Cytogenetics in the Diagnostic Laboratory

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Cytogenetics in the Diagnostic Laboratory

- Scottish pregnancy screening
- Conventional techniques
- Clinical Applications
- Array CGH (Comparative Genomic Hybridisation)
Scottish pregnancy screening

- Screening involving blood test
- Screening involving serum test
- Screening involving ultrasound test
Screening involving blood test

Routine blood test: as early as possible (8-10 weeks)

Haemoglobin, group, rhesus and antibodies, Syphilis, Hepatitis B, HIV and Rubella.
Scottish pregnancy screening

Screening involving serum test: 1st trimester combined test for Down’s syndrome (11-13 weeks)

- Biochemistry screening - PAPP-A (Pregnancy Associated Plasma Protein A), hCG (human Chorionic Gonadotropin)
- Ultrasound screening - Nuchal Translucency (NT)
Scottish pregnancy screening

Screening involving ultrasound test:

- **Dating scan** – 8-14 weeks
- **Fetal anomaly scan** – 18-21 weeks
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Conventional cytogenetic analysis involves examination of a G-banded karyotype to detect changes in the 46 human chromosomes by light microscopy.
Cytogenetics in the Diagnostic Laboratory

- Scottish pregnancy screening
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- CGH
Why do chromosome studies?

- Diagnosis
- Phenotype prediction
- Prognosis
- Recurrence risk
- Reproductive future
- Genetic counseling
- Prenatal diagnosis
Most common cytogenetic referrals

- Constitutional postnatal
  - Blood
  - Skin / other solid tissue
- Constitutional prenatal
  - Amniotic fluid
  - Chorionic villus sampling
- Molecular Cytogenetics
  - All samples above
  - DNA
Postnatal: blood referrals
Postnatal: blood referrals

- Congenital abnormalities (eg: heart defect) in neonates
- Failure to thrive/developmental delay
- Idiopathic mental retardation
- Sexual ambiguity & delayed puberty
- Recurrent miscarriage/infertility couples

• Lithium-heparin sample
  • Culture time ~ 3 days
  • Results up to 28 days
  • Urgent results within 10 days
Neonate with congenital abnormalities

Rounded prominent occiput, micrognathia
small posteriorly rotated “faun like” ears,
small mouth, high arched palate,
Short sternum, small thorax narrow pelvis
Severe IUGR
Profound MR ~ no response
CHD ~90%
Omphalocoele 60%
Rocker bottom feet, abnormal hand clenching
V poor prognosis, most die within 1 month, unless mosaic (10%)

Edward syndrome (+18) ~1:5,000 livebirths
Trisomy 18 in a neonate
47,XY,+18
• Mid face hypoplasia
• Short upturned nose
• Upward slanting palpebral fissures
• Epicanthic folds
• Small mouth and relative macroglossia
• Brachydactyly
• Single palmar crease ~50%
• Heart defects ~50%
• Duodenal/oesophageal, anal atresia~3%
• Premature ageing
• Most common cause of MR
• Mean IQ 50

Down Syndrome: 47,XY,+21
Rare and most lost before term

- IUGR & profound MR
- Moderate microcephaly
- All degrees of holprosencephaly (failure of forebrain cleavage)
- Lip and palate clefting
- Post-axial polydactyly of hand and feet
- Ears low set & flattened
- Scalp vertex anomalies
- Heart abnormality
- Kidney anomalies

Patau Syndrome: 47,XY,+13
Mental retardation & developmental delay

- Usually young children
- Often associated with dysmorphism
- Unbalanced karyotype causes phenotype
  - Unbalanced translocations
  - deletions
  - duplications
  - Additional “markers”
  - sometimes only detectable by FISH (Fluorescence in situ hybridisation)
Duplication

• 8 year old girl
• Global developmental delay
• Dysmorphic features
• Facial asymmetry
• Marked language delay

46,XX,dup(10)(q26.1q26.3)
Deletion

- 32 year old man
- Short stature
- Multiple exostoses
- Brachydactyly
- MR
- ?Trichorhino
  - Phalangeal syndrome

