Neurogenetics – Mechanisms of Inheritance and Disease

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Objectives

1) To recognize the inheritance patterns which underlie common neurogenetic disorders
2) To develop an appreciation of the diverse molecular mechanisms which cause neurogenetic disorders
Disclosure

• I have no conflicts of interest to disclose
Overview – Mechanisms of Inheritance

• Mendelian inheritance (AD, AR, XL)
• Modifiers of Mendelian inheritance - penetrance, expressivity, mosaicism and X-inactivation
• (Mitochondrial inheritance)
Overview – Mechanisms of disease

- (Metabolic diseases)
- (Mitochondrial diseases)
- Epigenetics
- Microdeletions/duplications
- Nucleotide repeat diseases
Mendelian inheritance - AD

• Usually affects every generation
• Each child of an affected parent has a 50% risk of inheriting the trait
• Unaffected individuals do not pass on the trait to their children
• Males and females are equally likely to pass on the trait to children of either gender
• Isolated cases are usually due to new mutations
Autosomal Dominant Inheritance

(One Parent Affected)

Affected Father

Affected Son (25%)
Normal Daughter (25%)
Affected Daughter (25%)
Normal Son (25%)

Affected Mother

D d

d d

D d

d d
Neurofibromatosis 1

- von Recklinghausen’s disease or “peripheral NF”
- (1) Multiple tumors of CNS, PNS
- (2) Altered skin pigmentation
- (3) Vascular and visceral lesions
- Variants: Familial spinal NF, segmental NF
NF-1 Genetics

- 1:3000 live births
- AD with variable expression
- Complete penetrance by 5 years
- ~50% sporadic
- Mutation rate ~1:10,000 gametes/generation
- Chromosome 17 q11.2
Neurofibromin

NF-1 gene

GTPase AP

p21ras

Astrocyte growth regulation

p21ras (i)
NF-1 Genetics

- > 1000 mutations
- deletions, frame shifts, point, stop
- net effect: truncated, nonfunctional neurofibromin
- complex expression and post-transcriptional modifications not yet understood
- poor clinical correlation
- mosaicism common
NF-1 Pathology = Tumors

- Neurofibromas > schwannomas
- Most are benign but may transform
- **Plexiform neurofibromas** --> neurofibrosarcoma (5%)
- PNS most common - ulnar, radial
- CNS: optic gliomas, pilocytic astrocytoma
- Other: leukemia, Wilm’s, neuroblastoma, pheochromocytomas
- “MEN syndrome” with café au lait
NF-1 Clinical Features - Skin

- Café au lait - (melanoblast hyperplasia)
- Freckling - diffuse and axillary/inguinal
- Melanoderma
- Pedunculated molluscum fibrosum
- Subcutaneous neurofibromas
NF-1 Clinical Features - CNS

- Cognitive - 60% normal, “UBO’s”
- IC tumors - optic glioma
- PN tumors - incl. ANS
- IS tumors - older, familial
- CVA’s - ICA occlusion
- seizures - <10%
- precocious puberty
- 50% have no CNS manifestations

NF1 MRI showing bilateral optic gliomas (A), basal ganglia UBO’s (B), and Sphenoid changes (C)
NF-1 Clinical Features - Other

- Short stature (1/3) - many are GH deficient
- Lisch nodules - iris hamartomas
- Kyphoscoliosis
- Tibial pseudoarthrosis
- Vertebral scalloping
- Hypertension - renovascular, pheochromocytoma (1-4%)
- Hydrocephalus
NIH diagnostic criteria (2/7)

• (1) Café au lait (6), > 5 or 15 mm
• (2) Optic glioma
• (3) Lisch nodules - 2 or more
• (4) Osseous lesion(s)
• (5) Relative - 1st degree with dx
• (6) Neurofibroma (2) or plex. neurofibroma
• (7) Freckling - inguinal or axillary
Figure 9: Timeline for detection of signs and complications of NF1

