DEMANDE DE RATTACHEMENT DES CENTRES DE REFERENCE CARAMMEL ET CALISSON A LA FILIERE FILNEMUS

Imagine Institute for Genetic Diseases
Hôpital Necker Enfants Malades and Université Paris Descartes
24 Boulevard du Montparnasse - 75015 PARIS arnold.munnich@inserm.fr

FONDATION imagine INSTITUT DES MALADIES GÉNÉTIQUES

Manifold missions of the Imagine Institute

Medical care, research and translational genomic within the same building

Rare genetic diseases (20-30.000 patients/year)

Innovative care: 22 clinical units (including genetics, neurology, metabolism)

Interaction between all pediatric specialists (neurology, MRI)

Rare disease reference centers (Carammel)

Research: 26 teams (including A. Rötig and J-M.Rozet)

Teaching: medical students, post-docs, MD PhD programs

Technology transfer to medical practice, partnership with companies

Ongoing clinical trials

Mitochondrial disorders

Human genome: 30,000-50,000 genes

1000-1500 genes encode mitochondrial proteins

Primary mitochondrial disorders

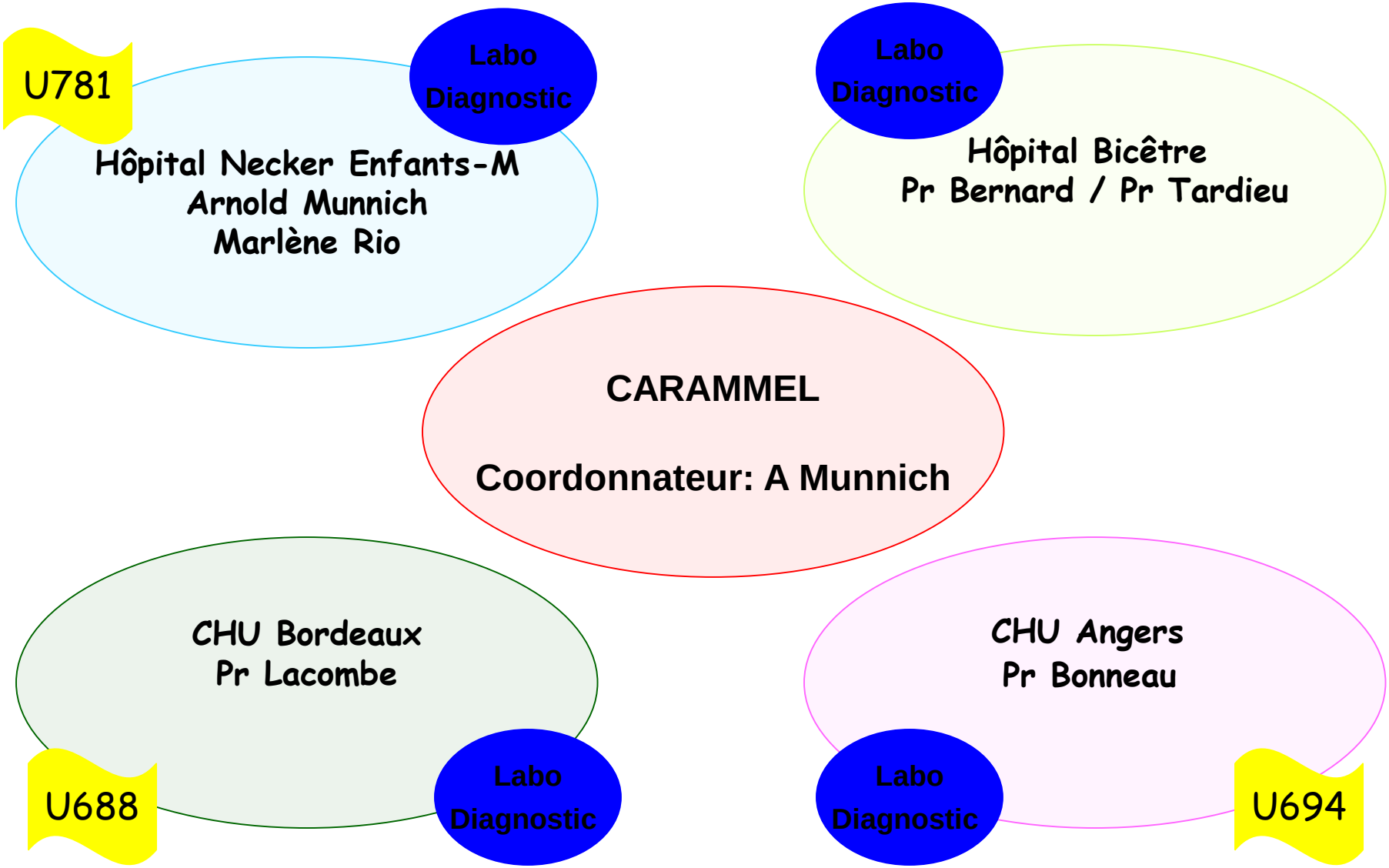
- **1/8,000 live birth**
- **all organs involved (nerves, muscles, CNS)!!**
- **mutations in 13 mitochondrial genes**
- **mutations in >200 nuclear genes**
- **the majority still to be found (lethality!)**

