Inherited Conditions

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Disclosure(s)

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The individual below has disclosed the following financial relationship(s) with commercial interest(s):

Elaine Lyon, PhD, FACMG

- Complete Genomics (advisory board)
- Asuragen (reagents/speaking)
- Life Technologies
- Invitae
Hemoglobinopathies and Thalassemias

- Hemoglobin: tetrameric protein composed of four globin polypeptides and four heme groups
- Abnormalities are qualitative or quantitative
- Predominantly Hemoglobin A: $\alpha_2\beta_2$
- Minor Form Hemoglobin A2: $\alpha_2\delta_2$
- Fetal Hemoglobin F: $\alpha_2\gamma_2$
Hb Disorders

• >5% of the world population carries an inherited hemoglobin disorder
  – 1 in 20 adults are carriers for a hemoglobin condition
• Most common human monogenic disorders
• Found most frequently (carrier rates) in
  • Mediterranean (3-30%)
  • Africa ~30%
  • Western/Southeast Asia (5-40%)
Two types of Hb mutations

Thalassemias

*Reduced synthesis* of structurally normal globin subunits resulting in *unbalanced synthesis* of beta and alpha globin chains

Hemoglobinopathies

Mutations in the globin genes resulting in the synthesis of *structurally abnormal* globin subunits
**α-Thalassemia: Genetics**

- **Alpha gene cluster** – Chr16 p13.3
  - ζ, ψζ, ψα2, ψα1, α2, α1, θ
  - Region covers >100K nucleotides
  - Combine with β gene products (ε, γ, β, δ)

- **Hemoglobin**
  - Adult: α2β2(HbA); α2δ2(HbA2)
  - Fetus: α2γ2(HbF)
  - Embryo:
    - ζ2ε2(Hb Gower); ζ2γ2(Hb Portland); α2ε2(Hb Gower 2)
\( \alpha \)-Thalassemia: Genetics

- Result from the **loss of \( \alpha \** gene expression
  - Deletions = 95%
    - 29 known deletions
  - Mutations (control regions, FS, NS, SPL)
    - 105 small deletions/mutations
## α-Thalassemia: Pathology

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα</td>
<td>Normal</td>
<td>4</td>
</tr>
<tr>
<td>α−/αα, α^T^α/αα</td>
<td>Silent Carrier</td>
<td>3</td>
</tr>
<tr>
<td>−α/−α, −−/αα</td>
<td>Mildly Affected</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mild anemia, reduced cell hemoglobin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−−/αα = Alpha zero trait</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−α/−α = Alpha plus trait</td>
<td></td>
</tr>
<tr>
<td>−−/−α</td>
<td>HbH disease</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Severe anemia requiring transfusions</td>
<td></td>
</tr>
<tr>
<td>−−/−−</td>
<td>Hb Barts</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hydrops fetalis</td>
<td></td>
</tr>
</tbody>
</table>
α-Thalassemia: Multiplex PCR Assay
α-Thalassemia: Point Mutations

• About 5% of alpha thalassemia is due to point mutations
• Often cause more severe thalassemia than gene deletions
• Hemoglobin Constant Spring -- most common point mutation
  – Stop codon mutated
  – results in large alpha globin protein
  – RNA unstable and little protein is made
# β-Thalassemia: Pathology

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β/β</strong> = Normal</td>
<td></td>
</tr>
<tr>
<td><strong>β\text{m}/β</strong> = Silent Carrier</td>
<td>Mild anemia, reduced cell hemoglobin, β Thalassemia minor or beta thalassemia trait</td>
</tr>
<tr>
<td><strong>β\text{m}/β\text{m}</strong> = Affected</td>
<td>Severe anemia requiring transfusions, Asymptomatic at birth, ~6mo for symptom</td>
</tr>
</tbody>
</table>

\[ β^0 = \text{No synthesis from affected allele} \]
\[ β^+ = \text{Reduced synthesis from affected allele} \]
β-Thalassemia: Genetics

• Beta gene cluster – Chr 11
  – ε, γA, γG, δ, β
  – Combine with α gene product

(b) Human β-globin gene cluster (chromosome 11)

• Hemoglobin
  – Adult: α2β2(HbA); α2δ2(HbA2)
  – Fetus: α2γ2(HbF)—no beta present
  – Embryo:
    \[ \zeta_2\varepsilon_2(\text{Hb Gower}); \zeta_2\gamma_2(\text{Hb Portland}); \alpha_2\varepsilon_2(\text{Hb Gower 2}) \]
β-Thalassemia: Genetics

- Result from the **loss of β** gene expression
  - Mutations = 99% (control regions, FS, NS, SPL)
    - >200 known mutations
  - Deletions = very rare

- Mutations classified into
  - No synthesis, $\beta^0$
  - Reduced synthesis, $\beta^+$
Hemoglobinopathies

- Mutations in the $\beta$ globin genes resulting in the synthesis of structurally abnormal $\beta$ globin subunits
- Missense mutations that change Hb function
- Autosomal recessive
- Can often be identified by Hb HPLC
- Hemoglobin variants
  - Were initially differentiated by their differing electrophoretic mobility
  - Variants may also produce changes in solubility and oxygen affinity
  - Can give rise to certain clinical manifestations
Hemoglobinopathies: S, C, E

• Most common mutations
• HbS (Glu6Val): Sickle cell anemia
• Hb C (Glu6 Lys): Significant in compound C/S
• Hb E (Glu26Lys): homozygous displays mild anemia
• Genotyping tests
  – Sequencing
  – Targeted mutation detection
HbS Mutation

- Beta globin gene, c.20A>T, Glu6Val
- Originated in Sub-Saharan Africa, the Middle East, and India
- Carried by ~10% of African Americans
- HbS/HbS = Sickle Cell Anemia
  - 1 in 500 African Americans
  - prone to aggregation, erythrocyte sickling, episodic pain crises
- HbS/other beta globin mutation
  - Sickle Cell Disease
  - Compound Heterozygote
HbC Mutation

- Beta globin gene, c.19G>A, p.Glu6Lys
- Originated in Western Africa
- Carried by 2-3% of African Americans
- HbC/HbC = mild hemolytic anemia, occasional joint pain
- HbS/HbC = sickle cell disease
HbE Mutation

- Beta globin gene, c.79G>A, p.Glu26Lys
- Originated in Southeast Asia
- Leads to decreased $\beta$ globin chain production
- HbE/HbE = mild hemolytic anemia
- HbS/HbE = milder form of sickle cell disease
- HbE/$\beta$ Thalassemia
  - Less than usual amount of hemoglobin
  - Can be life threatening: Severe anemia
Hemoglobinopathies: Molecular Assay: HbC Matched LS Probe

<table>
<thead>
<tr>
<th>Loci</th>
<th>E</th>
<th>S</th>
<th>WT</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coding</td>
<td>G</td>
<td>A</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>HbC (G→A)</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>HbE (G→A)</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>HbS (A→T)</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>
DNA Sequencing

DNA isolation from blood

PCR amplification

Gel to confirm PCR product

Product purification

Sequencing

Sequence analysis

Coding regions
Intron/exon boundaries
3 deep intronic known deleterious mutations
5’ and 3’ regulatory regions
Deletion(s)
Thrombophilia

• Tendency to develop deep venous thrombosis
  – Acquired
    • Pregnancy/puerperium, Hormone therapy, Surgery, Immobilization, Malignancy, Chemotherapy, Previous deep or superficial venous thrombosis, Heavy smoking, Obesity, Lupus anticoagulant
  – Genetic
  – Acquired + Genetic

• 2 million cases of DVT/year in the U.S.

