Genetics

Genes
- Coding - Exons → 1.5% of Human genome
- Non-Coding - INTRONS -

Regulation of genes.

i) Centromeres
ii) Telomeres
iii) Transposons (Jumping genes) (33%)
↓
func - chromatin organization

iv) Non-Coding RNAS

a) MTicro RNA - miRNA - Q 21-30 oligonucleotide
- gene silencing RNA (post-Transcriptional)
- 5% of human genome.

b) SiRNA (small interfering RNA)
↓
exogenous RNA (Invitro)
↓
To study gene function. - knock down technique

LINC-RNA - Long intervening non coding
>200 nucleotide.
- associated with Barr-Body formation

RNA → Gene (inactivation)

Barbodies

d) Pi-RNA - m/c - Non coding RNA in Human genome

piwi- Interacting.

Genetic disorders -

- Single gene
  - Mendelian
    - Classic
      - AR
      - AD
      - XR
      - XD.
    - Unclassic (Non Mendelian)
      - i) gonadal mosaicism
      - ii) mitochondrial inheritance
      - iii) genomic imprinting
      - iv) Trinucleotide Repeat - Gaut / HTN
- multiple gene
  - multifactorial inheritance / Complex polygenic.
  - (>2 mutant genes + environmental factors)
  - eg - Diabetes
  - Cleft lip/palate.
  - Congenital Heart disease.

- m/c pattern of inheritance - Autosomal dominant (Vertical)
- m/c pattern in inborn error of metabolism - AR - (Horizontal)

AR	AD
Aa - Carrier.	Aa - disease.
aa - disease.	aa - disease (die in utero)

AD : AR www.FirstRanker.com ADD

i) Reduced Penetrance

eg - Retinoblastoma ch-13q14

Rb - Two Hit Hypothesis - Homozygous

Loss of Heterozygosity - LHO. LOH

1st Hit - gamline Hit

2nd Hit - acquired Hit } Knudson's Two Hit

LOH : AD - PKD

Hirschsprung's disease

Wilm's Tumor

RetinoBlastoma.

ii) Dominant Negative effect.

eg Mutant - protein/gene

↓

affecting N genes/proteins.

MC inherited Connective tissue disorder - Marfan's Syndr

Fibrillin gene - C15q

Revised Ghents Criteria :

i) Family H/o Marfans

ii) Cardinal features

iii) Fibrillin gene - mutation

m/c - cause of death aortic dissection.

1) Ehlers Danlos Syndrome - Rubberman Syndrome.

Hyperextensibility of Joints + Skin Hyperextensibility

Collagen m/c collagen - III

m/c Subtype of EDS - Type - III

→ Rupture of colon/arteries - Type IV [Vascular type]

Worse prognosis - Type - IV.

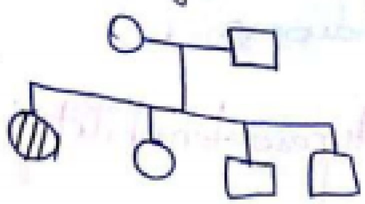
I/III - Scarring affected. Cigarette paper like Scarring

3) Osteogenesis imperfecta - Collagen - I.

Pedigree analysis -

- X-linked Recessive Carrier
- Proband/Index Case.
- deceased/dead.
- unknown gender/Hermaphrodite
- miscarriage/abortion.
- Divorce
- Infertility.
- emotional attachment.
- extramarital affair
- Adopted child
- 5 ③ - No. of siblings.
- Dizygotic
- monozygotic.

Pedigree analysis



- Both parents normal, one/variable child affected

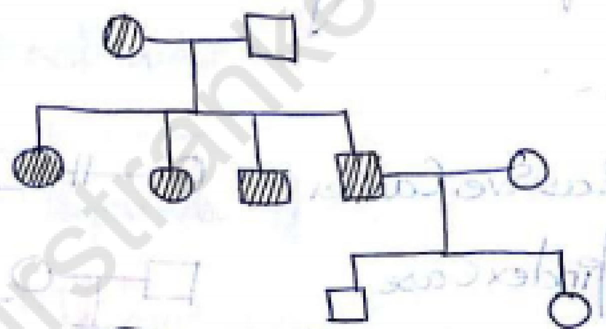
unclassic inheritance

① **Gonadal mosaicism** - Variant of Autosomal dominant

→ mutation occur in zygote level

eg- Osteogenesis Imperfecta, Tuberous Sclerosis, Achondroplasia

② Mitochondrial Inheritance (Maternal Inheritance)



ovum - Rich in mitochondria
↓
mt DNA
→ Normal
→ mutant
→ Heteroplasmy

When mutant allele > normal → disease

Sperms also contain mt-DNA - selectively degraded during fertilization
↓
ubiquitin proteasome pathway

