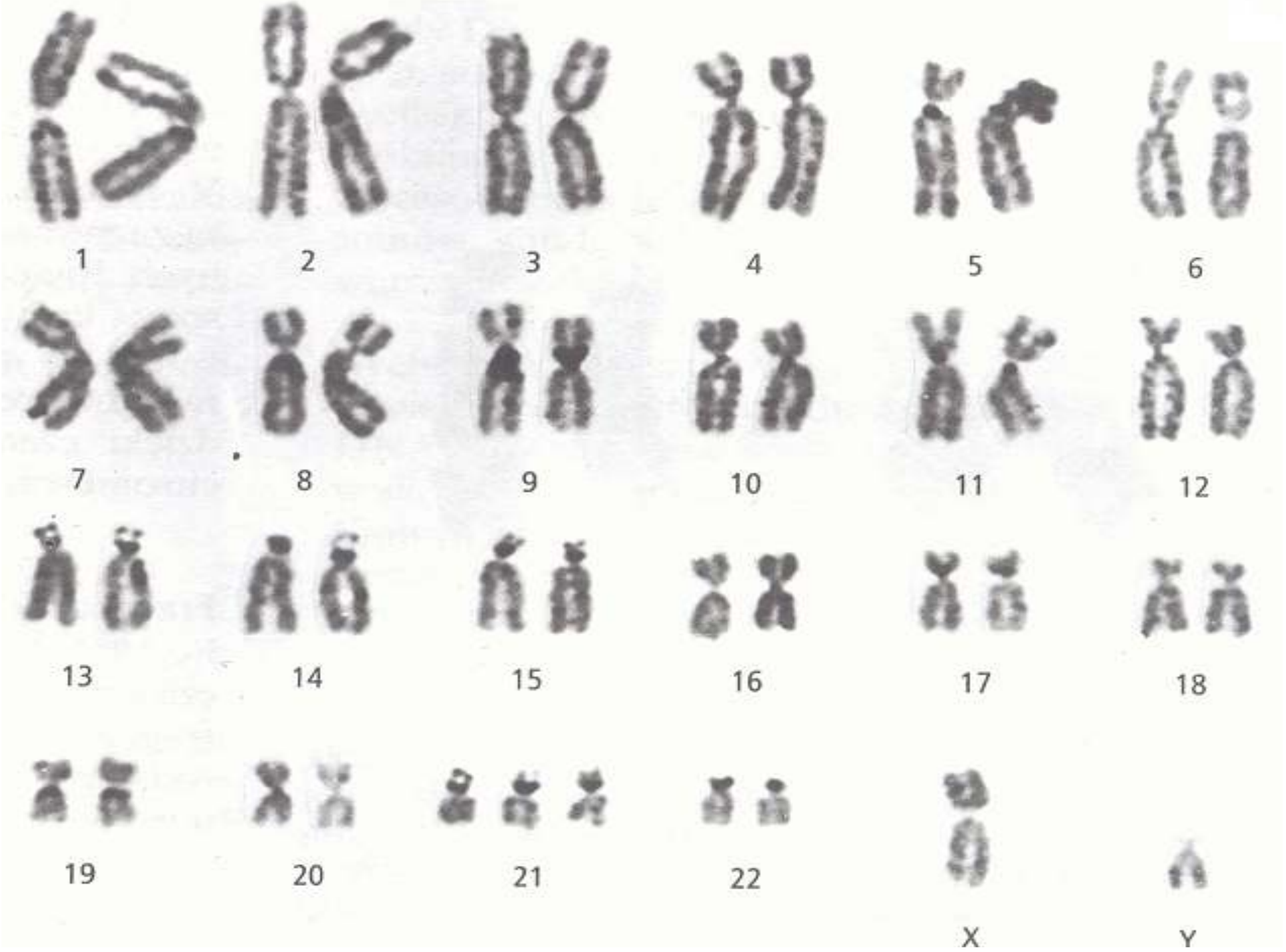
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X
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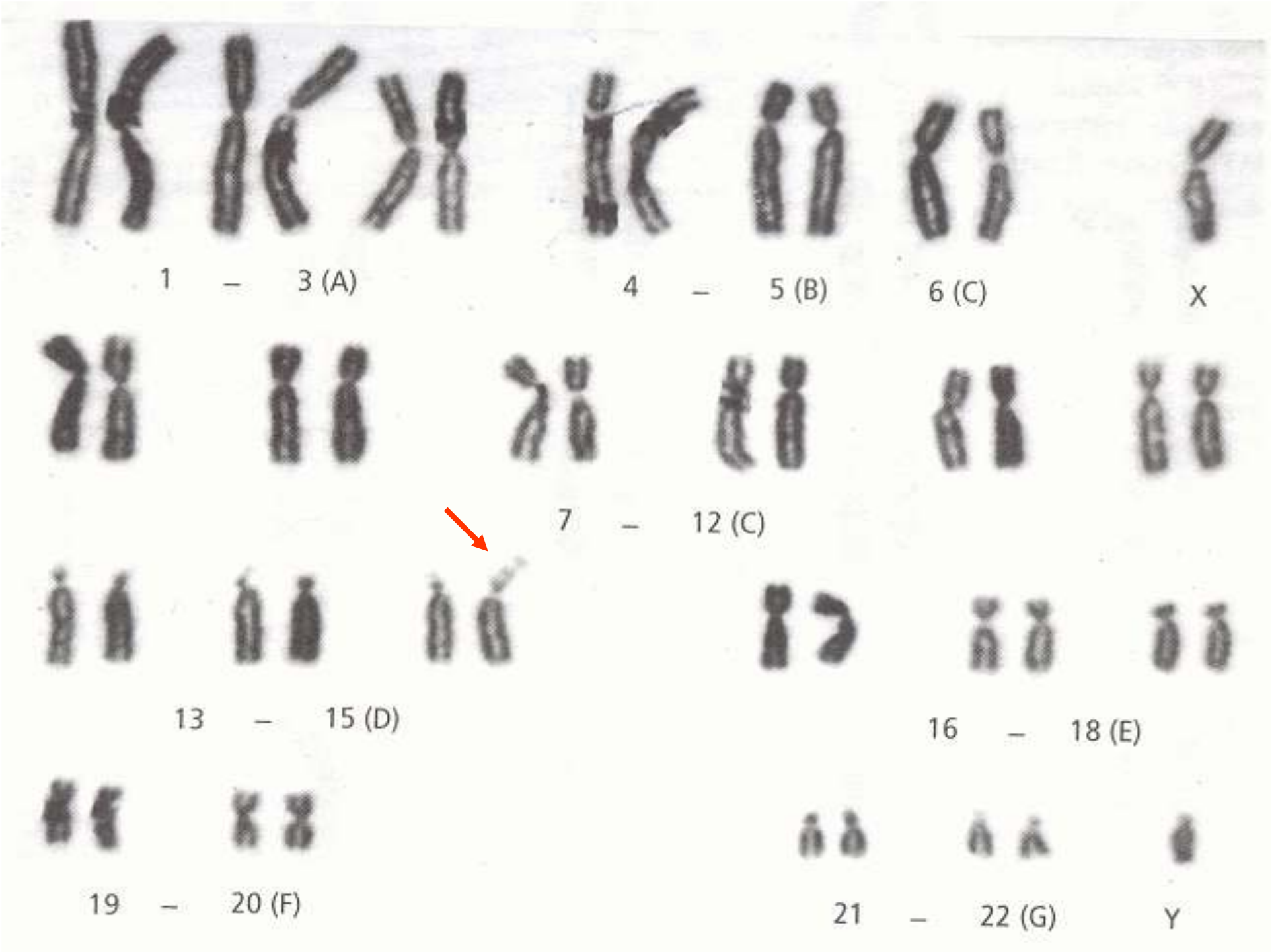
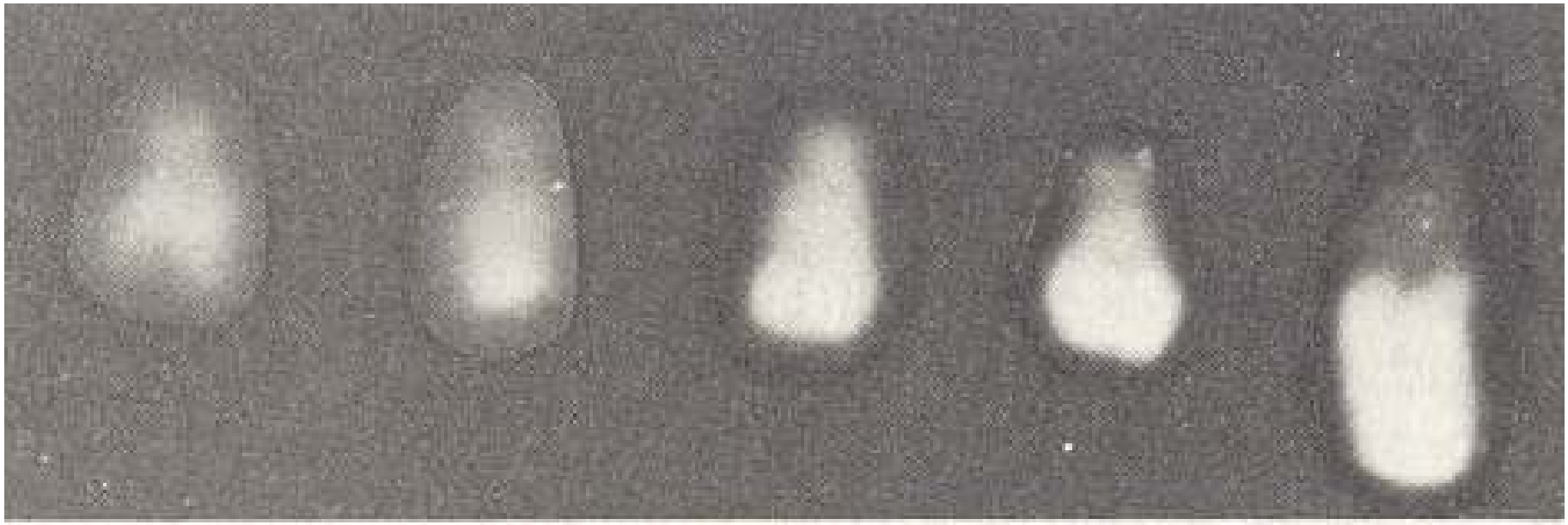

1 – 3 (A)
4 – 5 (B)
6 (C)
X
7 – 12 (C)
13 – 15 (D)
16 – 18 (E)
19 – 20 (F)
21 – 22 (G)
Y



Ryc. 3.8. Polimorfizm Yq.

Pathogenic changes

Chromosome abnormalities:

1) numerical
a) polyploidy
b) aneuploidy

2) structural
a) deletion
b) insertion
c) duplication
d) translocation
e) ring chromosome
f) isochromosome

Incidence of chromosomal abnormalities in live births

All chromosome abnormalities 1/160 live births

Frequencies of chromosome abnormalities

1 in 150 livebirths,

5% of stillbirths,

50% of spontaneous abortions.

The frequency of chromosome abnormalities at fertilization may be as high as 50%.

Humans are unique with regard to the high frequency of chromosome abnormalities when compared to other species. *mouse: < 1–2% Saccharomyces cerevisiae: 1/10,000, Drosophila melanogaster: 1/1,700- 6,000 (aneuploidy in fertilized eggs)*

Nat Rev Gen 2001

Human Chromosomes.pdf

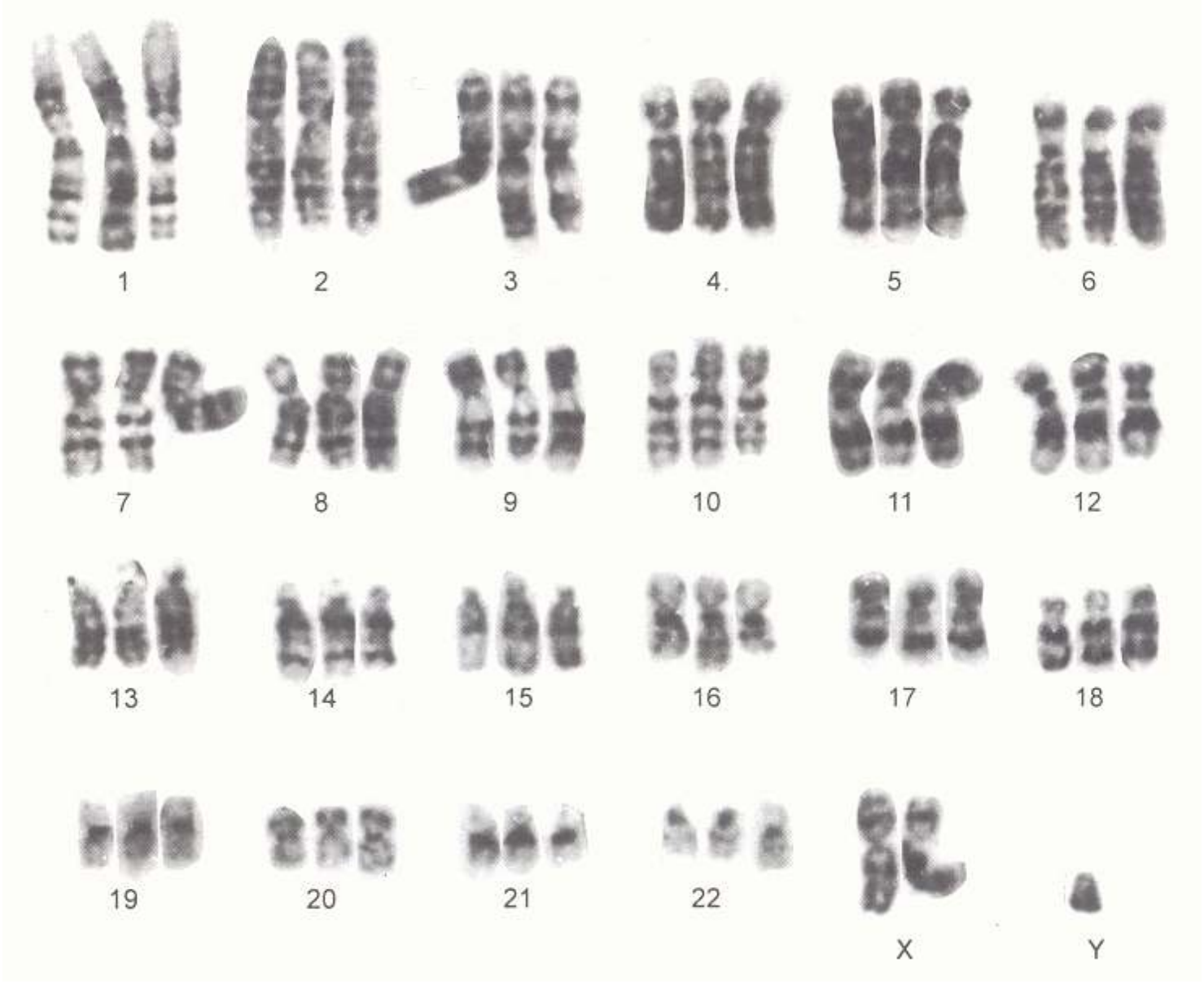
Numerical abnormalities - polyploidy

Change in the number of haploid sets of chromosomes

Triploidy (69 chromosomes) is one of the most frequent abnormalities in spontaneous abortions. Triploids arise when two haploid sperm fertilize a haploid egg. Triploidy is lethal, with most fetuses dying before birth.

