Nouvelles méthodes de diagnostic génétique des maladies mitochondriales
Mitochondrial disorders

1/8000 live birth

Primary mitochondrial disorders
  Mutations in 80 nuclear genes
  Mutations in 13 mitochondrial genes
  (cf V. Procaccio, V. Paquis)

Secondary mitochondrial disorders
  viral infection
  secondary RC deficiency (β oxidation)
  false diagnosis (cf M. Rio)

1000-1500 genes encoding mitochondrial proteins
Genetic diagnosis in mitochondrial disorders

Respiratory chain deficiency
  CI deficiency → CI subunits
  → CI assembly genes

Brain MRI
BN-PAGE

Clinical presentation
  Kearns-Sayre syndrome, Pearson syndrome → mtDNA deletion
  MELAS syndrome, diabetes-deafness → MELAS mutation

Liver failure + mtDNA depletion
no mtDNA depletion
Genotype/Phenotype correlations

Leigh syndrome
  CI deficiency (ND..., NDUF...)
  CII deficiency (SDHA)
  CIV deficiency (SURF1)
  combined deficiency

Liver insufficiency
  mtDNA depletion (DGUOK, POLG, PEO1)
  abnormal mt translation (TRMU, GFM1, TSFM)

POLG mutations
  PEO with multiple mtDNA deletion (AD)
  liver insufficiency with mtDNA depletion (AR)
228 Genes of Mitochondria-Localized Proteins Linked to Disease in Humans

Koopman et al, NEJM, 2012
Next Generation Sequencing (NGS)

Genome sequencing

Exome sequencing (3% of whole human genome)
- Mendelian disorders: mutations in genes encoding proteins
- Mutations usually disrupt protein coding sequences
- Rare non synonymous variants are predicted to be deleterious

Targeted genes sequencing

Human genome sequence
- Identification of genes
- Polymorphism databases (dbSNP, 1000 genomes)

Hybridization capture to isolate targeted DNA Sequencing platform

Sequence alignment
Variant detection
Annotation
Targeted genes sequencing
MitoExome

4.1 Mb DNA
16.6 kb mtDNA
>1600 nuclear genes (all coding and untranslated exons + splice sites)

1 individual ➔~2 billion bases of sequence

42 unrelated patients with mitochondrial disease
Results filtered against SNP databases
Selection of variants that were rare and predicted to be protein modifying

1 patient: 7.2 kb mtDNA deletion
10 patients with mutations in genes previously shown to cause OXPHOS disease
13 patients have variants in new candidate disease genes (to be validated)
2 patients: AGK (acylglycerol kinase) mutations (validated)
1 patient: NDUFB3 mutations (validated)

Calvo et al, 2012
Exome sequencing in mitochondrial diseases

Mitochondrial disorders:
  Clinically heterogeneous
  Genetically heterogeneous
  1500 nuclear genes encoding mitochondrial proteins
    mtDNA
    ~80 nuclear disease genes
  30% of patients with known mutation

Disease causing genes encodes
  - mitochondrial proteins
  - non-mitochondrial proteins
Exome Sequencing in a consanguineous family

- Trunk hypotonia
- Choreo-athetotic movements
- Complex III and IV deficiency in liver

P1 and P2

48,068 SNPs and Indels

- Exclusion of known SNPs
- Exclusion of intergenic variants
- Homozygous variations
- Shared by P1 and P2

- 25 genes
  - One encoding a mitochondrial protein PNPT1

Sanger sequencing

SureSelect Human All Exon Kit, Agilent (Illumina HISEQ2000, Illumina)
Exome Sequencing in a non-consanguineous family

Exclusion of known SNPs
Exclusion of intergenic variants
Compound heterozygous variations
Shared by P1 and P2

12 genes

Sanger sequencing

Neuronal brain iron accumulation
Conditions de réussite de l’exome

ADN de parfaite qualité
2 enfants atteints de la même famille
1 enfant avec tour de génome
plusieurs patients avec le même phénotype
Plate forme d’analyse

Les sources d’échec sont nombreuses :
un ADN de mauvaise qualité
une mauvaise couverture
une mauvaise profondeur
une hétérogénéité clinique
une méconnaissance des fonctions mitochondriales
………..
Exons 1 are usually GC-rich
False positive, Low depth

Not detectable by Sanger sequencing!
Disease-causing genes identified by exome sequencing

**AARS2** (mitochondrial alanyl-tRNA synthetase)
Cardiomyopathy (Götz, AJHG, 2011)

**ACO2** (aconitase)
Hypotonia, seizure (Spiegel, Am J Hum Genet 2012)

**AGK** (mitochondrial acylglycerol kinase)
Cataract, cardiomyopathy, myopathy (Mayr, AJHG 2012)

**AFG3L2** (m-AAA protease)
Spastic ataxia-neuropathy syndrome (Pierson, Plos Genet 2011)

**EARS2** (glutamyl tRNA synthetase)
Leukoencephalopathy (Steenweg, Brain 2012)

**FARS2** (mitochondrial phenylalanyl transfer RNA synthetase)
Alpers syndrome, (Elo, Hum Mol Genet 2012)

**LARS** Leucyl tRNA synthetase
Hepatopathy (Casey, MGM 2012)

**MRPL3** (mt ribosomal protein)
Cardiomyopathy and mental retardation (Galmiche, Hum Mut 2011)

**MTFMT** (mitochondrial methionyl-tRNA formyltransferase)
Leigh syndrome (Tucker, Cell Metab 2011)

**MTO1** (mitochondrial translation optimization 1)
Cardiomyopathy (Ghezzi, AJHG 2012)

**NDUFB3**
IUGR, failure to thrive (Calvo, Sci Tr Med 2012)

**PNPT1** (PNPase)
hypotonia, choreo-athetotic movements (Vedrenne, AJHG 2012)

**SERAC1** (phospholipid remodeling gene)
Dystonia and deafness with Leigh-like syndrome (Wortmann, Nat Genet, 2012)
Next generation sequencing: Research or diagnosis?

<table>
<thead>
<tr>
<th>Method</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td>3000 €/sample</td>
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<tr>
<td>Exome</td>
<td>1000-2000 €/sample</td>
</tr>
<tr>
<td>Targeted NGS</td>
<td>1000 €/sample</td>
</tr>
</tbody>
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Identification of mutations

in a gene with known function

- Mutation of polymorphism?
- Sanger sequencing
- Segregation of the mutations in the family
- Functional complementation

in a gene with unknown function

- Sanger sequencing
- Segregation of the mutations in the family
- Functional complementation
- Function of the protein

No mutation identified
Clinical presentation

Brain MRI

Metabolic investigations

RC analysis
BN-PAGE analysis
Gene sequencing

Gene mutation

Homozygosity mapping
Exome sequencing