Molecular Diagnosis of Mitochondrial Disorders

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**Case**

- **Clinical history:** Normal birth to unrelated Hispanic parents. Abnormal phenylalanine on newborn screening. Follow-up plasma amino acids showed elevated tyrosine, but normal phenylalanine while on a regular diet. At 7 wks he was noted to have conjugated hyperbilirubinemia, tyrosine and methionine high. He had significant failure to thrive associated with feeding difficulties and fat malabsorption.

- The progression of his liver disease with hypoglycemia and coagulopathy led to liver transplantation at 7 weeks of age. Blood mtDNA content was 62% of controls. Normal MRI of the brain

- His subsequent clinical course was dominated by hypotonia and psychomotor regression. He died at 23 months from a cardiac arrest.
Mitochondria

A mitochondrion (singular of mitochondria) is part of every cell in the body that contains genetic material. Mitochondria are responsible for processing oxygen and converting substances from the foods we eat into energy for essential cell functions. Mitochondria produces energy in the form of ATP, which is then transported to the cytoplasm of a cell for use in numerous cell functions.
Mitochondrial Functions

(A.) Glycolysis
- Glucose → Pyruvate → Lactate
- Promotes Glycogen
  - Carbohydrates
  - Dichloroacetate
- Inhibits Glycolysis/Promotes Lactate
  - Anaerobic environment
  - NADH
  - Acetyl CoA
  - ATP

(B.) Fatty Acid Oxidation
- Fatty Acids → Acylcarnitine
- Promotes Fatty Acid Oxidation
  - Carnitine
  - Fatty Meal
  - Prolonged Exercise
- Inhibits Fatty Acid Oxidation
  - Carnitine deficiency
  - MCAD/LCAD deficiency
  - Deficiencies of CPT/II
  - Carnitine Translocase deficiencies

(C.) Oxidative Phosphorylation
- From glycolysis and tricarboxylic acid cycle NADH
  - I
  - Oxidative Phosphorylation
  - ATP
- From β-fatty acid oxidation FADH2
  - II
  - Oxidative Phosphorylation
  - ATP
- Inhibits oxphos (and increases NADH)
  - Sedation
  - Alcohol
  - Fever
  - Cold
  - Nucleoside analogs
  - Infection

Mitochondrial Functions

- >1500 genes
- Nuclear DNA
- mtDNA
- ATP generation
  - ATP production via oxidative phosphorylation
- Energy resource:
  - supplies 90% of energy for the body
Mitochondrial Genome

- Double stranded, circular 16.5Kb
- No intron, 80 - 93% coding gene
- No repeat
- Lack histone and DNA repair mechanism damage, mutations (free radicals)
- 37 gene: 22 tRNA, 2 rRNA & 13 protein
- Heteroplasmy
mtDNA Encodes for

- 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

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<th>mtDNA</th>
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<td>AUA</td>
<td>Met</td>
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Mitochondrial DNA Inheritance

Maternal Inheritance

sperm

ovum
Mitochondrial Genome

- Highly polymorphic
  - >1000 polymorphisms
    (http://www.mitomap.org/MITOMAP)
  - 200 mutations

Genealogy and Ancestry

Romanov Family
Mitochondrial Disease - Clinical Heterogeneous

Definition
Clinically heterogeneous disorders that are due to mitochondrial respiratory chain dysfunction, caused by mutations in the mtDNA OR nDNA that encodes for any of the following:

1. structural protein of the OXPHOS complexes
2. protein required for assembly of OXPHOS complexes
3. proteins involved in mtDNA translation
4. proteins involved in mtDNA maintenance
5. proteins involved in mitochondrial fusion and fission

Mitochondrial Disease Prevalence

- Incidence of 1:5000 live births (Smeitink 2006)
- 20% are due to mtDNA mutations (200 pathogenic mutations), 80% to nuclear DNA mutations
Phenotype Recognition

- Very Difficult Disorders to Diagnose
- Several hundred clinical presentations
- Frequency: as low as 1:8000 (1:3000)
Mitochondrial disorders

- Multisystem or single organ

- Affect organs with high energy usage
  - Brain and neurons, heart, retina, muscle, liver, kidneys, respiratory system, endocrine organs

- Wide scope of presentation > family members; same mutation
# Mitochondrial Disease: Clinical Heterogeneous