Mitochondrial diseases through mitochondrial DNA

→ Leber's Hereditary optic neuropathy - Prototype

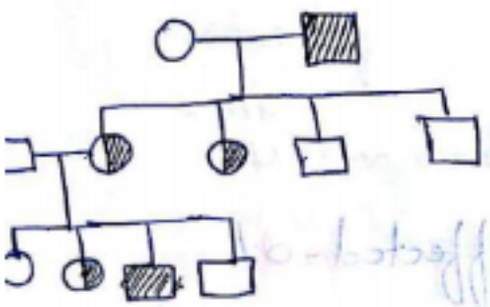
→ NARP - Neurogenic weakness
Ataxia
Retinitis Pigmentosa.

Retinitis pigmentosa m/c pattern of inheritance

60% Sporadic 40% Hereditary

AR (60%) 30-40% AD Digen
inherit

X-linked Recessive.



→ X-chromosome of father transmitted to daughter only.

→ mostly males are affected.

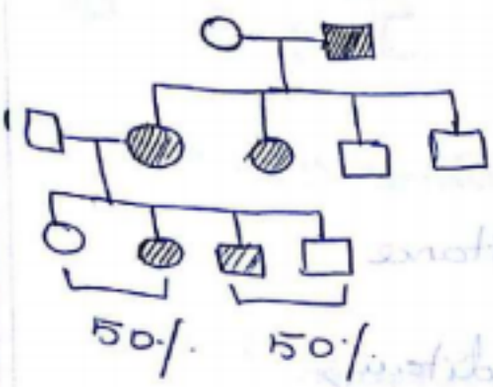
→ females also affected when Carrier mother + affected father.

Y-chromosome - Y linked Disorders

- only males are affected : Retinitis pigment
- Hairy ears.

Fragile X Syndrome ← XLR (m/c) ← X-D

→ Hemophilia - A/B - XR Hemophilia-C - AR.



X Linked dominant

Father
↓
all daughters
↓
Sons spared.

from affected daughters

50% Sons 50% Daughters

Alports - 85% → X linked Dominant
m/c

Autosomal Recessive-

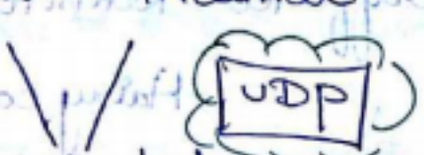
Aa AA - Affected - 0%

AA AA aA aA. Carrier - 50%.

Galactosemia → AR.

Uniparental Disomy - one parent giving both genes.

Carrier — Normal



- mechanism

Affected - 1

Genomic imprinting - imprinting - inactivation

→ Functional gene inherited from 1 parent only

Mono imprinting - epigenetics

Chromosome-15q

Prader-Willi
- Obesity.
- MR
- Hypogonadism
Ghrelin ↑ - orexegenic.

Angelman.
- Ataxia
- Laughter.
Happy puppet.

m/c i) deletion

ii) uniparental disomy.

maternal gene  Prader-Willi.

Paternal gene  - Angelmans

UPD associations: i) Angelmain
ii) Prader-Willi.
iii) Russel-Silver
iv) Bloom - Rare (m/c - AR)
v) Beckwith-weidman (UPD of WT-1 Ch 11

Trinucleotide Repeat

Codons - normally Triplets - Repeat normally

Times - 29 (6-55)

55 - 200 - Premutation - asymptomatic III - Carrier.

>200 Repeats - Disease

∴ No. of Repeats correlate with severity of disorder.

No. of Repeats ∝ Severity.

Dynamic mutation - No. of Repeats - gain of mutation

- Oogenesis
  - affecting Noncoding areas -
  - eg - 1) Fragile - X - Syndrome
  - 2) Freduich's ataxia
  - 3) Myotonic Dystrophy
- Spermatogenesis
  - Coding areas
  - exons
  - 1) Huntington's chorea
  - 2) Spino Cerebellar ataxia.

Huntington's Disease: Father → 300 Repeats

↓

Son - ↑ Repeats 600 Repeats (earlier age); ↑ Severity.

↓

next generation - earlier onset

Anticipation: ↑ Severity, years - earlier onset.

→ X-Chromosome shows fragile sites, when grown on folate deficient medium ++++

Large Testis (macroOrchidism) > NTR [Hallmark]

Large face
mandible
everted ears
} Small / Normal-rose

KARYOTYPING

→ Study of chromosome.

