Molecular Genetics of Neurological Disorders

Said Ismail
Faculty of Medicine
University of Jordan
Outline:

- **Neurodegenerative disorders:**
  - affecting movement
  - memory loss and Dementia
  - Tri-nucleotide repeat disorders:
    (CAG repeat disorders)
  - Infantile
  - Parkinson's disease (PD)
  - Alzheimer
  - Huntington’s Disease (HD)
  - Spinal Muscular Atrophy (SMA)

- **Other disorders:**
  - Epilepsy
  - Schizophrenia
  - Fragile X syndrome (Also a Tri-nucleotide repeat disorder)

- **Future prospective**
  - Diagnosis & Therapy
WHO numbers: March 2007

Hundreds of millions worldwide are affected by neurological disorders:

Examples:

- 50 million: epilepsy
- 62 million: cerebrovascular disease
- 326 million: migraine
- 24 million: Alzheimer disease & other dementias

- 6.8 million people die every year as a result of neurological disorders
Neurodegenerative disorders
Huntington’s Disease (HD):

- Major example of inherited neurological disorders (ND)

- Unlike other common NDs: single genetic cause in all patients (ie single disease mechanism)
  Gene:  Huntingtin

- Symptoms:
  - abnormal body movements called chorea and a lack of coordination
  - Also involves behavioral and intellectual problems

- Age of onset:
  all ages but majority in middle age

- Death typically occurs 15 years after motor symptoms onset
HD Inheritance and Genetics:

- All cases: due to expansion of the CAG tri-nucleotide repeat in the HD gene

- HD gene is found on short arm of chromosome 4

- Normally: 6 – 34 CAG units (>34 → HD)

- In rare HD Heterozygotes, the longer repeat determines the age of onset (the second repeat has no effect):

  HD is **autosomal dominant** (at least in regard to age of onset)
Inheritance of Huntington disease
The extended CAG repeat:

- **CAG repeat:** In coding sequence near 5’ end of HD gene
  - > 34: Inheritance is meiotically **not** stable (specially from father)
  - Instability is greater in spermatogenesis **than** oogenesis
    → Child has longer CAG region than father (earlier onset) called → **Anticipation**

- **Age of Onset:** depend largely on length of CAG repeat
  - (Longer CAG repeat = Younger age of onset)

- **Survival:** NO correlation bet. CAG length & Duration (onset → death)

- **Progression of symptoms:** Little correlation with CAG length

**Conclusion:** Mechanism of disease onset **might** be different from mechanism of disease progression
Correlation of HD CAG-repeat length with age at onset
- CAG repeats: Encodes poly-Glutamine tract beginning 18 aa of the N-terminus

- HD protein: “Huntingtin” large (> 3,100 aa)

  - expressed in both neuronal and non-neuronal tissues

  - expression not limited to neurons affected in HD !!

- Polyglutamine tract: causes misfolding of protein → loss of function

- HD is caused by:
  - Loss of function of Normal protein
  - toxic (bad !) function of mutated protein
Toxic effect of HD polyglutamine tract:

- Still not very clear

- Only the N-terminus of Huntingtin is necessary for HD symptoms

- General hypothesis:
  - HD is a conformational disease in which the pathogenic pathway is triggered in some manner by protein mis-folding and its consequences including formation of aggregate structures
Alzheimer’s disease:

- Most common cause of Dementia (50-60 %)
- First described 100 years ago by Alois Alzheimer,

-Dementia:
  - < 1% in people aged 60-64 years
  - 24 – 33% in people over 85 years

- World wide:  
  - 26.6 million in 2006
  - 81 million in 2040 (due to increased life expectancy)

- Mostly Sporadic……Rarely Familial

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American President: Ronald Regan
1911 - 2004
Genetics of Alzheimer’s:

- “Familial” type:
  - Autosomal dominant disorder
  - Onset before 65 years
  - Rare: prevalence below 0.1%
  - Genes involved:
    - Presenilin 1 (PSEN1) & (PSEN2): most familial cases
    - Amyloid precursor protein (APP): only few cases
“Sporadic” type:

- Linked to genetic risk factors

- Best known genetic risk factor: Apolipoprotein E (APOE) ε4 allele

- 80% of cases has at least one apoE4 allele

- APOE: is a cholesterol transporter in Brain
  (APOE4: low efficiency variant in reuse of membrane lipids and repair)

- Hetero APOE ε4: 3 fold increase in risk
- Homo APOE ε4: 15 fold increase in risk

- APOE ε4 allele affects mainly the age of onset:
  - each allele copy lower age of onset by 10 years

- NO other gene has been shown to have similar significance
  (Yet: several other genes + environment are implicated)
### Genetic Causes of Alzheimer's Disease