Tetraploidy (two extra sets of chromosomes -92 chromosomes) is more rare and also lethal.

Human Chromosomes.pdf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X
Y

Numerical abnormalities - aneuploidy

Loss or gain of a single chromosome(s)

Results from errors in division during meiosis, where a daughter cell receives both pairs of a particular chromosome (nondisjunction errors).

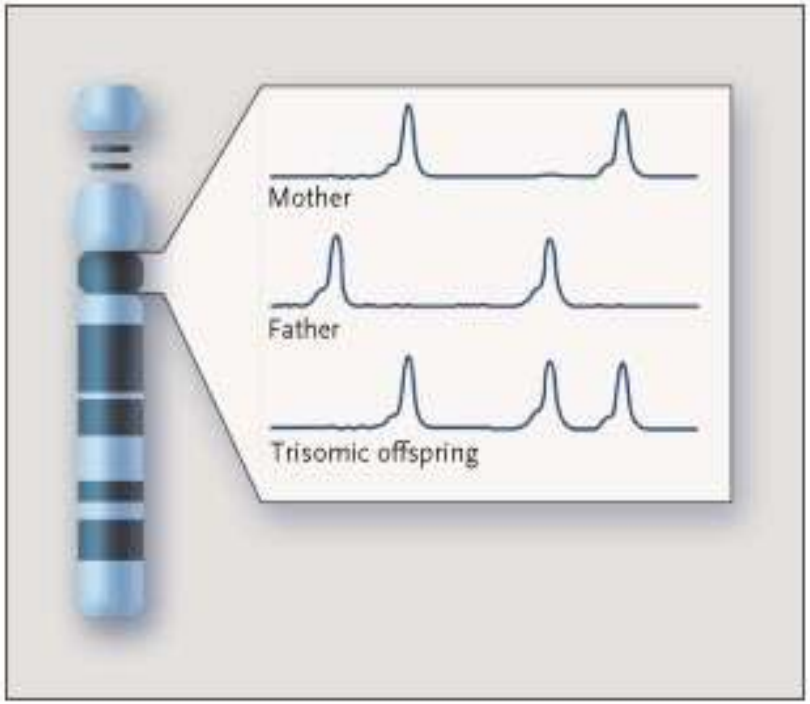
Addition of an extra chromosome, trisomy, has been described for all the chromosomes but only three autosomal trisomies survive to birth. Those are trisomies for chromosomes 21, 18 and 13. The remaining autosomal trisomies are miscarried.

Trisomy for chromosomes 13 and 18 are much more severe than 21 and those that survive to term usually die shortly after birth.

Chromosomes 13 and 18 are the two chromosomes composed of the least amount of GC-rich/gene-rich DNA whereas the chromosome 21 is one of the smallest autosomes.This may be why they can survive to term when other autosomal trisomies are miscarried.

Human Chromosomes.pdf

Nondisjunction types (meiosisi I or II) can be diagnosed with the use of polymorphic genetic markers



Analysis of inheritance of polymorphic DNA markers can be used to determine the meiotic stage and parent of origin of aneuploidy. In this example, different alleles of a chromosome 21 centromeric locus are visualized as peaks in sequencing electrophoretograms. The trisomic offspring (+21) has inherited one paternal allele and two different maternal alleles; thus, the extra chromosome originated from an error at maternal meiosis I.

The origin of human trisomy

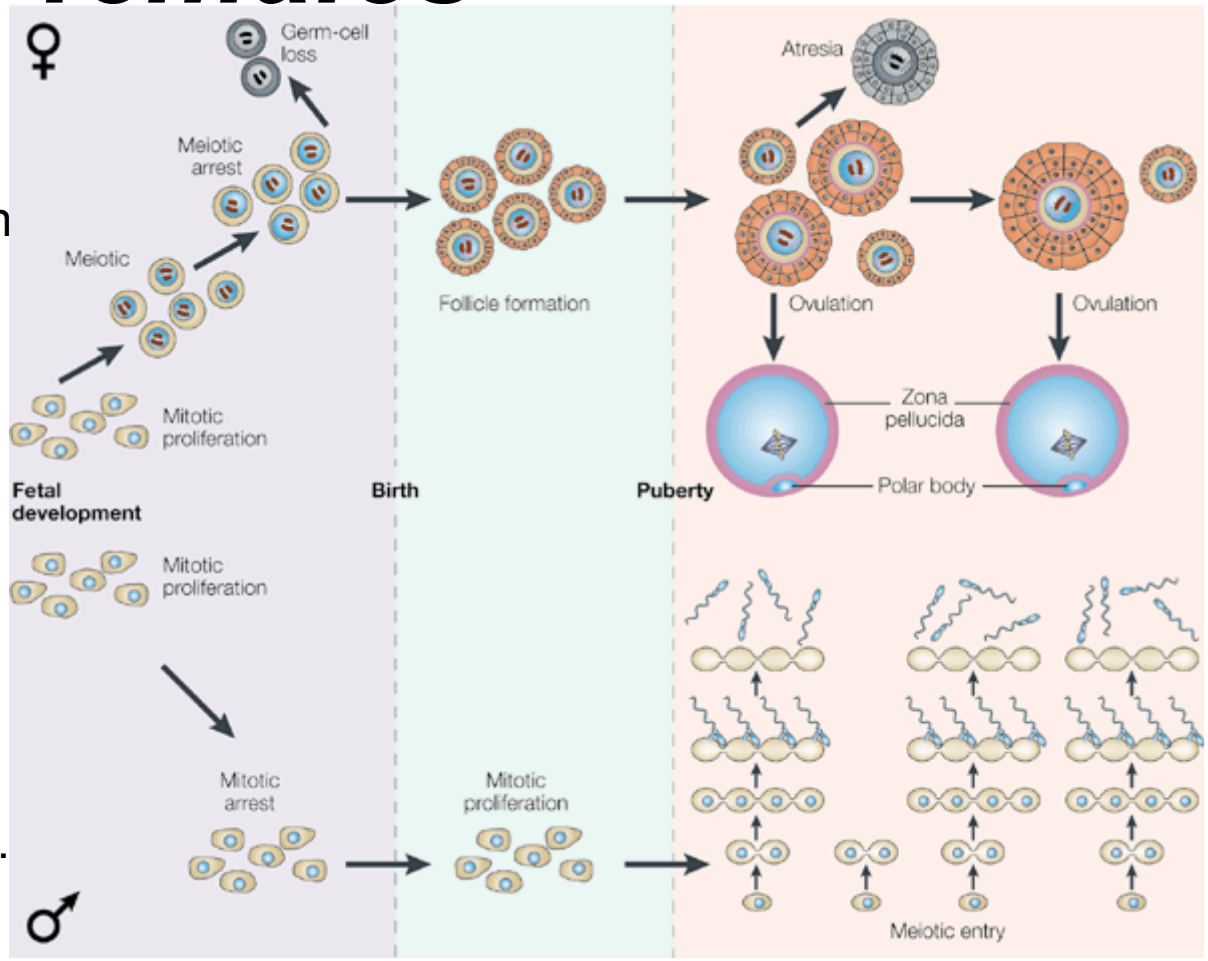
MI, meiosis I; MII, meiosis II

Nat Rev Gen 2001

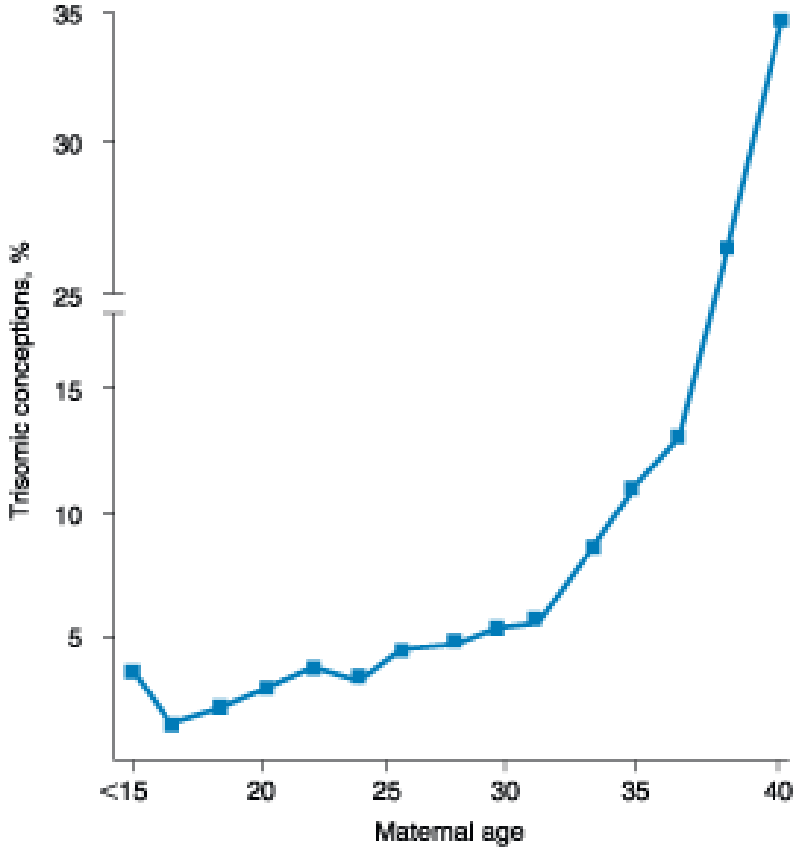
Meiosis in in males vs. females

In male, meiosis begins with puberty and the important events are sequential: in the adult testis, cells progress from prophase to metaphase I and on to metaphase II without an intervening delay, and each cell that enters meiosis produces four sperm.

In female is extraordinarily protracted: all oocytes initiate meiosis during fetal development, but after homologous chromosomes undergo synapsis and initiate recombination, the oocyte enters a period of meiotic arrest. Resumption of meiosis and the completion of the first division occur years later, just before the oocyte is ovulated. After the completion of MI, the oocyte arrests at the **metaphase of MII** and the second division is completed only after the egg is



Nat Rev Gen 2001

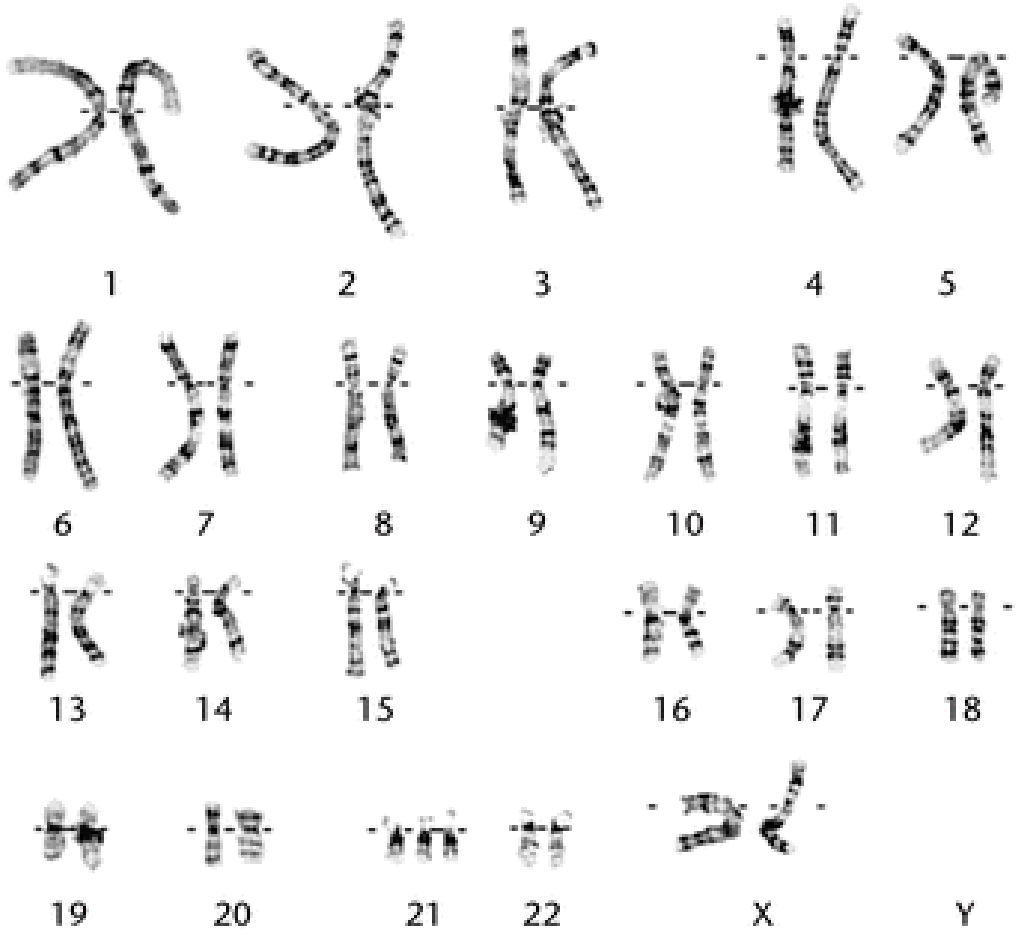


Maternal Age	Risk of Down Syndrome	Risk of Any Chromosomal Abnormality*
20	1/1667	1/526
22	1/1429	1/500
24	1/1250	1/476
26	1/1176	1/476
28	1/1053	1/435
30	1/952	1/384
32	1/769	1/323
34	1/500	1/238
35	1/385	1/192
36	1/294	1/156
37	1/227	1/127
38	1/175	1/102
39	1/137	1/83
40	1/106	1/66
41	1/82	1/53
42	1/64	1/42
43	1/50	1/33
44	1/38	1/26
45	1/30	1/21
46	1/23	1/16
47	1/18	1/13
48	1/14	1/10
49	1/11	1/8

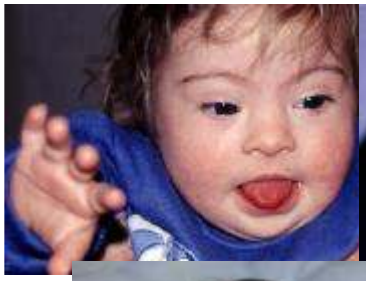
*47,XXX excluded for ages 20 to 32 (data not available).

Data from Hook EB: "Rates of chromosome abnormalities at different maternal ages." *Obstetrics and Gynecology* 58:282–285, 1981; and Hook EB, Cross PK, Schreinemachers DM: "Chromosomal abnormality rates at amniocentesis and in live-born infants." *Journal of the American Medical Association* 249:2034–2038, 1983.