→ paired chromosome

↓ length.

xy - last

Sample - i) Amniotic fluid - shedding of epithelium (skin, UGT, GIT, RT)

ii) Chorionic villi Biopsy.

iii) Fetal tumor Sample. m/c - fetal tumor - Teratoma.

iv) Cordocentesis - umbilical cord Blood

vi) Peripheral blood - lymphocytes

other cells reversed - dont proliferate in Test tube

Fixative of choice -

Karyotyping - Carnoy's Fluid (3 parts Methanol + 1 part glacial acetic acid)

Histopathology - 10% neutral buffered formalin

Electron microscopy → glutaraldehyde > Best

Osmium Tetraoxide

Cytology → 95% Ethanol (cytology) - Pap Smear.

Hematopoietic cells → mercuric Chloride - Zenker's fluid

Picric acid - Bouin's fluid → Glycogen/Lipid storage disorders.

Testicular biopsy → Bouin's fluid - Ideal (preserves architecture)

Routine Karyotyping - neutral buffer formalin - universal

Cells

↓

Proliferate in Test

↓

metaphase arrest Colchicine

Resolution - 800 bands/Haploid set

To increase Resolution - prophase arrest

Resolution - 1500 bands/Haploid set

Routine Cytogenetic analysis → Light micro → Resolution of chromosome 5Mb

Best stain for Karyotyping - Giemsa stain
∴ Karyotyping known as G-band

p(short) q-Next to 'p' (arbitrary)

p-petit

Types of chromosomes

i) Metacentric - p=q - Rest all chromosomes metacentric

ii) Sub-Metacentric - X-chromosome q>p

iii) Acro-centric - Y-chromosome q>>p.

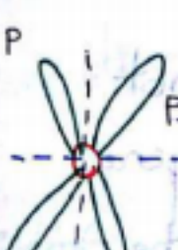
i) associated with pathogenesis

Down-Syndrome - Robertsonian Translocation

t → 21 → 13, 14, 15 } All are Acrocentric chromosomes

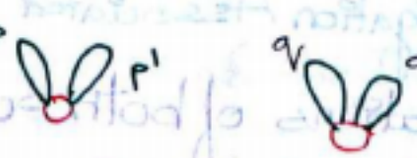
Acrocentric group - 21, 13, 14, 15, 22, Y

iv) Telocentric - not seen in Humans



P' normal division - vertical.

⊥r to normal axis - Horizontal plane



Isochromosome (i)

m/c isochromosome - X_q - x long arm

m/c in Human Cancer - 17q (i)

12p — germ cell tumors of ovary & Testis

17q — m/c cytogenetics in Medulloblastoma → Poor prognosis.

Uses of Karyotyping:-

i) Numerical disorders < Euploid – 23x
Aneuploidy – odd

m/c Chromosomal disorder – Trisomy 16 (But die in utero)

m/c chromosomal disorder in live birth } Down Syndrome

Limitations – i) microdeletion
ii) Amplification
iii) Complex Translocation } Cannot be seen

→ FISH – can overcome this

Best for known gene loci – FISH – limitation – Cannot detect small genes.

∴ for point mutation – PCR limitation – Cannot detect larger genes.

MLPA – Multiplex Ligation Associated Probe Amplific
– overcome limitations of both PCR & FISH
– Both small & large genes
– Best for cystic fibrosis detection

Down's Syndrome

Mech - i) Meiotic - Nondisjunction - Meiosis - I (In all trisomies, except for Edward Syndrome - Meiosis - II) 95%

Rocker Bottom feet - more Edward Syndrome

Extrachromosome in Down's → Maternal in origin

ii) Robertsonian - 4% } No Relation with advanced maternal age

iii) Mosaicism - 1% }

Robertsonian

→ t 21 → 13, 14, 15; 22.

t (21, 21) - 100% chance of 2nd Baby

Mosaicism

Normal + abnormal no. of chromosomes.

ALL - m/c leukemia associated with Down's.

AMTLM7 - Acute Megakaryocytic - m/c leukemia in children < 3 years.

> 40 years - Alzheimer's - 100%

m/c Cardiac defect - endocardial cushion defect > VSD > ASD

Turner's Syndrome i) 45 XO m/c

ii) 46 X (r) (Ring chromosome)

iii) 46 ~~X~~ Xp

~~46 X q~~

Histology - Absence of ovarian Tissue.

Histology - Breast Tissue Normal

m/c Cardiac - Bicuspid aortic valve

Tumors - i) Colon Ca
ii) AITL.

AITL - Down's
Turner
Klinefelter

Klinefelter Syndrome -

Trisomies - ≥2x and ≥1y

m/c - 47xxy - meiotic nondisjunction (meiotic ↑)

→ Associated with both Advanced Maternal/Paternal age

→ Testosteron ↓↓ ; ↑FSH, oestrogen.

