CYTOGENETICS –PERIPHERAL BLOOD CULTURES & COMMON DISORDERS

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PERIPHERAL BLOOD KARYOTYPING

Peripheral blood samples is the most commonly used tissue for constitutional karyotyping. It can also be used for haematological malignancies in cases of high blast counts noted in the peripheral blood counts.

In normal healthy adults, PB lymphocytes are not dividing. Mitogens are added to stimulate the lymphocytes to divide.

Clinical uses of PB cultures are such as to determine constitutional karyotype of individuals requiring genetic diagnosis or genetic counselling, to determine carrier status and to determine constitutional karyotype of patients with haematological malignancies.

LIMITATION OF PB KARYOTYPING

Karyotyping can be used to identify numerical abnormalities (aneuploidy) or structural abnormalities. However, due to the resolution of the chromosomes, small structural abnormalities can be missed or be undetectable. In such cases, molecular methods such as FISH or microarray is recommended.

It may create problems in cases where mosaicism is present as some cell lines may be lost over time (Barch et al., 1997)[1].

In some disorders, the abnormality may only be presented in certain tissue types and may not be present in the blood[1].

COMPONENTS OF PERIPHERAL BLOOD

1. Erythrocytes (red blood cells)
2. Leukocytes (white blood cells)
3. Platelets
4. Serum proteins, lipids, fibrinogen, glucose, water, gases, salts, antibodies, hormones and excretory products.

LYMPHOCYTES

The cells targeted for routine cytogenetic studies - mononuclear leukocytes called lymphocytes.

In normal circulating peripheral blood, about 20-40% of the WBCs are lymphocytes.

There are B cells (bursa-dependent cells responsible of humoral immunity or antibody production) and T cells (thymus-dependent cells responsible for cellular immunity).

In the presence of mitogens, small lymphocytes undergo transformation in which cell enlarges and the staining properties of the nucleus change.

The transformed cells are capable of cell division.

LYMPHOCYTES

The most common mitogen for cytogenetic studies is Phytohaemaglutinin (PHA).

When exposed to PHA, mature T cells dedifferentiate to a T-lymphoblastic cell. In this immature form, T cells synthesize DNA to undergo mitosis[1].

During the first 24-hour of exposure to PHA, the T cells undergo transformation and undergo cell division. Peak mitotic activity is reached after 60-70 hours of culture and is considered to be optimum harvesting time[1].

PB from newborn infants may yield sufficient mitoses at 48-hour which makes them convenient as urgent specimens[1].

PB CULTURE

Blood is collected in sodium heparin tubes as the anticoagulant of choice. Optimally, the blood should not be clotted or kept at extreme temperatures as this would affect the cells viability.

There are three types of PB cultures:

- 48hour
- Synchronised (SYNC)
- High resolution (HI-RES)

The type of cultures to set up is dependent on the clinical indicator of the patient and can yield different banding resolutions.

48hour cultures typically yield up to 400bphs, SYNC cultures can yield up to 550 bphs while HI-RES can yield >550 bphs. However, the challenge of obtaining HI-RES metaphases are that the chromosomes are thread-like and may be overlapping making it difficult to identify the chromosomes.

INDICATIONS FOR URGENT BLOOD CULTURE[7]

Newborn (<3 months) or young child (<12 years) with stigmata of a known syndrome

Characteristics that indicate a chromosome disorder of a usually seen syndrome

When patient is in ICU

Patient awaiting surgery

To confirm the abnormality in newborns

Prenatal fetal blood

Cultures are set up as 48hour and SYNC for these indications

Extracted from Task procedure:
SJMC TP/LAB-308-11

INDICATIONS FOR SYNC AND HI-RES CULTURES[8]

Couple with infertility problem of a history of multiple miscarriages

Characteristics that indicate a chromosome disorder different from a usually seen syndrome

Young adult with delayed or otherwise abnormal sexual development

Follow up or family studies where a relative has a chromosomal rearrangement that may be inherited.

Parents of unexpected prenatal structural abnormality.