Secondary mitochondrial disorders

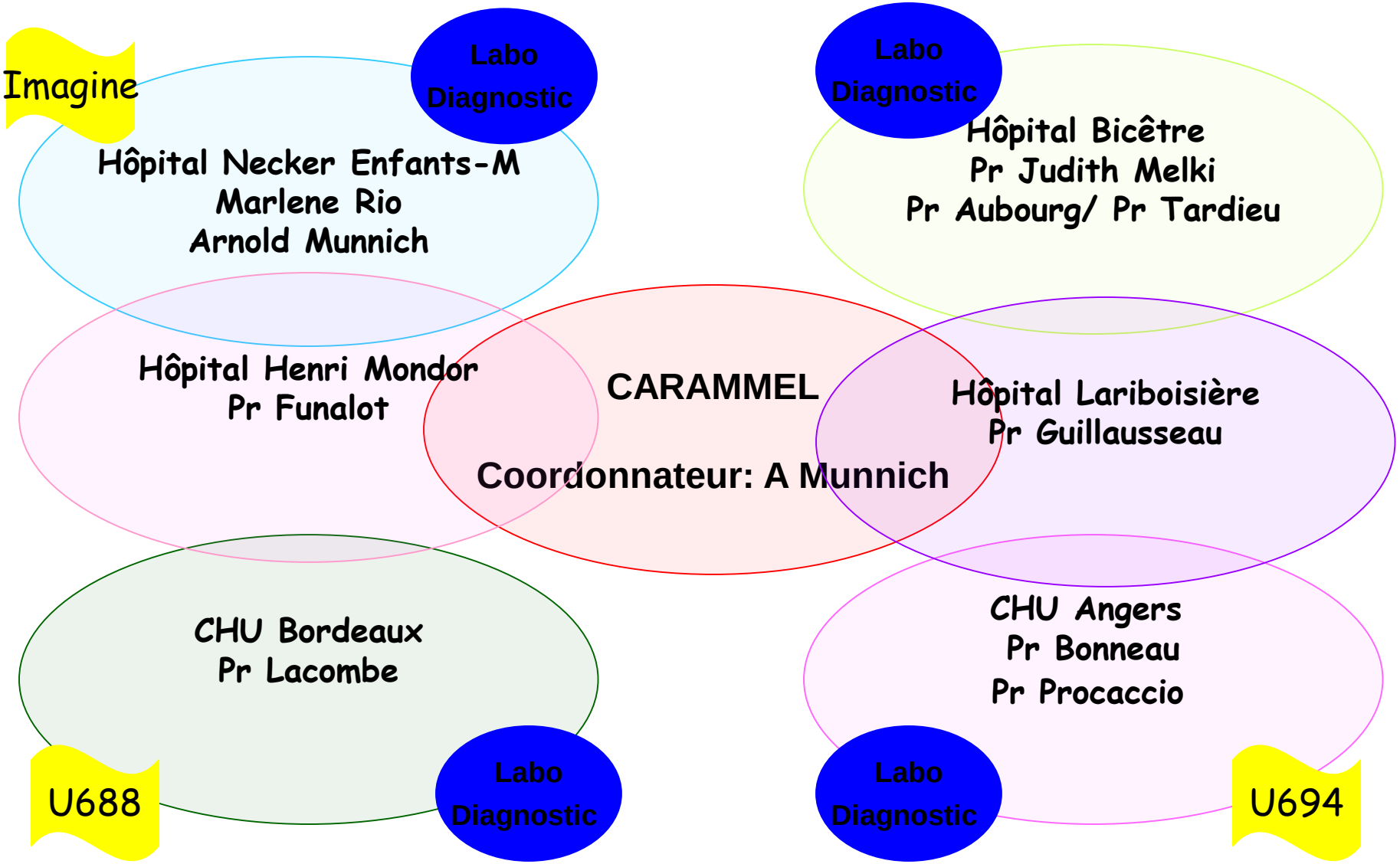
- **bacterial/viral infection**
- **secondary Oxphos deficiency (β oxidation)**
- **false positive diagnoses**

1200 patients in our database (mostly children)