- **Congenital (0-2 yrs)**
  - Cafe au lait spots
  - Flexiform neurofibromas
    - Diffuse
    - Superficial or nodular
  - Tibial dysplasia
  - Skinfold freckling

- **Preschool years (2-6 yrs)**

- **Late childhood & adolescence (6-16 yrs)**
  - Optic pathway tumors
  - Learning disabilities
  - Hypertension
  - Headaches
  - Dermal neurofibromas

- **Adulthood (16 yrs +)**
  - Scoliosis
  - Malignant peripheral nerve sheath tumors
Mendelian inheritance - AR

- Typically seen in the sibship of the proband – other relatives not usually affected
- Males and females are equally affected
- Parents of an affected child are obligate carriers for the trait
- Increased incidence in consanguineous families
- Recurrence risk for each pregnancy is 25%
- Unaffected sibs have a 66% risk of being carriers
AR
Autosomal Recessive Inheritance
(Both Parents Carriers)

Carrier Father
    Rr

Carrier Mother
    Rr

RR
Rr
Rr
rr

Normal (25%)
Carrier (50%)
Affected (25%)
Genetic Testing

- *C5orf42* (now called *CPLANE1*) → compound heterozygous for c.3626A>C (Q1209P) and c.424G>A (E142K) pathogenic variants
Mutations in C5orf42 Cause Joubert Syndrome in the French Canadian Population

Myriam Sroud,1,11 Jeremy Schwartzentruber,2,11 Fadi F. Hamdan,1 Luis H. Ospina,3 Lysanne Patry,1 Damian Labuda,4 Christine Massicotte,4 Sylvia Dobrzenecka,1 José-Mario Capo-Chichi,1 Simon Papillon-Cavanagh,4 Mark E. Samuels,4 Kym M. Boycott,5 Michael I. Shevell,6 Rachel Laframboise,7 Valérie Désilets,4 FORGE Canada Consortium,12 Bruno Maranda,8 Guy A. Rouleau,9 Jacek Majewski,10 and Jacques L. Michaud1,9

Initial report: AJHG 2012

Original Investigation

C5orf42 is the major gene responsible for OFD syndrome type VI

Estelle Lopez · Christel Thauvin-Robinet · Bruno Reversade · Nadia El Khartoufi · Louise Devisme · Muriel Holder · Iléaène Ansart-Franquet · Magali Avila · Didier Lacombe · Pascale Kleinfinger · Irahara Kaori · Jun-Ichi Takanashi · Martine Le Merrer · Jelena Martinovic · Catherine Noël · Mohammad Saboul · Lena Ho · Yeliz Güven · Ferecét Razavi · Lydie Burglen · Nadège Gigot · Véronique Darmency-Stamboul · Julien Thevenon · Bernard Arat · Hülya Kayserili · Frédéric Huet · Stanislas Lyonnet · Cédric Le Caignec · Brunella Franco · Jean-Baptiste Rivière · Laurence Faivre · Tania Attié-Bitach

Hum Genet (2014) 133:367-377
DOI 10.1007/s00439-013-1385-1
Joubert syndrome

- Prevalence: 1/80,000 → may be underestimate
- Relatively high prevalence in French-Canadian and Ashkenazi Jewish populations
- 50% of individuals with JS have mutations identified in one of the clinically available genes
- To date pathogenic variants in 34 genes are known to cause JS; 33 of these are AR and one is X-linked
Joubert Syndrome - clinical

• Neurological features:
  – Hypotonia
  – Ataxia
  – Developmental delay, intellectual disability
  – altered respiratory pattern in neonatal period
  – ocular motor apraxia
  – MRI - “molar tooth sign”
Joubert Syndrome - clinical

- Retinal dystrophy (30%)
- Renal disease (25%)
- Ocular coloboma (19%)
- Occipital encephalocele
- Hepatic fibrosis (15%)
- Polydactyly (20%)
- Oral hamartoma
- Others
Joubert syndrome
(Romani et al, Lancet Neurology 2013)
Mendelian inheritance - XL