Family history of chromosome abnormality referred in the 2nd trimester

Microdeletion syndrome

Young adult with developmental delay and/or subtle dysmorphic features

Expected subtle chromosome rearrangements

Extracted from Task procedure:
SJMC TP/LAB-308-10

CULTURE MEDIA CONTAINS:

RPMI 1640 (basal media containing essential components such as amino acids, vitamins and other additives)

Hepes buffer (to maintain pH level of 6.6-7.8)

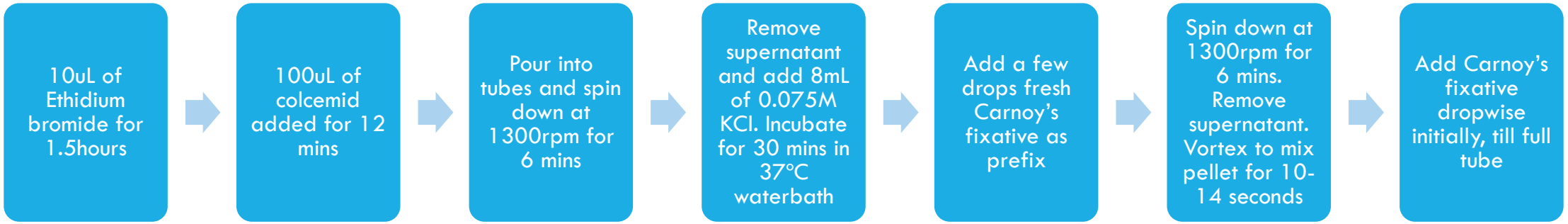
Inactivated fetal bovine serum (provide growth factors)

L-glutamine (usable form of amino acid. In storage, it becomes D-glutamine which the cells cannot use)

Penicillin-streptomycin (antimicrobial agent)

Phytohaemaglutinin (PHA) (mitogen)

HARVESTING PROTOCOL



- For SYNC and Hi-RES cultures, blocking reagent is added the day before the harvest at 5.30pm. Releasing reagent is added on the day of the harvest at 9am.
- For Hi-RES cultures, the harvest steps will start at 1pm while for SYNC cultures, the harvest step will start at 2.30pm starting at the second step (omitting the Ethidium bromide step)

PURPOSE OF THE CHEMICALS ADDED FOR HARVESTING

1. Blocking & releasing reagents – 2-Deoxycytidine and thymidine are added to synchronise the cell cycles to obtain higher and better yield of metaphases.
2. Ethidium bromide – Intercalates with DNA to produce long, uncoiled chromosomes
3. Colcemid – arrests cells at metaphase of mitosis
4. Hypotonic solution (0.075M KCl) – causes cellular swelling and allows chromosomes to be well dispersed within the membrane. Lyses red blood cells.
5. Carnoy's fixative – denaturation and precipitation of proteins and nucleic acids. Hardens chromation, enhances morphology and prevents membrane bursting and scattering of chromosomes.

PB ANALYSIS

At the end of the harvesting step, the harvested patient tubes go through a series of Carnoy's fixative change until they are ready to be dropped onto slides. Subsequently, the slides are aged and undergo banding and staining until they are ready to be analysed.

Karyotype analysis:

- Analysis of at least 20 metaphases are required for each case.
- More cells will be analysed in cases of suspected mosaicisms.

Turnaround time:

- According to MS ISO 15189, urgent blood karyotyping should be reported out within 7 days while routine blood karyotyping should be reported out within 18 days.

