

LSB509 Lecture 6 (31/3/03)

Chromosome Abnormalities

Lecture Outline

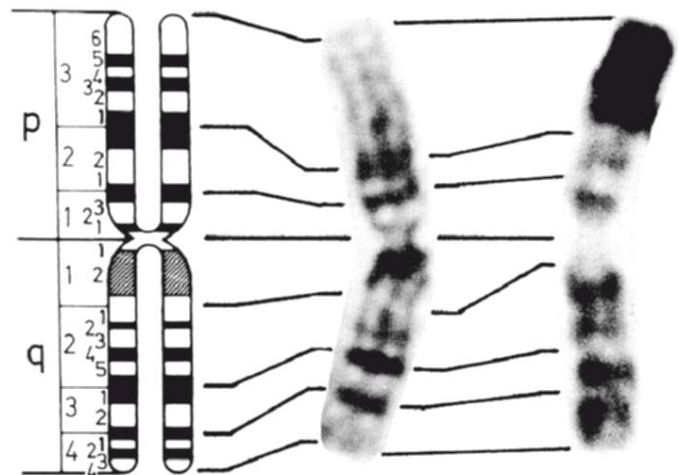
- ❖ The human karyotype
- ❖ Chromosome structure and staining
- ❖ Chromosome defects
 - ◆ defects of chromosome structure
 - ◆ defects of chromosome number
- ❖ Chromosome abnormality syndromes
- ❖ Mechanisms of chromosome disorders
- ❖ Modern molecular cytogenetics

The Human Karyotype

- ❖ Cytogenetics is a new discipline
 - ◆ first observations of human chromosomes were reported early this century
- ❖ Initially the number of human chromosomes was incorrectly estimated to be 48
 - ◆ the same as in the gorilla
- ❖ The correct number of 46 chromosomes was not reported until 1956
 - ◆ due to improvements in chromosome spreading techniques
- ❖ There are 46 chromosomes
- ❖ 22 pairs of autosomes
- ❖ One pair of sex chromosomes (XX in females, XY in males)
- ❖ A karyotype is the entire chromosome complement seen during mitotic metaphase
 - ◆ chromosomes are sorted according to chromosome size and position of centromere
- ❖ Karyotyping is difficult without chromosome banding techniques

Chromosome Banding

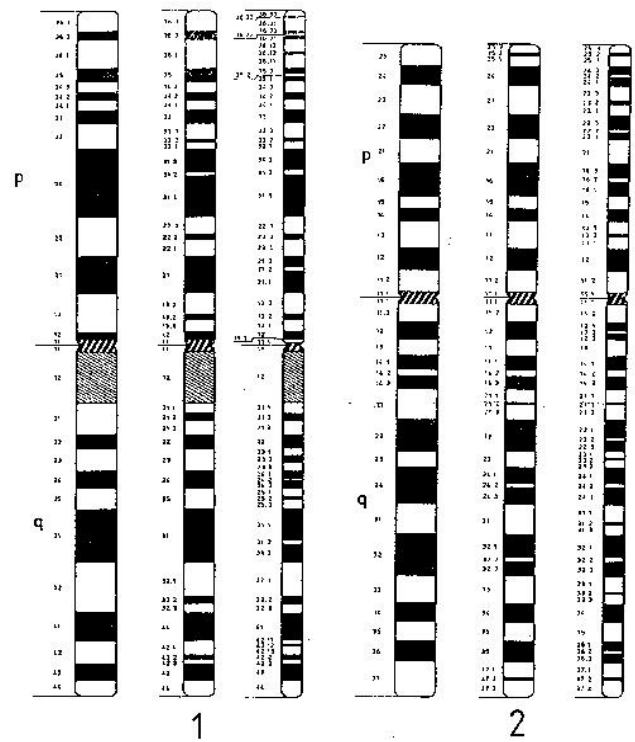
- ❖ G-banding (Giemsa stain)
 - ◆ techniques are used such that only the most readily stained chromosome segments take up stain
 - ◆ same as Q-banding (quinacrine)
- ❖ R-banding (reverse)
 - ◆ controlled heat denaturation results in 'negative' staining
 - ◆ R-bands (dark) are G interbands (light) and vice versa
- ❖ C-banding (constitutive heterochromatin)
 - ◆ heterochromatin - densely stained condensed chromosome segments
 - ◆ mostly associated with inert DNA
 - ◆ generally located near the centromere



Chromosome Nomenclature

- ❖ Chromosomes are numbered according to their size
- ❖ The arms of the chromosomes are labelled p or q
 - ◆ p is the short arm
 - ◆ q is the long arm
- ❖ Chromosome bands are numbered according to the degree of banding
 - ◆ The bands closest to the centromere are labelled 1, eg 12q1 or 12p1
 - ◆ sub-bands are labelled in the same way, eg 12q11 or 12q12

