Lecture-5

Single Gene Disorders

Single Gene Disorders

- When a certain gene is known to cause a disease, it is called a single gene disorder or Mendelian disorder.
- More than 10,000 single-gene traits and disorders have been identified.
- Most of these are individually rare, but they affect between 1-2 % of the general population.
- Examples are cystic fibrosis, sickle cell disease, Fragile X syndrome, muscular dystrophy, and Huntington disease.

Huntington Disease

- Dr. George Huntington, first described multiple affected individuals in a large North American family in 1872.
- The natural history of HD is characterized by slowly progressive selective cell death in the central nervous system.
- There is no effective treatment or cure.
- The prevalence in most parts of the world is approximately 1 : 10,000.
- The onset is mostly between 30 and 50 years, but it can start at any age, including a rare juvenile form with different clinical features.

Clinical Features of HD

- Characterized by slowly progressive movement disorder and insidious impairment of intellectual function with psychiatric disturbance and dementia.
- The mean duration of the illness is approximately 15 to 20 years and chorea is the most common movement abnormality.
- This takes the form of subtle involuntary movements such as facial grimacing, twitching of the face and limbs, folding of the arms, and crossing of the legs.
- As the disease progresses the gait becomes very unsteady and speech unclear.
- **Juvenile HD:** Mostly paternally inherited. Up to 5% of HD cases present before the age of 20 years and instead of chorea there is rigidity, with slowing of voluntary movement and clumsiness. A decline in school performance indicates the onset of a severe progressive dementia, often in association with epileptic seizures.

Genetics of HD

- HD is an **autosomal dominant disorder**, which means that a person needs only one copy of the defective gene to develop the disorder.
- HD is caused by a mutation in **HD gene (HTT gene)** located at 4p16.3
- HD gene encodes for a protein, **huntingtin**, whose exact function is unclear, but it appears to be important to nerve cells (neurons) in the brain.
- Almost all individuals with HD possess an expansion of a CAG polyglutamine (triplet) repeat sequence located in the 5′ region of the HD gene.
- There is a direct relationship between repeat length and disease severity.
- The average age of onset at 57 y (40 repeats), 37 y (45 repeats), and 26 y (50 repeats). The juvenile cases often have an expansion greater than 55 repeats.

Normal alleles	26 or fewer CAG repeats. Stable in meiosis.
Mutable alleles	27 to 35 repeats. Do not cause disease but may show meiotic instability to increase or decrease in size.
Reduced penetrance alleles	36 to 39 CAG repeats. Late-onset disease or complete absence of disease expression (non-penetrance).
Disease alleles	40 or more CAG repeats.

Genetics of HD

GCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACCTGCGGCCCAGAGCCCCATTC
ATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGGCCTCCGGGGACTGCCGTGCCGGGCGGGAGA
CCGCCATGGCGACCCTGGAAAAGCTGATGAAGGCCTTCGAGTCCCTCAAGTCCTTCCAGCAGCAGCAGCA
GCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAACAGCCGCCACCGCCGCCG
CCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCCGCAGGCACAGCCGCTGCTGCCTCAGCCGCAGC
CGCCCCCGCCGCCGCCCCCGCCGCCACCCGGCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGT

23 glutamine residues

/translation="MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQQQQQQPPPP
PPPPPPPQLPQPPPQAQPLLPQPQPPPPPPPPPPGPAVAEEPLHRPKKELSATKKDRV
NHCLTICENIVAQSVRNSPEFQKLLGIAMELFLLCSDDAESDVRMVADECLNKVIKAL
MDSNLPRLQLELYKEIKKNGAPRSLRAALWRFAELAHLVRPQKCRPYLVNLLPCLTRT
SKRPEESVQETLAAAVPKIMASFGNFANDNEIKVLLKAFIANLKSSSPTIRRTAAGSA

Clinical Applications & Future Prospects

- Predictive genetic testing is part of routine clinical genetic practice, but this should be offered only as part of a careful counseling package.
- Prenatal diagnosis is possible for those couples who find this acceptable.
- One therapeutic approach is based on observation that large CAG repeats result in intracellular accumulation of *huntingtin* aggregates, which are cleaved by a protease (**caspase)** to form a toxic product that causes cell death (apoptosis). Caspase inhibitors showed beneficial effects in HD mouse model.
- Another therapeutic approach under consideration is fetal neuronal cell transfer into regions of the brain, such as the caudate nucleus and putamen, which become atrophic in the early stages of the disease.