Deletion at 8q24
Problems of sexual development
Klinefelter syndrome
47,XXY

• ~1:500-1,000 males
• Prepubertally normal
  12-14 yrs testosterone plateaus
• Testes remain small and firm
• Tall stature (? Due to extra stature genes) but eunochoid body habitus
• Fat distribution female
• Gynaecomastia in ~30% (mechanism unknown)
• Most common genetic cause of male infertility
• This phenotype is also variable – many men never know they have KS until routine investigations for infertility
Turner Syndrome: 45,X & variants

- Prenatal: Cystic hygroma, heart defects
- Newborn:
  - redundant neck webbing,
  - peripheral lymphoedema
- Later childhood:
  - short stature,
  - broad chest
  - low hairline to nape
  - neck webbing
  - cubitus valgus
  - 20% co-arctation of aorta or ASD
- Adults: primary or secondary amenorrhoea
  - (gonadal dysgenesis or failure)
  - Many mosaic (may ameliorate phenotype)
Recurrent miscarriages

- 29 year old woman
- Mother had several pregnancy losses
- Carries balanced translocation
- Patient has had losses & abnormal neonatal deaths due to unbalanced meiotic segregants
- Prenatal diagnosis by CVS or AF
Maternal blood: balanced t(4;18) translocation
Prenatal: CVS and amniotic fluid samples
Prenatal: CVS and amniotic fluid samples

Reasons for referral include:

• Abnormal ultrasound scan
• Carrier of a structural rearrangement
• Elevated risk of a chromosome abnormality indicated by biochemical and/or ultrasound maternal screening
• Previous chromosome anomaly
• Maternal age >35
• FH of chromosome abnormality

Prenatal diagnosis is normally carried out using one or more of the following sample types:

• Amniotic fluid
• Chorionic villi
• Fetal blood
Prenatal: CVS and amniotic fluid samples

- Amniotic fluid:
  ~15ml at 16/40 gestation

  Culture time 2-3 weeks
  Results in 2-3 weeks
  RTG – 14 days

  Rapid aneuploidy Screening by QF-PCR
  (1-2 days)
Prenatal: CVS and amniotic fluid samples

- **CVS:**
  - ~10-25mg at 10-13/40 weeks gestation

  **Long term** culture time:
  - 2-3 weeks
  - Results: 2-3 weeks
  - RTG – 14 days

  **Rapid aneuploidy screening** by QF-PCR
  - (1-2 days)

  **Direct preparations:**
  - 1-2 days
Amniotic fluid with unbalanced translocation
Trisomy 21 in amniotic fluid

[Diagram of chromosomes]
Cytogenetics in the Diagnostic Laboratory

- Department Workload
- Conventional techniques
- Clinical Applications
- Array CGH
Principle of CGH

- Comparative Genomic Hybridisation
- Global karyotype assay - does not require informed probe choice
- Hybridise to a slide with clones or oligonucleotides dotted on
- Differentially label test DNA green & control reference DNA red
- Compare fluorescence ratios using software to give a CGH profile
- Analyse profile to demonstrate amplifications or deletions of test DNA relative to control
Differential labelling

Hybridisation under competitive conditions: specific binding

Washing to remove unbound material

Scan
The basic assumption of a CGH experiment is that the ratio of the binding of test and control DNA is proportional to the ratio of the concentrations of sequences in the two samples.
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Array CGH in the Diagnostic Lab

- Improved resolution over conventional cytogenetics for the detection of copy number changes
  - Confirmation of cytogenetically visible abnormality
  - Elucidation of cytogenetically visible copy number change
  - Detection of Microscopically Invisible Copy Number Change

- Patients with developmental delay + dysmorphism

- Initially used in retrospective cases with normal conventional cytogenetics

- Increasingly used as a front line test
Example 1

- **Case 1: Confirmation of cytogenetically visible abnormality**
Example 1

- Case 1: Confirmation of cytogenetically visible abnormality
Example 2

Case 2: Elucidation of cytogenetically visible copy number

[Chromosome image with various bands and labels from 1 to 22, X, and Y.]
Example 2

- Case 2: Elucidation of cytogenetically visible copy number change
Example 3

- **Case 3:** Detection of a microscopically invisible copy number change