- ♦ finer divisions of bands are given a decimal point, eg 12q12.3 or even 1q42.13



Structural Chromosome Alterations

- ❖ Inversions
- ❖ Interstitial deletions
- ❖ Terminal deletions
- ❖ Ring chromosomes (centric or acentric)
- ❖ Reciprocal translocations
- ❖ Insertional translocations
- ❖ Centric translocation (Robertsonian)
 - ◆ often results in dicentric chromosomes



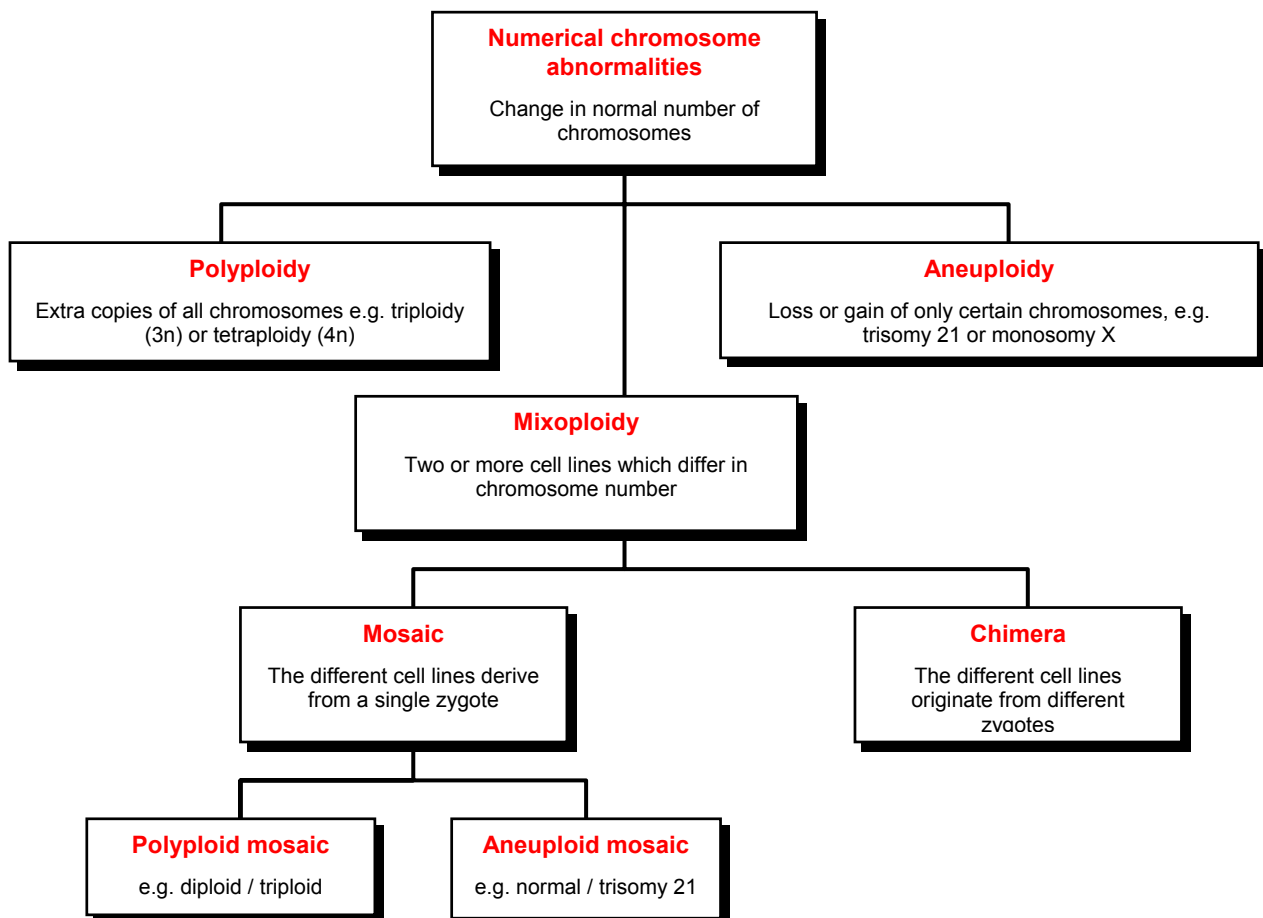
Numerical Chromosome Abnormalities

Polyploidy

- ❖ Increase in the number of sets of chromosomes
 - ♦ one set is 23 chromosomes,
 - ♦ normal diploid humans have 46 chromosomes
- ❖ Triploidy (3 sets of chromosomes) 69 chromosomes
 - ♦ 2 sperm fertilising a single egg
 - ♦ or fertilisation involving a diploid gamete and a haploid gamete
- ❖ Tetraploidy (4 sets) 92 chromosomes
 - ♦ failure to complete the first zygotic division

