Chromosomes
(part 2)
FISH

Fluorescence In Situ Hybridization

Figure by MIT OpenCourseWare.
Images of different types of FISH: whole-chromosome paint, multicolor FISH (spectral karyotyping), and rainbow FISH (Rx).
Unbalanced Structural Abnormalities

- Deletions
- Duplications
- Isochromosomes
- Rings
- Markers
Deletions (a.k.a., contiguous gene syndromes, segmental aneusomy syndromes)

- Loss of chromosomal segment; hemizygosity
- Classified as terminal or interstitial
- Phenotype dependent on number, function of genes
- Deletion must be larger than 3 – 5 mb for classical cytogenetic detection
- May be unbalanced result of balanced chromosome rearrangement in parent
Deletion Syndromes

• Classically detectable terminal deletions
  – Wolf-Hirschhorn - 4p-, Cri-du-chat - 5p-, 9p-, 18p-, 18q-
• Microdeletions (detectable by FISH)
  – Williams syndrome – 7q11.2
  – WAGR syndrome - 11p13
  – Angelman s. / Prader-Willi s. – 15q11-q13
  – Rubinstein-Taybi syndrome – 16p13.3
  – Smith-Magenis s. – 17p11.2
  – Miller-Dieker s. - 17p13.3
  – DiGeorge syndrome / VCFS – 22q11.2
  » Many more....
Image removed due to copyright restrictions.
Karyotype of an individual with genotype 46,XX,del(4)(p16).
Wolf Hirschhorn syndrome (4p- syndrome)

- Growth deficiency of prenatal onset
- Microcephaly, skull asymmetry
- Prominent forehead (Greek helmet)
- Hypertelorism, Epicanthal folds
- Developmental delay, seizures
- Cleft lip and/or palate, short upper lip
- Low set ears
- Cryptorchidism and/or hypospadias
- Heart defects
Photographs of children with Wolf Hirschhorn syndrome.
Cri-du-chat (5p- syndrome)

- One of the most common deletion syndromes
  del(5)(p15.2) (critical region)
- Characteristic high-pitched cry - diagnostic
- Low birth weight, hypotonia
- Microcephaly, micrognathia
- Round face, low set ears
- Feeding difficulties
- Mental retardation
Cri-du-chat syndrome

Images removed due to copyright restrictions.

Photographs of children with Cri-du-chat syndrome, from specialchild.com and criduchat.asn.au.

Image from
www.specialchild.com

Image and wav from
www.criduchat.asn.au
DiGeorge syndrome / velo-cardio-facial syndrome (DGS/VCFS)

- high arched or cleft palate
- thymus aplasia or hypoplasia
- conotruncal cardiac defects
- mildly dysmorphic features
- developmental delay

VCFS phenotypically milder than DGS
Images removed due to copyright restrictions.
Photographs of young people with DiGeorge syndrome.
ish del(22)(q11.2q11.2)(TUPLE1-)

Image removed due to copyright restrictions.
Image of fluorescent chromosomes; ish del(22)(q11.2q11.2)(TUPLE1-).
• The deletions which cause DGS are most frequently caused by unequal crossover between repeat sequences at positions A and D which are ~3 megabases apart.
Mechanisms of chromosome rearrangements mediated by low copy repeats