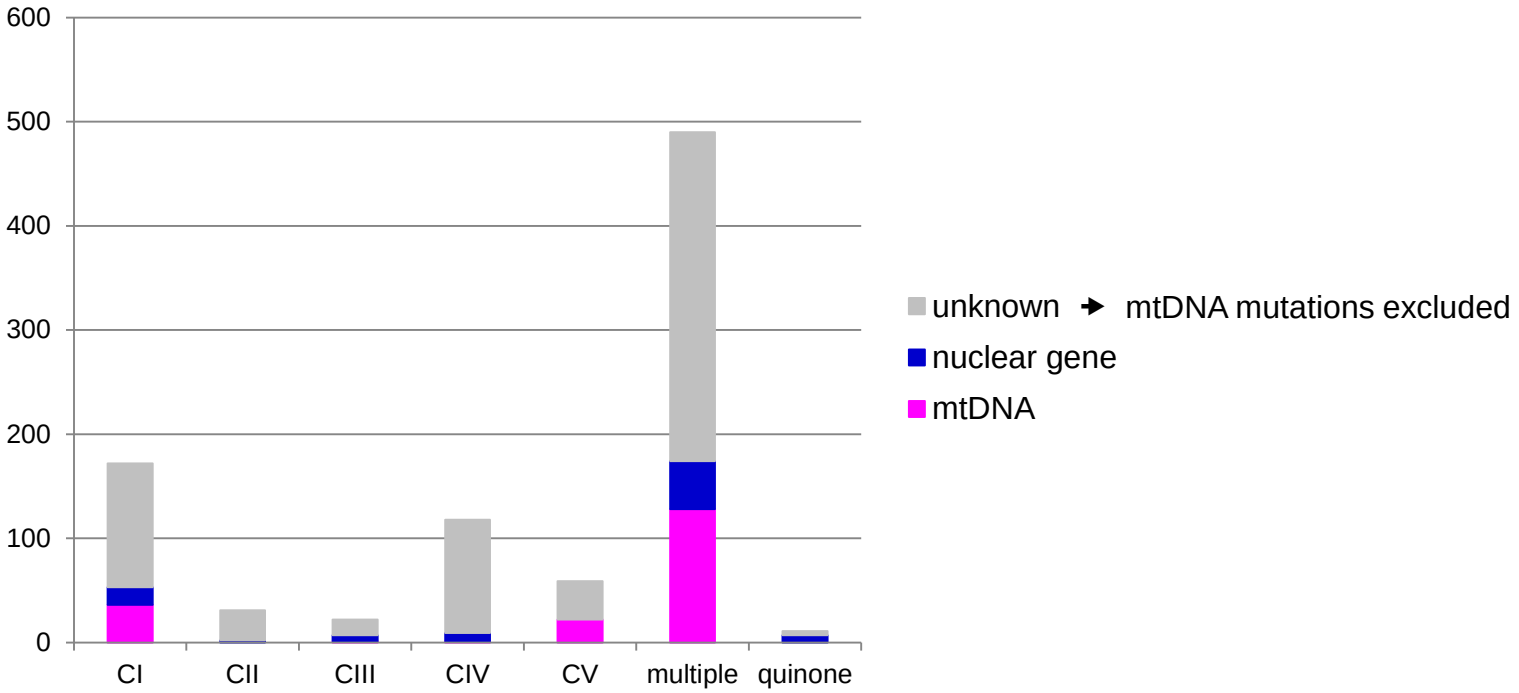
CARAMMEL - 2005



CARAMMEL - 2015

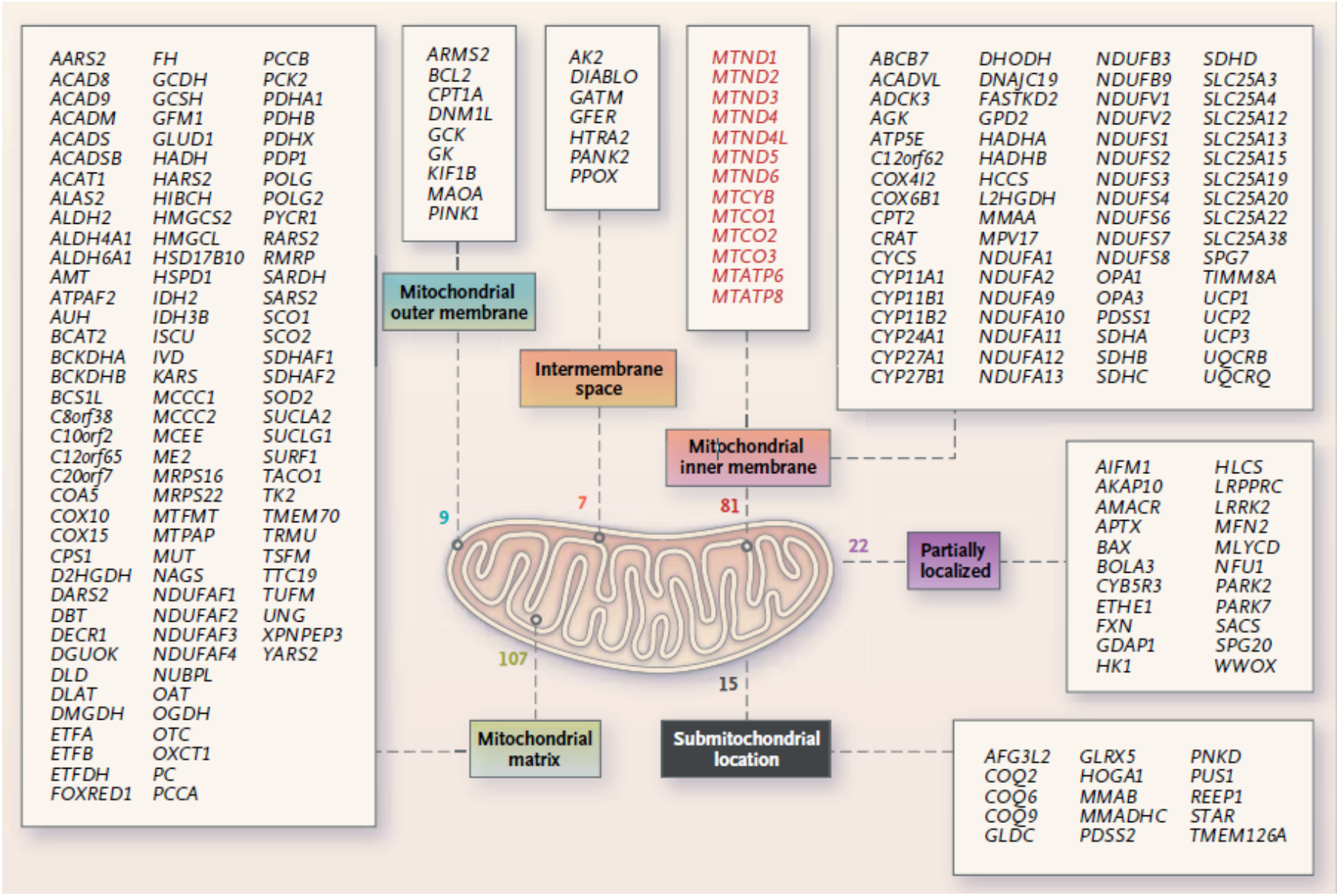


Mitochondrial disorders



Genetic heterogeneity
Clinical heterogeneity

More than 200 genes of mitochondria-localized proteins linked to disease in humans



Koopman et al, NEJM, 2012

Genotype/Phenotype correlations ? Yes and no !!

Kearns-Sayre syndrome, Pearson syndrome
(mtDNA deletions)

Liver insufficiency
mtDNA depletion (*DGUOK, POLG, PEO1*)

Barth syndrome
cardiolipin deficiency, *TAZ* mutations

Leigh syndrome
CI deficiency (mtND, nuclear *NDUF*… genes)
CII deficiency (*SDHA*)
CIV deficency (*SURF1*)
multiple oxphos deficiency

Absence of genotype/phenotype correlations: the rule, not exception

Private/unique disease genes

Pipeline from suspected mitochondrial disease phenotypes to the corresponding genotypes

The starting point is a sick child with unknown condition
Is it a mitochondrial disease?