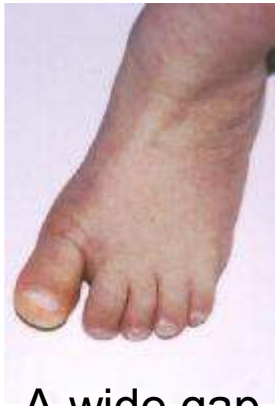
47,XX,+21 female Down syndrome karyotype (trisomy 21)



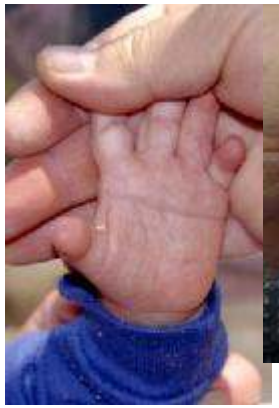
Down syndrome: morphology



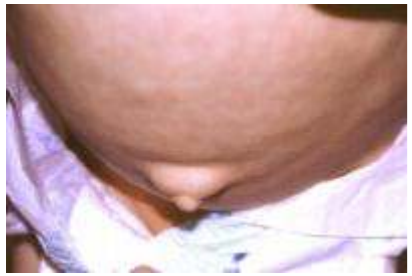
Flat face with hypertelorism, depressed nasal bridge, protrusion of the tongue



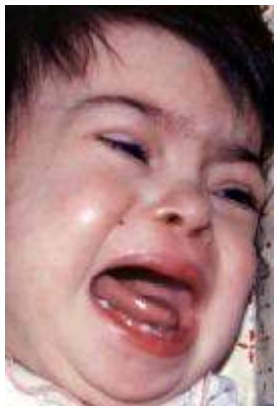
A wide gap between the first and second toes and onychomycosis



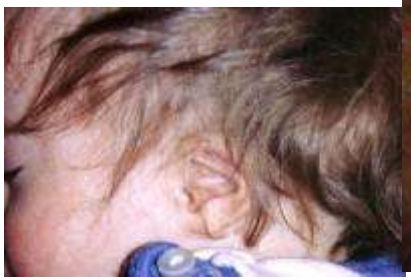
A palmar simian crease

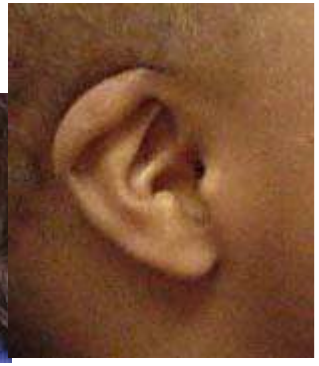


A protuberant abdomen and an umbilical hernia



Hypodontia





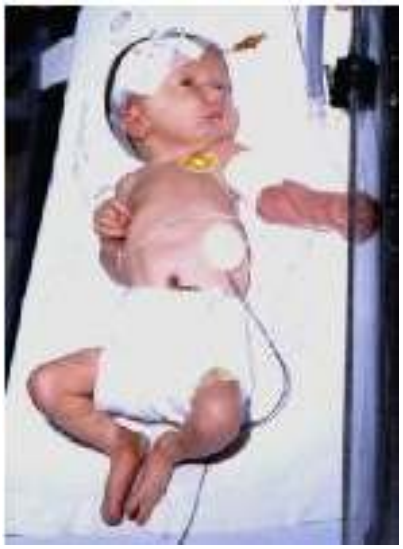
Small and misshapen auricle and anomalies of the folds

http://medgen.genetics.utah.edu

http://www.emedicine.com

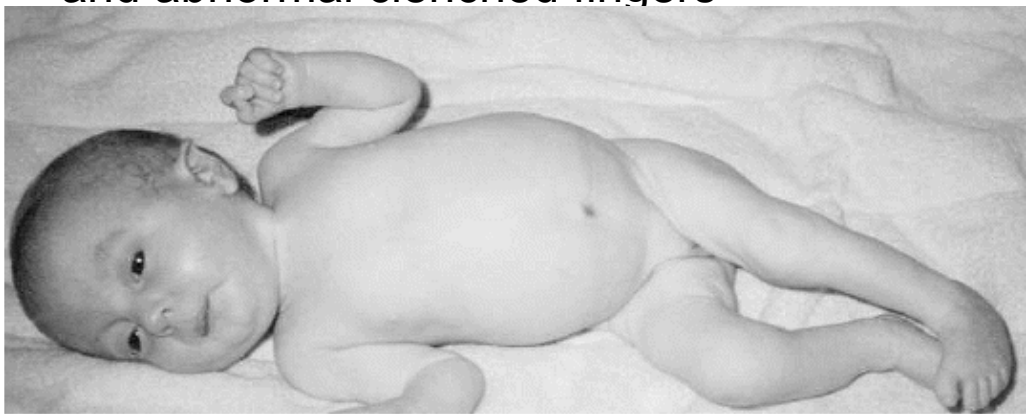
Trisomy 18- Edward's Syndrome

1 in 6 000-8 000 live births

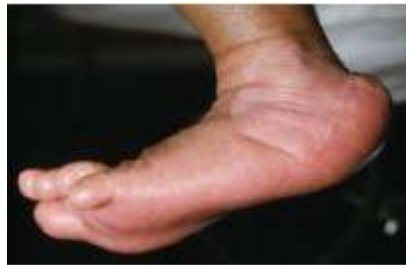


microphthalmia, micrognathia/retrognathia, microstomia, low set/malformed ears, short sternum, and abnormal clenched fingers

clenched hand with the index finger overriding the middle finger and the 5th finger overriding the 4th finger



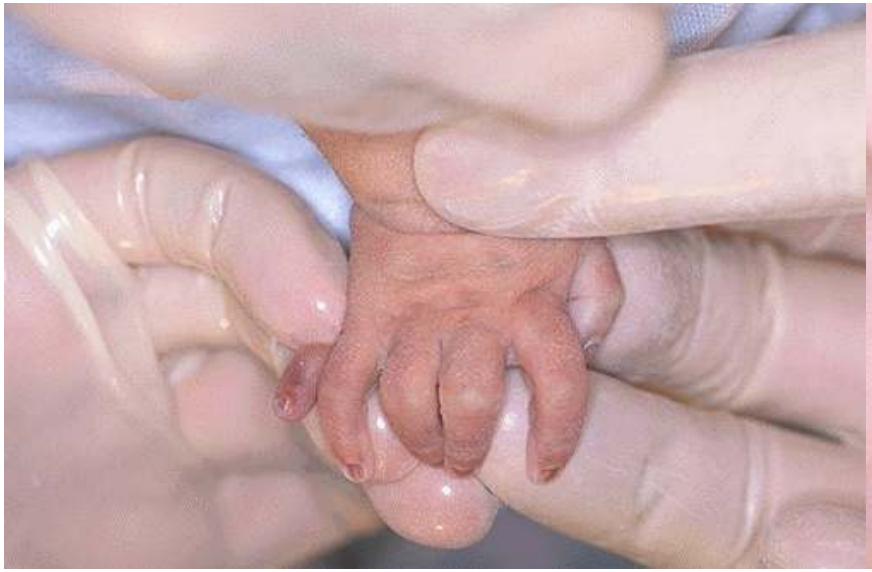
rocker-bottom foot with prominent calcaneus

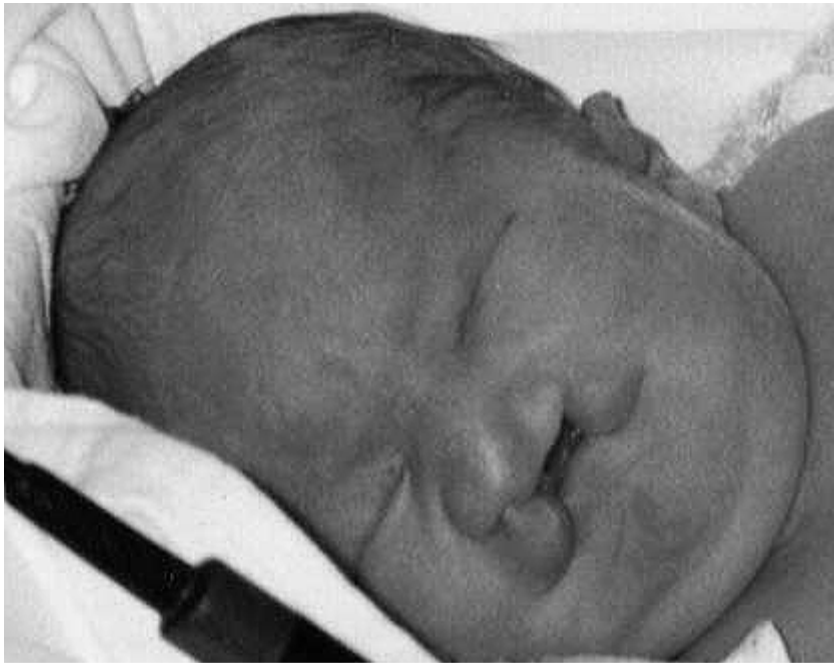


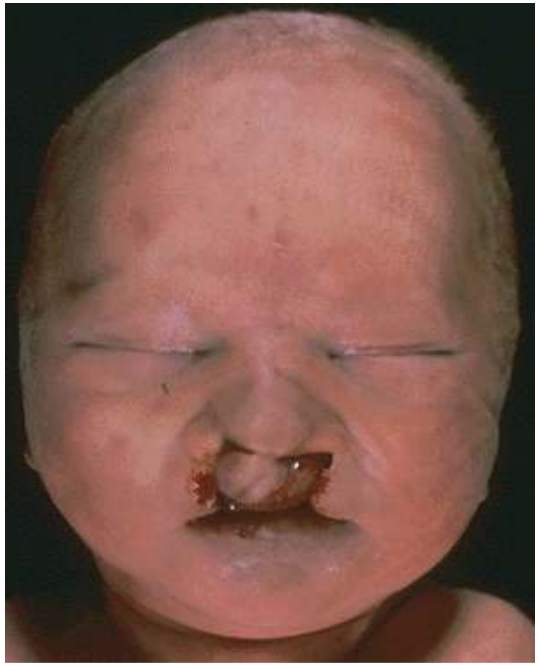
http://medgen.genetics.utah.edu

Patau Syndrome. Trisomy 13

1 case per 8,000-12,000 live births







13 is the largest autosomal imbalance that can be sustained by the embryo and yet allow survival to term

Sex chromosome aneuploidy

Much more frequent among live births than autosomal aneuploidy

Reasons:

The Y chromosome is relatively gene poor with the exception of the sex-determining genes;

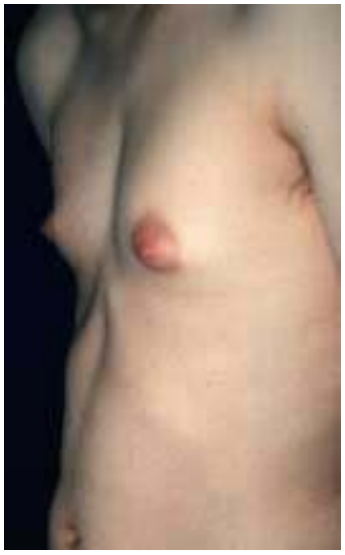
All but one X in females and individuals with more than one X, are inactivated.

Klinefelter Syndrome (XXY males): Pathogenesis

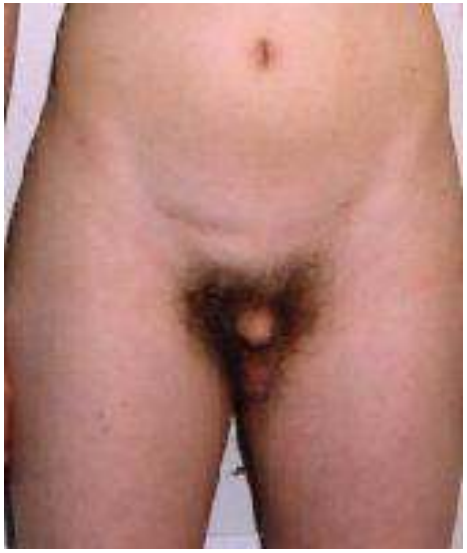
- XXY individuals are slightly feminized. A small part of the X chromosome near one telomere the pseudoautosomal region is not inactivated. In XXY males, this region is active at twice the level of the pseudoautosomal region in XY males.

Klinefelter syndrome

Disproportionately long arms and legs



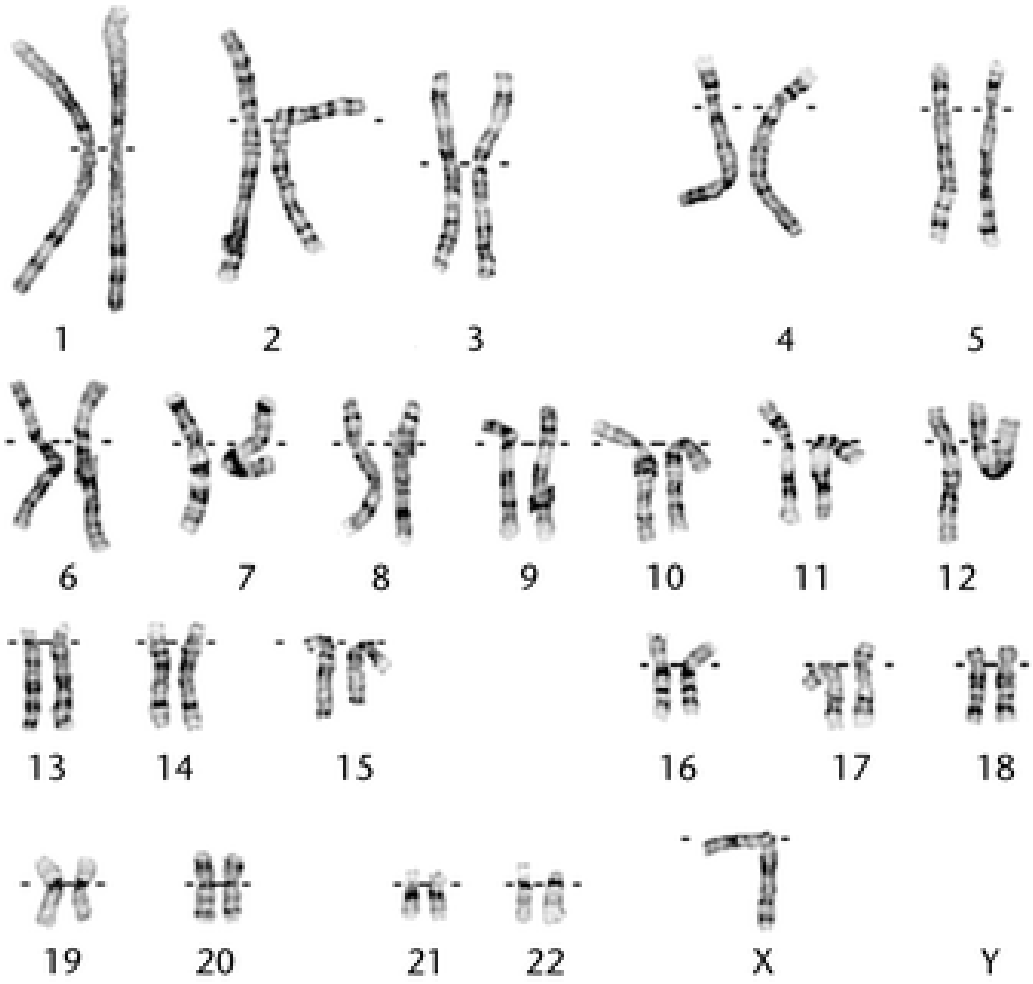
Gynecomastia (increased risk of breast cancer though still much less than in females).