• Complications
  – Pulmonary embolus
    • 100,000 deaths/year
  – Post-thrombotic syndrome in 60% of cases
    • Chronic vessel obstruction/valve
Inherited thrombophilia

• Mutations in the major anticoagulants
  – Protein C (200 mutations described)
    • Prevalence: 0.2---0.4%
    • Heterozygous – thrombophilia (RR 3-8)
    • Homozygous – neonatal purpura fulminans
  – Protein S (> 100 mutations)
    • Prevalence: 0.03-0.1%
    • Heterozygous – thrombophilia (RR 2.4-20.0)
    • Homozygous - neonatal purpura fulminans
  – Antithrombin III
    • Prevalence: 0.07-0.2%
    • Heterozygous – thrombophilia (RR 5-10)
    • Homozygous - severe, early onset thrombotic syndrome, lethal before birth
APC Resistance Factor V Leiden

- 1691G>A substitution
- 95% of Activated protein C (APC) resistance
- Arg506Gln at one of 3 APC cleavage sites
  - Required for subsequent cleavage at R306 and R679
  - Down-regulation of the procoagulant activity of FV
- Inactivated 10X slower than normal Factor V
- Mild hypercoagulable state
- Heterozygous
  - Prevalence 1-5% RR 3-8, Lifetime risk 10%
- Homozygous
  - Prevalence 0.02%, RR 50-100, Lifetime risk >80%
Laboratory Diagnosis of Factor V Leiden

• Coagulation screening test
  – Modified functional APC resistance assay
  – Positives followed up DNA analysis to confirm zygosity

• DNA analysis
  – Single mutation detection - multiple technologies
  – Recommended by ACMG for testing family members of probands
Other Factor V mutations

- HR2 haplotype of the Factor V gene
  - Nine polymorphisms in exon 13 of FV
  - Present in 10% of the population
  - The haplotype has never been observed on the same chromosome as FVL
  - Increases risk for thrombotic event in individuals heterozygous for FVL

- Other mutations characterized so far are either rare or have mild/no effect on APC resistance
Prothrombin c. 20210G>A

• 3’ UTR (untranslated region)
  – Affects polyadenylation signal
  – Gain-of-function mutation

• Heterozygotes: 30% higher levels (RR 2 – 5)

• Homozygotes: 70% higher levels  RR unknown

• Higher potential for thrombin generation

• Analysis must distinguish G20210A from an uncommon polymorphism at 20209

• 2-3% of Caucasians
  – Very rare in non-Caucasians

• Compound het FV Leiden/PT
  – Prevalence in general population: 0.1%
  – RR: 20
Factor V Leiden should be performed after a venous thrombosis occurring:

- at <50 years
- during pregnancy or the puerperium
- with oral contraceptives or HRT
- at any age if strong family history
- in unusual sites unprovoked, at any age
- recurrently at any age

- Women planning a pregnancy or oral contraceptive use
- Unexplained 2nd or 3rd trimester pregnancy loss
- Unexplained severe preeclampsia, placental abruption or IUGR
- With first venous thrombosis related to using a selective estrogen receptor modulator
- Smokers <50 years with one provoked venous thrombosis
Consequences of positive test results

- FVL het, one thrombotic event
  - No change in immediate therapeutic approach for most patients - no long term OAT
- Risk for major bleeding (20% fatal) 1-2% per year
- Risk for thrombosis 1% per year
- Consider prophylaxis for surgery, prolonged immobilization, pregnancy+6 weeks postpartum
- FVL het, no thrombotic event (family history)
- Heightened awareness of presenting signs of DVT
- Decisions regarding oral contraceptives
- Other lifestyle choices
FVL and oral contraceptives

• FVL heterozygotes have a 35-fold increased risk for venous thromboemolisms (VTEs) while using oral contraceptives

• General screening of users of oral contraceptives is currently not recommended

• Screening of 400,000 women would identify
  – 20,000 FVL heterozygotes, which prevents
    • One death
Platforms for Single Base Mutations

• Restriction Enzyme/Gel
• Hybridization Probes/ LightCycler
  – FDA approved
  – PCR (Target amplification)
• Invader
  – Signal amplification
FV Leiden Detection by RFLP

*Mnl I* Restriction Digest

5' GGCGAGGAA 3'

A

400 bp  200 bp

Caveats

False positive result: Irrelevant mutations within the enzyme recognition site may abolish cutting

False negative result: Other (relevant) mutations that do not abolish the cut site are not detected

Wildtype
Heterozygous
Homozygous Leiden
No template control
Light Cycler Melting Curve Analysis

Mismatch

Perfect match

Temperature

low

high

medium
Factor V Genotyping by Fluorescent Melting Peaks
Hydrolysis Probes
Other Probe Systems

- Molecular Beacon Probes
- Scorpion Probes
Aberrant peak → sequence

C1690T melting peaks

A second novel mutation has a $T_m$ of 59.44 ± 0.02 °C and $\Delta T_m$ of 5.49 ± 0.03 °C. In this sample, T replaces C at position 1690. C1690T encodes an OPA stop codon instead of arginine at amino acid 506 (R506X), truncating the protein. The patient is heterozygous for the C1690T and negative for the Leiden mutation. No clinical information was provided regarding this patient.
Factor II (c.20210G>A)

c.20209C>T in African Americans
Causative or benign?
Hyperhomocysteinemia

- Associated with VTE and cardiovascular disease
- RR increases as fasting plasma conc. >10 µmol/l
- Underlying mechanisms unclear
- Many causes for hyperhomocysteinemia
  - Renal failure, hypothyroidism, leukemia, psoriasis, drug therapy
  - MTHFR mutations
- Spuriously elevated homocysteine
  - Non-fasting
  - ex-vivo release from rbc
MTHFR mutations

• C677T
  – 30-40% het, 10-15% homo
  – Mild elevation of total plasma homocysteine
  – Accounts for 1/3 of hyperhomocysteinemia cases
  – Plasma homocysteine more informative

• A1298C
  – Probably of little or no value by itself

• Compound het C677T; A1298C
  – Increased levels of homocysteine

• Hyperhomocysteinemia + FVL RR 20
Invader assay
(Third Wave Technologies, Madison, WI)

• Available for PT (G20210A), FVL, MTHFR(C677T)
• Non-PCR method
  – No royalty costs
  – “Clean/dirty room” not required
• Microtiter plate format
• Total time 4-5 hours, hands-on time <1 hr
• Probe hybridization only – lacks the melting curve feature - less sensitive than LightCycler
Invader Technology

- Isothermic
- With or without PCR
- Allele specific primary probe + Invader® probe hybridize to target DNA
- Overlap structure recognized, cleaved by Cleavase® enzyme, 5’-Flap released
- 5’-Flap oligo “invades” a (FRET™) cassette
- Cleavase® action on the FRET™ cassette releases fluorophore from quencher, generates fluorescence signal
Hereditary Hemochromatosis