COMMON ANEUPLOIDY DISORDERS

1. Down syndrome
2. Edwards syndrome
3. Patau syndrome
4. Klinefelter syndrome
5. XXX syndrome
6. Turner syndrome

1. DOWN SYNDROME

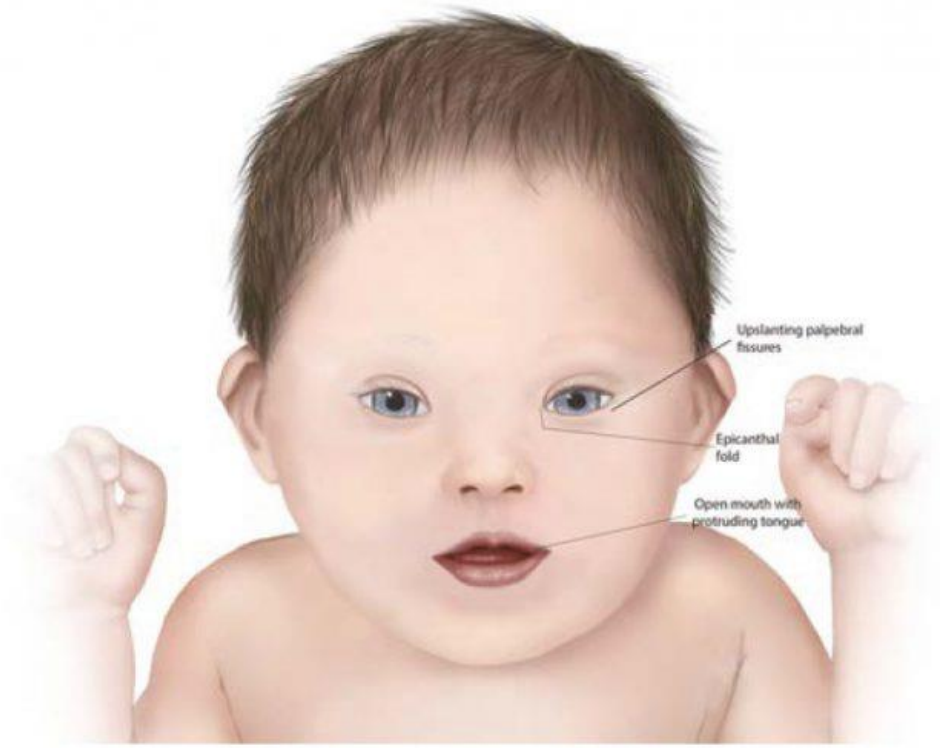
Trisomy of chromosome 21.

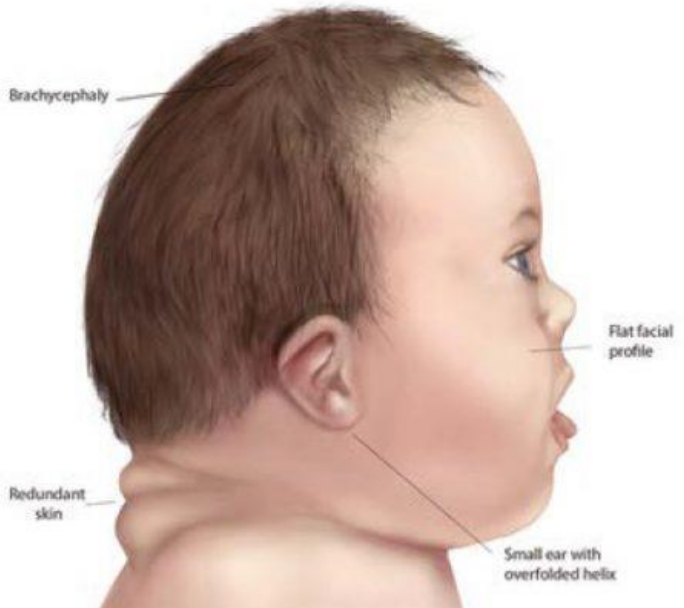
Three types:

- Trisomy 21: About 95% of people with Down syndrome have Trisomy 21. Each cell in the body has 3 separate copies of chromosome 21 instead of the usual 2 copies.[2]
- Robertsonian translocation : Accounts for ~3% of people with Down syndrome. This occurs when an extra part or a whole extra chromosome 21 is present, but it is attached or “trans-located” to a different chromosome rather than being a separate chromosome 21.
- Mosaic trisomy 21: Affects about 2% of the people with Down syndrome. For children with mosaic Down syndrome, some of their cells have 3 copies of chromosome 21, but other cells have the typical two copies of chromosome 21. Children with mosaic Down syndrome may have the same features as other children with Down syndrome. However, they may have fewer features of the condition due to the presence of some (or many) cells with a typical number of chromosomes.

Common physical features:

- Flattened face especially at bridge of nose, almond-shaped eyes that slant up, short neck, small ears, tongue tends to stick out of mouth, poor muscle tone (Centers for Disease Control and Prevention, 2021)[2].





2.EDWARDS SYNDROME

Caused by trisomy 18

Mostly causes fetal demise (>80%) in the first 8 weeks of pregnancy,. For affected liveborns, significant cardiac and renal malformations and respiratory difficulties contribute to a mortality rate of 50% in the first week of life. Only 5-10% survive beyond 1 year (Geno et al., 2021)[3].

Common physical features: high nasal bridge, short palpebral fissures, ptosis, small mouth, narrow palate, micrognathia, clenched hands with overriding fingers, rocker-bottom feet, short sternum, small pelvis (Berg et al., 2007)[4].