<table>
<thead>
<tr>
<th>Organs</th>
<th>Presentations</th>
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<tr>
<td>Nervous system</td>
<td>visual/hearing loss, fit, myoclonus, migraine, stroke, encephalopathy, focal deficit, ataxia, hypo/hypertonia, peripheral neuropathy, antibiotic-induced ototoxicity, cataracts, mental retardation/degeneration</td>
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<tr>
<td>Musculoskeletal</td>
<td>Myopathy, rhabdomyolysis, ptosis, exercise intolerance, ophthalmoplegia, chronic fatigue</td>
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<tr>
<td>Cardiac</td>
<td>Cardiomyopathy, conducting defect</td>
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<tr>
<td>Endocrine</td>
<td>Endocrine diabetes, pancreatic insufficiency, hyperthyroidism, systemic lipomatosis</td>
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<tr>
<td>Blood and bone marrow</td>
<td>Sideroblastic anemia, pancytopenia, petechia, acrocyanosis</td>
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<tr>
<td>Liver</td>
<td>Hepatitis, cirrhosis</td>
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<tr>
<td>GIT</td>
<td>diarrhea, dysmotility, intestinal obstruction, FTT, vomiting</td>
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</tbody>
</table>
Mitochondrial Diseases Prevalence

- Minimum prevalence of pathogenic mtDNA mutations: 1:8000
- Maternal inheritance
- Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Myoclonic epilepsy with ragged red fibers (MERRF)
- Neuropathy, ataxia, retinitis pigmentosa (NARP)
- Deafness
- Leber hereditary optic neuropathy (LHON)
- Kearnes Sayre syndrome (KSS)
- Pigmentary retinopathy, chronic progressive external ophthalmoplegia (CPEO)

Chinnery et al. Lancet 2000
Mitochondrial Mutations Associated with Disease

- MELAS: 3243A>G
- LHON: 3460G>A
- MERRF: 8344A>G
- NARP: 8393T>G
- LHON: 11778G>A
- LHON: 14484T>C

Areas deleted in KSS
Detection of NARP Mitochondrial Point Mutation (ATPase VI 8993 T→C or G) by PCR-RFLP

U = Uuncut, no Mspl
C = Cut, with Mspl

The presence of the mutation creates an Mspl restriction enzyme site in the amplicon.

551 bp → Agarose gel → 345 bp
206 bp

Mutation present
Detection of KSS Mitochondrial Deletion Mutation by Southern Blot

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

- **M** = Mutant
- **+** = Normal
- **U** = Uncut, No \textit{Pvu}II
- **C** = Cut with \textit{Pvu}II

**Autoradiogram**

16.6 kb (normal)

Heteroplasmy

Deletion mutant
New Class of Mitochondrial Disease

- Nuclear genes
  - Nuclear genes which affect mtDNA levels: POLG; MPV17, EFG1
  - Nuclear gene which affects mito protein assembly: SURF1

- Inheritance:
  - Autosomal Recessive
  - Autosomal Dominant
  - X-linked
Diagnostic Criteria in Adults and Children

**Major Criteria**
- Clinical presentation, ↑lactate
- Histology
  - >2% RRF
  - 2-5% COX-negative fibers
- Enzymology
  - <20% RC in a tissue or <30%RC>=2 tissues
  - <30# RC in a cell line
- Functional
  - Fibroblast ATP synthesis rates >3 SD below normal
- Molecular
  - Nuclear or mtDNA mutation of undisputed pathogenicity

**Minor criteria**
- Clinical presentation, +/-
- Histology
  - >2% RRF age 30-50y
  - >2%SSMA (<16y)
  - Abnormal mitochondrial (EM)
- Enzymology
  - 20-30% RC in a tissue or 30-40% RC>=2 tissues
  - 30-40% RC in a cell line
- Functional
  - Fibroblast ATP synthesis rates 2-3 SD below normal
- Molecular
  - Nuclear or mtDNA mutation of undisputed pathogenicity

Bernier et al. Neurol 2002 59:1406-11
Red Ragged Fibers
ARUP Approach

- Next Generation Sequencing (NGS) (Shale Dame and Bob Chou)
  - Mitochondrial genome sequencing
  - 128 Mito Nuclear genes sequencing
  - Large deletions and duplications in mitochondrial genome and >100 nuclear gens by high density exonic CGH Microarray (Tracey Lewis)

- Point mutations and small ins/del
- Low heteroplasmy

- 20% of del in mito DNA
- 5-10% large del/dup in nuclear genes
Mitochondrial Genome NGS Assay

