Animal models in mitochondrial disorders

Massimo Zeviani, MD, PhD

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Paediatric Patients with Respiratory Chain Deficiency

- mtDNA mutation: 18%
- nDNA mutation: 21%
- Unknown: 61%
Discovering new disease genes

**LINKAGE/HOMOZYGOSITY**

Families with (autosomal recessive) mitochondrial disorders

Linkage analysis or homozygosity mapping

Candidate genes

Mutational screening

**NGS**

Small families and single individuals

Exome sequencing

Coding variants

Filtering strategies

Candidate genes
In silico

Filtering genes

Structure

Integrated genomics

Phylogenetic analysis

Omic- analysis

Systems analysis
Cell models

- patients
- MEFs
- yeast
- recombinant E. coli
- iPS
- HeLa/cybrids
In vivo
In vivo pathogenesis

Experimental therapy

Protein factory

Clinical phenotype
Organ pathophysiology
Cell biology
Gene and protein interactions
Systems biology and omics

Gene replacement
Cell replacement
Organ replacement
Drugs
Other bioreactors

Mutant proteins
Tagged proteins
Complexes
Disease models

SURF1 constitutive KO
MPV17 constitutive KO
ETHE1 constitutive and conditional KO
COX15 constitutive KO (embryonic lethal) and conditionals (ACTA-COX15; GFAP-COX15)
SCO2 constitutive KO
NDUFS1 constitutive KO
NDUFS4 constitutive KO
FASTKD2 constitutive KO
TTC19 constitutive and conditional KO
PARP1 constitutive KO
FASTKD2 constitutive and conditional KO

Transgenic mice

PGC1α
TG CRE: general deleter
ALB CRE: liver specific
MYOD CRE: (muscle specific, early expressed)
ACTA CRE: (skeletal muscle specific)
TG FLPe (general deleter)
GFAP-CRE: (brain specific)
AOX (knockin in locus ROSA26)
NDI1 (knockin in locus ROSA26)
Ethylmalonic Encephalopathy (EE)

CNS
- early-onset hypotonia, developmental delay
- later spasticity, then global failure

Vascular system
- acrocyanosis
- petechiae, microematuria, internal bleedings

Gastrointestinal system
- chronic diarrhoea

Biochemistry
- COX deficiency
- lactic acidosis
- ethylmalonic aciduria

![Brain MRI Image](image1.png)
![Skin Lesion Image](image2.png)

![Chemical Reaction Diagram](image3.png)
**ETHE1** is the EE disease gene

- **HeLa**
  - merge
  - Mitotracker

- **ETHE1p**
  - hom.
  - sup.
  - mitos
  - matrix
  - memb

- **Works as a homodimer**
- **Binds 1 atom of iron/dimer**
Creating an *ETHE1*⁻⁻ mouse

**Genotypes**

- KO
- WT
- HET

**Behavior**

- Ethylmalonic acid (urine)
  - CTR mouse
  - KO mouse
  - CTR human
  - EE human

**Lifespan**

- Survival probability (%)
  - genotipo
    - ctr
    - ko
Respiratory chain complex activities

**p<0.0005
ETHE1-like proteins are present in operons containing Rhodanese-like proteins or as ETHE1/Rhodanese chimaeric proteins.
Sulfur metabolism

Thiosulfate in urines

and tissues

Szabo, Nat Rev Drug Discov 2007
H$_2$S concentration in tissues of *ETHE1* knockout mice

**MUSCLE**

CTR  | KO
---|---
p<0.0007

**LIVER**

CTR  | KO
---|---
p=0.00007

**BRAIN**

CTR  | KO
---|---
p<0.0016

H$_2$S concentration in tissues of *ETHE1* knockout mice.
H$_2$S is a metabolically active compound
H$_2$S inhibits COX