Female-type distribution of pubic hair and testicular dysgenesis

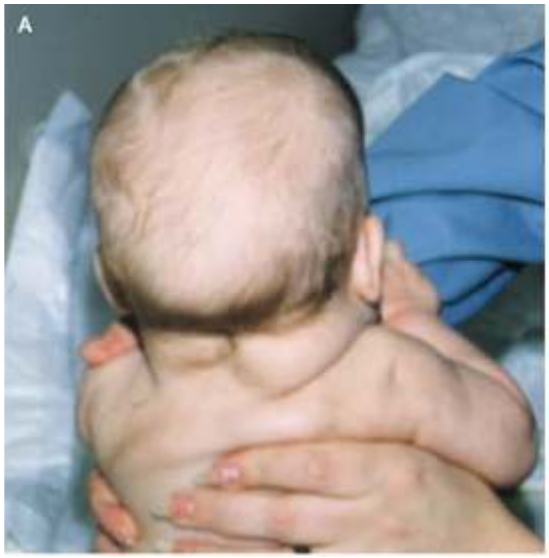
http://www.emedicine.com

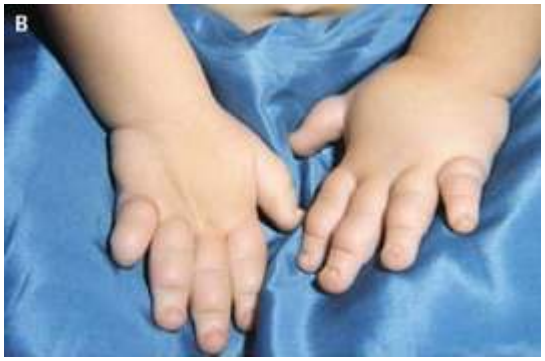
45,X Turner syndrome karyotype (monosomy X)



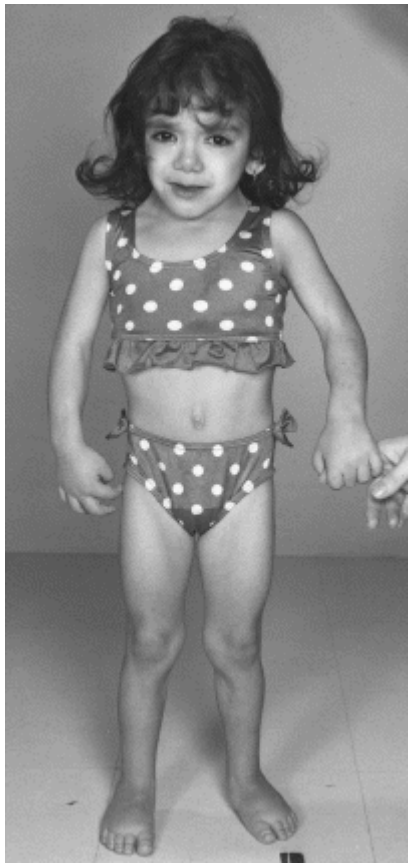
Monosomy X: Turner Syndrom

1 in 2 500 -3 000 live-born girls





Redundant Nuchal Skin and Puffiness of the Hands and Feet



Reduced stature. Broad, "webbed" neck. Swelling (edema) is in the ankles and wrists.

http://www.emedicine.com/

Advanced maternal age is not associated with an increased incidence

Turner's Syndrome.pdf

Turner Syndrome: patogenesis

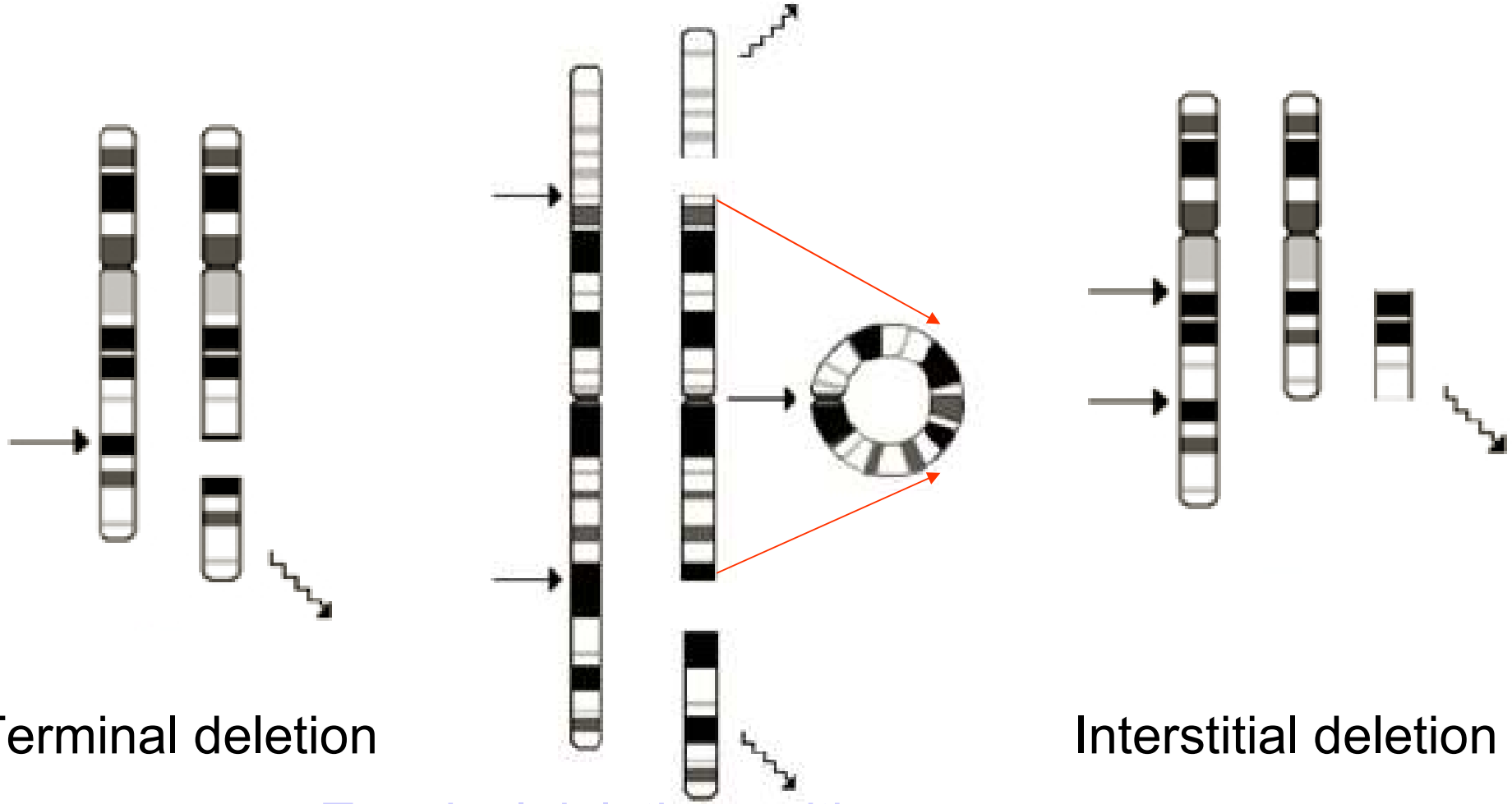
- Monosomy for the pseudoautosomal region of the X ?
- Absence of two normal sex chromosomes before X-chromosome inactivation ?
- Loss of the testis-determining factor (*SRY*) gene on the short arm of the Y chromosome (phenotype of Turner's syndrome even without a 45,X cell population).

XYY males and XXX females

- The XYY males are usually fertile. Their meioses are of the XY type; the extra Y is not transmitted, and their gametes contain either X or Y, never YY or XY. Attempts have been made to link the XYY condition with a predisposition toward violence.
- The XXX individuals are phenotypically normal and fertile females; In XXX females. Meiosis is of the XX type, producing eggs bearing only one X. In XXX females the pseudoautosomal region is active at only 1.5 times the level that it is in XX females. This relatively lower level of functional aneuploidy, plus the fact that the pseudoautosomal genes appear to lead to feminization, may explain the phenotype.

Structural abnormalities

Unbalanced structural rearrangements: Deletions

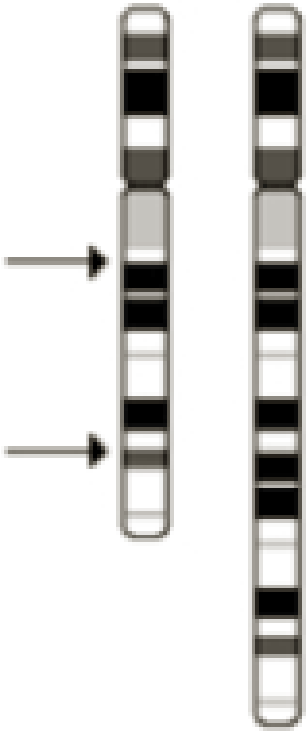


Terminal deletion

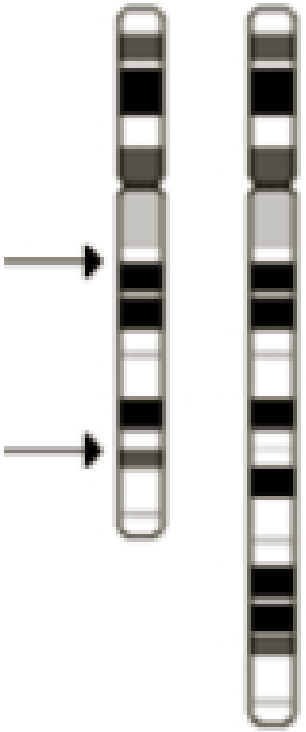
Interstitial deletion

Terminal deletions with
ring chromosome formation

Unbalanced structural rearrangements: Duplications

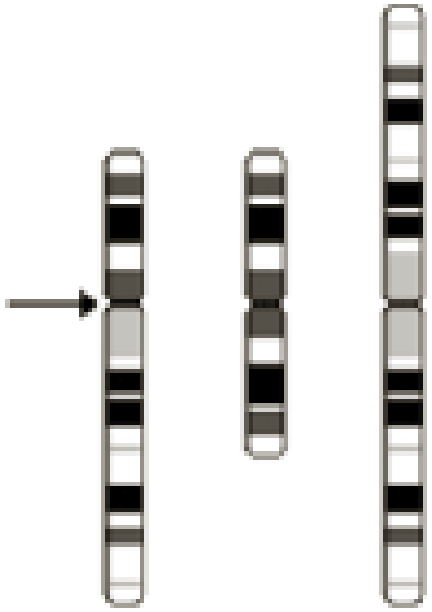


Direct duplication



Inverted duplication

Unbalanced structural rearrangements: Isochromosomes



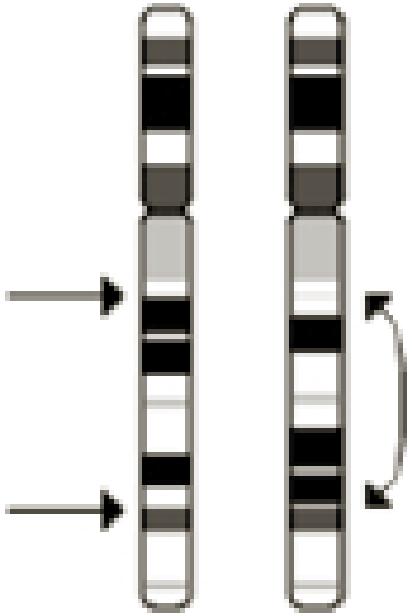
http://www.tokyo-med.ac.jp

An isochromosome is a chromosome consisting of two identical copies of one arm of a single chromosome and none of the other.

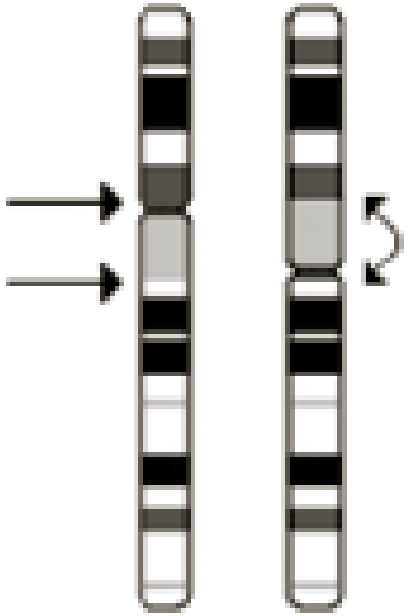
By cytogenetic techniques impossible to distinquish from homologous ROB

In a person with 46 chromosomes, an isochromosome results in partial monosomy and partial trisomy.

Balanced structural rearrangements: Inversions



Paracentric inversion



Pericentric inversion

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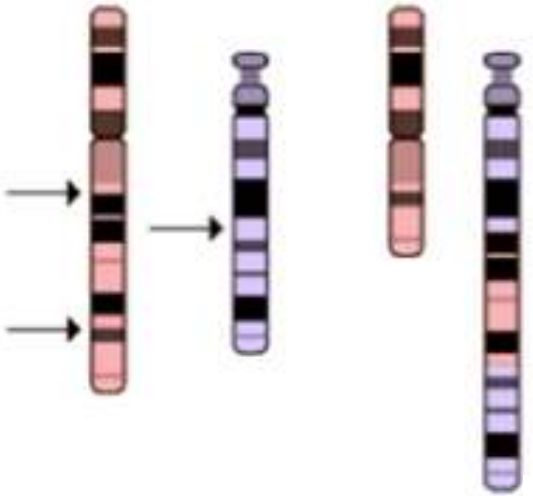
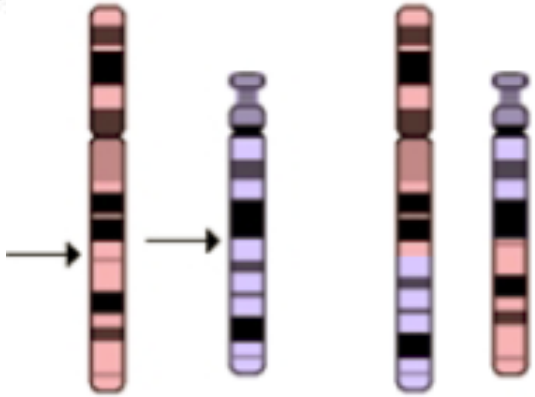
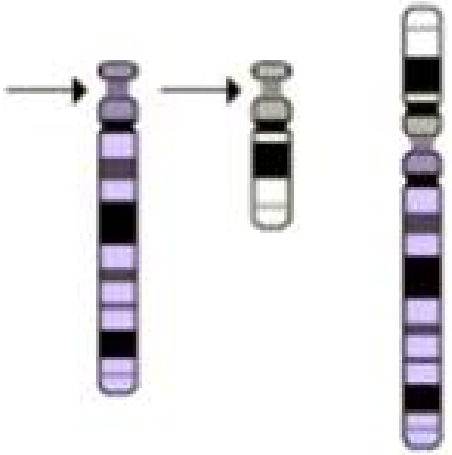
Balanced structural rearrangements: Translocations