- Iron storage disease
- Autosomal recessive (later onset)
- ~1/500 individuals affected
- HFE (HLA-H) gene
- High penetrance allele (C282Y)
- Low penetrance allele (H63D)/polymorphism?
## Hemochromatosis

### Allele Frequencies

<table>
<thead>
<tr>
<th></th>
<th>C282Y/H63D</th>
<th>HH patients</th>
<th>Random controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT/WT</td>
<td>WT/WT</td>
<td>8.0%</td>
<td>64.0%</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>WT/WT</td>
<td>80.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>C282Y/WT</td>
<td>WT/WT</td>
<td>1.1%</td>
<td>8.6%</td>
</tr>
<tr>
<td>C282Y/WT</td>
<td>H63D/WT</td>
<td>5.2%</td>
<td>1.3%</td>
</tr>
<tr>
<td>WT/WT</td>
<td>H63D/H63D</td>
<td>1.1%</td>
<td>3.0%</td>
</tr>
<tr>
<td>WT/WT</td>
<td>H63D/WT</td>
<td>3.4%</td>
<td>23.0%</td>
</tr>
</tbody>
</table>

Average calculated from published European Caucasian populations.
Hemochromatosis Multiplex

**C282Y**

- **Cys282Tyr**
- **Wild-type**

**H63D/S65C**

- **Wild-type**
- **Ser65Cys**
- **His63Asp**

Probes complementary to mutant sequence
X-linked Disorders

Hemophilia
Muscular Dystrophy
Hemophilia Symptoms

- Easy bruising
- Hemorrhage into joints
- Prolonged bleeding from wounds
- Severe disease = spontaneous bleeds
- Moderate disease = bleeds following minor trauma
- Mild disease = bleeds following major trauma
# Hemophilia Severity

- **Hemophilia A**
  - Severe: $<1\%$, 50% of patients
  - Moderate: 1-5%, 10% of patients
  - Mild: $>5\%$, 40% of patients

- **Hemophilia B**
  <50% normal activity of F9
F8 Gene

Int22h-1

Int22h-2

Int22h-3

26..23

22...2

1

145 kb

500 kb

600 kb

qter
## Hemophilia A Mutations in Severe Disease

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Intron 22 Inversions</td>
<td>45%</td>
</tr>
<tr>
<td>Intron 1 Inversion</td>
<td>2%</td>
</tr>
<tr>
<td>Large Deletions (&gt;50bp)</td>
<td>5%</td>
</tr>
<tr>
<td>Small Deletions (&lt;50bp)</td>
<td>1%</td>
</tr>
<tr>
<td>Unknown</td>
<td>1%</td>
</tr>
<tr>
<td>Point Mutations</td>
<td>46%</td>
</tr>
</tbody>
</table>

HAMSTeRS: The Haemophilia A Mutation, Structure, Test and Resource Site
http://europium.csc.mrc.ac.uk/WebPages/Main/main.htm
Inverse PCR, wild type

1. Cut with Bcl1

2. Self-ligate

3. PCR
Inverse PCR, Inversion Positive

1. Cut with Bcl1

2. Self-ligate

3. PCR

- IU primer
- ED primer
- 20.0 kb ring
- 460 bp
- 99 bp
- 559 bp product
Inversion Results

[Image of gel electrophoresis with labeled bands: WT band, Inversion band, WT, + Control, Sample, NTC]
**F8 Gene Sequencing**

- **26 Exons**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amplicon, bp</th>
<th>Exon</th>
<th>Amplicon, bp</th>
<th>Exon</th>
<th>Amplicon, bp</th>
<th>Exon</th>
<th>Amplicon, bp</th>
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<tr>
<td>1</td>
<td>514</td>
<td>8</td>
<td>518</td>
<td>14A</td>
<td>601</td>
<td>15</td>
<td>333</td>
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<td>295</td>
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<td>2</td>
<td>333</td>
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<td>334</td>
<td>14B</td>
<td>462</td>
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<td>592</td>
<td>22</td>
<td>321</td>
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<tr>
<td>3</td>
<td>324</td>
<td>10</td>
<td>284</td>
<td>14C-1</td>
<td>490</td>
<td>16B</td>
<td>328</td>
<td>23</td>
<td>328</td>
</tr>
<tr>
<td>4</td>
<td>406</td>
<td>11</td>
<td>396</td>
<td>14C-2</td>
<td>511</td>
<td>17</td>
<td>431</td>
<td>24</td>
<td>284</td>
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<tr>
<td>5</td>
<td>293</td>
<td>12</td>
<td>332</td>
<td>14D</td>
<td>624</td>
<td>18</td>
<td>396</td>
<td>25</td>
<td>334</td>
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<tr>
<td>6</td>
<td>292</td>
<td>13</td>
<td>398</td>
<td>14E</td>
<td>604</td>
<td>19</td>
<td>282</td>
<td>26</td>
<td>591</td>
</tr>
<tr>
<td>7</td>
<td>434</td>
<td></td>
<td></td>
<td>14F-1</td>
<td>411</td>
<td>20</td>
<td>262</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>14F-2</td>
<td>516</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **34 Amplicons**
Principle of MLPA

1. Denature and Hybridization

2. Ligation

3. Amplification
MLPA for Factor 8 (Hemophilia A)

- Covers all 26 exons
- 33 different probes
  - Exons 1, 3, 7, 12, and 26 = 2 probes each
  - Exon 14 = 3 probes
Testing Scheme

Severe
Inversion Testing
Int22 Inversion
Positive 47%

Int1 Inversion
Negative

Moderate or Mild
Full Gene Sequencing
Positive 46%
95%

Del/Dup
Positive 5%
Negative

Negative
Triplet Repeat Diseases

Fragile X
Huntington Disease
Myotonic Dystrophy
Trinucleotide Repeat Expansion Disorders

• Fragile X Syndrome - (CGG)n 5’UTR
• Myotonic Dystrophy - (CTG)n 3’UTR
• Huntington Disease - (CAG)n coding
• Kennedy's Disease - (CAG)n coding
• Spinocerebellar Ataxias - (CAG)n coding
• Machado-Joseph Disease - (CAG)n coding
• Friedreich Ataxia - (GAA)n intron 1
Diseases associated with Trinucleotide repeat expansions

Trinucleotide repeat expansions have been shown to occur in 5’ and 3’ untranslated regions (UTR), coding regions (exons), and introns of several genes. The expansion of trinucleotide repeats interferes with the expression of the gene or the encoded protein.
Fragile X Syndrome

• Most common inherited form of mental retardation.
• Incidence 1:4000 males and 1:8000 females.
  – Carrier 1.3% (Strom et al. Genet in Med 2007;9:46-51)
• Affected males have mental retardation, characteristic physical features and behavior.
• Affected females exhibit a less severe phenotype.
• Found in all populations.
• RNA binding protein widely expressed (nerve, brain, etc.)
  – Protein expression by immunohistochemistry (IHC)
• Pre-mutation – normal protein, increased mRNA
• Full mutation – no protein produced
Fragile X: Repeat Number Classification