Myotonic Dystrophy

- Myotonic dystrophy (MD) is the most common form of muscular dystrophy seen in adults, with an overall incidence of approximately 1 : 8000.
- It shares many features in common with HD—both show **autosomal dominant inheritance**. However, in MD the early-onset form is transmitted almost exclusively by the mother and presents at birth, in contrast to juvenile HD, which is generally paternally transmitted with an age of onset in the teens.
- **MD** is a long term genetic disorder that affects muscle function.
- Symptoms include gradually worsening muscle loss and weakness. Muscles often contract and are unable to relax. Other symptoms may include cataracts, intellectual disability and heart conduction problems.

Types of MD

- There are two main types: type-1 (DM1) and Type-2 (DM2).

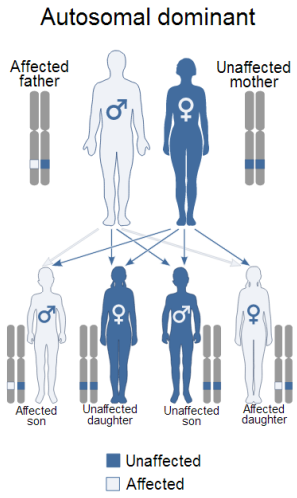
Type-1	Type-2
Caused by mutations in myotonic dystrophy protein kinase (DMPK) gene	Caused by mutations in CNBP (cellular nucleic acid binding protein) gene Also known as ZNF-9 (zinc finger protein-9) gene.
Expansion of CTG triple trinucleotide repeat at 3' un-transcribed region (UTR)	Expansion of CCTG tetranucleotide repeat in Intron 1 of the gene.
May be present at birth (congenital)	Appears later in age
Symptoms are moderate to severe	Symptoms are mild to moderate

Clinical Features of MD

- Symptoms include gradually worsening muscle loss and weakness. Muscles often contract and are unable to relax, which can manifest as a delay in releasing the grip on shaking hands.
- Other clinical features include cataracts, cardiac conduction defects, disturbed gastrointestinal peristalsis (dysphagia, constipation, diarrhea), weak sphincters, increased risk of diabetes mellitus and gallstones, somnolence, frontal balding, and testicular atrophy.
- The age of onset is very variable and in its mildest form usually runs a relatively benign course. However, as the age of onset becomes earlier, so the clinical symptoms increase in severity and more body systems are involved.
- In 'congenital' form, affected babies present with hypotonia (low muscle strength), talipes (foot deformatoin), and respiratory distress that can prove life threatening. Children who survive tend to show a lack of facial expression (myopathic facies) with delayed motor development as well as learning and intellectual difficulties.

Genetics of MD

- It follows autosomal dominant inheritance.
- There is a chance of 50% children to be affected if one of the parents is normal and other is affected (heterozygous).



Genetics of MD

- Affected individuals have an expansion of at least 50 repeats of the CTG.
- There is a close correlation between disease severity and the size of the expansion (repeats), which can exceed 2000 repeats.
- The severe congenital cases show the largest repeat copy number, with almost invariable inheritance from the mother. However, expansion of a relatively small number of repeats appears to occur more commonly in the male.
- Thus, meiotic or germline instability is greater in the female for alleles containing large sequences.
- One possible explanation is that mature spermatozoa can carry only small expansions, whereas ova can accommodate much larger expansions.