Aneuploidy

- ❖ Loss or gain of one specific chromosome
- ❖ Trisomy
 - ♦ eg trisomy 21, Down syndrome
 - ♦ trisomy 13, 18 etc
- ❖ Monosomy
 - ♦ monosomy 12
 - ♦ monosomy X (XO, Turner syndrome)
- ❖ Causes of Aneuploidy
 - ♦ nondisjunction -
 - the failure of paired chromosomes to separate at meiosis I
 - or failure of sister chromatids to separate at meiosis II or during mitosis
 - results in loss and gain of chromosomes
 - ♦ Anaphase lag
 - delayed movement of chromosome into daughter cell during anaphase
 - results in loss of chromosome
- ❖ Polyploid and aneuploid mosaics can also be formed



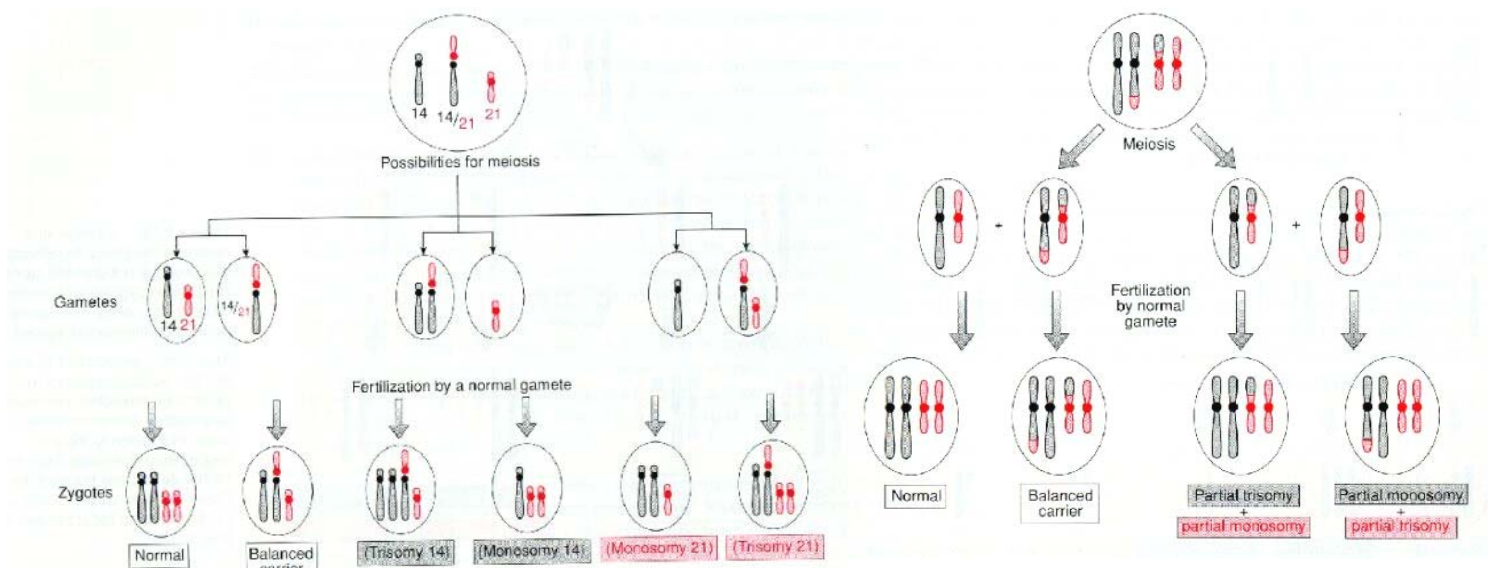
Chromosome Abnormality Syndromes

Deletions

- ❖ Wolf-Hirschhorn syndrome
 - ◆ deletions of 4p16
 - ◆ dominant phenotypic effect
 - ◆ involves loss of many genes
 - Huntington disease
 - MPS I etc.
 - ◆ multiple symptoms affecting many organ systems
 - growth retardation, mental retardation, unusual facial features
- ❖ Other deletions
 - ◆ Prader Willi
 - ◆ Angelman's syndrome
 - ◆ etc.
- ❖ The syndrome will depend on the genes deleted
- ❖ May get contiguous gene syndrome
 - ◆ ie multiple genes involved resulting in complex combination of symptoms
- ❖ Very small deletions may be harmless, even if they involve genes (recessive)

Translocations, Inversions, etc.