3. PATAU SYNDROME

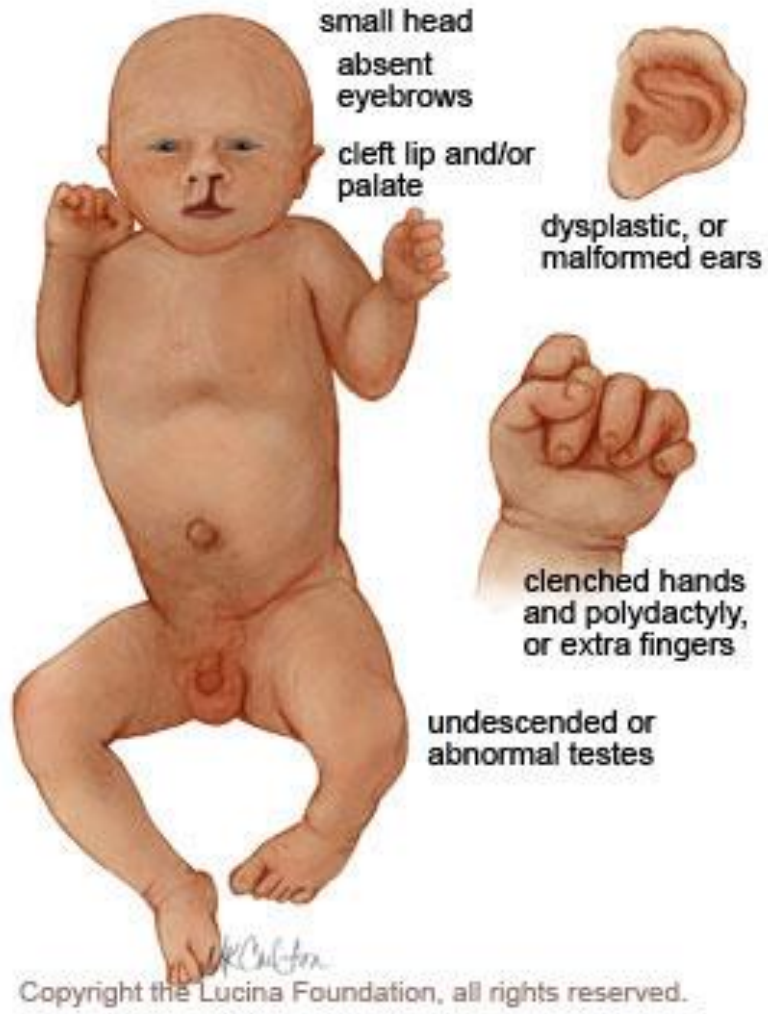
Caused by trisomy 13

Mostly causes miscarriage, stillbirth or baby dying shortly after birth

- About 1 in 10 babies with less severe forms (partial or mosaic trisomy 13) live for more than a year (NHS UK, 2019)[5].

Physical features

- Low birth weight, severe heart defects, holoprosencephaly, cleft lip and palate, hypotelorism, polydactyly, rocker-bottom feet