- The incidence of the trait is usually much higher in males than females
- Carrier females are usually unaffected but may express the trait with variable severity
- All daughters of an affected man are obligate carriers
- Male to male transmission is never seen
- Affected males in a family are related through females
Pedigree 8. X-linked recessive inheritance.
X-Linked Recessive Inheritance
(Carrier Mother)

Father

Carrier Mother

X Y

X X^r

X Y

X X^r

X Y

Normal Daughter (25%)
Normal Son (25%)
Carrier Daughter (25%)
Affected Son (25%)
Duchenne Muscular Dystrophy

• Described by Guillaume Benjamin Amand Duchenne in 1868
  – ‘pseudohypertrophic muscular paralysis’
• Gene discovery by Louis Kunkel in 1986
• Dystrophin gene locus is Xp21.2-p21.1
• XL condition affecting 1/3,500 – 6,000 males
• Life-limiting neuromuscular disorder
Dystrophin gene has 79 exons

Genes are made of exons and introns

Exons contain instructions to make protein

Every 3 letters code for an amino acid

Dystrophin Protein
Dosage Mutations

- **Normal**
  - 1
  - 2
  - 3

- **Deleted**
  - 1
  - 3

- **Duplicated**
  - 1
  - 2
  - 2
  - 3
Mutation Types

http://emedicine.medscape.com/article/1173204-overview
The Protein: Dystrophin

- 4 domains
- Expressed in skeletal, cardiac, and smooth muscles
  - brain to a lesser extent
- Critical role in maintenance of muscle integrity (mechanical link between muscle fibers and basement membrane)
- Functions as a membrane stabilizer during contraction or transducer of signals from ECM to muscle cytoplasm

Dystrophin protein: 3,685 amino acids
Dysytophinopathy Spectrum

- Elevated creatine phosphokinase (CK)
  - Increased serum CK + muscle cramps + quadricep myopathy

- Dilated Cardiomyopathy (DCM)
  - Heart is primarily affected

- Becker Muscular Dystrophy (BMD) & Duchenne Muscular Dystrophy
  - Progressive symmetrical muscle weakness (proximal greater > distal), often with calf hypertrophy

Elevated CK  DCM  BMD  DMD
Clinical Features of DMD

Early childhood signs of DMD

• Delayed milestones: motor, speech, learning
• Progressive muscle weakness causes waddling gait, difficulty climbing, Gower sign
• Calf hypertrophy
• Elevated CK

Childhood

• Wheelchair assistance needed before age 13
• Cognitive impairment

Late childhood/early adolescence

• Heart – Cardiomyopathy, arrhythmia
• Respiratory complications
# BMD versus DMD

<table>
<thead>
<tr>
<th></th>
<th>Becker Muscular Dystrophy (BMD)</th>
<th>Duchenne Muscular Dystrophy (DMD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>1/3,500</td>
<td>1/35,000</td>
</tr>
<tr>
<td>Onset</td>
<td>&gt;8y</td>
<td>&lt;5y</td>
</tr>
<tr>
<td>Wheelchair</td>
<td>&gt;16y</td>
<td>&lt;13y</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>~100% by 18y</td>
<td></td>
</tr>
<tr>
<td>Cognitive</td>
<td>Not common</td>
<td>working memory &amp; executive function</td>
</tr>
<tr>
<td>Death</td>
<td>Mid 40s (cardiomyopathy)</td>
<td>30s (respiratory/cardio-myo-pathy)</td>
</tr>
<tr>
<td>Other</td>
<td>Preservation of neck flexor muscle strength</td>
<td></td>
</tr>
</tbody>
</table>
Females

• Spectrum of phenotypes:
  – Muscle weakness (75-80%)
  – Myalgia/Cramps (5%)
  – LV dilation (15-20%)
  – DCM (0-10%)
Female Carriers

• 19% DMD and 14% BMD female carriers will present with symptoms in their life (Hoogerwaard 1999)

• Average age of presentation 33 years, mild symptoms: myalgias, muscle weakness and mildly elevated CK