- Normal: **5-44 repeats**: Rules out Fragile X syndrome/carrier status.
- Intermediate: **45-54 repeats**: Not affected but unstable, could expand to a pre-mutation, then full mutation.
- Pre-mutation: **55-200 repeats**: Carrier and at risk for expansion in next generation (females). At risk for premature ovarian insufficiency (POI) or ataxia.
- Full mutation: **>200-230 repeats**: Gene is methylated and inactive; confirms diagnosis of Fragile X syndrome.
- Mosaic: Both pre-mutation (un-methylated) and full mutation (methylated) present. Severity of symptoms cannot be predicted, but may be milder.
Fragile X: Transmission

• Female pre-mutation carriers
  – 50/50 chance of transmitting unstable allele
    • May stay within pre-mutation range
    • May expand to full mutation (higher pre-mutations more likely to fully expand in one generation)

• Male pre-mutation carriers
  – Will transmit pre-mutation to all daughters
  – Unlikely to expand

• Intermediate
  – May expand to pre-mutation, but not full mutation, in one generation
Fragile X: Risk of Expansion by Pre-mutation Size

<table>
<thead>
<tr>
<th>Number of Maternal Pre-Mutation CGG Repeats</th>
<th>The allele will expand into a full mutation range</th>
</tr>
</thead>
<tbody>
<tr>
<td>56-59</td>
<td>14%</td>
</tr>
<tr>
<td>60-69</td>
<td>20%</td>
</tr>
<tr>
<td>70-79</td>
<td>58%</td>
</tr>
<tr>
<td>80-89</td>
<td>72%</td>
</tr>
<tr>
<td>90-99</td>
<td>94%</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>100%</td>
</tr>
</tbody>
</table>

Information from GeneReviews at [www.genetests.org](http://www.genetests.org),
Fragile X-associated Conditions

- **Tremor/Ataxia Syndrome (FXTAS)**
  - Late-onset, progressive cerebellar ataxia/intention tremor
  - Short-term memory loss, executive function deficits, cognitive decline
  - Lower-limb proximal muscle weakness, and autonomic dysfunction

- **Fragile X: Premature Ovarian Insufficiency**
  - Cessation of menses before age 40
  - 21% of females with POI have FX pre-mutations

- **Genetics**
  - FMR1 pre-mutation
  - mRNA accumulation
Fragile X: Indications for Testing

• Fragile X syndrome testing
  – Diagnosis for mental retardation especially with physical/behavioral characteristics, family history, fetal testing

• Premutation Testing
  – Carrier status for those with family history, PIO, FXTAX

• Population screening?
  – Newborn screening (full mutation)
  – Carrier testing (pre-mutations)
Fragile X Testing

- **PCR**
  - Sizes normal/pre-mutation allele
  - Difficult to amplify through CGG repeat
  - Amplification into full mutation range possible

- **Southern blot analysis (concurrently or reflexed)**
  - 80-800+ repeats
  - Full mutations
  - Methylation
  - Difficult to size accurately
Fragile X: PCR Challenges

- Difficult to amplify large CGG repeats with PCR
- Preferential amplification of normal allele in females
- Difficult to distinguish: One allele/undetected expanded allele from two normal homozygous alleles in females
- Sizing difference between laboratories
Fragile X: Southern Blot

- Restriction Digest
- Electrophoresis
- Transfer to Membrane
- Anneal Probe
- Detect
The FMR-1 gene region with the CGG trinucleotide repeats is flanked by Eco RI sites and an Nru 1 site.

Full mutation has been shown to methylate the gene and prevent Nru 1 restriction of the DNA.

DAKO's Fragile X probe is specific for the 1.1 kb Pst1 fragment 3’ to the CGG trinucleotide repeat region within the FMR1 gene on human chromosome Xq27.3.
Fragile X: Southern Schematic

Normal females show
- one methylated allele (5.2 kb)
- one un-methylated allele (2.1 kb)

Normal males
- one un-methylated allele (2.1 kb)
Fragile X: Technology Advances

- Improved PCR – into full mutation range
- Triplet-repeat primed PCR
  - Stuttering only up to the largest allele size present in the sample
  - Expanded alleles (detected if present)
  - Females (resolve “apparent homozygous”)
  - Males
  - Mosaics
    - Pre-mutation/full mutation
    - Normal/full mutation
- Methylation-specific PCR
  - Detect methylated alleles in males
  - Determine whether borderline pre/full expansions are methylated
  - Methylation and size determined simultaneously
- Commercially available ASR’s
20/31 CGG repeats

29/103 CGG repeats

140/800 mosaic CGG repeats
Spinocerebellar Ataxia, Type 1

- Progressive incoordination of walking
- Poor coordination of hand and eye movements & poor speech
- Autosomal dominant
- 6% of dominant ataxias in North America
- Average age of onset = 30s
- Average duration of disease = 15 yrs.
Friedreich Ataxia

- Most common type of inherited ataxia
- Symptoms appear between 5-15 years of age
- Lose ability to walk by mid-20s
- Loss of sensation in extremities
- Poor speech, hypertrophic cardiomyopathy
- Autosomal recessive
Friedreich’s Ataxia

- FXN gene
  - GAA repeat
  - Normal = 7-22 repeats
  - Expanded = 66-1000 repeats
    - 66-300 repeats have later appearance of symptoms (after age 25)
Huntington Disease (HD)

• Autosomal dominant
• 1 in 10,000 in the U.S.
• Symptoms
  – Psychiatric, Motor, Cognitive
• Adult onset (mean age 35-44)
• Progressive degeneration, eventually fatal
  – Autopsy shows severe loss of brain areas
• 5% of cases are juvenile onset
Huntington Disease: Symptoms

- Psychiatric changes
  - Depression, Personality and mood changes, Apathy, Delusions, paranoia
- Chorea - uncontrolled movements
- Progressive problems with coordination, judgement and thinking
- Result from selective neuronal loss from caudate nucleus and putamen
- Death 15-20 years after symptoms begin
Huntington Disease: Management

• NO CURE – treatment cannot slow progression
• Psychiatric disturbances
  – Psychotropic medications (SSRI)
• Motor symptoms
  – Neuroleptics for chorea
  – Anti-Parkinsonian agents for hypokinesia
  – Prone to side effects
• Cognitive Impairment – no treatment
• Movement training or speech therapy
Huntington Disease: IT-15 Gene Function

• Normal
  – Housekeeping
  – Widely expressed in neuronal & non-neuronal tissues

• HD mutation leads to toxic gain of function
  – overactivity of normal or novel function
  – novel interaction with other proteins
  – multimerization of the protein; large insoluble aggregates leading to cell death
Huntington Disease: Mutant Huntington Protein

Expansion of polyglutamine tract (CAG)
Paternal Bias

Normal: teens-20s
Normal Huntington protein

HD-causing: 40 or more
Mutant Huntington protein
## Huntington Disease: Effect of CAG Repeat Length