- Long range PCR (LRPCR) enrichment
- Library prep, barcode/pooling
- Single end HiSeq reads (100bp)
- CLCBio data analysis
Mito Genome NGS: Long Range PCR Enrichment
Illumina Library Prep

- Sonicated samples are placed in the SPRI-TE for library prep
  - Blunted
  - Adenylated
  - Ligation of adapters
- Post SPRI-TE, samples are PCR amplified with multiplex PE primers and one of 12 index primers (4, 6 and 8 samples pooling)
Illumina

Sequencing by reversible dye terminators

Adaptor modified DNA strand hybridized to oligonucleotide anchor

Cluster generated by bridge amplification

Denature, Cleave

Sequencing of forward strands

Template Strand

Incorporation

Fluor cleavage

Block removal

Sequencing by reversible dye terminators
Sequencing

4 reversible dye terminator NTPs \rightarrow \text{Incorporate one nt, Image} \rightarrow \text{Terminator & dye cleaved}

Three step cycle

Read 100 bases
Mitochondrial Genome NGS

SPRI-TE

Illumina HiSeq 2000

Day 1

- Long Range PCR

Days 2-3

- Amplicons equimolar pooled
- SPRI-TE and index

Days 4-9

- Illumina HiSeq
- Sequence Alignment

Days 10+

- Variant calls
Data Analysis

- Raw HiSeq files converted to FastQ
- CLCbio
  - Alignments
  - SNP/DIP calls
  - Sequence annotation
    - Reference sequence dependant
    - Manual
- Data report
  - Excel spreadsheet and .html
Mitochondrial Genome NGS

- CLCbio Genomics Workbench

Mito genome: 16.5KB

Genes

mt genome NC_012920

Coverage: 1827
Mito Genome NGS Data Analysis

- **Alignment/variant call parameters**
  - Aligned to fully annotated reference sequence
  - Minimum coverage: 200-fold
  - Minimum minor allele frequency: 3%
  - Report nonsynonymous single nucleotide polymorphisms (SNP) and deletion/insertion polymorphisms (DIP) variants
  - Filter out common polymorphisms
Sample ID: NA11605
Fastq file: NA11605_7_1
Start date of run: 022111
Date of analysis: 03082011
Flowcell ID: 81C03ABXX
Cluster kit ID: 0745788 L/N 5836181
Index sample, Single read
Technician: S. Dames

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<th>Frequencies</th>
<th>Coverage</th>
<th>Clinical Significance</th>
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### Results

All SNPs/DIPs filtered >200-fold coverage and > 10% heteroplasmy, 16 variants calls


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Mitochondrial Genome NGS Validation

Results:

- Reproducibly detected all reported SNP/DIP variants in Coriell samples (8/8)
- Currently sequencing 18 additional samples
- Can detect low levels of heteroplasmy (<10%)
  - All selected variants Sanger verified with >30% heteroplasmy
  - Low level heteroplasmy has been verified by “variant-specific” PCR
Mitochondrial 128 Gene Nuclear Panel

- RainDance enrichment
- Library prep
- Single end HiSeq reads
- CLCBio data analysis
### mt 128 Gene Nuclear Panel

<table>
<thead>
<tr>
<th>Mito Nuclear Genes</th>
<th>Number of Genes</th>
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<tbody>
<tr>
<td>Mitochondria DNA integrity</td>
<td>12</td>
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<tr>
<td>Complex assembly</td>
<td>22</td>
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<tr>
<td>Fatty acid metabolism</td>
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<td>Coenzyme Q10</td>
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<tr>
<td>Respiratory chain disorders</td>
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<tr>
<td>OXPHOS subunits</td>
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<td>Carriers</td>
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<td>Mitochondria maintenance</td>
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RainDance Library Prep

RDT Reagent Inputs
- Primer Libraries
- Genomic DNA Template

RDT 1000 Consumables
- RDT 1000 Input/Output Vials
- RDT 1000 Chip

Courtesy of Take Ogawa, RainDance
RainDance RDT 1000-Emulsion PCR

Advantages
- Evenness of PCR
- Specific primer, No pseudogene amp

Limits
- Primer design
- Chip expensive

Example: 128 nuclear genes for mito disorder in 1304 amplicons

Courtesy of Take Ogawa, RainDance
mt 128 Nuclear Gene Panel

RainDance Enrichment

- Automated
- Emulsion PCR based
- 1,304 Amplicons
- All exons and splices site junctions
- Single tube amplification