Clinical Applications & Future Prospects

- Presymptomatic genetic testing and prenatal diagnosis can be offered to those families for whom it is appropriate and acceptable.
- This is particularly relevant for couples who have had a child with the severe congenital form, for whom the risk of recurrence is relatively high.
- There is currently no cure for myotonic dystrophy. Therefore, the focus is on managing the complications of the disease.
- Important components of the management of MD include regular surveillance for cardiac conduction defects and the provision of information about risks associated with general anesthesia.
- Pacemaker insertion may be required for individuals with cardiac conduction abnormalities.
- Complications relating to the cardiopulmonary system account for 70% of deaths due to DM1.

Cystic Fibrosis

- CF is one of the most common **autosomal recessive** disorders encountered in individuals of western European origin (1 in 2000 to 1 in 3000).
- The incidence is much lower in African Americans (1 in 15,000) and Asian Americans (1 in 31,000).
- There is no cure for cystic fibrosis.
- Although antibiotics and physiotherapy have been very effective in increasing the average life expectancy of a child with CF from less than 5 years in 1955 to at least 30 years, CF remains a significant cause of chronic ill health and death in childhood and early adult life.

Clinical Features of CF

- The organs most commonly affected in CF are the lungs and the pancreas.
- The main signs are poor growth, accumulation of thick, sticky mucus, frequent chest infections, and coughing or shortness of breath.
- Epithelial cells have a mutated protein that leads to viscous mucus production.
- Chronic lung disease caused by recurrent infection eventually leads to fibrotic changes in lungs with secondary cardiac failure, also known as **cor pulmonale**.
- In 85% of people with CF, pancreatic function is impaired, with reduced enzyme secretion from blockage of the pancreatic ducts by inspissated (thick) secretions. This leads to malabsorption with an increase in fat content of the stools.
- Around 10% of children with CF present in the newborn period with obstruction of the small bowel from thickened meconium, known as **meconium ileus**.
- Almost all males with CF are sterile because of congenital bilateral absence of the vas deferens (CBAVD)

Genetics of CF

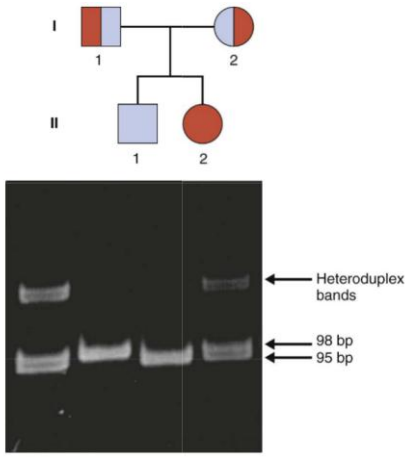
- CF shows autosomal recessive inheritance.
- CF is caused by a mutation in the **Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)** gene.
- CFTR gene is 230 kb long and is located at 7q31.2
- The primary role of the *CFTR* protein is to act as a chloride channel.
- CFTR protein helps to maintain the balance of salt and water on many surfaces including lung.
- When the protein is not working correctly, chloride becomes trapped in cells.
- Without the proper movement of chloride, water cannot hydrate the cellular surface. This leads the mucus to become thick and sticky, causing many of the symptoms associated with cystic fibrosis.

Mutations in CFTR Gene

- The first mutation identified in *CFTR* was a deletion of three adjacent base pairs at the 508th codon which results in the loss of a phenylalanine residue.
- This mutation is known as **Phe508del** and accounts for approximately 70% of all mutations in *CFTR* gene.
- Homozygotes for Phe508del almost always have severe classical CF.

PCR Diagnosis

- PCR amplification of 98- and 95-bp DNA fragments surrounding the Phe508del mutation site in the CFTR gene from a child with cystic fibrosis and her parents.
- The child, II-2, is homozygous for Phe508del.
- Her parents, I-1 and I-2, are heterozygous.
- Her brother, II-1, is homozygous for the normal allele.
- Heterozygotes are identified by the presence of heteroduplex bands formed between 95- and 98-bp products and electrophoresed on a non-denaturing gel.