<table>
<thead>
<tr>
<th>Category</th>
<th># of CAGs</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;27</td>
<td>Normal</td>
</tr>
<tr>
<td>Mutable Normal</td>
<td>27-35</td>
<td>Normal</td>
</tr>
<tr>
<td>Reduced Penetrance</td>
<td>36-39</td>
<td>Normal/HD</td>
</tr>
<tr>
<td>Affected</td>
<td>40+</td>
<td>HD</td>
</tr>
</tbody>
</table>
Huntington Disease: Juvenile HD

- Age of onset < 20 years, faster progression
- 5% of HD patients have juvenile form
- Symptoms
- Expanded repeat size 80-100 CAG
- 70-90% Juvenile HD paternally inherited
Huntington Disease: PCR Assay
Huntington Disease:

- Unaffected
- Reduced Penetrance
Huntington Disease: Apparent Homozygote CAG

- Which one?
  - True homozygote
  - Heterozygote: second allele did not amplify
  - Heterozygote: second allele is too big to detect
Huntington Disease: Additional PCR
Huntington Disease: Southern Blot

22 / 66
22 / 101
HD Society of America
HD Approved Testing Centers

• Pre-test discussion with genetic counselor
  – Assessing patient’s risk perception, expectations and support systems
  – Explaining implications of testing vs not testing
  – Medical management and reproductive options

• Performance of a neurological examination

• Psychologist/psychiatrist evaluation for depression, or other psychiatric illness
Huntington Disease:
1996 ACMG /ASHG HD Recommendations for Labs

- Document informed consent for diagnostic and predictive testing
- Consent must note whether the pt permits sample to be stored &/or used for research
- Confirm HD molecularly in an affected family member if possible
Huntington Disease: Predictive Testing in Minors

• General consensus: contraindicated
  – Adult-onset condition
  – No current treatment
• HDSA: not recommended, but each center should develop their own policy
• International HD Association: not recommended
• NSGC: consider that many adults (80%) would choose NOT to have testing
Huntington Disease: HD Pedigree

60 yo

30 yo

5 yo

25 yo

2 yo
Myotonic Dystrophy, Type 1

- Autosomal dominant
- Pleiotrophic
  - Vision, bone, respiratory, endocrine, cardiovascular, GI, muscle
- DMPK gene
  - Little known about the protein function
  - Signal transduction pathway
  - Highest expression in skeletal muscle and heart
- (CTG)n expansion in the 3’UTR
Myotonic Dystrophy, Type 1:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CTG Repeat Size</th>
<th>Age of Onset</th>
<th>Avg Age of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutable normal (premutation)</td>
<td>35 to 49</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mild</td>
<td>50 to ~150</td>
<td>20 to 70 yrs</td>
<td>60 yrs to normal life span</td>
</tr>
<tr>
<td>Classic</td>
<td>~100 to ~1000</td>
<td>10 to 30 yrs</td>
<td>48 to 55 yrs</td>
</tr>
<tr>
<td>Congenital</td>
<td>&gt;2000</td>
<td>Birth to 10 yrs</td>
<td>45 yrs</td>
</tr>
</tbody>
</table>
Anticipation

• A feature of pedigrees in which a disease is seen at earlier ages or with increased severity in more recent generations
  – Triplet expansion commonly occurs during meiosis
  – Repeat count expands over successive generations
  – Children thus have more severe symptoms at an earlier age than their parents

• At the DMPK locus, preferential transmission of expanded repeats (premutation) over smaller alleles (normal)
Myotonic Dystrophy Testing

- PCR sizing
  - Normal alleles
  - Minimally expanded alleles
- Southern blot
  - Large affected alleles

Somatic instability
  - Increases with age
Myotonic Dystrophy, Type 2

- Autosomal dominant
- Less than 2% of myotonic dystrophy cases
- Milder signs and symptoms than type 1
- Main symptoms =
  - Myotonia
  - muscle dysfunction
  - cardiac conduction defects
- CNBP gene
  - Little known about the protein function
  - Signal transduction pathway
  - Highest expression in skeletal muscle and heart
- (CCTG)n expansion in intron 1
  - Complex motif
Myotonic Dystrophy, Type 2: Intron 1 Complex Repeat Region

- Each region is polymorphic
- Interruptions in the (CCTG)n tract
- Complex repeat length
  - Normal 107-177 bp 11-26 repeats
  - Premutation 177-372 bp 27-74 repeats
  - Affected 372 bp->44,000 bp 75->11,000

Expansion Region
Myotonic Dystrophy, Type 2: Instability of the (CCTG)n repeat

• Intergenerational Instability
  – Typically increase in size (anticipation)
  – Decrease in allele size has been observed

• Somatic Instability
  – Increase
  – reduction
Non-Mendelian Inheritance: Mitochondrial & Imprinting
mtDNA Genes

• Each human cell contains 50-5000 mitochondria
• In one oocyte ~100,000 mtDNA molecules (amplification of mtDNA)
• Each mitochondrion contains 2-10 mtDNA molecules.
• mtDNA – approx. 1% of total cellular DNA.
• Circular, 16,569 bp
  — ~93% coding DNA. Introns absent.
  — Continuous transcription of multiple genes
  — No recombination
  — High mutation rate
    • ten-fold increase as compared to nuclear DNA
    • 37 genes
• 13 protein subunits of the respiratory chain (of a total of approx. 67)
• 16S and 12S mt rRNAs
• 22 mt tRNAs
• Genetic code differs slightly
  — standard mtDNA
    — UGA stop Arg
    — AGA Arg stop
    — AGG Arg stop
    — AUA Ile Met
Mitochondrial Inheritance

- 99.9% of mtDNA is maternal
- 100 mitochondria in sperm, >100,000 in egg. Most of sperm tail does not enter egg
- Eventually sperm mitochondria are deleted or diluted out
- Thus any mutations in mtDNA are inherited from the mother
- During mitotic cell division, the mtDNA molecules segregate in a purely random way to the two daughter cells.
  - Homoplasmy: In normal individuals approx. 99.9% of the mtDNA molecules are identical
  - Heteroplasmy: If a new mutation arises and spreads in the mtDNA population, there will be two significantly frequent mtDNA genotypes
  - The severity of the disease phenotype depends on the amount of mutant and normal mitochondria present
Mitochondrial Disorders

• Approximately 1:8500
• maternal inheritance
• respiratory chain components
• rRNA and tRNA
• heteroplasmy
• mutations must exceed a threshold for expression
• Usually affect the most energy demanding tissues
• At cell division, the proportion of mutant mtDNA in daughter cells can shift. The level of heteroplasmy may differ between cells and tissue
• A minimum critical number (threshold) of mutant mtDNAs must be present before tissue dysfunction and clinical signs become apparent.
Mitochondrial Disorders: Common Features

Think mitochondrial disorder when 3 or more organ systems are involved

- **Brain**  Developmental delay, seizures, strokes, dementia
- **Nerves**  Weakness, absent reflexes, dysautonomia
- **Muscles**  Weakness, GI problems, muscle pain
- **Kidneys**  Renal tubular acidosis or wasting
- **Heart**  Cardiomyopathy, cardiac conduction defects
- **Liver**  Hypoglycemia, liver failure
- **Eyes/Ears**  Visual loss, hearing loss
Human Disorders Due to Mitochondrial Mutations

- Kearnes Sayre syndrome (KSS)
- Pigmentary retinopathy, chronic progressive external ophthalmoplegia (CPEO)
- Leber hereditary optic neuropathy (LHON)
- Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Myoclonic epilepsy with ragged red fibers (MERRF)
- Deafness
- Neuropathy, ataxia, retinitis pigmentosa (NARP)
- Subacute necrotizing encephalomyelopathy with neurogenic muscle weakness, ataxia, retinitis pigmentosa (Leigh with NARP)
mtDNA Disorders: KSS

- **Kearns-Sayre syndrome (KSS)**
  - sporadic condition with onset before age 20.
  - Deletions in mtDNA
  - Symptoms:
    - Progressive external ophthalmoplegia.
    - Retinal pigment abnormalities
    - Heart block
    - Cerebellar ataxia
    - Diabetes
    - Kidney failure
Detection of KSS Mitochondrial Deletion Mutation by Southern Blot

The restriction enzyme, \( Pvu \text{II} \) cuts once in the circular mitochondrial DNA.