Interchromosomal

Intrachromosomal

Figure by MIT OpenCourseWare.
<table>
<thead>
<tr>
<th>Genomic disorder</th>
<th>Chromosomal rearrangement</th>
<th>Chromosomal location</th>
<th>Rearrangement size (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charcot-Marie-Tooth disease type 1A (CMT1A)</td>
<td>Interstitial duplication</td>
<td>17p12</td>
<td>1.5</td>
</tr>
<tr>
<td>Hereditary neuropathy with pressure palsies (HNPP)</td>
<td>Deletion</td>
<td>17p12</td>
<td>1.5</td>
</tr>
<tr>
<td>Smith-Magenis syndrome (SMS)</td>
<td>Deletion</td>
<td>17p11.2</td>
<td>5</td>
</tr>
<tr>
<td>Duplication 17p11.2</td>
<td>Interstitial duplication</td>
<td>17p11.2</td>
<td>5</td>
</tr>
<tr>
<td>Neurofibromatosis type1 (NF1)</td>
<td>Deletion</td>
<td>17q11.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Prader-Willi syndrome (PWS)</td>
<td>Deletion</td>
<td>15q11-15q13</td>
<td>4</td>
</tr>
<tr>
<td>Angelman syndrome (AS)</td>
<td>Deletion</td>
<td>15q11-15q13</td>
<td>4</td>
</tr>
<tr>
<td>Inverted duplication 15 (inv dup (15))</td>
<td>Supernumerary marker chromosome</td>
<td>15q11-15q14</td>
<td>4</td>
</tr>
<tr>
<td>Williams-Beuren syndrome (WBS)</td>
<td>Deletion</td>
<td>7q11.23</td>
<td>1.6</td>
</tr>
<tr>
<td>DiGeorge and velocardiofacial syndromes (DGS/VCFS)</td>
<td>Deletion</td>
<td>22q11.2</td>
<td>3</td>
</tr>
<tr>
<td>Cat eye syndrome (CES)</td>
<td>Supernumerary marker chromosome</td>
<td>22q11.2</td>
<td>3</td>
</tr>
<tr>
<td>X-linked ichthyosis</td>
<td>Deletion</td>
<td>Xp22</td>
<td>1.9</td>
</tr>
<tr>
<td>Haemophilia A</td>
<td>Inversion</td>
<td>Xq28</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure by MIT OpenCourseWare.
Markers (a.k.a. ESACs)

• By definition, unknown chromosomal origin
• With FISH, most “markers” can now be described
• Phenotype depends on size, copy number, % cells w/ marker, chromosomal origin
  – X markers can be more severe than supernumerary X chromosome
Ring Chromosomes

- **Formation**: breakage in both arms followed by fusion at breakpoints with loss of distal fragments (deletion)

- **Mitotic Instability (Recombination)**
  - increase in size leads to increased instability

- Small rings can be supernumerary; phenotype can depend on copy number
Ring Chromosomes

• Ring chromosomes are formed when a chromosome undergoes two breaks and the broken ends of the chromosome reunite in a ring structure.
• Have been detected for every human chromosome.

Taken from Thompson & Thompson Genetics in Medicine 6th ed.
Fragile sites

• Appear as constriction or unstained region
• Inducible by specific culture conditions
• Only 3 fragile sites assoc. w/ pathology
  – FRAXA  Xq27.3  Fragile X syndrome
  – FRAXE  Xq28  X-linked mild MR
  – FRA11B  11q23  ??Jacobsen syndrome
• >100 fragile sites exist as normal variants
• May be implicated in cancer
Image removed due to copyright restrictions.
Differentiation of male and female gonads; see http://herkules.oulu.fi/isbn951426844X/html/graphic11.png
Image removed due to copyright restrictions.
X and Y chromosome maps.
Y Chromosome

- Yp pseudoautosomal region
- SRY
- USP9Y
- DAZ genes
- Satellite DNA
- Yq pseudoautosomal region

- q
  - 11.2
  - 11.21
  - 11.22
  - 11.23
  - 12
- p
  - 11.3
  - 11.2

Region present in XX males
Region deleted in XY females

AZFa
AZFb
AZFc

Regions deleted in azoospermia

Figure by MIT OpenCourseWare.

Adapted from Thompson and Thompson; Genetics in Medicine
Important Y Chromosome Genes

• **SRY gene (TDF)**
  - Maps just proximal (5 kb) to PAR1; Yp11.3
  - Not sufficient for normal male sexual differentiation

• **AZF (azoospermia factor) genes on Yq**
  - 3 non-overlapping AZF regions (e.g., *DAZ, USP9Y*)
  - 10% males with idiopathic azoospermia/severe oligospermia deleted in this region
  - Necessary, but not sufficient for spermatogenesis
Dosage Compensation (Mammals)

• Inactivation of all but one X chromosome per cell

• Lyon Hypothesis (1961)
  – In phenotypically normal females only a single X chromosome is active
  – X-inactivation occurs early in development (blastocyst)
  – Inactivation is random
  – Inactivation is clonal and irreversible in somatic cells
Numerical Abnormalities of the Sex Chromosomes
Frequencies of sex chromosome aneuploidies