Insertion



Reciprocal translocation

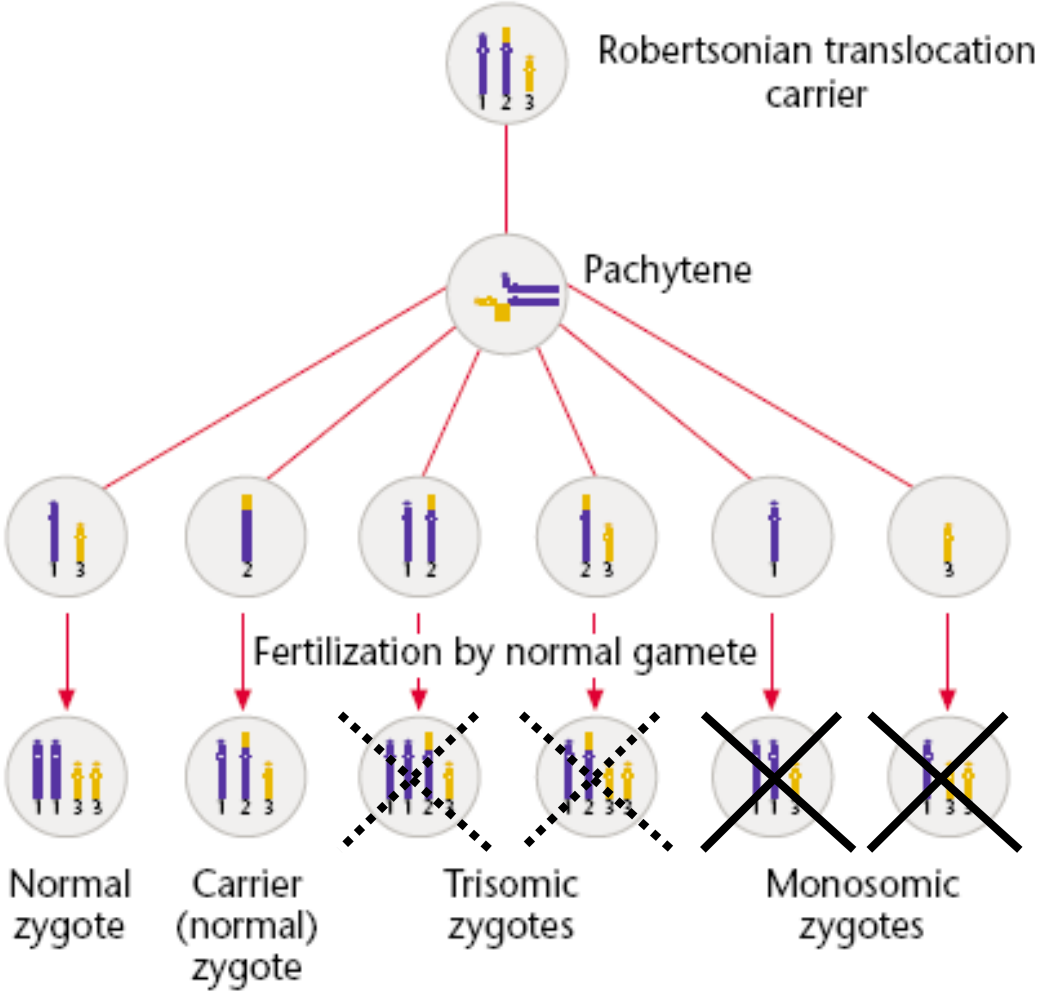


Robertsonian translocation

http://www.tokyo-med.ac.jp

(whole arm rearrangements of the acrocentric chromosomes, often creating a dicentric,chromosome)

Genetic counselling in Robertsonian translocation carrier

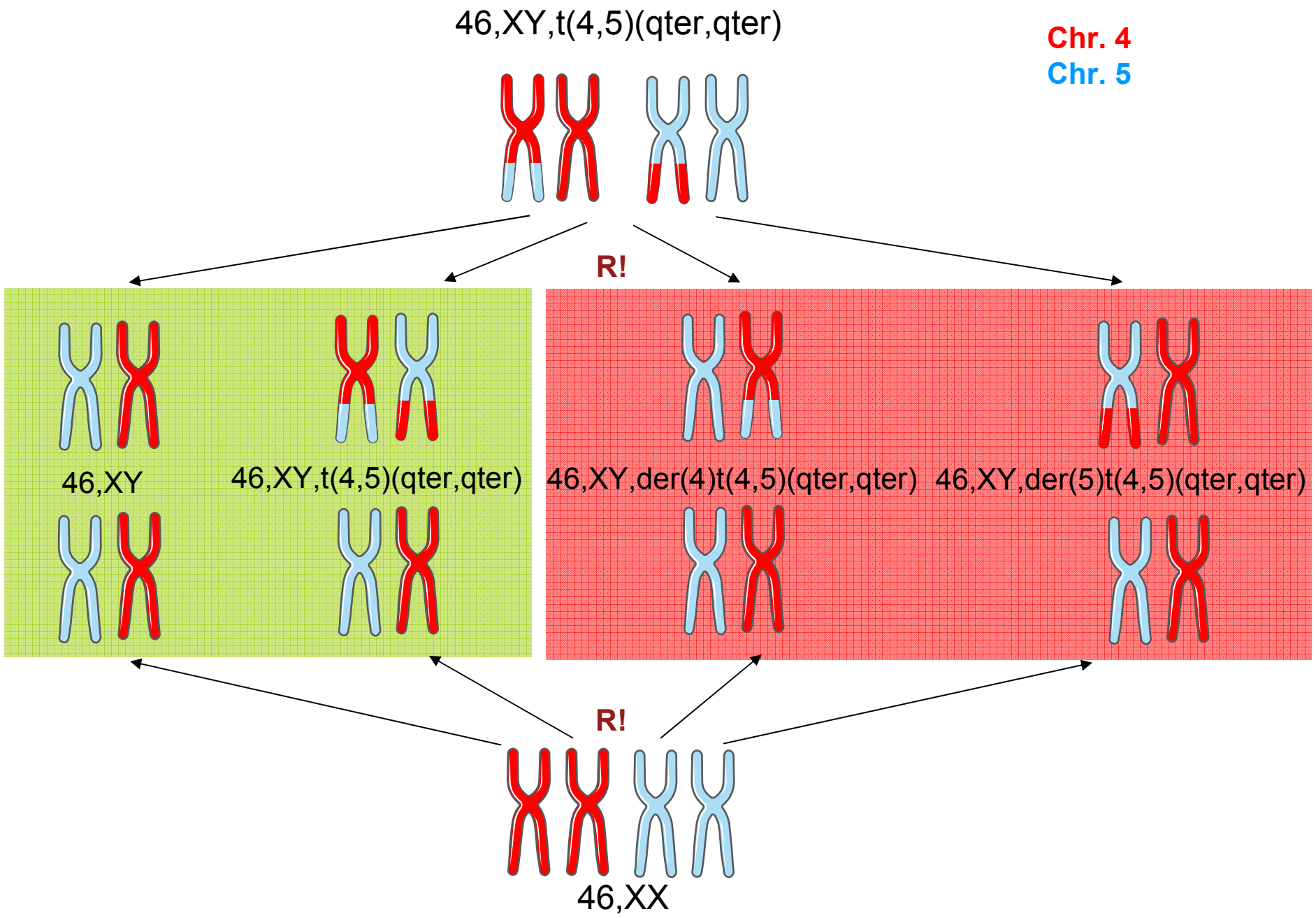


Karyotype nomenclature

47, XY,+8 [15] / 46,XY [15]
45,X [85] / 46,XX [15]
45,XY,der(1)t(1;3)(p22;q13),-3
46,XY,dup(12)(q13)
46,XY,der(2)t(2;?)(q37;?)
46,XY,der(1)t(1;3)(p22;q13)
46,XX,del(7)(p11)
45,XY,der(13;21)(p11;p11)
46,XY,der(13;21)(p11;p11) +21
46,XX t (7;14)(q11;p22)
69,XXX
46,XX,inv(3)(q21q26)
46,XY,del(5)(q13q33)

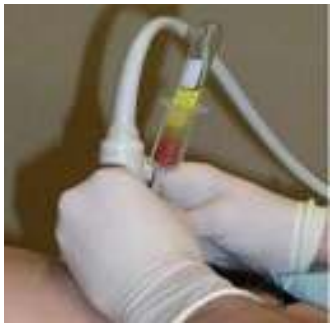
Karyotype nomenclature

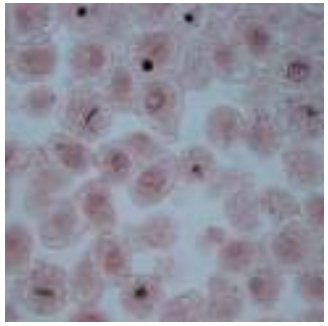
Abbreviation	Meaning	Example
cen	centromere	
del	deletion	46,XX,del(5p)
der	derivative chromosome	der(1)
dic	dicentric chromosome	dic(X;Y)
dup	duplication	
i	isochromosome	46,X,i(Xq)
ins	insertion	
inv	inversion	inv(3)(p25;q21)
mat	maternal origin	47,XY,der(1)mat
p	short arm	
pat	paternal origin	
q	long arm	
r	ring chromosome	46,X,r(X)
t	translocation	46,XX,t(2;8)(q21;p13)
ter	terminus	46, XX, del(Xq21;qter)
upd	uniparental disomy	
+	gain of a chromosome	47,XX,+21
-	loss of a chromosome	45,XY,-14
::	break and join	
/	mosaicism	46,XX/47,XX,+8

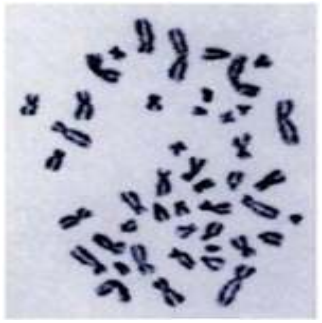

46,XY,t(4,5)(qter,qter)
Chr. 4
Chr. 5
R!
46,XY
46,XY,t(4,5)(qter,qter)
46,XY,der(4)t(4,5)(qter,qter)
46,XY,der(5)t(4,5)(qter,qter)
R!
46,XX

METHODS

Cytogenetic testing







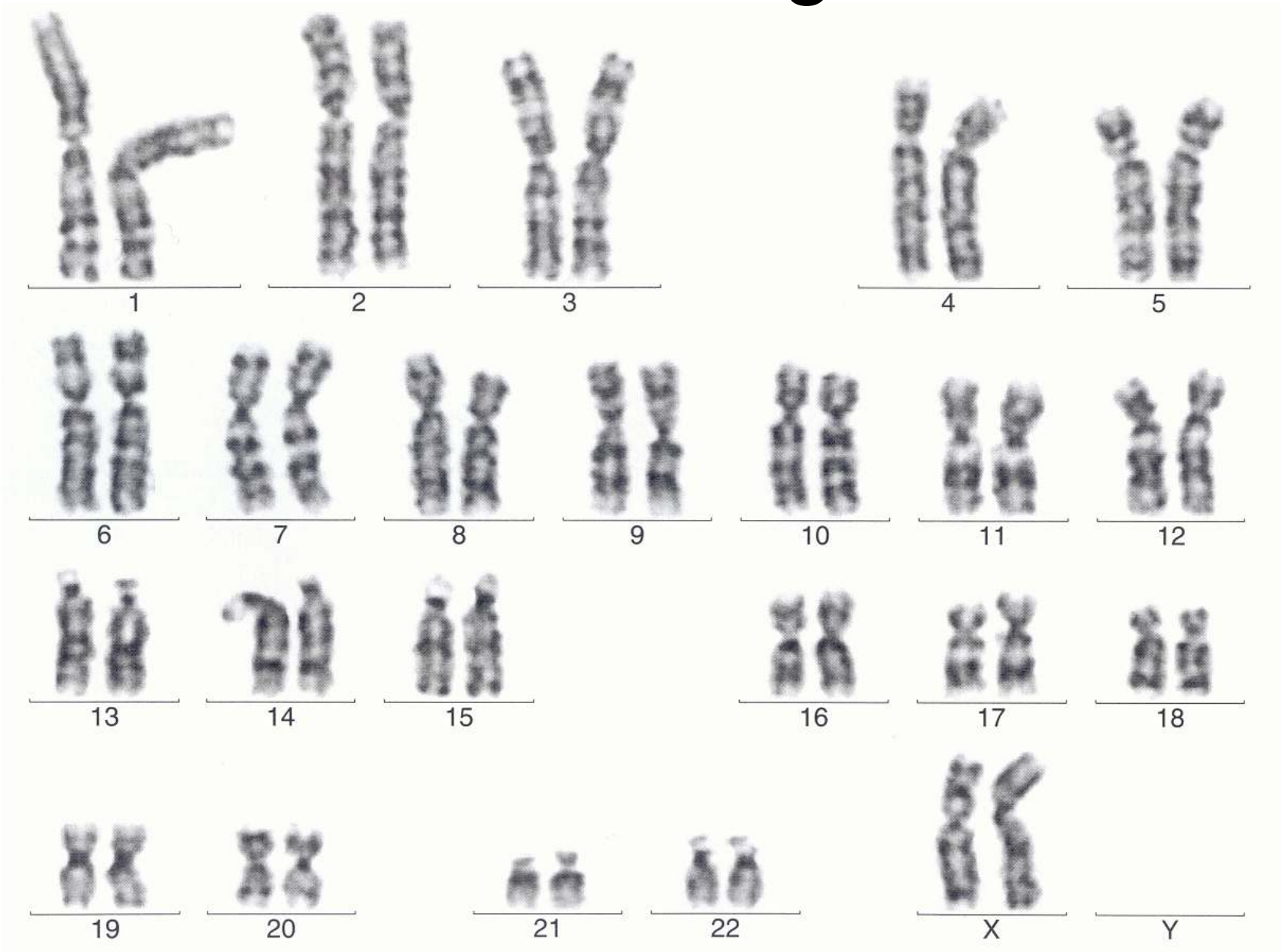
peripheral blood
amniotic fluid
chorion / trophoblast
dermal fibroblasts
bone marrow
umbilical cord blood
fetal tissues
blastomeres

Chromosome banding techniques

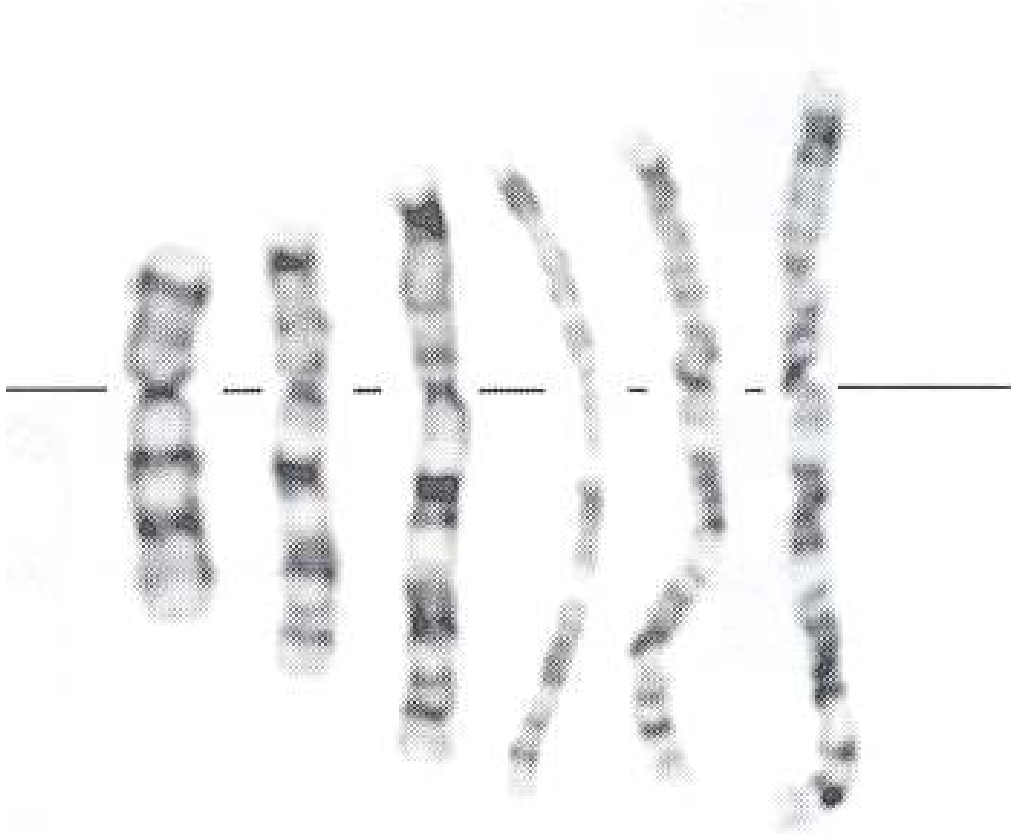
Type of banding	Method	Properties of bands
G banding	Controlled digestion with trypsin followed by staining with Giemsa	Series of bands along the chromosomes, late replicating, gene poor, condensed, AT rich
R banding	Heat denaturation of chromosomes followed by staining with Giemsa	Reverse of G banding, early replicating, gene rich, less condensed, GC rich
C banding	Denaturation of chromosomes with BaOH followed by staining with Giemsa	Stains constitutive heterochromatin, esp. centromeres, highly condensed, late replicating, no genes, not transcribed
NOR banding	Chromosomes are stained with a silver nitrate solution	Stains the active nucleolar organizer regions at the stalks
Q banding	Fluorescent stain quinacrine dihydrochloride is used which binds preferentially to AT-rich DNA	Late replicating, gene poor, condensed, AT rich. Similar to G banding
Replication banding	Incorporation of the thymidine analogue BrDU into either late replicating (G bands) or early replicating (R bands) followed by treatment with UV light to destroy BrDU incorporated DNA and staining with Giemsa	Stains DNA based on replication timing, highest band levels
T banding	Controlled heat denaturation followed by staining with Giemsa	Stains the telomere regions, very GC rich, high gene density, early replicating