- \( M \) = Mutant
- \(+\) = Normal
- \( U \) = Uncut, No \( Pvu \text{II} \)
- \( C \) = Cut with \( Pvu \text{II} \)

The autoradiogram shows an 16.6 kb (normal) and a Deletion mutant. The presence of heteroplasmic bands indicates a mixed population of normal and mutated mitochondrial DNA.
mtDNA Disorders: NARP

- Neuropathy, ataxia, and retinitis pigmentosa (NARP)
- **MT-ATP6 gene mutation**
  - MT-ATP6 mutation in 70 percent to 90 percent of mitochondria.
  - When than 90 percent to 95 percent, it causes a more severe condition known as maternally inherited Leigh syndrome

- **Symptoms:**
  - Learning disabilities and developmental delays
  - Seizures
  - Hearing loss
  - Cardiac conduction defects
mtDNA Disorders: CPEO & MERRF

• **Chronic progressive external ophthalmoplegia (CPEO)**
  – Symptoms: paralysis of the muscles responsible for eye movement, ptosis
  – *Deletions in mtDNA*
  – AD type also described

• **Myoclonic epilepsy with ragged red fibers (MERRF)**
  – Symptoms: myoclonus, epilepsy, ataxia presenting in childhood or adolescence. May involve hearing loss, dementia, optic nerve atrophy.
  – In 85% of cases, *base substitution mutation in tRNA-lysine* leading to defects in complexes I and IV
  – Usually heteroplasmic
mtDNA Disorders: MELAS

• Mitochondrial encephalomyopathy, lactic acidosis and stroke–like episodes (MELAS).
  – Dysfunction of brain tissue (often causing seizures, transient regional paralysis and dementia) combined with mitochondrial myopathy and a toxic buildup of acid in the blood.
  – Progressive disease, with the first symptoms occurring between 5 and 15 years of age. Cortical blindness may develop.
  – Nearly 80% of individuals are heteroplasmic.
  – Base substitution mutation in tRNA – leucine, leading to a deficit in the necessary mitochondrial-coded protein subunits that carry out oxidative phosphorylation.
mtDNA Disorders: LHON & Nonsyndromic deafness

- Leber’s hereditary optic neuropathy (LHON)
  - Acute or subacute loss of vision around the age of 20.
  - Several missense mutations in a gene coding for the protein ND4. *Often homoplasmy.*
  - Increased penetrance in males.

- Nonsyndromic deafness.
  - Irreversible hearing loss that is associated with the use of aminoglycoside antibiotics (e.g., streptomycin, gentamicin, kanamycin).
  - Antibiotics bind to mutant mitochondrial rRNA (mainly missense mutation), thus interfering with translation within the mitochondria and leading to a loss of mitochondrial function.
Genomic Imprinting

• Process whereby genes are expressed differently depending upon the parent of origin.
• Occurs during gametogenesis.
• Epigenetic, functional (potentially reversible) inactivation of allele by methylation.
• Applies to less than 5% of human genes.
• Proves the importance of having genes from both parents.
Genomic Imprinting

• Gene silencing due to methylation of C residues and other modifications.
• Genomic imprinting occurs during production of egg and sperm.
• The phenotypic effects of imprinting are revealed in diseases in which the maternal or paternal allele is lost (uniparental disomy/deletion).
• Abnormal DNA methylation can cause imprinting problems.
Genomic Imprinting: Disorders

- **Prader-Willi Syndrome:** caused by regional deletion or mutation in the paternally inherited chromosome 15

- **Angelman Syndrome:** a different disease phenotype caused by regional deletion or mutation in the maternally inherited chromosome 15
Genomic Imprinting: Disorders: Chr 15q11-13

• Genetic information from the maternal and paternal chromosome 15q11-13 regions is expressed differently
• The parental “imprint” is set during formation of the egg and the sperm
• An intact maternal and paternal chromosome 15q11-13 are needed for normal development
Genomic Imprinting: Prader-Willi

Prader-Willi
• prevalence ~ 1/10,000
• Hypotonia and failure to thrive in infancy, rapid weight gain 1 to 6 years,
• round face, almond shaped eyes, hypogonadism, 40% borderline IQ and 40% mild MR
• Behavior problems including voracious appetite (elevated ghrelin), food hoarding
• skin picking, sleep disturbance/sleep apnea, short stature, hypopigmentation, hypofunctioning of the hypothalamus

Angelman
• Prevalence of 1/12,000
• Severe developmental delay, little or no speech, movement or balance disorder, ataxia, most have seizures, hyperactive, wide mouth, usually happy
• Often use augmentative communication device.
Genomic Imprinting: The Prader-Willi / Angelman Region on Chr15q11-13

- **Maternally imprinted** (genes active only on male chromosome)
  - MKRN3 (ZNF127)
  - MAGEL2
  - NDN
  - C15orf2
  - SNURF / SNRPN
  - HBII-13
  - HB2-85
  - IPW
  - PAR-1
  - HB2-52
  - UBE3A-AS
  - UBE3A
  - HERC2

- **Paternally imprinted** (genes active only on female chromosome)
  - PFATP
  - GABRB3
  - GABRA5
  - GABRG3
  - P

**Breakpoint**

**Centromeric**

**Telomeric**

**Chromosome 15q11-q13 region**
Genomic Imprinting: The Prader-Willi / Angelman Region on Chr15q11-13
Genomic Imprinting: Chr.15q11-13

Prader-Willi/Angelman syndrome deletion region, ~4 Mb

- Gene – imprinted (silent on paternal chromosome)
  - Angelman syndrome results if active maternal chromosomal region deleted
  - Maternally imprinted (silent on maternal chromosome)
- Prader-Willi Syndrome results if active paternal chromosomal region is deleted
Genomic Imprinting: Angelman Syndrome: Uniparental Disomy

- Two paternal chromosomes
- No maternal 15
- ~ accounts for 5% AS
Genomic Imprinting: The Prader-Willi / Angelman Syndromes: Defect in Imprinting Center

- UBE3A gene
- duplicon = repetitive sequences of DNA
- bp common breakpoints
- imprinting centre (IC)
Genomic Imprinting: Defects in the UBE3A gene