Tiranti et al, Nat. Med. 2009
A mitochondrial pathway for H$_2$S detoxification

Jorns MS et al, 2012
Liver homogenate

ETHE1

SDH

Liver

ETHE1

SDH

Purified

ETHE1

25kDa

Liver

-/-

+/+
A mitochondrial pathway for H$_2$S detoxification

Jorns MS et al, 2012
H$_2$S production: multiple sources

**Exogenous:**
a by-product of anaerobes in the large intestine

**Endogenous:**
a “gasotransmitter” produced in trace by many organs
METRONIDAZOLE, A BACTERICIDAL AGENT

N-ACETYL CYSTEINE: A GSH PRECURSOR

**Ethe1-/- mice**

<table>
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<tr>
<th>Treatment</th>
<th>Median Survival (Days)</th>
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<tr>
<td>Metronidazole</td>
<td>27</td>
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<tr>
<td>NAC</td>
<td>49</td>
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<td>Metronidazole+NAC</td>
<td>72</td>
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**Ethe1-/- children**

**PRE**

**POST**

**Diagram: Metronidazole and NAC survival curves.**
Liver-targeted AAV-mediated gene replacement
Genetic classification of OXPHOS disorders

Defects of Mitochondrial DNA

- Protein synthesis genes (rRNAs, tRNAs)
- Protein-encoding OXPHOS subunit genes
- Large deletions

Nuclear DNA mutations

- OXPHOS subunits
- OXPHOS assembly factors
- mtDNA maintenance and transcription
- Mitochondrial protein synthesis
- Biosynthesis of phospholipids or cofactors
- Mitodynamics, mitophagy, apoptosis
- Mitoprotein quality control
- Mitobiogenesis
- Mitochondrial detoxification mechanisms
Real-time quantitative PCR to estimate mtDNA depletion

mtDNA multiple deletions in muscle of MNGIE patients detected by Southern blot hybridization

<table>
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<th>MN1-1</th>
<th>MN2-1</th>
<th>MN2-2</th>
<th>MN3-1</th>
<th>MN4-2</th>
<th>MN7-2</th>
<th>Cont.</th>
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</table>
Why is mtDNA affected in MNGIE?

- thymidine
- deoxyuridine
- deoxycytidine
- dTMP
- dUMP
- dCMP
- mtDNA
- thymidine
- deoxyuridine
- deoxycytidine
- TK2
- dNT2
- TK1
- dTMP
- nDNA
- TP
- thymine
- uracil
- de novo synthesis
Vector: TBG-TYMP AAV2/8. Two-month old mice were treated at different doses:
- \(2 \cdot 10^{11}\) gc/kg (N=8)
- \(10^{12}\) gc/kg (N=8)
- \(2 \cdot 10^{12}\) gc/kg (N=6)
- \(10^{13}\) gc/kg (N=2)

Follow-up during 8 months after treatment and killed after 8 months. Endpoints:
- Monitoring of plasma nucleosides concentration
- Monitoring of plasma ALT levels
- TP activity in liver and other tissues (8 months)
- Nucleosides in plasma, liver and small intestine (8 months)

To be determined:
- Mitochondrial dNTP pools in liver and brain (8 months)
- Histological analysis of liver tissue (8 months)

By today, the following mice reached 8 months of life:
- \(2 \cdot 10^{11}\) gc/kg (N=5)
- \(10^{12}\) gc/kg (N=4)
- \(2 \cdot 10^{12}\) gc/kg (N=4)
- \(10^{13}\) gc/kg (N=2)
Liver TP activity
Nucleosides 8 months after treatment (intracardiac puncture)
Liver TP activity vs. plasma nucleosides after 8 months of treatment
AAV-mediated gene replacement

- Liver targeting of suitable Ethe1-expressing recombinant AAV vectors is specific
- Ethe1p liver-specific expression restores SDO activity
- *Ethe1*−/− mice treated with >$10^{10}$ AAV/gm of body weight show significant prolongation of the lifespan; the oldest treated animal is now 180 days old and well
- Liver-targeted, AAV-based gene therapy is potentially transferable to patients
- Suitable mitodisorders include conditions due to accumulation of toxic compounds such as Ethylmalonic Encephalopathy and MNGIE, as well as liver-specific mtDNA depletion
Path Towards Clinic Translation: Timeframe

1. Preclinical studies
   - Orphan Drug Designation
   - Pre-inquiry IMPD/IND (ISS: Italian Institute of Health)
   - Protocol assistance (EMA)

2. Clinical and molecular characterization of specific patients

3. GMP manufacturing of AAV

4. AAV8-ARSB GMP certified

5. GLP Safety/Tox studies

6. GMP vector

7. Clinical Trial (5 years)

Month:
- 0
- 6
- 12
- 24