- **clinical information, brain MRI and metabolic workup**
- **oxphos enzyme assessment (muscle, liver biopsy)**
- **respiratory chain assembly in cultured fibroblats**
- **mtDNA mutations/deletions/quantification**
- **DNA repository for all patients (cell repository)**

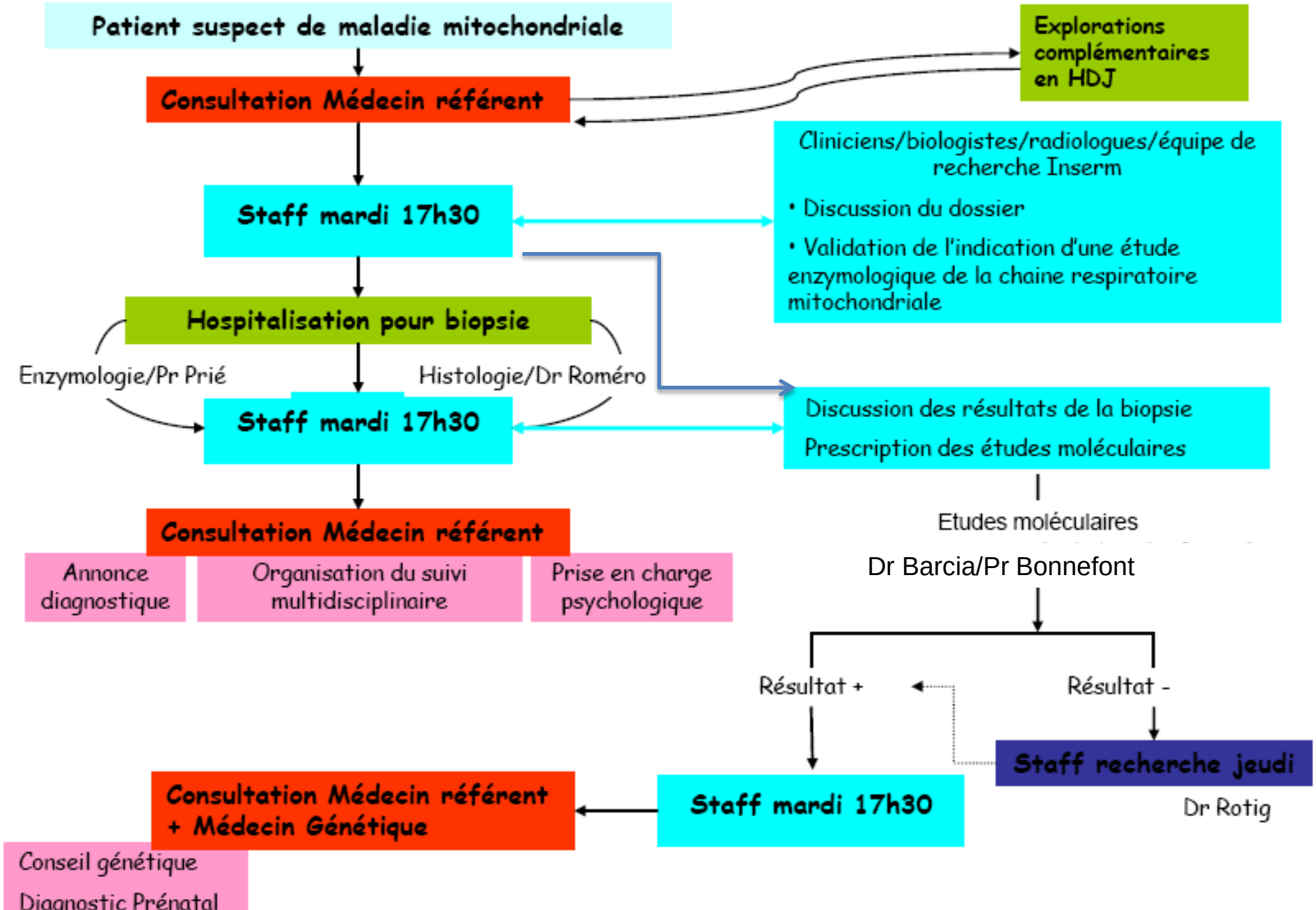
Sanger sequencing of a specific gene

Targeted sequencing
(known disease genes)

Exome sequencing
(all coding sequences)

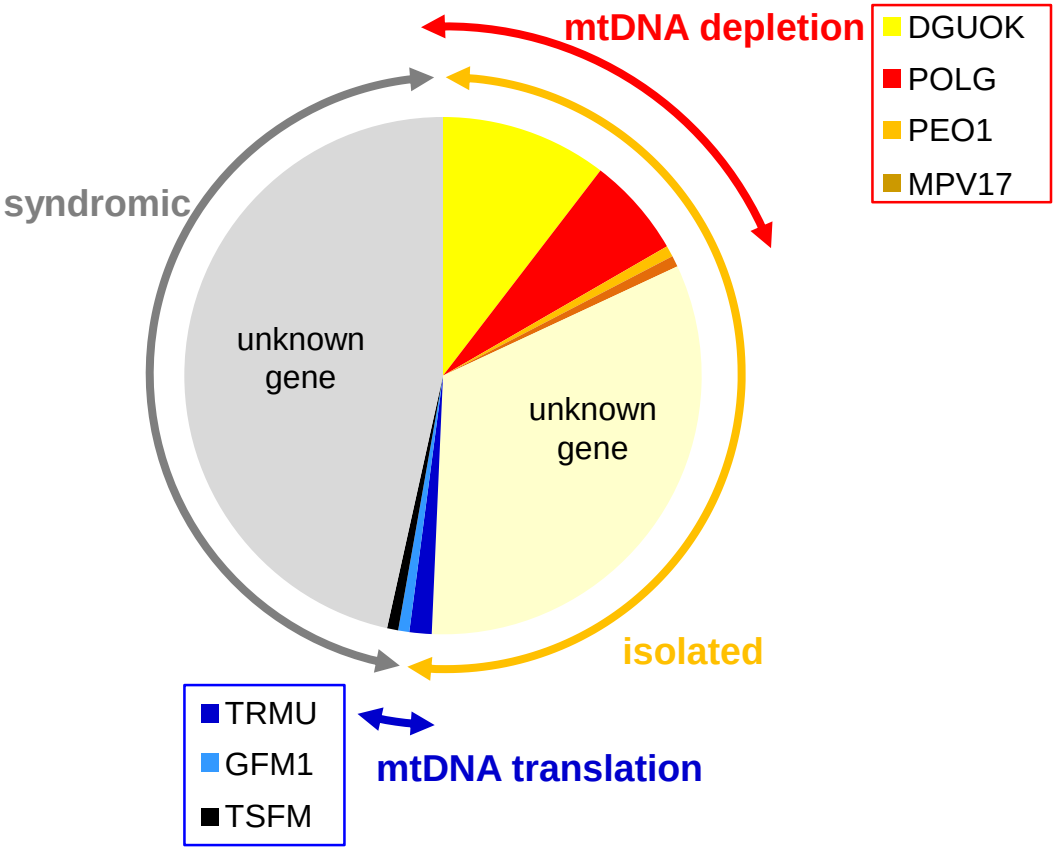
Next Generation Sequencing (NGS)