Days 1-3

Sonicate + RainDance

PCR amplify → ligation

Days 4

Sonicate and SPRI-TE

Days 5-10

Illumina HiSeq

Sequence Alignment

Days 11+

Variant calls
Data Analysis

- Whole genome, NG, artificial chromosome, or masked genome?
- Quality metrics
  - Minimum coverage
  - Q scores
  - Heterozygous frequencies
  - Seed/window length
  - Cost to open gaps
Masked alignments are useful
Polymorphism database

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<td>CDS filtered</td>
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<td>nonsynonymous filtered</td>
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Data Analysis

- Minimum coverage: 50-fold
- Q score: 30
- Heterozygous allele frequencies: 30-70%
- Seed/window length: +/- 11

Are these quality metrics reasonable?
mt 128 Nuclear Gene Panel

- CLCbio Genomics Workbench

Gene

Coverage

- Alignment/variant call parameters:
  - Aligned to dbSNP132 annotated and masked reference sequence
  - Minimum coverage: 50-fold
  - Heterozygous allele frequency range: 30-70%
  - Report all CDS SNP/DIP variants
  - Filter out common polymorphisms
# Mito Nuclear Gene Panel - Results

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<th>Gene</th>
<th>Reference</th>
<th>Variants</th>
<th>Allele</th>
<th>Frequencies</th>
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<th>Amino Acid Change</th>
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<td>rs2286963</td>
<td>Lys333Gln</td>
<td></td>
<td>Clinical source, LCAD DEFICIENCY</td>
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<tr>
<td>DBT</td>
<td>100672060</td>
<td>C</td>
<td>100</td>
<td>2377</td>
<td>rs12021720</td>
<td>Ser384Gly</td>
<td></td>
<td>Clinical source, MAPLE SYRUP URINE DISEASE, INTERMEDIATE, TYPE II</td>
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<tr>
<td>NDUFV2</td>
<td>9117867</td>
<td>T/C</td>
<td>50.6/49.3</td>
<td>2767/2696</td>
<td>rs906807</td>
<td>Val29Ala</td>
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<td>Clinical source, VARIANT OF UNKNOWN SIGNIFICANCE (PD)</td>
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<tr>
<td>TRMU</td>
<td>46731689</td>
<td>G/T</td>
<td>53.3/46.7</td>
<td>340/298</td>
<td>rs11090865</td>
<td>Ala10Ser</td>
<td></td>
<td>Clinical source, DEAFNESS, MITOCHONDRIAL, MODIFIER OF [TRMU, 28G-T, ALA10SER]</td>
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<tr>
<td>ETFDH</td>
<td>159603550</td>
<td>C/T</td>
<td>52.9/47.1</td>
<td>880/782</td>
<td>not reported</td>
<td>Leu127Phe</td>
<td>CM093456</td>
<td>Leu127Arg and Leu127His are disease causing</td>
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<td>ETFDH</td>
<td>159605751</td>
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<td>59.3/40.6</td>
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<td>Leu138Arg</td>
<td>CM024518</td>
<td>disease causing mutation</td>
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<td>HADHB</td>
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<td>POSS1</td>
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<td>T/C</td>
<td>64.5/34.2</td>
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<td>59.1/38.9</td>
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<td>Asp51Ala</td>
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<td>53.2/46.7</td>
<td>1431/1256</td>
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<td>Gly105Gly</td>
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<td>unknown</td>
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<tr>
<td>SDHA</td>
<td>225593</td>
<td>C/T</td>
<td>51.0/49.0</td>
<td>1506/1446</td>
<td>not reported</td>
<td>Tyr124Tyr</td>
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<td>unknown</td>
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<tr>
<td>SDHA</td>
<td>229645</td>
<td>G/A</td>
<td>55.3/44.7</td>
<td>1177/917</td>
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<td>Met142Val</td>
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<td>225646</td>
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<td>Val66Leu</td>
<td>rs6818847</td>
<td>polymorphism</td>
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<td>SDHA</td>
<td>225656</td>
<td>G/T</td>
<td>54.8/45.1</td>
<td>2715/2235</td>
<td>rs7991</td>
<td>Thr14Thr</td>
<td>rs7991</td>
<td>polymorphism</td>
</tr>
<tr>
<td>COX10</td>
<td>14095309</td>
<td>G</td>
<td>76.8</td>
<td>3886</td>
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<td>Pro233Pro</td>
<td>rs2230354</td>
<td>polymorphism</td>
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<td>COX10</td>
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<td>52.1/47.9</td>
<td>2152/1979</td>
<td>rs34362247</td>
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<td>COX15</td>
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<td>G</td>
<td>100</td>
<td>421</td>
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<td>Phe374Leu</td>
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<tr>
<td>COX6B1</td>
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<td>C/T</td>
<td>54.8/45.1</td>
<td>2715/2235</td>
<td>rs7991</td>
<td>Thr14Thr</td>
<td>rs7991</td>
<td>polymorphism</td>
</tr>
<tr>
<td>CPT1A</td>
<td>68549340</td>
<td>G</td>
<td>99.9</td>
<td>3920</td>
<td>rs2228502</td>
<td>Phe417Phe</td>
<td>rs2228502</td>
<td>Minor allele frequency &lt;1%</td>
</tr>
</tbody>
</table>