- ❖ These may be balanced
 - ◆ ie there is the correct amount of genetic material present
 - ◆ they may result in offspring with specific syndromes, eg monosomy or trisomy
 - ◆ The break point(s) may disrupt an essential gene and result in a dominant disorder
 - ◆ or contribute to the development of a recessive disorder



Changes in Chromosome Number

- ❖ Triploids and tetraploids lead to early embryonic death
 - ◆ rarely, triploids may survive to birth
 - ◆ tetraploids may form cystic intrauterine or ovarian growths
- ❖ **Trisomy 21 (Down syndrome)**
 - ◆ characteristic facial features
 - ◆ growth retardation
 - ◆ mental retardation
 - ◆ congenital heart disease, increased infections etc. etc.
 - ◆ reduced life expectancy (~40, may live to 60s)
 - ◆ frequently increases with age of mother (about 1 in 400 births above age 35)
- ❖ Other trisomies - all have been recorded (1-22)
- ❖ Trisomy 13 and trisomy 18 are relatively common
 - ◆ both result in intrauterine or neonatal death

Sex Chromosome Abnormalities

- ❖ Klinefelter syndrome (47 XXY; trisomy for sex chromosome) 1 in 700 males
 - ◆ impaired IQ
 - ◆ tall stature
 - ◆ sparse hair
 - ◆ infertile
 - ◆ mild feminisation
- ❖ Turner syndrome (45 XO; monosomy X) 1 in 2500 females at birth
 - ◆ normal IQ
 - ◆ low set ears, webbed neck
 - ◆ poorly developed gonads
 - ◆ behaviour disorders
- ❖ Other abnormalities
 - ◆ XXXY (1 in 2500) Klinefelter variant also rare XXXXY
 - ◆ XXX (1 in 1000) normal to mild retardation
 - ◆ XXXX and XXXXX (rare) severe retardation but physically normal
- ❖ XYY (super male) (1 in 800)
 - ◆ increased stature
 - ◆ behaviour abnormalities?
- ❖ XXYY (rare) like Klinefelter
- ❖ Y chromosome defines a male; X defines number of Barr bodies (inactive X chromosomes)

Mechanisms of Chromosome disorders

- ❖ Structural abnormalities like deletions, insertions and translocations may result in
 - ◆ gene loss
 - ◆ gene disruption
 - ◆ gene inactivation (altered chromosomal context)
 - ◆ gene imbalance (partial monosomy or partial trisomy)
- ❖ Polyploidy results in gene imbalance due to increased numbers of all chromosomes
- ❖ Monosomy results in loss of heterozygosity for all genes on a chromosome (except for XO)
- ❖ Trisomy results in imbalance of the genes from one chromosome

- ❖ In monogenic recessive disorders homozygotes are affected but there is no difference between heterozygotes and homozygous normals

Chromosome abnormalities tell us that the number of copies of each gene is very important

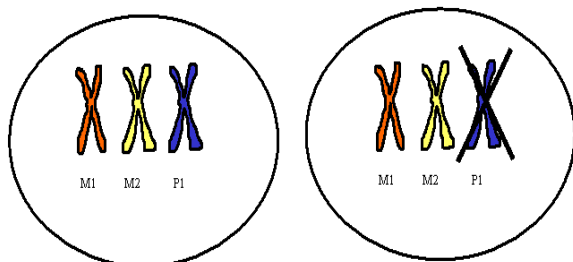
Uniparental Diploidy/Disomy

- ❖ Uniparental diploidy
 - ◆ normal chromosome constitution (46, XX or 46, XY)
 - ◆ both chromosomes sets are derived from one parent
 - ◆ the zygote does not develop properly and can form a chorio-carcinoma or ovarian teratoma
 - ◆ normal number of chromosomes, why so severe?
 - ◆ due to maternal or paternal imprinting of some genes, ie some genes receive an imprint that makes them active or inactive depending on their parental origin
 - ◆ due to degeneration of pronucleus and division of remaining pronucleus in fertilised egg
- ❖ Uniparental disomy and isodisomy
 - ◆ two copies of a specific chromosome are inherited from a single parent
 - ◆ inheritance of both homologues from one parent is uniparental disomy
 - ◆ inheritance of two copies of one homologue is uniparental isodisomy
 - ◆ uniparental disomy is thought to arise by loss of one of the extra chromosomes in inviable trisomies
1 in 3 chance of producing uniparental disomy
 - ◆ uniparental isodisomy is thought to arise by selective duplication of a monosomic chromosome in a monosomic embryo