Site Necker – Parcours du patient



Targeted sequencing

Hepatic failure and oxphos deficiency



Targeted panel resequencing of known mitochondrial disease genes

- **FOR SERVICE ONLY, NOT RESEARCH, COVERED BY HEALTH SYSTEM**

- **RESEARCH TEAM LEADER HELPS TRANSLATION (A. Rötig)**

- **mtDNA mutations excluded by NGS**
- **215 nuclearly-encoded disease genes** (256 transcripts)
- **mutated in at least two unrelated families**
- **total probes size: 633 Kb**
- **XL: 9 genes, AD: 12 genes, AD/AR: 9 genes, AR: 154 genes, mtDNA**
- **minimum sequencing per sample (200X)**
- **100% of positive controls detected**

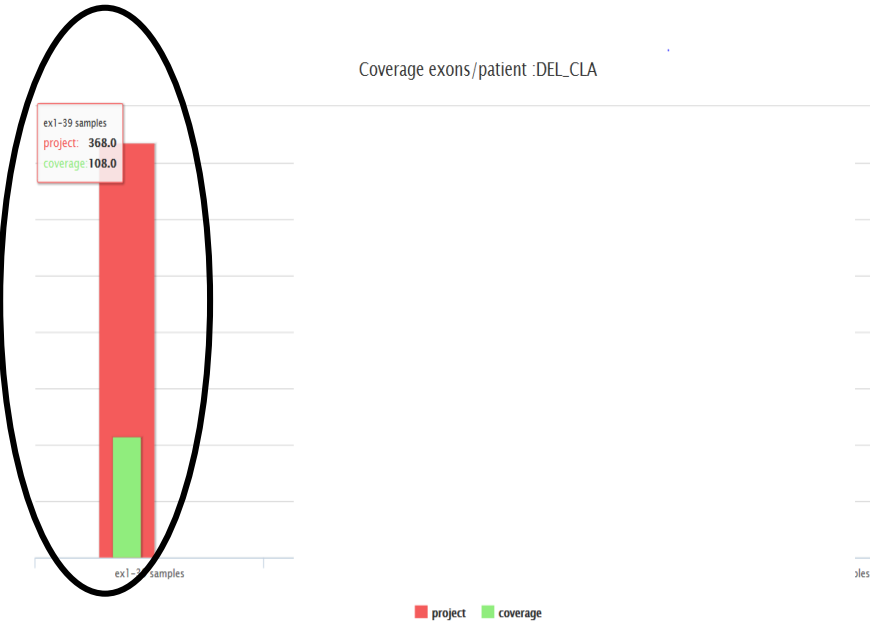
- **112 patients tested**
- **diagnostic yield: 35%**
- **25 different genes**
- **full costs≈ 1200 €/patient**

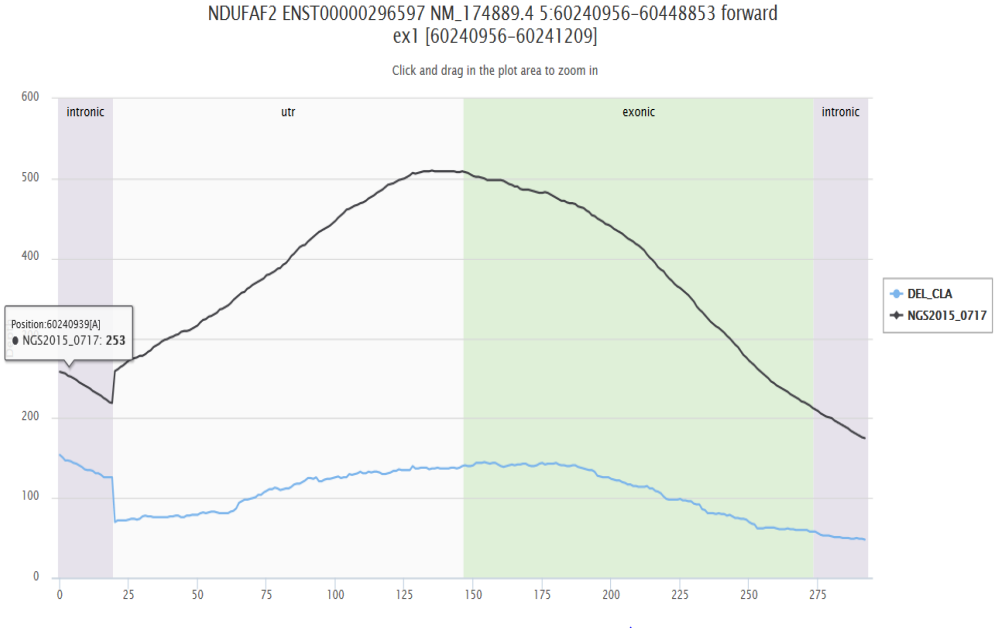
NDUFAF2 NM_174889

1. 5:60368963; c.139C>T; p.Arg47*
2. Possible deletion de l'exon 1

NDUFAF2

	exons	multi	CLA_CIN	CLA_SAN	DEG_MAT	DEL_CLA
ctrl-1_chr5_60240911	ex1	1	0.96	1.38	1	0.51
ctrl-1_chr5_60368861	ex2	1	1.02	1.04	1	0.93
ctrl-1_chr5_60394727	ex3	1	1.25	0.55	1.07	0.97
ctrl-1_chr5_60448490	ex4	1	1.03	0.62	1.08	1