BaOH, barium hydroxide; BrDU, bromodeoxyuridine

G banding

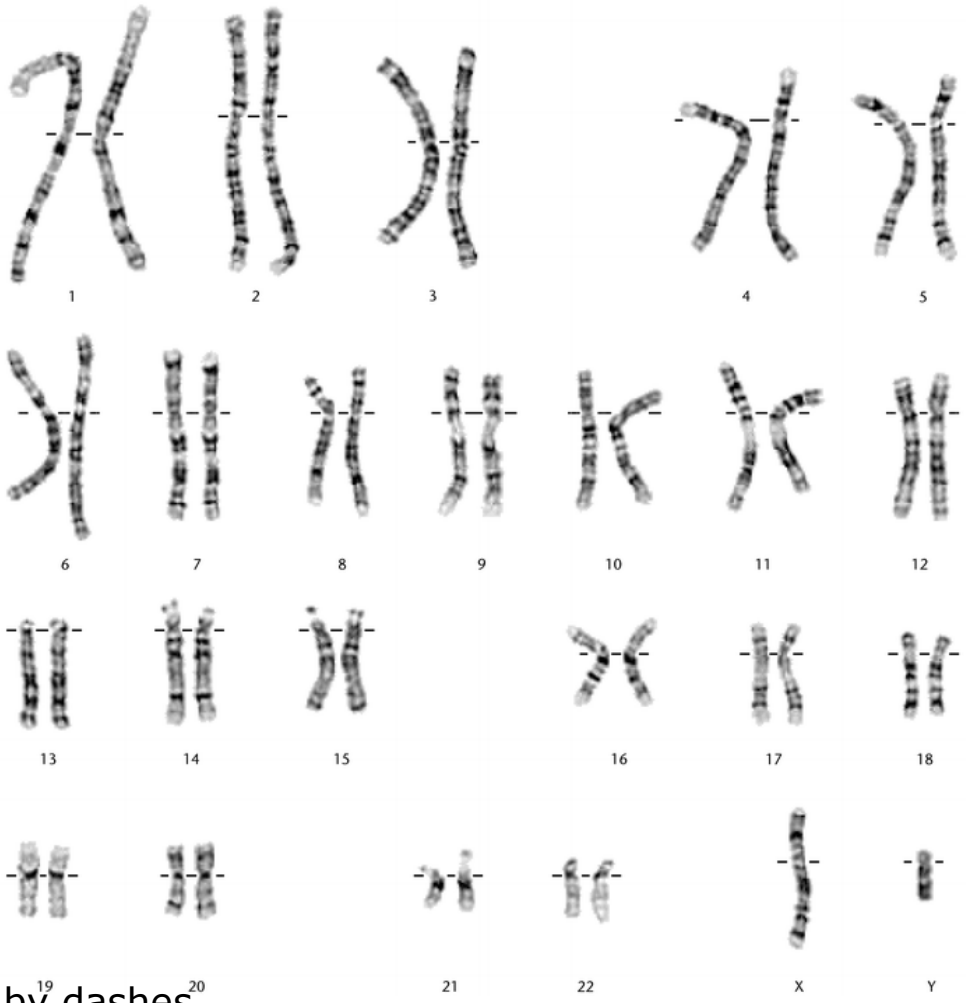


Same chromosome prepared six different times

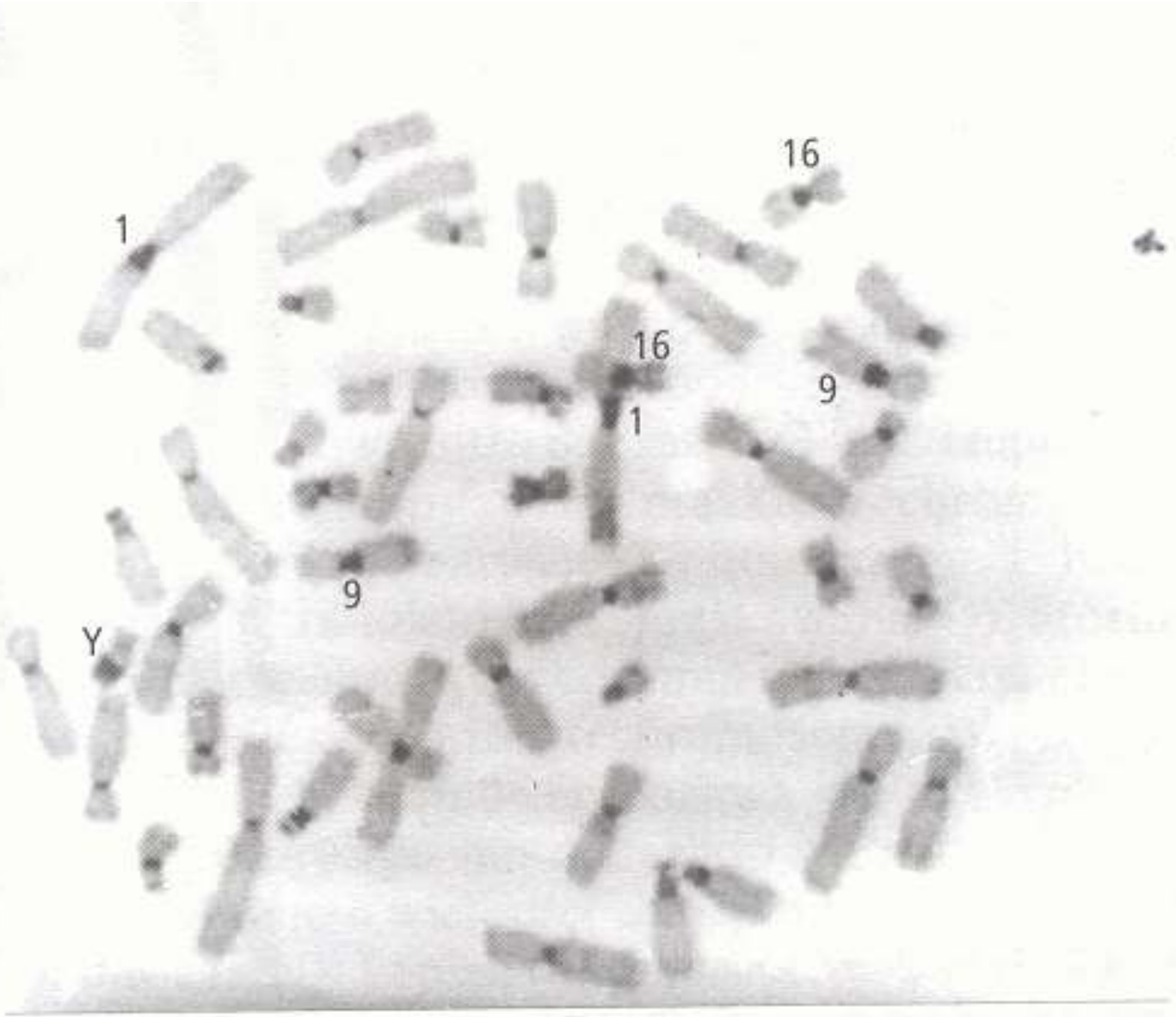


G-banded HRT normal male karyotype

Human Chromosomes.pdf

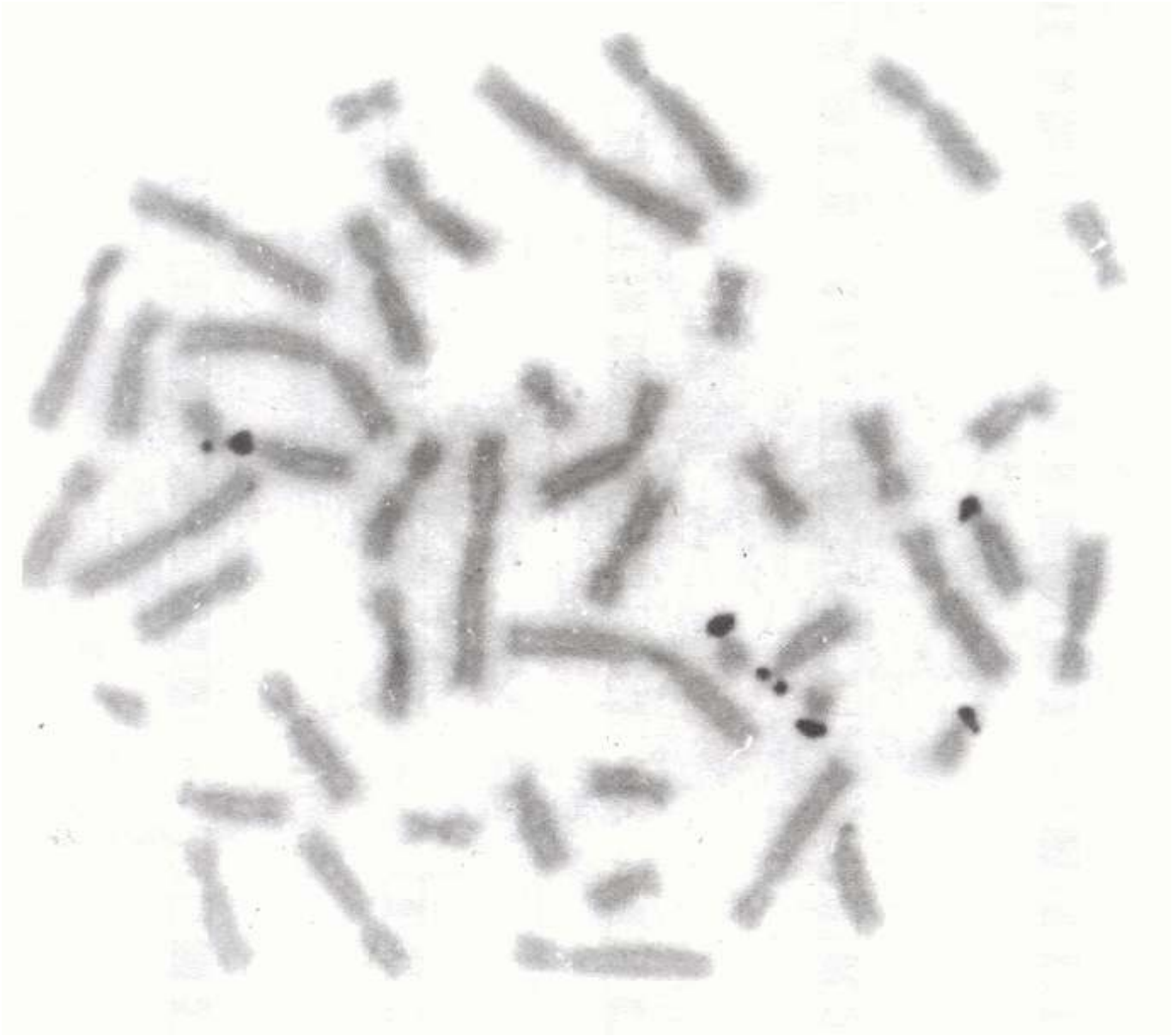


Centromeres marked by dashes.
Chromosomes 13, 14, 15, 21 and 22 are acrocentric chromosomes with the secondary constriction, composed of stalks and satellites, above the centromere.



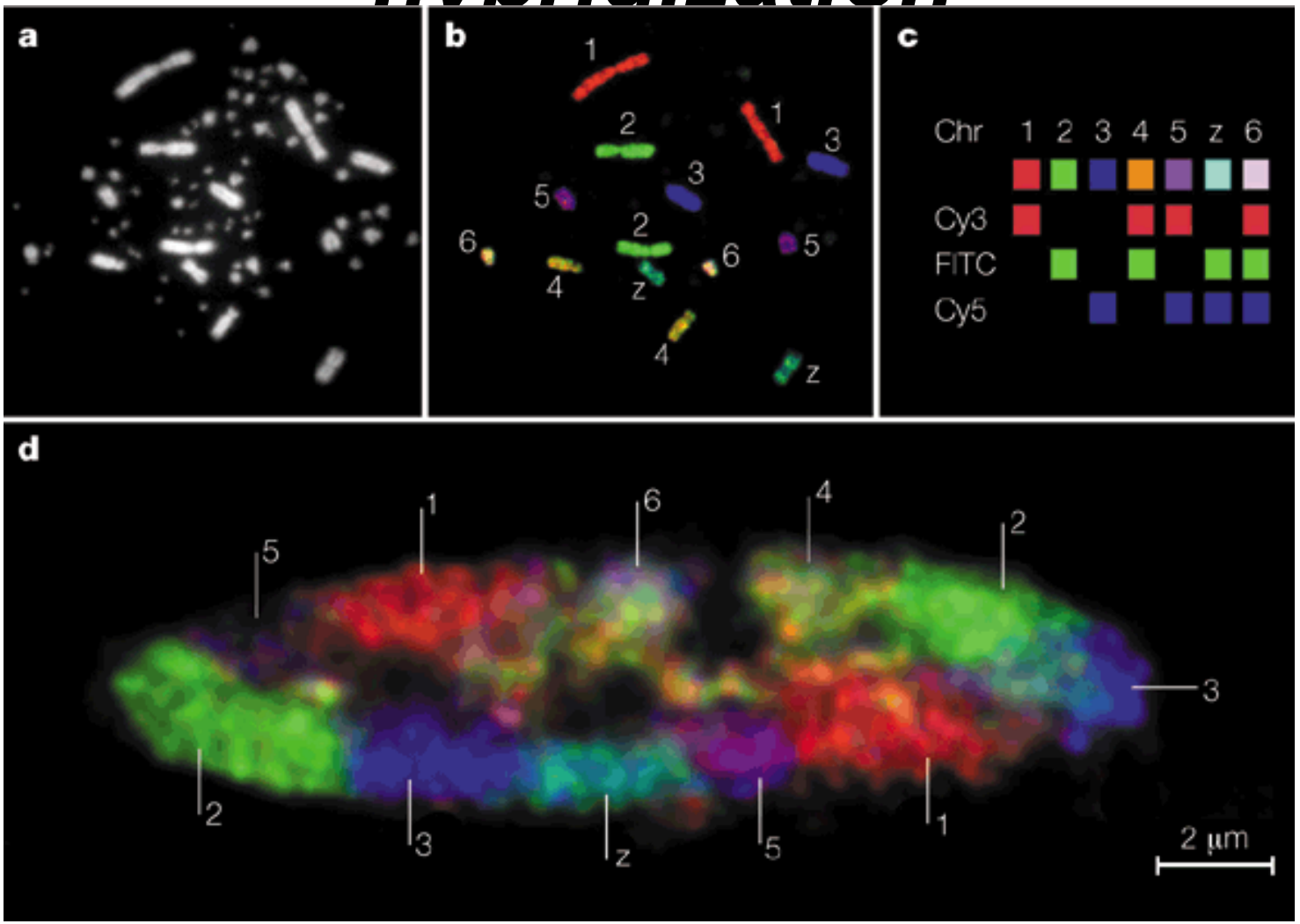
Ryc. 4.4. Prawidłowy kariotyp męski (prążkowanie – C).