→ gynaecomastia

m/c Cancer Breast Cancer.

m/c germ cell tumor - Extragonadal > gonadal
GCT GCT

Teratoma

m/c site - mediastinum

m/c Site EGCT in Brain - pineal gland

Genetic polymorphism - 0.5% variation among individuals (

↓

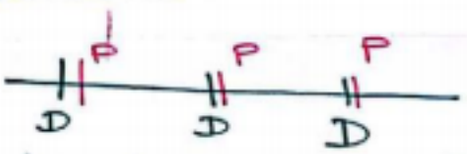
15-million Base pairs

- polymorphic genes / marker genes - candidate

multifactorial / unknown gene mutation,

Polymorphic genes - (characters)

i) always close to disease causing allele.



ii) disease.

Polymorphic genes - always stably transmitted to next generation.

↓

Linkage - Disequilibrium

m/c type of polymorphic gene - SNP - Single nucleotide Polymorphi

- 1 nucleotide difference / 1000 base pair

iii) Repeat length polymorphism -

a) microsatellite

Q (2-6) Base Pair repeats.

macrosatellite

Q 15-70 Base Pair repeats.

Uses.

Genetic

Single gene

Direct Sequencing with PCR / FISH

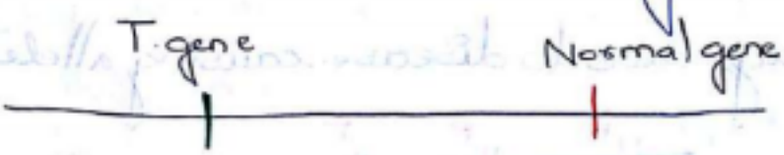
multifactorial

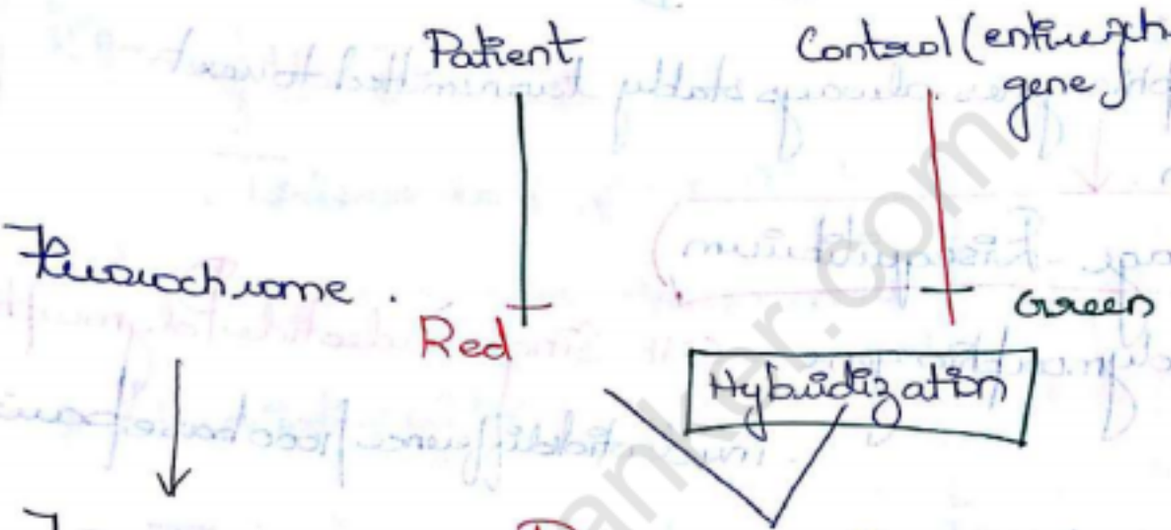
Polymorphic genes - Indirect Sequencing

Linkage Analysis - Study of polymorphic gene for a disease in family

Large Population - GWAS (genome wide Analytical Study)

To detect Same chromosome - Tumor gene / Normal gene





} Microarray Analysis.

Microarray based Comparitive, genomic Hybridization CGH

Uses of FISH

Breast Cancer.

i) gene expression profiling eg ER+; PR+ve; HER. neu.

ii) molecular classification of Cancer.

→ Molecular classification of Breast Ca based on gene expression profiling

Q FISH Congenital malformation - metaphase

Oncological Specimens - Interphase

2 variants- i) Chromosomal painting (entire human genome on same chromosome)

fluorochrome painted on entire genome of one chromosome

Q 2) Multicoloured FISH: entire genome on all gen chromosome simultaneously

Spectral Karyotyping

Spectacual Karyotyping → fluorochromes & Computer generalized.

Immunology

Immunity

Innate	Acquired
- Birth / 1st line of defence	- memory ⊕
- Nonmemory	- Specific ⊕
- Non-Specific	