- ubiquitin protein ligase which is involved in protein degradation within the brain
- Only the maternal copy is active in brain
- specific regions of the brain involved in learning, memory and movement
- Point mutation within the UBE3A gene leads to lack of normal expression of gene
Genomic Imprinting: PWS inheritance risk depends on the molecular cause

• The vast majority of PWS deletion and UPD patients are sporadic and recurrent risk is <1%.
• For imprinting defect, the risk can be as high as 50%.
• The risk for the recurrent for a parent carries a balanced translocation is 25%, otherwise, the de novo chromosomal rearrangement will be <1%.
Genomic Imprinting: AS inheritance risk depends on the molecular cause

- The recurrent risk for the AS deletion and UPD patients is <1%.
- The imprint defect can be familial at a risk as high as 50% if the mother carries the same imprint defect, as well as the UBE3A mutation.
- Paternal UPD with Robertsonian translocation is approaching 100% risk to transmit to the offspring.
Genomic Imprinting: Diagnosis of PW/AS with Bisulfite-treated Methylation specific PCR (MSP-PCR)

• Principle: Promoter region of SNRPN gene contains CpG islands which are heavily methylated in the maternally-derived allele and unmethylated in the paternally-derived allele.
  – PW = only methylated (maternal) allele present
  – AS = only unmethylated (paternal) allele present

• Method: Genomic DNA is treated with sodium bisulfite, converting cytosine to uracil except where cytosine is methylated. Then PCR and melting analysis are performed.
Genomic Imprinting: Diagnosis of PW/AS with Bisulfite-treated Methylation specific PCR (MSP-PCR)

Bisulfite treatment converts unmethylated C residues to U.

...GTCMeGATCMeGATCMeGTG...  

Bisulfite treatment converts unmethylated C residues to U.

...GTCGATCGATCGTG...

...GTCMeGATCMeGATCMeGTG...  

←G CTAG CTAG CAC

PCR primer

Product

...GTUAGATUGATUGTG...  

CTAGCTAGCACG

PCR primer

No product

G

PCR
Genomic Imprinting: Diagnosis of PW/AS with Bisulfite-treated Methylation specific PCR (MSP-PCR)

Lower Tm for unmethylated
(≈83°C)
= Angelman Syndrome

Higher Tm for methylated
(≈87°C)
= Prader-Willi Syndrome

Dectets >99% of causes PWS

Dectets 75-80% of causes for AS
Other Methods for Detection of DNA Methylation

- Methylation-sensitive single-nucleotide primer extension
- PCR-RFLP with methylation sensitive restriction enzymes
- Southern blot with methylation-sensitive restriction enzymes
- MLPA
Rett Syndrome

- X-linked disease, 1/10,000 females
- Normal early development, loss of motor skills and communication
- Variability in disease severity and progression
- MECP2 mutation identified in 90-95% classic RTT
- MECP2 mutations can result in non-classic RTT phenotypes
- Test: gene sequencing and deletion/duplication

Mutation types in females:
- Frameshift: 19.40%
- Complex deletion: 6.40%
- Missense: 39.40%
- Nonsense: 35.10%
MECP2 Gene

• Two main isoforms/ MECP2B and MECP2A

  MECP2B (brain, thymus, lung)

  MECP2A

• Many isoforms with different 3’UTR lengths

Regulates genes involved in neuronal maturation

MECP2 Slides Courtesy of Dr. Pinar Bayrak-Toydemir, Molecular Genetics, University of Utah
MECP2 Duplications in Males

- severe MR
- progressive neurological findings
- hypotonia
- microcephaly
- dysmorphic features, e.g. large ears, hypospadias, flat nasal bridge
- autistic-like features
- 6 duplications and 1 triplication
  - 0.4-0.8 Mb duplications (10 different cases)
  - 0.2-2.2 Mb duplications (including L1CAM gene)
Rett-like Syndrome

• CDKL5 related disorders
  – Intellectual disability
  – X-linked infantile spasm syndrome
  – Seizures
  – Males and females reported
  – Sequence variants/deletions/duplications
  – De novo mutations
Newborn Screening

MS/MS: Several analytes can be detected and quantified simultaneously.

The pattern of analytes (amino acids/acylcarnitines) is characteristic of specific metabolic disorders.
MS/MS Analysis

• Two main classes of metabolites
  – Amino acids.
    • The level of one or more amino acids increases in disorders of amino acid metabolism (metabolic block close to the actual amino acid).
  – Acylcarnitines.
    • In disorders of the intermediary metabolism of amino acids or of fatty acid oxidation the abnormal metabolites are conjugated with carnitine to facilitate their excretion and to balance the Coenzyme A pool.
Fatty Acid Oxidation (FAO) Defects

• Episodic and clinically silent when fat is not utilized
• Fat burned to produce energy when glucose sources are not sufficient
• Environmental triggers
  – infections or other illnesses, fasting, prolonged exercise
• Present shortly after birth, or at any time in life
• In the symptomatic child,
  – measure urine organic acids, plasma carnitine and plasma acylcarnitine profile
  – confirmed by enzyme assay or DNA studies.
  – First attack may be fatal or cause irreversible brain damage.
FAO Defect: MEDIUM CHAIN ACYL-CoA DEHYDROGENASE (MCAD) DEFICIENCY

- Most common fatty acid oxidation defect (frequency: 1:6,500-1:17,000 live births).
- Hypoketotic hypoglycemia triggered by fasting. Between episodes MCAD patients are normal.
- First episode can be fatal and resemble SIDS.
- Unidentified asymptomatic cases may result in unexpected death even in adult life.
- Treatment consists in avoidance of fasting, low fat diet, carnitine supplementation, and aggressive treatment with IV glucose of triggering conditions (infections, etc.).
Common MCAD Mutations

- **K304E - lysine to glutamate**
  - Classic” MCAD mutation
  - 80% of MCAD patients are homozygous A985G
  - An additional 18% are heterozygous

- **T199C polymorphism (?)**
  - To date never observed in patients with clinically manifested disease (2001)
  - Mild missense mutation affecting protein folding
  - 1/500 carrier frequency
  - Present in acylcarnitine-positive samples
Galactosemia: Clinical Findings

• Poor feeding, vomiting, diarrhea, jaundice, lethargy progressive to coma
• Poor weight gain and cataracts in patients surviving the neonatal period.
• Abdominal distention with hepatomegaly a few days after birth (usually at 3-14 days of age) followed by progressive liver failure.
• Increased risk for *Escherichia coli* or other Gram negative neonatal sepsis.
• Progressive liver disease and brain damage (mental retardation) become irreversible.
• Routine neonatal screening tests for galactosemia have been instituted in many parts of the world.
Galactosemia

Galactose + ATP → Gal-1-P + ADP

Galactokinase (GALK)

Gal-1-P + UDPGlu → Glu-1-P + UDPGal

galactose-1-phosphate uridyl transferase (GALT)

UDPGalactose → UDPGlucose

Uridine diphosphate galactose-4-epimerase (GALE)

DIET

Galactitol + Galactonate

Aldose reductase

Galactose dehydrogenase

Endogenous production

Aldose reductase

Galactose dehydrogenase
Galactosemia: Newborn Screening

- Primary screen: Metabolites
  - Total galactose (Galactose + Galactose-1-phosphate)
- Secondary screen: Enzyme activity
  - Galactose-1-Phosphate uridyltransferase