Clinical Applications and Future Prospects

- Population screening for carriers of CF and neonatal screening for CF homozygotes have been widely implemented.
- CF is a prime candidate for gene therapy because of the relative accessibility of the crucial target organs (i.e., the lungs).
- Gene transfer studies carried out using adenoviruses and *CFTR* complementary DNA (cDNA)–liposome complexes have resulted in the restoration of chloride secretion in CF transgenic mice.

Spinal Muscular Atrophy

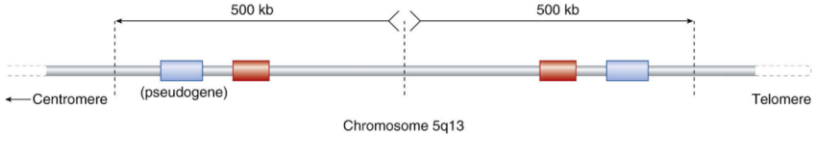
- SMA is the term used to describe a clinically and genetically heterogeneous group of disorders that are among the most common genetic causes of death in childhood.
- The disease is characterized by degeneration of the anterior horn cells of the spinal cord leading to progressive muscle weakness and ultimately death.
- Three common childhood forms of SMA have the incidence of approximately 1 : 10,000 and carrier frequency of about 1 : 50.
- There are 3 types of SMA: Type-1, Type-2 and Type-3.
- SMA type-1 is most common and most severe.

Clinical Features of SMA

	Type-I	Type-II	Type-III
Other name	Werdnig-Hoffmann Disease	-	Kugelberg-Welander Disease
Onset	0-6 months	6-18 months	>18 months
Life expectancy	< 2 years	Early adult	Adult
Grade	Severe	Moderate	Mild
Features	Severe hypotonia (low muscle tone)	Hypotonia and muscle weakness	Slowly progressive muscle weakness
Activity	Lack of spontaneous movements	Can sit unaided but never able to achieve independent locomotion	Able to walk without support but need a wheelchair by early adult life

Genetics of SMA

- All three types of childhood-onset SMA show **autosomal recessive inheritance**.
- The disease is caused by mutations in survival of motor neuron (***SMN1***) gene, located at 5q13 and codes for SMN protein.
- ***SMN1*** is the telomeric copy of the gene; ***SMN2*** (the pseudogene of *SMN1* that shares ~99% homology) is the centromeric copy.
- *SMN1* and *SMN2* are part of a 500 kb inverted duplication.
- *SMN1* shows homozygous deletion of exons 7–8 in 95-98% of patients with SMA.
- The presence of *SMN2* modifies the phenotype, causing milder forms of SMA.
- Mutations in *SMN2* alone do not cause disease.



Duchenne Muscular Dystrophy

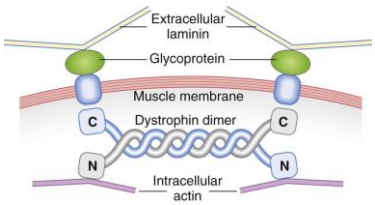
- DMD is the most common and most severe form of muscular dystrophy.
- A similar but milder condition, Becker muscular dystrophy (BMD), is caused by mutations in the same gene.
- The incidences of DMD and BMD are approximately 1 : 3500 males and 1 : 20,000 males, respectively.
- There is no cure for DMD or BMD, although physiotherapy is beneficial for maintaining mobility and preventing muscle spasm and joint contractures.
- Orthopedic appliances (such as braces and wheelchairs) may improve mobility and the ability for self-care.
- Gene therapy offers a realistic hope of a cure in near future.

Clinical Features of DMD

- Males with DMD usually present between the ages of 3 and 5 years with slowly progressive muscle weakness resulting in an awkward gait, inability to run quickly, and difficulty in rising from the floor.
- Most affected boys have to use a wheelchair by the age of 11 years because of severe proximal leg muscle weakness. Subsequent deterioration leads to lumbar lordosis, joint contractures, and cardiorespiratory failure, resulting in death at a mean age of 18 years without aggressive supportive measures.
- In BMD, the clinical picture is very similar but the disease process runs a much less aggressive course. The mean age of onset is 11 years and many patients remain ambulant until adult life. Life expectancy is only slightly reduced.