- 1/400-1/500 liveborns
- Most common:
  - 47,XXX (~1/1000 females)
  - 47,XXY (~1/1000 males)
  - 47,XYY (~1/1000 males)
- Turner syndrome: 45,X (~1/5000 females)
  »~ 1-2% of all conceptuses
  »~20% of all SAB
Image removed due to copyright restrictions.
Karyotype of a 45,X woman (Turner syndrome).
Turner Syndrome Phenotype

- **Short stature** (under 5 ft): *SHOX* gene maps in PAR
- **Gonadal dysgenesis** (usually streak)
- Fetal **cystic hygroma** (lymphedema)
  - Post-natal neck webbing
- Low posterior hairline, shield chest with widely spaced nipples, cubitus valgus, **coarctation of aorta** and renal anomalies
- Deficiencies in spatial perception, perceptual motor organization and fine motor skills
- Fully viable, though **99% of 45,X conceptuses are lost**
Turner syndrome

Images removed due to copyright restrictions.
Photographs of a girl with Turner syndrome.

www.endocrineonline.org/ ts.htm
Karyotypes in Turner Syndrome

- ~50% show 45,X
- Remaining 50%:
  - 46,X,i(Xq) ~15%
  - 45,X/46,XX ~7%
  - 45,X/46,X,i(Xq) ~5%
  - 45,X/46,X,r(X) ~16%
  - 45,X/46,X,del(Xp) ~5%
  - Other ~2%

- 45,X/46,XY ascertained through clinic visits
  - Phenotype ranges from females with classical TS to infants with ambiguous genitalia to normal but infertile males
  - Risk for gonadal tumors

- 45,X/46,XY ascertained prenatally
  - Phenotypically male infant 90-95% of the time
  - ? Normal fertility
Image removed due to copyright restrictions.
Karyotype; 47,XXX.
47,XXX: Phenotype

- Physically normal with normal sexual development and usually **normal fertility**
- Many taller than average
- IQ 10-15 points lower than siblings
- Language delay, learning disabilities and impaired gross motor skills often exist
- Increased frequency of psychosocial disorders
Sex chromosome abnormalities--males

47 XXY--Klinefelter syndrome • 1/1,000
48 XXXY--Klinefelter syndrome • 1/25,000
47 XYY • 1/1,000
Other X or Y abnormalities • 1/1,500
XX males • 1/20,000
Overall incidence Males ~ 1/400
Image removed due to copyright restrictions.
Karyotype; 47,XXY.
Klinefelter Syndrome: Phenotype

- Hallmark features: hypogonadism, androgen deficiency and **impaired spermatogenesis**
- Variable, but classic features:
  - Tall, thin, long legs
  - Feminine distribution of body fat, gynecomastia
  - Underdeveloped, secondary sexual characteristics with small, firm testes and sparse body hair
- Reduced IQ (particularly verbal), dyslexia, ADD
Slides removed due to copyright restrictions.
Illustration and photographs of people with Klinefelter syndrome.
Karyotype; 47,XYY.
47,XYY: Phenotype

- Increased height
- Increased risk of behavioral problems
- Possibly some reduction in IQ
- **Normal fertility**
Sex Chromosome Tetrasomy and Pentasomy

- Rare
  - case reports only (no unbiased ascertainment studies)
- Phenotype more severe with each additional chromosome
  - more severe for X than for Y
- Supernumerary X chromosomes
  - Reduced IQ
  - Skeletal and cardiovascular abnormalities
  - In males, malformed genitalia (and infertility)
  - In females, effect on fertility unclear
Y Chromosome Structural Aberrations

• Sterility
  – Most of the euchromatic region must be present for germ cell development

• No phenotype
  – Most of the heterochromatic region can be deleted, rearranged without phenotypic effect
PGD - Preimplantation Genetic Diagnosis

Take one cell from 8 cell embryo after IVF.
Perform diagnostic test for specific condition or disease.
Slides removed due to copyright restrictions.
Single-cell FISH assays to determine chromosome balance in the blastomere.