4. KLINEFELTER SYNDROME

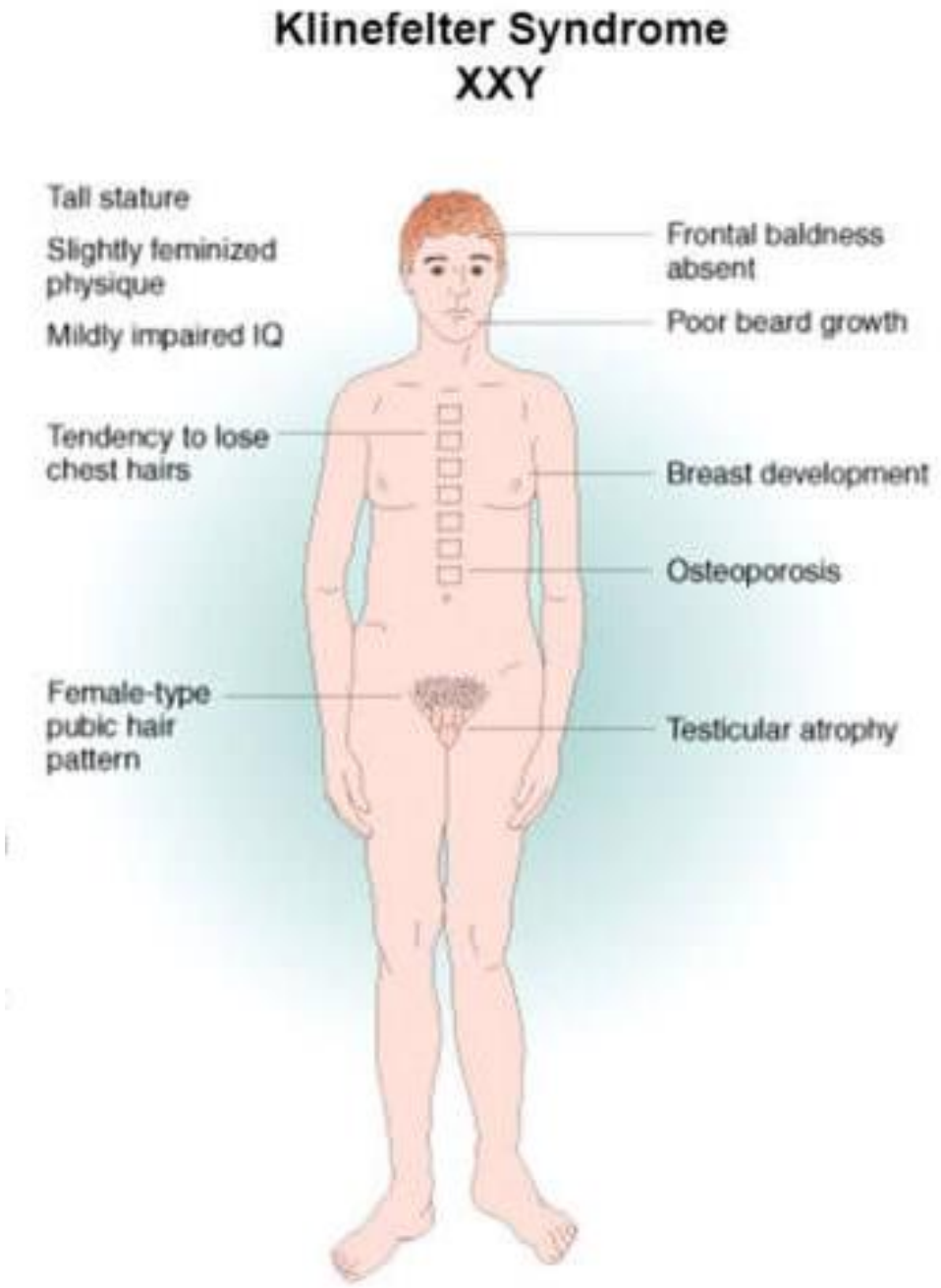
XXY syndrome.

In some cases can have more than one X chromosome (variants of Klinefelter) eg 48,XXXY or 49,XXXXY.

Signs vary among affected people, some men have no symptoms of the condition or are only mildly affected.

Some symptoms may include:

- Small, firm testicles, delayed puberty, gynecomastia, infertility, tall stature, learning disability, cryptorchidism, hypospadias (National Library of Medicine, 2020)[6].