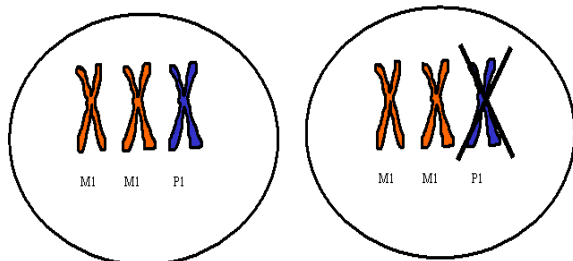
Cytogenetic Techniques

- ❖ Mid 1970s - in situ hybridization
 - ◆ preparation of metaphase spreads on glass slide
 - ◆ hybridize a radioactively labelled gene probe
 - ◆ apply a photographic emulsion and expose (weeks)

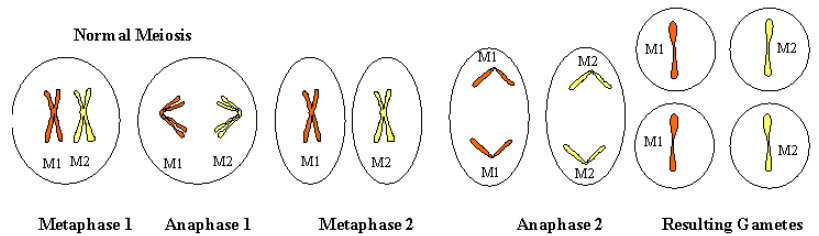
Uniparental disomy



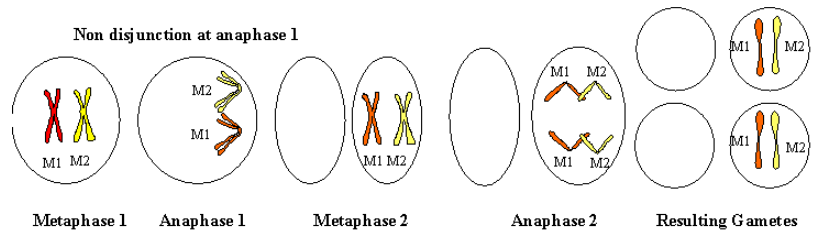
Uniparental isodisomy



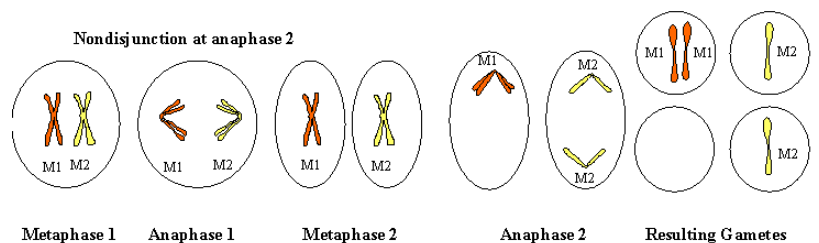
Normal Meiosis



Non disjunction at anaphase 1



Non disjunction at anaphase 2



Cytogenetic Techniques

- ❖ Mid 1970s - in situ hybridization
 - ◆ preparation of metaphase spreads on glass slide
 - ◆ hybridize a radioactively labelled gene probe
 - ◆ apply a photographic emulsion and expose (weeks)
 - ◆ develop and count individual silver grains
 - ◆ all silver grains on many metaphases must be scored
 - ◆ gives a fair measure of the cytogenetic location of unique or repetitive genes
- ❖ Late 1980s - fluorescent in situ hybridization (FISH)
 - ◆ metaphase spreads on glass slide
 - ◆ hybridize fluorescently labelled gene probe
 - ◆ excite fluorophore and visualise in a fluorescence microscope (instant result)
 - ◆ all metaphases should show the same result
 - ◆ only a few metaphases need to be examined
 - ◆ different fluorophores can be used on different gene probes
 - ◆ can use chromosome specific probes (chromosome libraries)
 - ◆ repetitive DNA probes to label centromeres or telomeres etc.

Learning Objectives

By the end of the lecture students should be able to:

- Briefly describe the basis underlying the use of banding techniques to identify chromosomes
- Identify chromosome bands on an idiotype when given the band location (e.g. Xq28.3) and *vice versa*.
- Be able to use specific examples to illustrate the pathology associated with chromosome disorders and, where possible, describe the mechanisms underlying the disorders, including:
 - Deletions
 - Translocations and inversions
 - Changes in chromosome number
 - Polyploidy
 - Aneuploidy
 - Sex chromosome abnormalities
- Be able to describe the mechanisms underlying various chromosome abnormalities