<table>
<thead>
<tr>
<th>GENE</th>
<th>CHROMOSOME</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenilin-1</td>
<td>14</td>
<td>S182</td>
</tr>
<tr>
<td>Presenilin-2</td>
<td>1</td>
<td>STM2</td>
</tr>
<tr>
<td>Amyloid Precursor Protein</td>
<td>21</td>
<td>APP</td>
</tr>
<tr>
<td>ApoLipoprotein e</td>
<td>19</td>
<td>APOE</td>
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Parkinson’s Disease (PD):

- The second most common adult onset neurological condition
- Affects over 1% of people over 65 years
- Main symptoms: bradykinisia, tremor, and rigidity

**Sporadic PD:** Toxins + genetics

- 15% of patients have family history:
  - example genes: LRRK2, PARK2, & 7, PINK1, SNCA

- Toxins acting via inhibition of mitochondrial respiratory chain leading to increase in oxidative stress, impairing dopamine-producing neurons
celebrity patients: Michael J. Fox and Muhammad Ali
**Familial PD:**

- Rare (and not all gene mutation effects in **understood**)
- Different gene mutations → different inheritance patterns

- **DJ-1:**
  - autosomal recessive
  - Normal DJ-1 appears to provide neuro-protection against oxidative stress caused by mitochondrial toxins

- **PINK1**
  - PTEN Induced Kinase 1
  - autosomal recessive

- **PARK2 and PARK7:**
  - autosomal recessive

- **LRRK2 and α-synuclein:**
  - involved in juvenile and early-onset PD
  - **LRRK2**: autosomal dominant
  - **α-synuclein**: autosomal dominant
Autosomal recessive inheritance where both parents carry the faulty parkin gene.
Autosomal **dominant** inheritance when one parent either has PD or has the faulty \(\alpha\)-synuclein gene copy.
Gene: Glutamic Acid Decarboxylase (GAD)

- GAD: increases production of neurotransmitter GABA which quiets neurons in subthalamic nucleus (similar effect to electrical deep brain stimulation DBS)

- Injection site: subthalamic nucleus

Vector: Adeno-Associated Virus (AAV2).
Surgeon drilling a hole in the skull of a patient with Parkinson’s disease
### Spinal muscular atrophy (SMA)

| Incidence: | 1:10,000 | **Most** common genetic cause of **infant death** |
| gene frequency: | 1:100 | (i.e.: **1 in 50** is a carrier) |

**Pathology:** Death of neuronal cells in the anterior horn of spinal cord followed by general muscle wasting (atrophy)

**Inheritance:** Autosomal Recessive  *(Can appear de novo (Not hereditary) in 2-4%)*

**Gene:** Survival of Motor Neuron (**SMN1**)

**Mutations:**
- **95 %:** exon 7 deletion in **both** SMN1 alleles
- **5 %:** **One** allele has exon 7 deletion
  One allele has point mutation  *(65 SNPs identified)*

**SMN Protein:** Found in high levels in spinal cord  
Important for maintenance of motor neurons
Autosomal recessive inheritance of SMA
**Severity:**
- **Variable** depending on similar gene called SMN2
- **SMN2** has a SNP (840 C→T) causing alternative splicing of exon7 giving a truncated unstable protein

<table>
<thead>
<tr>
<th>Type</th>
<th>Age</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0–6 months</td>
<td>Severe form, manifests in first months of life, usually with quick unexpected onset. Pneumonia-induced respiratory failure is the most frequent cause of death. Babies do not generally live past two years of age, with death occurring as early as within weeks in the most severe cases. With proper respiratory support, milder SMA type I phenotypes are known to live well into adulthood.</td>
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<tr>
<td>II</td>
<td>6–18 months</td>
<td>Children are never able to stand and walk but able to maintain a sitting position at least some time in their life. Onset of weakness is noticed between 6 -18 months. Progress vary greatly, some gradually grow weaker over time while others through careful maintenance avoid progression. Body muscles are weakened, and respiratory system is a major concern. Life expectancy is somewhat reduced but most SMA II patients live well into adulthood</td>
</tr>
<tr>
<td>III</td>
<td>&gt;18 months</td>
<td>Usually manifests after 18 months of age and describes patients who are able to walk without support at some time. Life expectancy is normal or near normal</td>
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<tr>
<td>IV</td>
<td>&gt;35 years</td>
<td>Usually manifests after 35 years of age with gradual weakening of muscles. The disease progress mainly affects proximal muscles of the extremities, frequently rendering the patient wheelchair-bound. Life expectancy is normal</td>
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**Explanation:**

- **Two SMN genes** (chromosome 5):

  - **SMN1**: functional protein
  - **SMN2**: 80-90% **Non functional** (exon 7 splicing defect)
    - 10-20% functional protein