Leigh/complexe 1

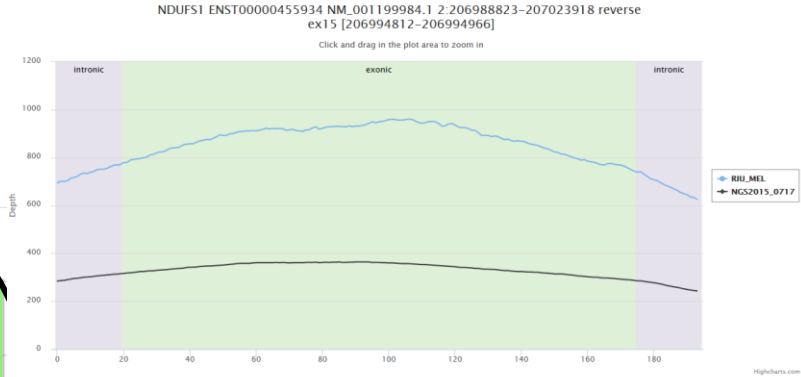
NDUFS1 NM_001199984

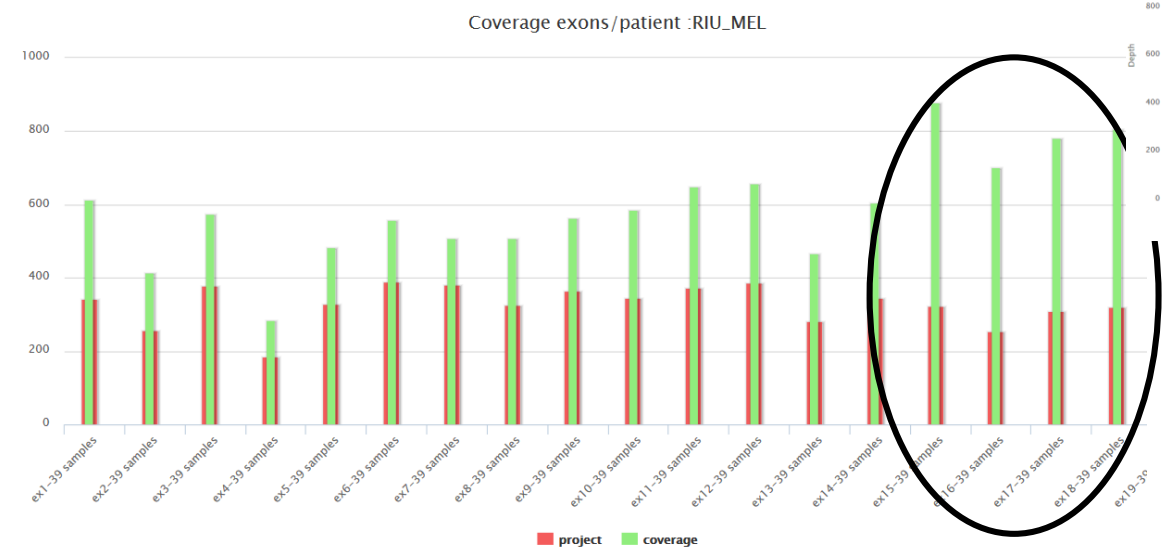
1. c.496C>T; p.Arg166*

2. Duplication Exons 15 à 19

NDUFS1

	exons	multi	OUK_MAL	PEL_CIN	PRO_MAR	RA_CHA	RIU_MEL
ctrl-1_chr2_206988181	ex19	1	0.97	0.95	0.93	0.96	1.43
ctrl-1_chr2_206991163	ex17;ex18	1	0.95	0.96	0.97	0.97	1.54
ctrl-1_chr2_206992472	ex16	1	0.98	0.98	0.98	0.98	1.62
ctrl-1_chr2_206994717	ex15	1	1	1.05	0.98	0.81	1.62
ctrl-1_chr2_206997577	ex14	1	0.96	1	1	1	1.04
ctrl-1_chr2_207003113	ex13	1	1	1.08	0.95	0.91	0.98
ctrl-1_chr2_207006569	ex12	1	0.99	0.9	0.92	0.98	1.04
ctrl-1_chr2_207007310	ex11	1	0.96	1.01	0.98	1.04	1.05
ctrl-1_chr2_207008651	ex10	1	0.95	0.9	0.94	1.02	1.02
ctrl-1_chr2_207009523	ex9	1	0.97	0.97	1.07	0.99	0.95
ctrl-1_chr2_207011583	ex8	1	0.9	0.97	1.05	1.02	0.93
ctrl-1_chr2_207012160	ex6;ex7	1	1.06	0.98	0.98	1.04	0.85
ctrl-1_chr2_207013646	ex5	1	1.06	0.97	0.99	0.95	0.9
ctrl-1_chr2_207014447	ex4	1	0.88	0.91	1.06	0.95	0.9
ctrl-1_chr2_207017052	ex3	1	0.99	0.93	0.98	1.04	0.92
ctrl-1_chr2_207018250	ex2	1	1.1	0.89	0.91	0.88	0.95
ctrl-1_chr2_207023721	ex1	1	0.92	1.07	0.92	0.93	1.09





Targeted panel resequencing of known disease genes

Ciliome (Ciliopathies)

Leber's Congenital Amaurosis

Hirschprung disease
~120 genes, ~100 samples

Callosome (corpus callosum defects)
~423 genes, ~100 samples

Alport syndrome, FSGS, PKD

SureSelect

Intellectual disabilities

Skeletal dysplasias

Osteogenesis imperfecta

Mitochondrial disorders

Epilepsies

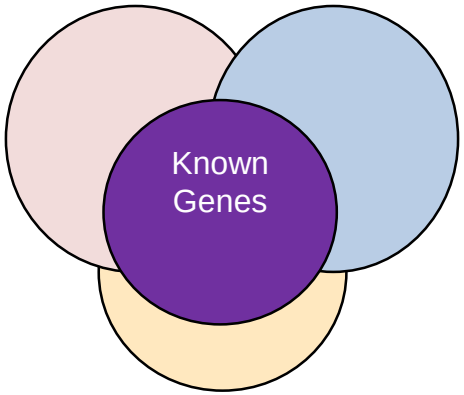
Genetic deafness

Brain malformations

Neural crest diseases

Ichtyoses

SCID



NGS resequencing of known disease genes for specific presenting symptoms

Molecular Genetic Service Lab of the Necker hospital

Targeted panel resequencing of known mitochondrial disease genes

SHARING RESOURCES FOR THRIFTY DIAGNOSTIC SERVICE TO PATIENTS

Access to NGS platforms:	**Imagine Research Institute**
Supervision:	**Researchers and Imagine BioInformatics**
Reagents:	**Health Care system/Hospital**
Technical/medical genetic staff:	**Health Care system/Hospital**

MUTUAL BENEFITS OF RESEARCHERS, DOCTORS, PATIENTS !!