- Primary screen: Enzyme activity
  - Galactose-1-Phosphate uridyltransferase

- Primary screen: Metabolites and Enzyme activity
  - Total galactose (Galactose + Galactose-1-phosphate)
  - Galactose-1-Phosphate uridyltransferase
GALT Mutations

• Q188R (Arginine > Glutamine)
  – most common G allele in Caucasians with a frequency~ 50 to 60%
  – associated with classical galactosemia (severe)

• K285N
  – second common mutation –10%, e prevalent in South Germany, Austria, and Croatia
  – Severe in either homozygous or heterozygous with Q188R

• S135L
  – founder mutation in African American~ 50%
  – associated with milder phenotype, Residual enzyme activity in liver

• Duarte 1 and Duarte 2 both have N314D
  – Duarte 1 (Los Angeles, type): increased enzyme activity with a silent variant (L218L) in cis associated with increased translation efficiency.
  – Duarte 2: reduced enzyme activity due to 5’UTR-119_-116delGTCA deletion in the promoter region associated with decreased gene expression.
# Galactosemia Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Enzyme Activity</th>
<th>Clinical Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/N</td>
<td>100%</td>
<td>None</td>
</tr>
<tr>
<td>N/G or D/D</td>
<td>50%</td>
<td>None or <strong>Carrier</strong></td>
</tr>
<tr>
<td>D/N</td>
<td>75%</td>
<td>None</td>
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<tr>
<td>D/G</td>
<td>25%</td>
<td><strong>Uncertain</strong></td>
</tr>
<tr>
<td>G/G</td>
<td>0%</td>
<td><strong>Severe</strong></td>
</tr>
<tr>
<td>LA/N</td>
<td>112%</td>
<td>None</td>
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</table>
Galactosemia: Methodology-Single Nucleotide Extension (SNE)

- Multiplex PCR
- pooled SNE primers and fluorophore-labeled ddNTPs
- Separate fragments based on size and fluorophore

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS2-2 A&gt;G</td>
<td>5’CCTATCCTTGTCGGT 3’</td>
</tr>
<tr>
<td>S135L C&gt;T</td>
<td>5’AGGTCAATGTGCTTCCACCCCTGGT 3’</td>
</tr>
<tr>
<td>T138M C&gt;T</td>
<td>5’GGATCTCAGGGACCGACATGAGTGCGCAGC 3’</td>
</tr>
<tr>
<td>Q188R A&gt;G</td>
<td>5’ATGATGGGCTGTGTCAACCCACCCCCACCTGCC 3’</td>
</tr>
<tr>
<td>L195P T&gt;C</td>
<td>5’CTGCTTTTGCCCCCTTGACAGGTATGGGCGACAGTTTCC 3’</td>
</tr>
<tr>
<td>Y209C A&gt;G</td>
<td>5’GGCGGCTGTACTCCATAGCAGGGGCTCTCCATGCTGACTCTTA 3’</td>
</tr>
<tr>
<td>L218L C&gt;T</td>
<td>5’TTGGGCTCTCTCCACCTTCCTGAGTAGCTCCTGGCCGCTGTA 3’</td>
</tr>
<tr>
<td>K285N G&gt;T</td>
<td>5’GAGTCAGGCTCTGATCCAGATCTAGCTCGCTCCATCATGAAGAA GCTCTTGACCAA 3’</td>
</tr>
<tr>
<td>N314D A&gt;G</td>
<td>5’CAGCGTCAGGCTCTCTTCTGTCGAGGGCTCCCATGAGATCG ACTGGCTGGGCGCAACTGG 3’</td>
</tr>
</tbody>
</table>
Galactosemia Mutation Panel

5'UTR-116-119delGTCA

active site

Q188R
L195P
Y209C
N314D
K285N

c.940 A>G

IVS2-2A>G

S135L
T138M
L218L

5' UTR
Galactosemia: Examples
Galactosemia: A Combination of Enzymatic Assay and DNA Test

Newborn Screening

Positive

Biochemical enzyme assay in erythrocytes

Results concordant

Stop

Molecular genotyping
Target mutation Analysis > 85%

GALT Panel

Results inconclusive

Full Gene Sequencing

5 KB Deletion
Spinal Muscular Atrophy (SMA)

- Lower motor neuron disease
- Onset predominantly infancy/childhood
- Symmetrical limb and trunk weakness
- Muscular atrophy
- Degeneration and loss of the anterior proximal horn cells in the spinal cord
- Autosomal recessive
- Incidence ~1:10,000
- Carrier frequency ~1:100
- SMA1 is the most common monogenic cause of death in infancy
SMA: Clinical Classification

• **Type I (Werdnig-Hoffman disease):**
  - most severe form
  - age onset: within first 6 months
  - severe generalized weakness at birth or shortly after born
  - never able to sit without support
  - death in first two years in most cases

• **Type II:**
  - do not sit, stand, or walk unaided
  - more prolonged survival

• **Type III:**
  - stand, walk unaided
  - highly variable age of onset
  - usually show proximal muscle weakness in early childhood
SMA: SMN GENE-2

- **SMN1 (SMN\(^T\))**:  
  - Contains 9 exons  
  - Coding 294 aa  
  - 82-96% of normal individuals have 1 copy on each chromosome; 4-18% have 2 copies, complicating the process of heterozygous testing.

- **SMN2 (SMN\(^C\))**:  
  - Strong homology to SMN1: 5 nucleotide differences  
  - Exon 7 skipped during transcription

**Diagram**:  
- **SMN1**:  
  - Genomic DNA  
  - Exon 6  
  - Exon 7  
  - Exon 8  
  - mRNA  
  - SMN1 - 90% of pre-mRNA spliced to full length SMN

- **SMN2**:  
  - Genomic DNA  
  - Exon 6  
  - Exon 7  
  - Exon 8  
  - mRNA  
  - SMN2- 80% of pre-mRNA spliced to SMN without exon 7, unstable & rapidly degraded
SMA: SMN Genes

- SMN1: Deleted in > 95% of SMA patients
- SMN2: Numbers of copies related to severity of SMA

SMN1 Gene:
- *Deletion*: More severe
- *Conversion* to SMN2 gene: Milder SMA

SMN2 gene: Variations
- *More copies*: Correlates with milder SMA.
- *SMN2 mutations* alone: Do not produce SMA
SMA: PCR assay to detect homozygous deletions in the SMN1 gene

- Aneasy DNA-based diagnostic test for SMA
- Exon 7 and 8 each have a single base pair difference between SMN1 and SMN2
- PCR of exons 7 and 8 amplifies both SMN1 and SMN2
- Restriction enzyme distinguishes SMN1 from SMN2
  - Cut SMN2 exons, not SMN1
SMA Screening Test

Normalized Melting Peaks

Temperature °C

0 SMN1

0 SMN2

...TTACTTCTTTTATTTTCTTACAGGGTTTC/TAGACAAAAATCAAAAAAGAAGGAAGGTGCT...
...AATGAAGGAAATAAAAGGAATGTCCCAAAG/ATCTGTTTTTATTTTTTCTTCTTTCAAGA...