- **SMA patients**:
  - SMN1 is mutated
  - one copy of SMN2 is **not** enough
  (many have 2-4 copies: better survival of neurons)

- **So, severity depends on**:
  - performance of SMN2 genes (splicing variants %)
  - No. of SMN2 gene copies
Splicing of SMN1 and SMN2 mRNAs

Unstable truncated protein

Functional protein
- SMA I: 1-2 SMN2 copies
- SMA II, III: ≥ 3 SMN2 copies
- SMA IV: ≥ 4 SMN2 copies
Other Neurological disorders
Epilepsy:

- 40% of cases are associated with genetic factors

- Genetic Epilepsies:

  1. **Mendelian** disorders:  
     single major locus account for segregation of disease

  2. **Non-Mendelian** (complex) diseases:  
     several gene loci implicated in addition to environmental factors  
     or by maternal inheritance pattern of mitochondrial DNA

  3. **Chromosomal** disorder:  
     Gross cytogenetic abnormality is present
**Mendelian:**

- Over 200 Mendelian diseases include epilepsy in their phenotype
- BUT, all are rare (1% of all patients)
- Channelo-pathies: arise from mutation in both voltage-gated and ligand-gated ion ion channels

**complex:**

- Most common familial type of epilepsy

**Chromosomal:**

- Trisomy 21 (Down Syndrome)
- Trisomy 12p
Schizophrenia:

- Chronic & severe mental illness with delusions & hallucinations, apathy & social withdrawal along with specific cognitive failures

**Strong Genetic influence:** Heritability of 65-80%

- mode of **inheritance**: complex: SNPs + environment

  Multigene: non-Mendelian

- Last 5 years: extensive **GWAS** (SNP profiling): dozens of gene associations:

  **Big names**
  - Dysbindin (DTNBP1): Controls Synaptic Homeostasis
  - Neuregulin: Neuronal growth factor
  - DISC1 “Disrupted In SCizphrenia” Neuronal Structural development

  **Other genes**
  - MHC (HLA):
  - NOTCH4: involved in neurodevelopment
  - SOX11: neuronal differentiation

  **More…**
  - ZNF804A, MYO18B/ADRBK2, AGAP1 (CENTG2), NTRK3, EML5, ERBB4, NRGN, TCF4, CCDC60, RBP1, PTPN21, CMYA5, PLAA, ACSM1, ANK3, SULT6B1, ASTN1, CNTNAP1 and GABBR1. ASTN2, OPCML, PSD3, RYR3, TMCC2, GRID1, A2BP1, CACNA1C, CNTN5, CRYBB1, EML5, CSMD1, FAM69A, LRP8, PTPRG1, SLIT3, TMEM17 and VGCNL1/NALCN
Genetic association of Schizophrenia

Gottesman, 1991
Fragile X syndrome:

- Most common known single-gene cause of **autism** (or autism like disorders)
- Most common inherited cause of intellectual disability
- Inherited in an X-linked dominant pattern

- **Males** are most affected: (1 in 4,000-9,000)  (1X)
- **Females** are less vulnerable: (1 in 7,000-15,000)  (2X)

- Physical characteristics: elongated face, large ears
- Behavioral characteristics: stereotypic movements (e.g. hand-flapping)
‘Sindrome X Fragil’
- DNA level:

- Expansion of CGG repeat
  - Affects fragile X mental retardation 1 (FMR1) gene on X chrom.
  - FMR protein (FMRP): required for normal neural development

- Depending on length of CGG repeat:
  - normal allele: unaffected (5-40)
  - pre-mutation: Risk of fragile X related disorders (50-200)
  - full mutation: Affected by (>200)

- Diagnosis:

- genetic testing of the no. of CGG repeats.
  - Testing for pre-mutation carriers: Good for genetic counseling
Chromosomal Analysis

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Pre-mutation: expansion is small

Full mutation: expansion is large and accompanied by abnormal methyllation, leading to low or NO production or absence of FMRP
Future prospective
Main Expected Developments:

1. Discovering new genes/mutations:
   Using new DNA technologies such as:
   - Genome-wide association studies (GWAS):
     “SNP profiling” of controls vs patients: “SNP Microarrays”

2. New Therapeutic approaches:
   - Gene Therapy: only for single gene disorders
   - Stem cell Therapy: will have a major impact!
Genome-wide association studies (GWAS)
Genome-wide association studies (GWAS)
Stem cell research:

- Promising animal model and human experiments

- Which stem cell:
  - Embryonic: Ethics..!!!!
  - Adult: Cord, BM, Neural
  - iPS: Vector ?? Quality ??

- Example Target Disorders:

  - Neurodegenerative disorders:
    - Parkinson’s & Huntington’s: localized degeneration: easier cell therapy
    - Alzheimer: affected neurons are less defined

  - Spinal cord injuries:

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THANK YOU