NOR staining



FISH -fluorescent in situ hybridization

Nature Genetics 2001



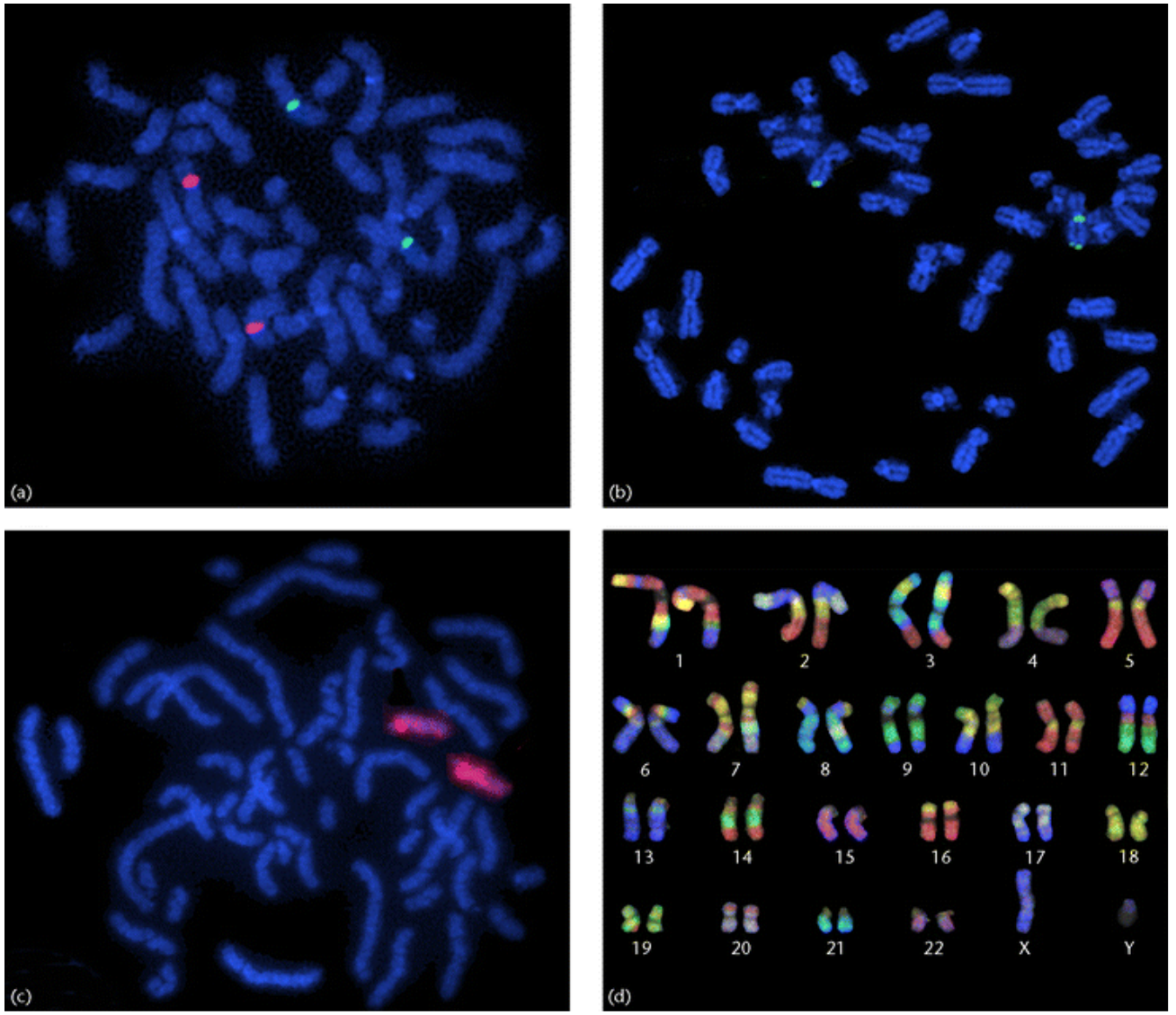
Mid-plane light optical section through a chicken fibroblast nucleus shows mutually exclusive chromosome territories (CTs) with homologous chromosomes seen in separate locations.

CHROMOSOME TERRITORIES.pdf

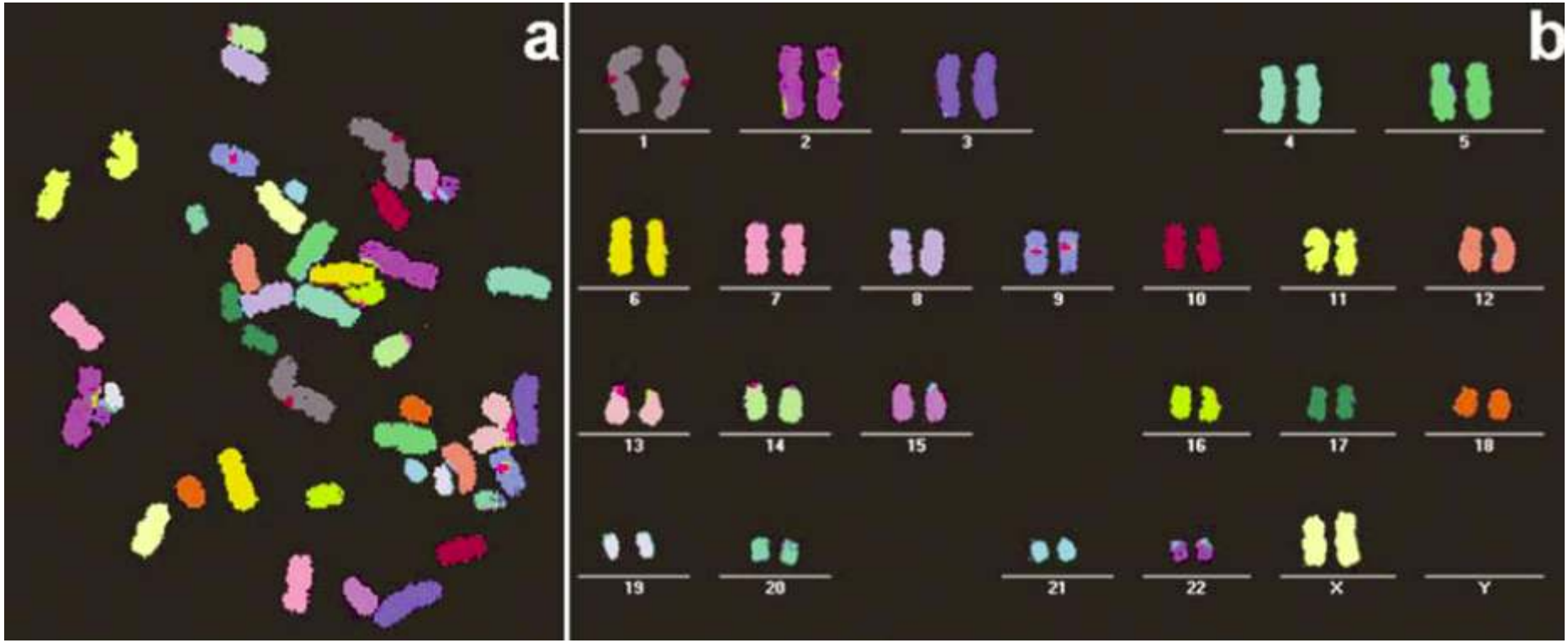
FISH examples

Preparation and Banding.pdf

(a) Normal human metaphase spread showing hybridization of the centromeric region of chromosomes 7 (green) and 8 (red) using α-satellite probes. Chromosomes counterstained in blue using DAPI. (b) Human male metaphase spread with deletion of the elastin (ELN) locus at the Williams syndrome critical region near the centromere on one homologue of chromosome 7. Cosmid probe for the ELN locus and a control probe are visible on the normal homologue (right); however, only the control probe can be seen on the deleted homologue (left). (c) Normal human metaphase spread with whole-chromosome paint probe for chromosome pair number 15 in red. (d) Cross-species colour banding (RX-FISH®) on normal human male chromosomes arranged in standard karyotype format.



Chromosome painting with mFISH



(a) Staining of all 46 chromosomes of a human female cell simultaneously in different colors by M-FISH. This analysis is based on an adaptive spectral classification approach for seven fluorochromes. The automated analysis results in computer-generated false colors for each chromosome.
(b) Multicolor classified karyogram of the normal female metaphase spread shown in (a).

Multicolor chromosome painting

Interphase FISH

useful in situations where it is difficult to obtain metaphase spreads for karyotyping,such as cancer tissue, preimplantation embryos and foetal material after miscarriage.

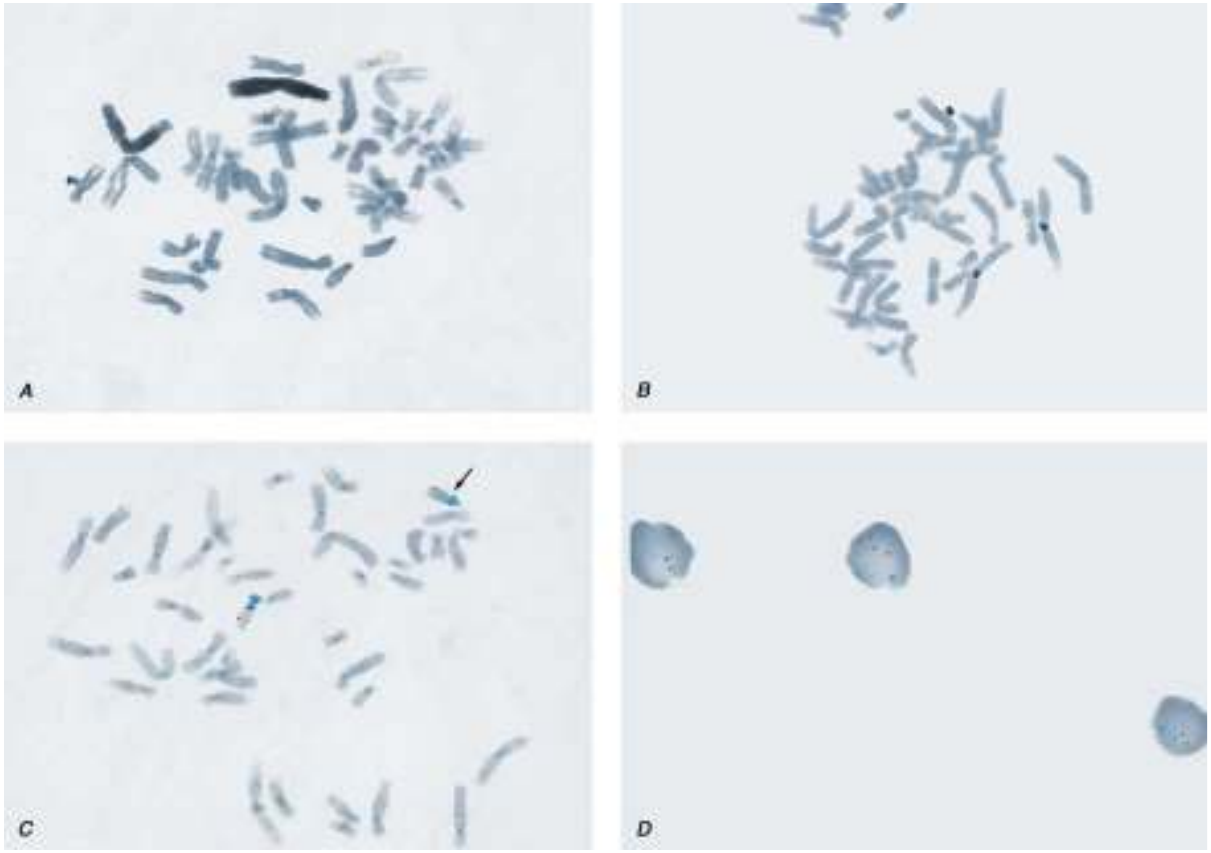
Applications of fluorescence in situ hybridization (FISH) to metaphase or interphase preparations

A. **A chromosome 1-specific "paint" probe hybridizes to both normal chromosomes 1 as well as to a marker chromosome derived from chromosome 1.**

***B*. A repetitive DNA probe specific for centromeric -satellite sequences on chromosome 1 hybridizes to the centromeric region of both normal chromosomes 1 as well as to a marker chromosome derived from chromosome 1.**

***C*. Two-color FISH used to detect a microdeletion of chromosome 15 associated with Prader-Willi syndrome. A repetitive classic satellite probe hybridizes to the short arm of chromosome 15 (large blue dots) and a probe for PML (a locus on the distal portion of chromosome 15, visualized as small black dots) are observed on both chromosomes. However, a probe for SNRPN (a locus within the PWS region of chromosome 15, small black dots) hybridizes only to the normal chromosome. The arrow points to the deleted chromosome.**