• Case reports of symptomatic females in the pediatric age range are rare
<table>
<thead>
<tr>
<th>Female Carriers</th>
<th>BMD families</th>
<th>DMD families</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>81%</td>
<td>76%</td>
</tr>
<tr>
<td>Muscle Weakness</td>
<td>14%</td>
<td>19%</td>
</tr>
<tr>
<td>Myalgia/cramps</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Heart - left ventricle dilation</td>
<td>16%</td>
<td>19%</td>
</tr>
<tr>
<td>Heart - dilated cardiomyopathy</td>
<td>0%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Source: Hoogerwaard et al. (1999)
Modifiers of Mendelian inheritance

- **Penetrance** – proportion of individuals with a disease-causing genotype who have signs/symptoms of the disease
- **Expressivity** – degree of severity of disease in individuals with a disease-causing genotype
- **Mosaicism** – arises when an individual/tissue has at least 2 genotypically different cell lines
- **X-inactivation** – random inactivation of genes on one X chromosome in female somatic cells
Mechanisms of disease

- (Metabolic diseases)
- (Mitochondrial diseases)
- Microdeletions/duplications
- Single nucleotide variants
- Epigenetics
- Nucleotide repeat diseases
Epigenetics

• Abnormal phenotype without change in genotype

• Imprinted genes are expressed only from either the maternal or paternal chromosome
  • majority of genes - inherit 1 copy from each parent
  • function only when inherited from either mother/father
  • imprinted in the male or female germline - retain molecular memory of parental origin
Imprinting

- **Mechanisms**
  - methylation of cytosine residues
  - chromatin structure
  - timing of DNA replication

- **Mutations**
  - deletions, uniparental disomy, imprinting centre
  - abN, specific gene mutations, rearrangements
  - mutation may have no obvious effect
  - (if inherited on the usually silenced chromosome)
Angelman Syndrome (AS)

- First reported in early 1980s
- Prevalence of 1/12,000-20,000
- Characterized by:
  - MR
  - severe speech impairment
  - gait ataxia, tremulousness
  - outbursts of laughter
  - characteristic facies
- age at diagnosis 3-7 years
- life span appears to be normal
AS - Neuro findings

• More than 80% of patients
  • delayed attainment of developmental milestones without loss of skills
  • delayed or disproportionately slow growth in HC, absolute or relative microcephaly by age 2
  • MRI usually normal
  • seizures, usual onset >3 years of age
  • abnormal EEG - characteristic pattern with large-amplitude slow-spike waves
AS clinical features

**Development**
- Developmental delay*
  - Speech
  - Motor Skills
  - Cognition
- Intellectual Disability

**Behavior**
- Happy/social demeanor*
- Reduced Sleep
- Characteristic behaviors*

**Physical**
- Microcephaly**
- Scoliosis
- Constipation, reflux
- Strabismus

**Neurological**
- Movement and Balance*
- Seizures**
- EEG changes**
- Feeding/swallowing concerns, Drooling

* Consistent in all children
** Seen in >80% children
AS Genetics

- AS results from absent or nonfunctioning maternal allele at 15q11-q15
- Genetic events leading to perturbation of maternal imprint
  - deletions
  - UPD
  - $UBE3A$ mutation
  - IC defects
  - Other (11%)
Deletions of 15q11-q13

- maternal deletions of 15q11-q13 accounts for 70-75% of cases
- deletion 3-5 Mb in size
- repeated copies of a large nonfunctional ancestral gene map to both ends of this region - unequal recombination
- Fluorescent in situ hybridization (FISH)
- Microarray
Green signal – control probe (15q31); red signal – probe containing loci including SNRPN gene (15q11.23 region)
Uniparental disomy

- arises when an individual inherits two copies of a chromosome pair from one parent and no copy from the other parent.
- Paternal UPD of chromosome 15 accounts for 2-5% of AS cases
SNRPN methylation analysis by southern blot

Restriction digestion of genomic DNA with XbaI and the methylation sensitive NotI is followed by DNA blot hybridization.