Two mutations found in ETFDH gene: Leu127Phe and Leu138Arg disease causing Multiple Acyl-CoA Dehydrogenase Deficiency, MADD

After filtered synonymous, known SNPs/DIPs and intronic sequence; 27 variant calls left
Copy Number Aberration in Mitochondrial Diseases

- Majority of the mutations: point mutations

- However, Deletions/ Duplications are:
  - 20% of mtDNA mutations
  - 5-10% of nuclear genes
Gene content:

- Mitochondrial DNA
- 101 nuclear genes:
  - 22 for OxPhos subunits
  - 11 genes for OxPhos assembly factors
  - 29 enzymes
  - 9 transcription/translocation
  - 11 carriers
  - 19 for mtDNA maintenance/mitochondria biogenesis
Mito aCGH design

- Mito genome: 16.5KB

- 101 Nuclear Genes:

Roche/NimbleGen
3X720K
1. Random fragmentation of DNA
2. Cy3 & Cy5 random prime label
3. Combine labeled test and reference DNA and hybridize
4. Scan array, Cy3 and Cy5 channels
5. Extract images and normalize Cy dye intensities
6. Calculate Log₂ Ratio and perform segmentation analysis
View the Probe Designs in SigMap

Chr 1 12 genes

SDHB gene

Exon1  Intron1  Exon2

Exonic level of SDHB gene
Gene Examples, SLC22A5

Gene = 25.9 Kb
Region = 35.9 Kb

Total Probes = 4724
Average Probe Spacing = 7.5 bp

Size, bp
Exonic Probes
656  103  154  171  126  100  214  182  135  1429
Exonic Probes
69  17  26  26  22  17  35  31  22  221

Size, bp
Intron Probes
5kb  8011  5664  1025  1524  1768  1667  1527  1059  372  5kb
Intron Probes
535  1096  836  157  193  284  278  249  177  62  372
Mito aCGH results - Chr2 Nine Genes
Deletion of **BCSL1** gene

<table>
<thead>
<tr>
<th>CHR</th>
<th>START</th>
<th>STOP</th>
<th>SIZE</th>
<th>PROBES</th>
<th>LOCATION</th>
<th>LOG2_RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr2</td>
<td>219231599</td>
<td>219237104</td>
<td>5505</td>
<td>385</td>
<td>BCSL1 entire</td>
<td>-0.63</td>
</tr>
</tbody>
</table>

**Table:**

- **CHR**: Chr2
- **START**: 219231599
- **STOP**: 219237104
- **SIZE**: 5505
- **PROBES**: 385
- **LOCATION**: BCSL1 entire
- **LOG2_RATIO**: -0.63

**Graph:**

- **chr2**
- **BCSL1**
- **Genes Exon Intron**
- **Segmental Duplications**
- **Structural Variants**

- **GM10918_ALigred_unavg_segMNT**: 0.00 (Del)
- **SampleGM07890_ALigred_unavg_segMNT**: 0.00 (Nor)
- **Sample83680_ALigred_unavg_segMNT**: 0.00 (Nor)
BCSL1 Mutations Causing Mitochondrial Disease

- BCSL1 gene encoding proteins necessary for assembly of Complex III in OXPhos

- Patients with BCSL1 mutations:
  - Mitochondrial encephalomyopathies
  - GRACILE syndrome = growth retardation, aminoaciduria cholestasis, iron overload, lactic acidosis, early death
## Deletion of the TIMM8A and NDUFA1 Gene

<table>
<thead>
<tr>
<th>CHR</th>
<th>START</th>
<th>STOP</th>
<th>SIZE</th>
<th>PROBES</th>
<th>LOCATION</th>
<th>LOG2_RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChrX</td>
<td>100485999</td>
<td>118889999</td>
<td>344</td>
<td>344</td>
<td>TOMM8A and NDUFA1</td>
<td>-0.74</td>
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</table>

Confirmed by 44K Constitutional CGH array
TIMM8A in Mitochondrial Disease

- TIMM8A protein mediate the import and insertion of hydrophobic membrane protein into the mitochondrial inner membrane.