5. XXX SYNDROME

Trisomy X syndrome

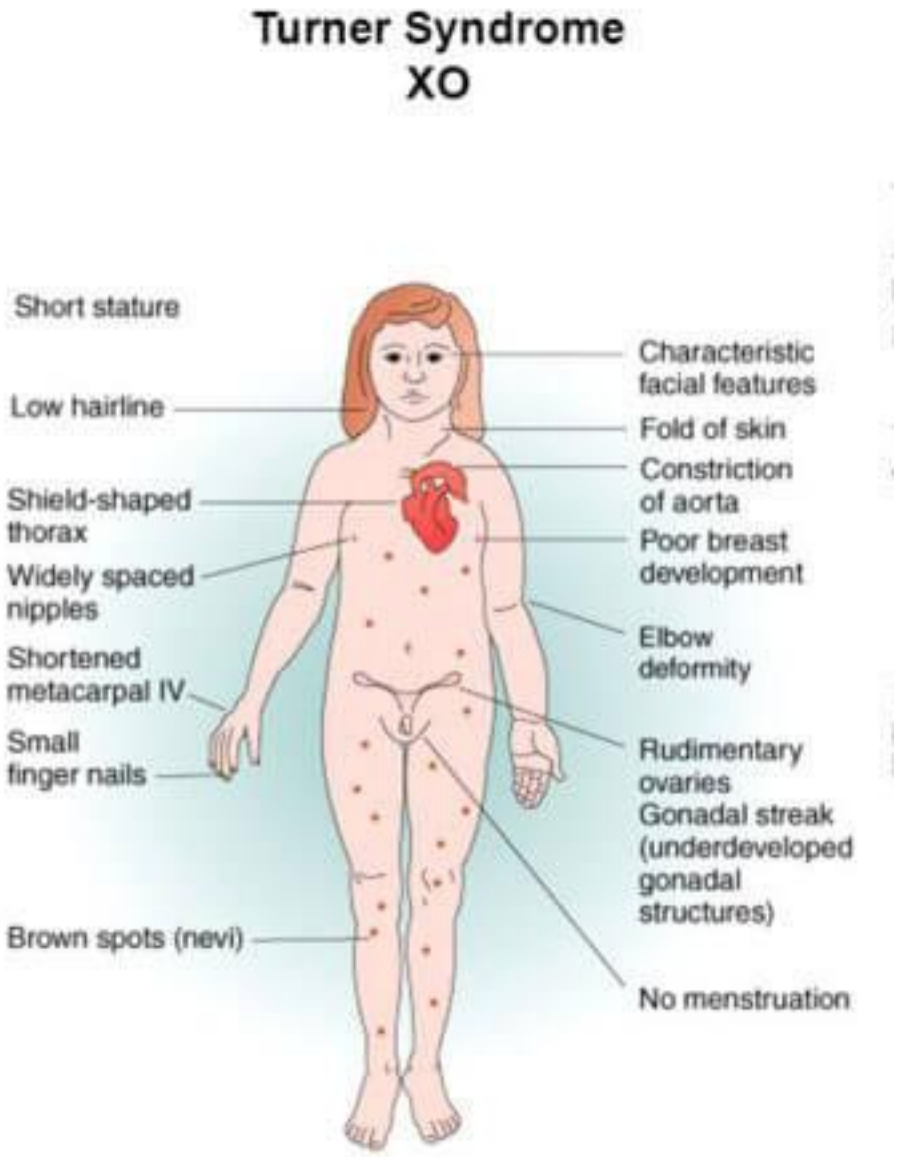
Symptoms vary in severity and may include epicanthal folds, hypertelorism, delayed development of speech, language skills and motor skills, hypotonia and behavioural difficulties.

6. TURNER SYNDROME

XO syndrome

Affects development in females.

Signs and symptoms may include short stature, premature ovarian failure, developmental delays, learning disabilities



COMMON STRUCTURAL ABNORMALITY SYNDROMES

Needs to span at least 5Mb to be visible karyotypically.

While most of the syndromes are caused de novo, they can also be inherited by parents carrying a balanced translocation.

As structural abnormalities are often difficult to detect through conventional karyotyping, further molecular testing are suggested such as FISH and microarray.

1. CRI DU CHAT SYNDROME

Caused by deletion of the short arm of chromosome 5 (5p) ranging from only involving band 5p15.2 to the entire short arm (OMIM.org, 2020)[9].

Most cases are de novo. Small percentage of cases are inherited by unaffected parents having balanced translocations.

Symptoms vary in severities and may include:

- Cat-like cry
- Microcephaly
- Round face
- Hypertelorism
- Micrognathia
- Epicanthal folds
- Low-set ears
- Hypotonia

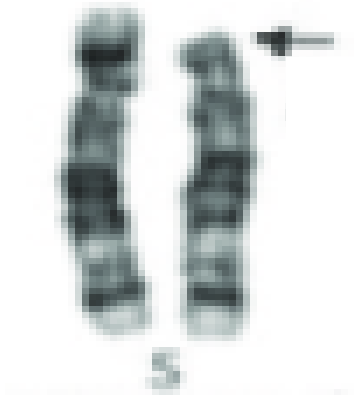


Image from: Hong, Yu & Choi, Eom & Ho, Yo & Lee, Oh & Kim, Young. (2018). Cri-du-chat Syndrome with Dysphagia. Perinatology. 29. 48. 10.14734/PN.2018.29.1.48.

2. WOLF HIRSCHHORN SYNDROME

Caused by a deletion at chromosome 4p16.3

May be difficult to diagnose based on karyotyping. Suggest for FISH using WHSCR probe or microarray studies which would be able to detect deletions involving WHSCR gene and defines the size of deletion (National Organization for Rare Disorders, 2020)[10].

Symptoms can vary based on size and location of the deleted region and may include:

- Ocular hypertelorism with broad or beaked nose
- Microcephaly
- low-set malformed ears
- growth deficiency
- cardiac defects
- intellectual disability
- Seizures.