Maternal

- XbaI
- 4.2 kb
- NotI
- Methylation sensitive

Paternal

- XbaI
- 0.9 kb

Normal PWS AS

- 4.2 kb
- 0.9 kb
UBE3A mutations

- 11% of AS patients have an identifiable UBE3A mutation
- encodes the E6AP-3A ubiquitin protein ligase
- 2.7 kb transcript, 865 aa, spans 120 kb
- UBE3A transfers activated ubiquitin molecules to proteins targeted for degradation via the 26S proteosome
- imprinted in certain regions of the brain
- stage-specific developmental role in fetal brain development
Imprinting centre defects

- defects in the mechanism(s) involved in the imprinting process during gametogenesis

- imprinting centre defects can change DNA methylation and transcription
AS Diagnosis

- Different tests detect different causes, no test can detect all causes of AS
  - DNA methylation analysis
  - FISH
  - High resolution chromosome analysis
  - UBE3A and IC mutation analysis
(Tri)nucleotide repeat disorders

- All cause neurological disease
- Most are autosomal dominant BUT exceptions eg. Friedreich ataxia and Fragile X
- CAG, CGG, CTG, GAA, GCG
Repeat Disorders

- Inverse correlation between repeat length and age of onset
- Repeat instability – germline and somatic
- Repeat length and composition, parent of origin influence stability
- Anticipation – earlier age of onset and more severe phenotype with each successive generation
- Intermediate repeat lengths
CAG (polyQ) repeat disorders

- SCA 1, 2, 3, 6, 7, 17
- DRPLA
- SBMA
- HD
Huntington Disease

• progressive disorder
• prevalence 3-7 per 100,000
• disturbances in both involuntary and voluntary movement
• cognitive decline and changes in personality, affective disorders, schizophrenic psychosis
• positive family history consistent with AD inheritance
HD - clinical

- mean age of onset 35-44 years
- median survival time 15-18 years
- average at death 54-55 years
- characteristic atrophy of caudate and putamen on CT/MRI
- decreased uptake and metabolism of glucose in the caudate nucleus
HD - early stage

- subtle changes in coordination
- minor involuntary movements
- difficulty with planning/other executive functions
- depressed/irritable mood
- oculomotor disturbances
HD - middle stage

- chorea becomes more prominent
- increasing difficulty with voluntary activity
- worsening dysarthria and dysphagia
- intermittent outbursts of aggressive behaviour and social disinhibition
- difficulties with fine motor control and gait
HD - late stage

- motor disability becomes severe
- patients often dependent for all ADLS
- mute and incontinent
- cachexia becomes prominent
- other manifestations include bradykinesia, rigidity, dystonia, and hyperreflexia
HD - Genetics

- **IT15/HD** gene on 4p16, 67 exons, 200 kb, codes for Huntingtin (348 kDa)
- polyGlu tract starts at residue 18 and is followed by a polyPro region
- disease caused by expansion of CAG/polyGlu tract in the **HD** gene
- expansion by anticipation
- normal range of CAG repeats is 10-26
- disease range of CAG repeats is 36 to >100
HD – Genetics

- alleles of 27-35 repeats are considered intermediate
- allele has not been shown to expand into the disease range when transmitted by mother
- risk of expansion into the disease range is 2.5% when transmitted paternally
- alleles of 36-41 repeats are associated with reduced penetrance for symptomatic HD
HD Diagnosis

Disease-causing

Normal
CAG pathogenesis

- Expanded polyglutamine (polyQ) tracts
- Misfolded proteins
- Unfolded protein response (UPR)
- Insoluble protein aggregates – inclusions
- Ubiquitin-dependent proteasome system (UPS)
- Ca homeostasis
- Mitochondrial stress and apoptosis
Teaching points

1) Neurogenetic disorders may be inherited via AD, AR, XL or maternal (mitochondrial) modes of inheritance

2) A vast array of mechanisms contribute to the pathogenesis of neurogenetic disorders