- TIMM8A mutation: a progressive neurodegenerative disorder (Mohr-Tranebjaeg syndrome).
Common CNS on Chr10

Common CNS, 1.6Kb in 1.5 probes, in PDSS1 gene
15 positive samples tested with 100% concordance

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chr</th>
<th>Gene(s)</th>
<th>Probes</th>
<th>Del/Dup</th>
<th>Size, kb</th>
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</thead>
<tbody>
<tr>
<td>559354</td>
<td>chr1</td>
<td>SDHB, PINK</td>
<td>2517</td>
<td>0.59</td>
<td>3634</td>
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<td>GM10918</td>
<td>chr2</td>
<td>BCS1L</td>
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<td>-0.63</td>
<td>6</td>
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<tr>
<td>130421</td>
<td>chr4</td>
<td>WFS1</td>
<td>2139</td>
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<tr>
<td>108377</td>
<td>chr6</td>
<td>BCKDHB</td>
<td>12280</td>
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<tr>
<td>300327</td>
<td>chr9</td>
<td>APTX</td>
<td>1449</td>
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<td>120968</td>
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<td>SURF1</td>
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<td>GM07890</td>
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<td>SLC25A15</td>
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<td>106561</td>
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<td>ATPAF2</td>
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<td>118417</td>
<td>chr18</td>
<td>NDUVF2</td>
<td>1765</td>
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<td>556003</td>
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<td>PDHA1, ABC</td>
<td>5537</td>
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<td>chrX</td>
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<td>700</td>
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aCGH for Mitochondrial DNA (Nexus)
SNPs Causing Probe Drop-off
aCGH for Mitochondrial DNA: D-Loop
Case cont:

- The patient’s sample has been tested for next generation sequencing for mitochondrial genome and 128 nuclear gene mutations.
- Two mutations have been detected in *DUGOK* genes.
### DGUOK Mut1:

<table>
<thead>
<tr>
<th>Mapping</th>
<th>Reference</th>
<th>Variation</th>
<th>Reference</th>
<th>Allele</th>
<th>Frequencies</th>
<th>Counts</th>
<th>Coverage</th>
<th>Amino</th>
<th>rs</th>
<th>Mutation</th>
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</thead>
<tbody>
<tr>
<td>DGUOK</td>
<td>74177859</td>
<td>SNP</td>
<td>G</td>
<td>A/G</td>
<td>53.7/46.3</td>
<td>2695/2327</td>
<td>5023</td>
<td>Gln197Gln</td>
<td>not reported</td>
<td>MDS compound het with AKA R202TfsX13.</td>
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</table>

**c.591G>A**  
**p.Gln197Gln**  
**Splice site**
### DGUOK Mut 2

![Reference Seq]

- **c.605-606delAG**
  - **p.Lys201fs**

### Table:

<table>
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<tr>
<th>Mapping</th>
<th>Reference</th>
<th>Variation</th>
<th>Reference</th>
<th>Allele</th>
<th>Frequencies</th>
<th>Counts</th>
<th>Coverage</th>
<th>Amino</th>
<th>rs</th>
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<td>AG/AG</td>
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<td>Lys201fs</td>
<td>not reported</td>
<td>MDS. AKA R202TfsX13. Introduces stop codon at aa position Glu214Ter (alt trans VCLKTVPEGQGGERN*)</td>
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</table>

Courtesy of Shale Dames
Conclusions

- Next generation sequencing technology provides opportunities for mutation detections in large gen panel
- The mitochondrial genome and 128 nuclear panel has been developed and will offer as the first clinical NGS assays in ARUP
- The NGS assay in accompany with aCGH for deletions and duplication will improve the sensitivity of the test
- Variants detected need confirmation and causality needs evidence
- Clinical and family information is critical in assessing significance
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- Kimberly Walker

ARUP Institute for Clinical & Experimental Pathology