Genetics of DMD

- Both DMD and BMD show **X-linked recessive inheritance**.
- Females are carriers while males are affected.
- DMD is caused by a mutation of the **dystrophin gene** at locus Xp21.
- Deletions that cause DMD usually disturb the translational reading frame.
- Deletions that cause BMD usually do not alter the reading frame. This means that the amino-acid sequence of the protein product downstream of the deletion is normal, explaining the relatively mild features in BMD.
- The 427-kDa ***dystrophin* protein** is located close to the muscle membrane, where it links intracellular actin with extracellular laminin. Absence of dystrophin in DMD leads gradually to muscle cell degeneration.

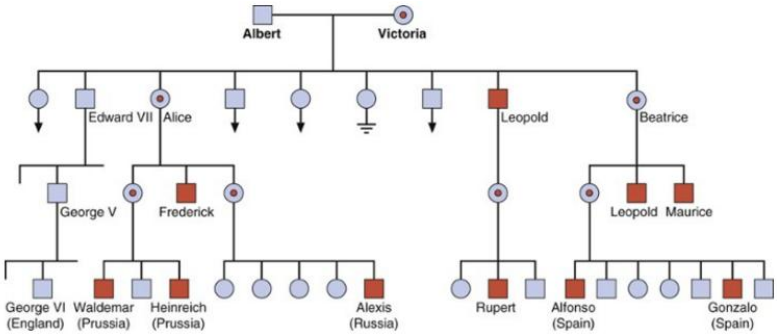


Hemophilia

- It is a bleeding disorder that impairs the body's ability to make blood clots.
- There are two main types of haemophilia: A and B.
- **Hemophilia A** is caused by the deficiency of factor VIII.
- Hemophilia A is also known as classic hemophilia.
- Hemophilia A is more common with an incidence of 1 : 5000 males.
- **Hemophilia B** is caused by the deficiency of factor IX.
- Hemophilia B is also known as Christmas hemophilia.
- Hemophilia B has an incidence of 1 : 40000 males.

Hemophilia in Royal Family

- Pedigree showing the segregation of hemophilia among Queen Victoria's descendants.



Clinical Features

- These are similar in both forms of hemophilia and vary from mild bleeding following major trauma or surgery to spontaneous hemorrhage into muscles and joints.
- The degree of severity shows a close correlation with the reduction in factor VIII or IX activity.
- Individuals with less than 1% active factor are classified as severe haemophilia, those with 1-5% active factor have moderate haemophilia, and those with 5-40% of normal levels as mild haemophilia.
- Hemorrhage into joints causes severe pain and swelling which, if recurrent, causes a progressive arthropathy (arthritis) with severe disability.
- Affected family members generally show the same degree of severity.

Genetics of Hemophilia

- Both forms of hemophilia show **X-linked recessive inheritance**.
- A mother who is a carrier has a 50% chance of passing the faulty X-chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters.
- Mutations in Factor-VIII (F8) gene (Xq28) cause hemophilia A.
- Mutations in Factor-IX (F9) gene (Xq27) cause hemophilia B.
- Deletions in factor-VIII gene account for 5% of all cases and usually cause complete absence of factor VIII expression.
- Inversions account for 50% of all severe cases with <1% factor VIII activity.

Treatment of Hemophilia

- Both forms of hemophilia have been treated successfully for many years using plasma-derived factor VIII or factor IX.
- Factor VIII is concentrated in the cryoprecipitate fraction of plasma and has been used widely for replacement therapy. It has a half-life of 8 h, so repeated infusions are necessary for elective surgery or major trauma.
- Half-life of factor IX is higher (18-34 h).
- Hemophilia A and B are excellent candidates for gene therapy as only a slight increase in the plasma level of the relevant factor is of major clinical benefit.