Targeted panel resequencing of known mitochondrial disease genes

the challenge of thrifty diagnostic service to patients

to test the right patient, for the relevant genes, with the right interpretation

the right patient = collegial inclusion

the right genes = only validated, published genes on the panels

the right, dialectic interpretation

combination of expertises (clinical, service, research)

Exome Sequencing (NGS)

Exome sequencing by research teams (Agnès Rötig, or *Nini*, Claude Besmond)

- Mendelian disorders: mutations in genes encoding proteins
- Mutations usually disrupt protein coding sequences
- Rare non synonymous variants are predicted to be deleterious

Human genome sequence

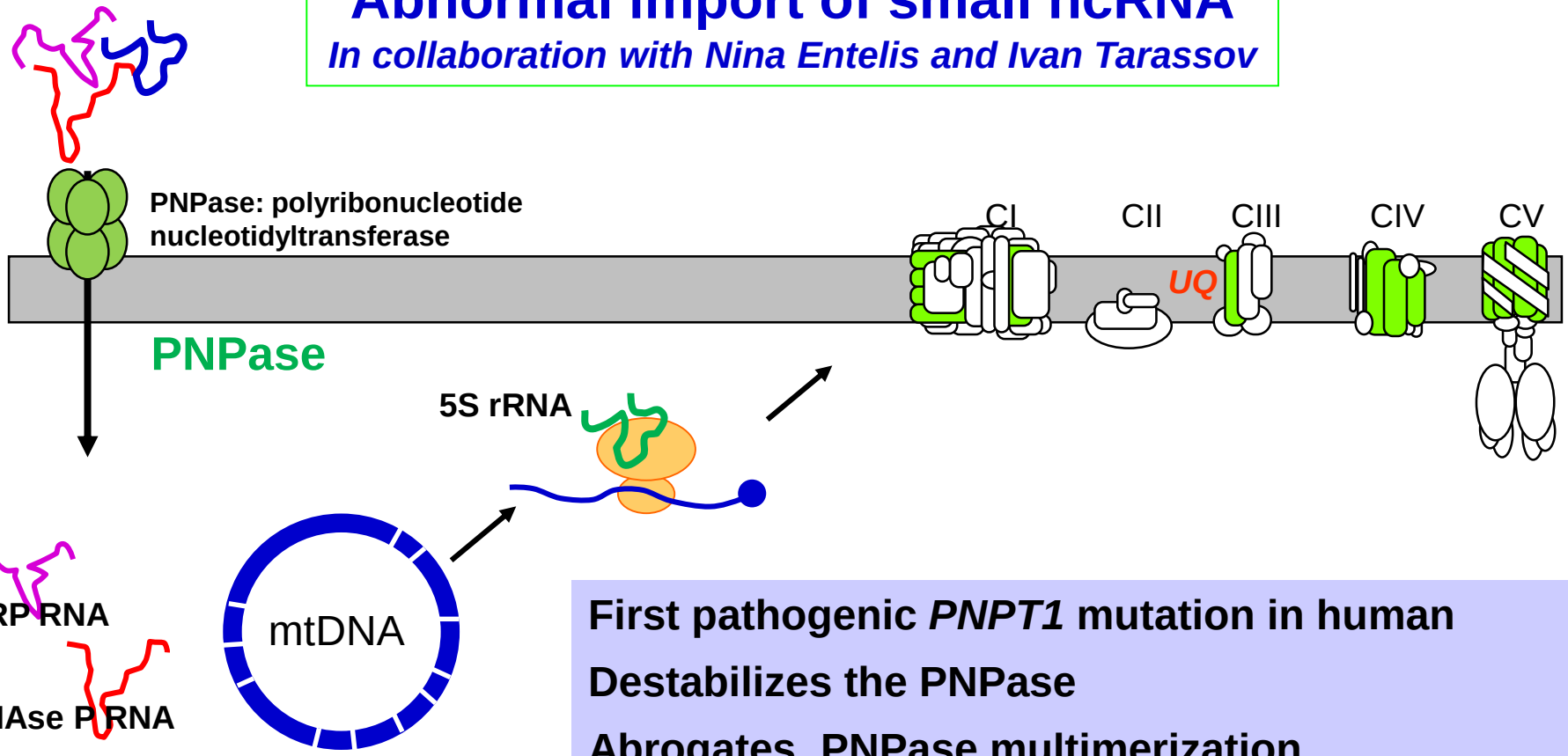
- Identification of genes
- Polymorphism databases (dbSNP, 1000 genomes)