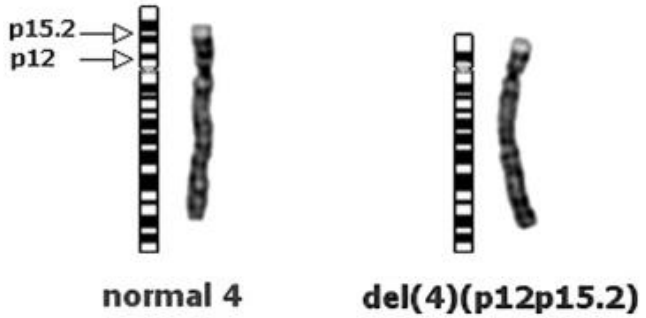


Image from: Pen-Hua Su, Inn-Chi Lee, Jia-Yuh Chen, Suh-Jen Chen, Ju-Shan Yu, Teng-Fu Tsao, Interstitial Deletions of the Short Arm of Chromosome 4 in a Patient With Mental Retardation and Focal Seizure, Pediatrics & Neonatology, Volume 52, Issue 3, 2011, Pages 165-168, ISSN 1875-9572, https://doi.org/10.1016/j.pedneo.2011.03.009.

3. DIGEORGE SYNDROME

Also known as velocardiofacial syndrome, 22q11.2 deletion syndrome

Caused by deletion at chromosome 22q11.2

May be difficult to diagnose with karyotyping. Suggest for FISH, whole genome array, SNP array, CGH array or MLPA.

Symptoms include:

- Congenital heart disease, palate abnormalities, immune system dysfunction, endocrine abnormalities, gastrointestinal problems, kidney abnormalities, hearing loss, seizures, skeletal abnormalities, learning and behavioural differences (National Organization for Rare Disorders, 2017)[11].
- Symptoms are extremely variable and affect almost any part of the body

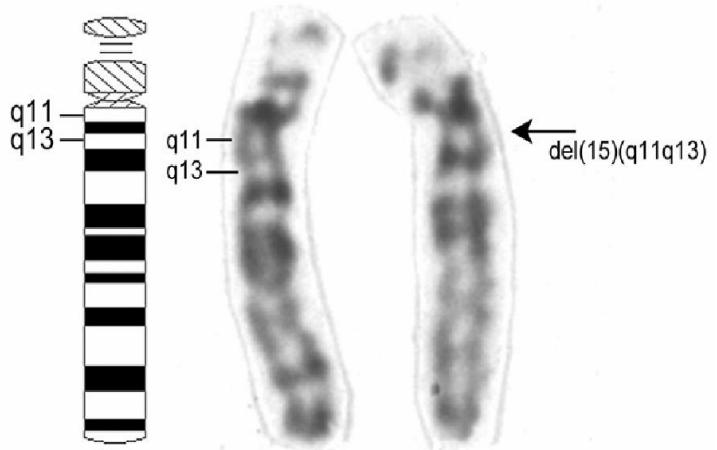
4. PRADER-WILLI SYNDROME

Caused by abnormalities of the paternally inherited chromosome 15 affecting the region of 15q11 to 15q13.

- Abnormalities such as deletion, maternal uniparental disomy and genetic imprinting errors (National Organization for Rare Disorders, 2018)[12].

Is a genetic multisystem disorder characterised during infancy by lethargy, hypotonia, weak suck and feeding difficulties with poor weight gain. Affected individuals do not feel satiated after meals and can lead to overeating and obesity.

Diagnosis of this syndrome can be enhanced by microarrays and has to be confirmed by certain tests including DNA methylation tests and imprinting defects to determine loss of paternal chromosome 15q11-13.



5. ANGELMAN SYNDROME

Caused by abnormalities of *UBE3A* gene of the maternally inherited chromosome 15 at the region of 15q11 to 15q13.

- Abnormalities such as deletion, gene mutation, imprinting error or paternal uniparental disomy (National Organization for Rare Disorders, 2018)[13].

Characterised by severe developmental delay and learning disabilities, absence or near absence of speech, inability to coordinate voluntary movements, behavioural pattern characterised by a happy disposition and unprovoked episodes of laughter and smiling.

Diagnosis needs to be confirmed through specialised test such as DNA methylation, FISH, microarray, *UBE3A* gene mutation studies.

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