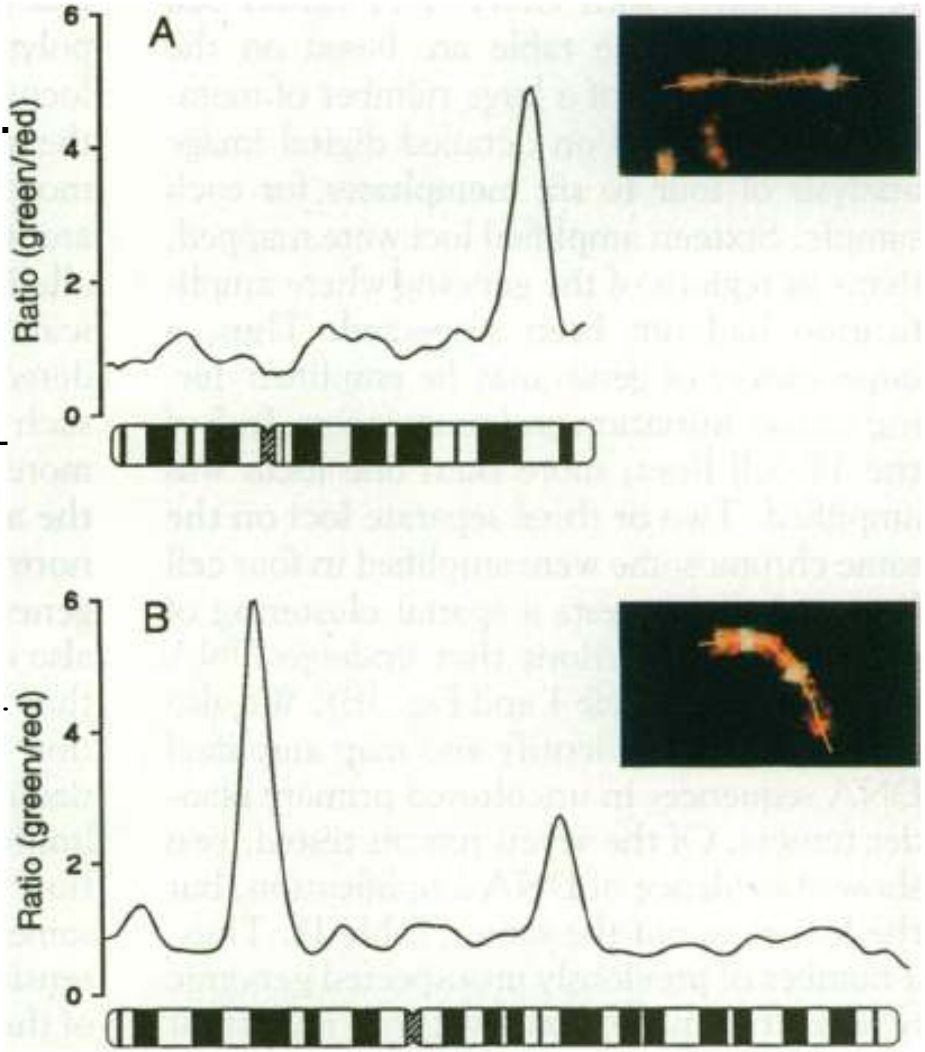
***D*. Interphase FISH using chromosome 13 (large blue dots) and chromosome 21 (small black dots) unique sequence probes on interphase cells from direct amniotic fluid preparations. Three chromosome 21-specific signals are observed, indicating the presence of trisomy 21 in the fetus.**



CGH - comparative genome hybridization

Kallioniemi et al. Science 1992

Green-to-red fluorescence ratio profiles of chromosome 8 (A) and chromosome 2 (B) after hybridization with COLO 320HSR and NCI-H69 cell line DNAs, respectively (green). Normal reference DNA included in the hybridization is shown in red. The inserts show the overlaid green and red fluorescence images of the chromosomes.



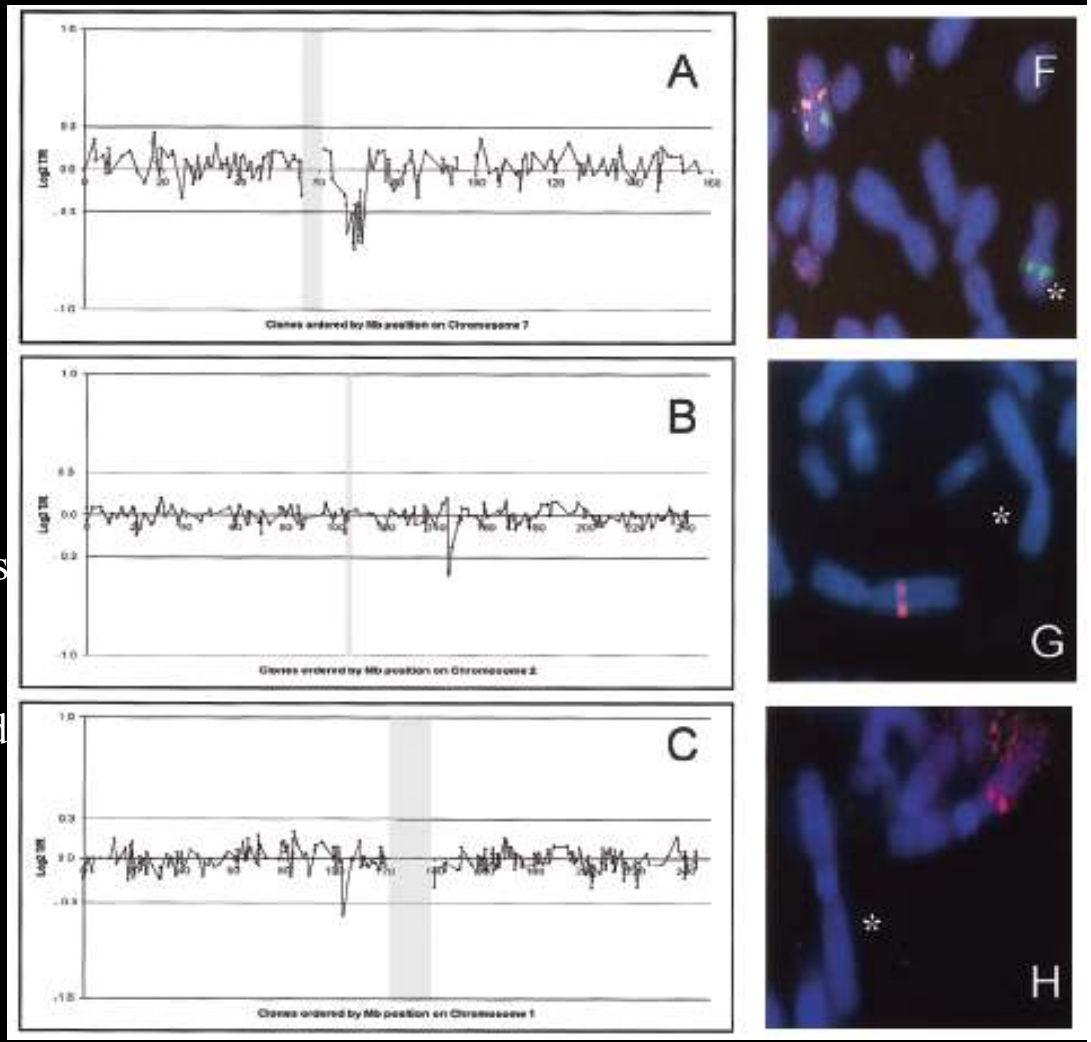
(A) the myc locus at 8q24 shows a highly elevated green-to-red ratio, which is compatible with the known highlevel amplification of myc in the COLO 320HSR cell line.

(B) 3 regions of amplification are seen on chromosome 2. The signal at 2p24 corresponds to the s location of N-myc known to be amplified in the NCI-H69 cell line. The two other regions with a highly increased fluorescence ratio, at 2p21 and 2q21, were not known

Array CGH:

Detailed genomic profiles and FISH validation of copy-number abnormalities identified in cases with unexplained mental retardation - deletions

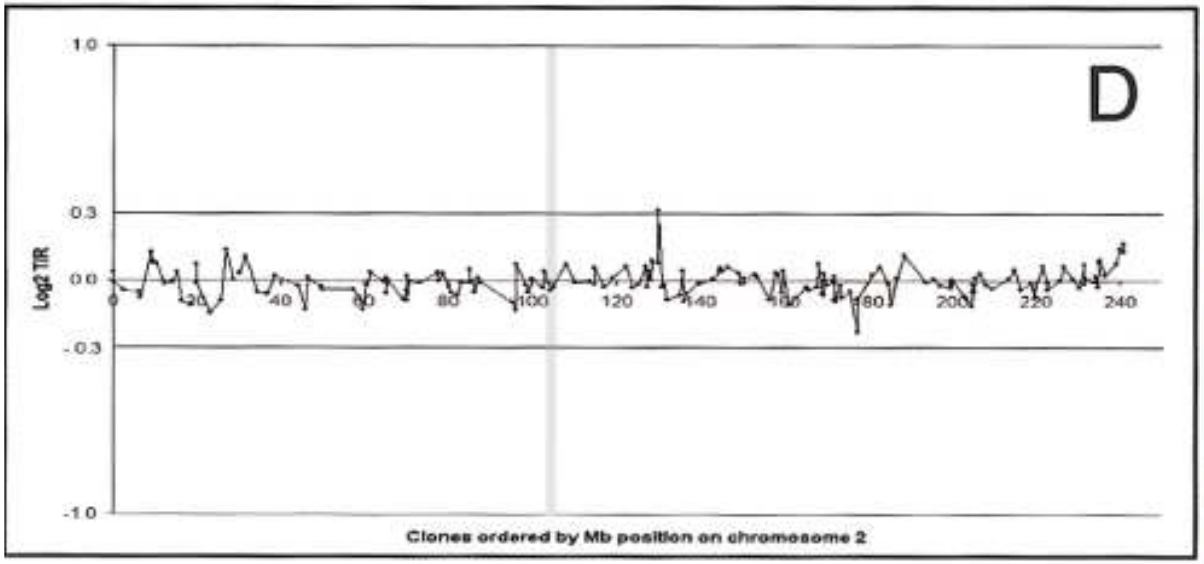
- Panels represent individual profiles of the affected chromosomes for each case, with clones ordered, for each chromosome, from pter to qter. The centromeric region is indicated by a vertical gray dash, the thresholds for copy-number gain (log2T/R value 0.3) and copy-number loss (log2 T/R value −0.3) are indicated by horizontal lines.
- Panels on the right represent the FISH validation using (one of) the target clone(s) identified by arrayCGH. Affected chromosomes are indicated by an asterisk (*).

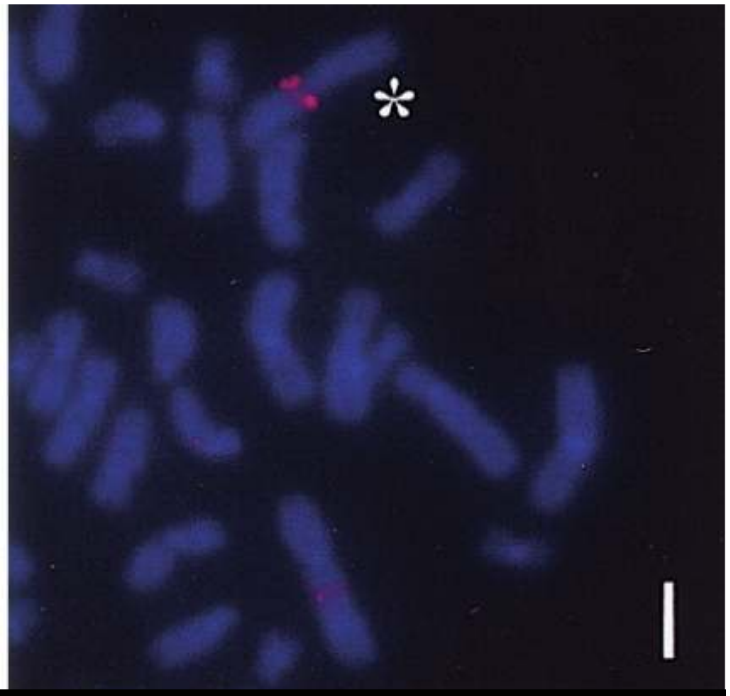


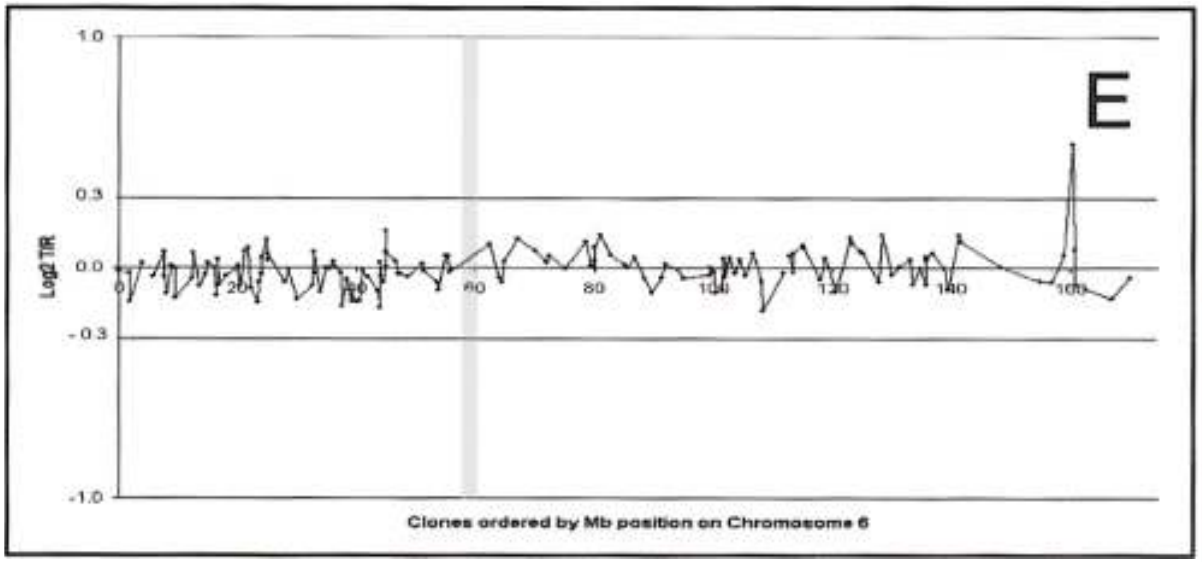
- *A* the deletion on 7q11 with 14 clones in this region showing an average log2 intensity ratio of −0.5. FISH validation of this case is shown in the adjacent panel*F,* in which one of the deleted clones on 7q11 is shown in red and an undeleted control probe is shown in green.
- *B* the microdeletion on 2q22 with a total of three clones crossing the threshold for copy-number loss, with FISH validation in the adjacent panel *G*.
- *C* Deletion of a single clone on 1p21

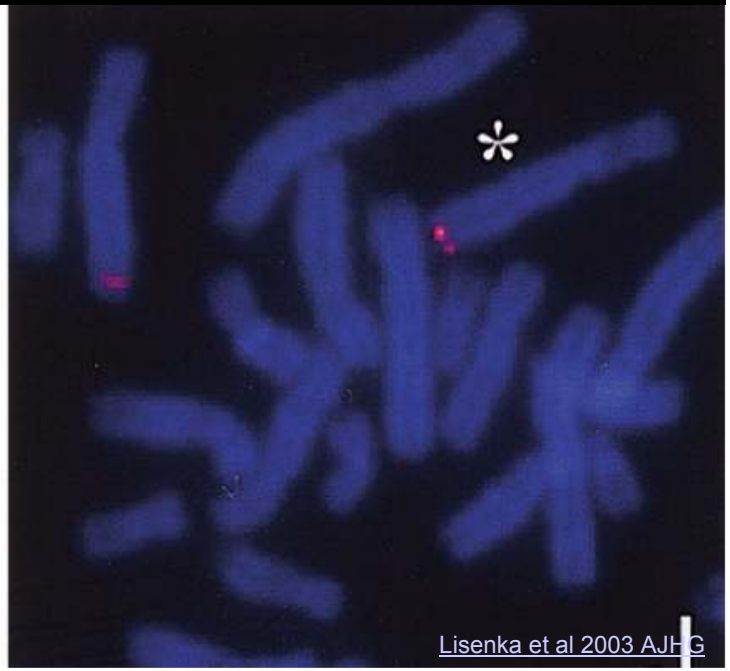
Lisenka et al 2003 AJHG

Array CGH: duplications









Lisenka et al 2003 AJHG

The Complete **Cytogenetics Arrays**

Whole-Genome 2.7M Array

Arrays

Simplified, streamlined, and all-inclusive

Reagents

Designed with input from cytogenetic researchers

Chromosome Analysis Suite (ChAS) Software

Instrumentation

THE END