Hybridization capture to isolate targeted DNA
Imagine Sequencing platform

Sequence aligment
Variant detection
Annotation

Abnormal import of small ncRNA

In collaboration with Nina Entelis and Ivan Tarassov



First pathogenic *PNPT1* mutation in human

Destabilizes the PNPase

Abrogates PNPase multimerization

Alters import of small non coding RNA

Alters mitochondrial translation

Clinical heterogeneity in PNPT1 mutations

- **choreo-athetotic movements**
- **myopathy+cataract**
- **isolated deafness**

Vedrenne et al Am J Hum Genet 2012

Novel disease-causing genes

AARS2	Cardiomyopathy (Götz, AJHG, 2011)
ACO2	Hypotonia, seizure (Spiegel, Am J Hum Genet 2012)
AGK	Cataract, cardiomyopathy, myopathy (Mayr, AJHG 2012)
AFG3L2	Spastic ataxia-neuropathy syndrome (Pierson, Plos Genet 2011)
CEP89	Intellectual disability, cystinuria, cataract, broad based walking pattern and deafness (van Bon, HMG 2013)
CLPP	Perrault Syndrome (Jenkinson, Am J Hum Gene. 2013)
COX7B	Microphthalmia with linear skin lesions (Zeviani AJHG 2012)
COX20	Ataxia and muscle hypotonia, Szklarczyk (Hum Mol Genet 2012)
DNA2	Progressive myopathy (Ronchi, Am j Hum Genet 2013)
EARS2	Leukoencephalopathy (Steenweg, Brain 2012)
FARS2	Alpers syndrome (Elo, Hum Mol Genet 2012)
LARS2	Perrault Syndrome (Pierce, Am J Hum Genet 2013)
IBA57	Myopathy and encephalopathy (Ajit Bolar, Hum Mol Genet 2013)
MGME1	PEO, emaciation and respiratory failure (Kornblum, Nat Genet 2013)
MRPL3	Cardiomyopathy and mental retardation (Galmiche, Hum Mut 2011)
MTFMT	Leigh syndrome (Tucker, Cell Metab 2011)
MTO1	Cardiomyopathy (Ghezzi, AJHG 2012)
NDUFB3	IUGR, failure to thrive (Calvo, Sci Tr Med 2012)
POP1	Skeletal dysplasia (Glazov, Plos Genet 2011)
PNPT1	Hypotonia, choreo-athetotic movements (Vedrenne, AJHG 2012)
SERAC1	Dystonia and deafness with Leigh-like syndrome (Wortmann, Nat Genet, 2012)
SLC25A1	Agenesis of corpus callosum and optic nerve hypoplasia (Edvardson J Med Genet 2013)
VARS2	Microcephaly and epilepsy (Diodato, Hum Mut 2014)
TARS2	Axial hypotonia and severe psychomotor delay(Diodato, Hum Mut 2014)

Conclusion: flow chart of genetic diagnosis in mitochondrial disorders

Sanger sequencing of specific genes: service laboratory

- **Testing obvious candidate genes**

Targeted sequencing of known mt disease genes: Imagine/service laboratory

- **testing known disease genes (current efficiency : 30%)**

Exome sequencing: research laboratory

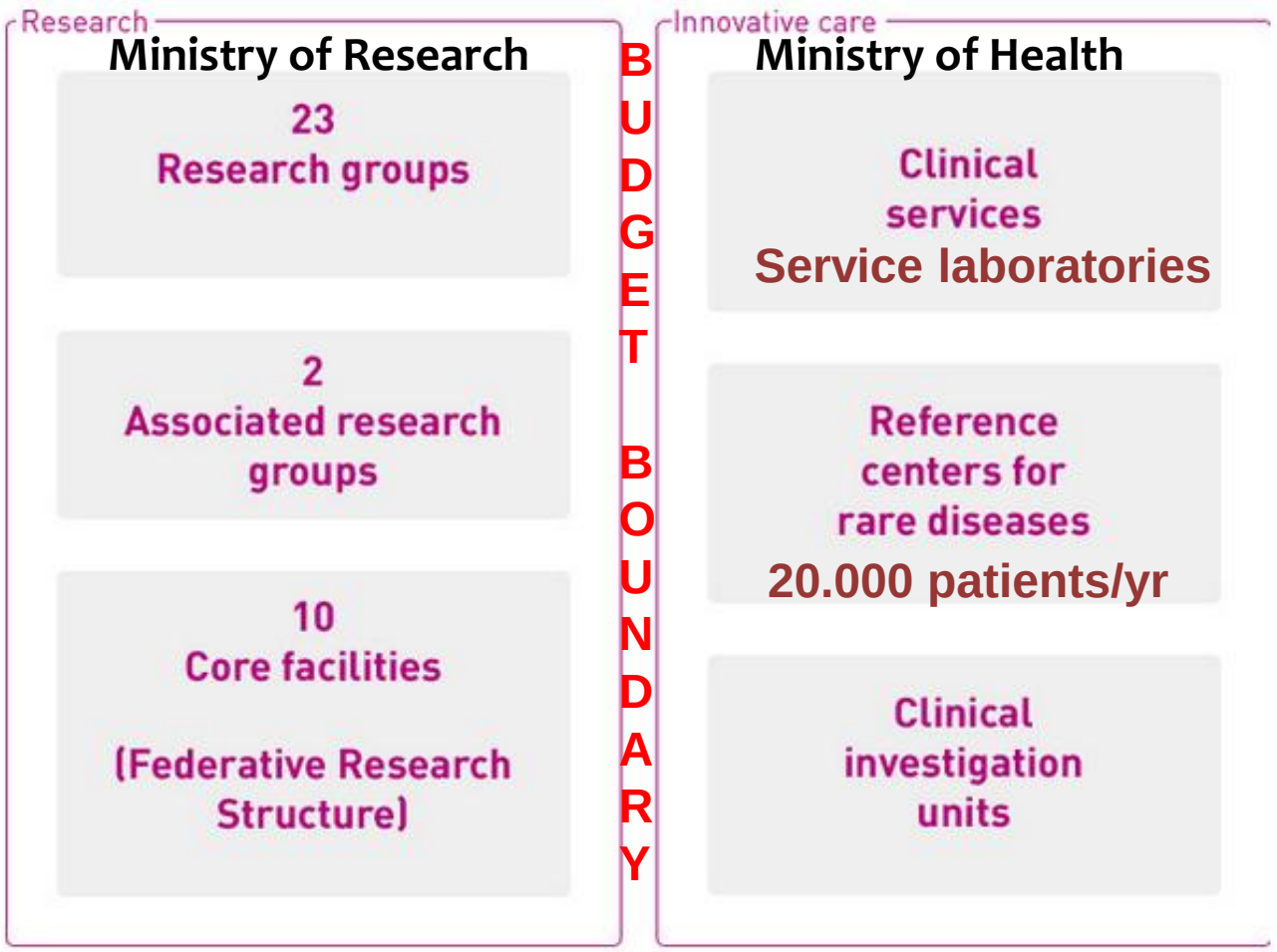
- **Identification of novel disease genes**
- **Functional validation**

Merci…

INSERM U1163

Service de Génétique

Research and clinical cares within the same building



Research program at *Imagine*

Patient-centered approach