Coexistence of nuclear genes mutations and mtDNA mutations in mitochondrial diseases

Digenism in mitochondrial diseases

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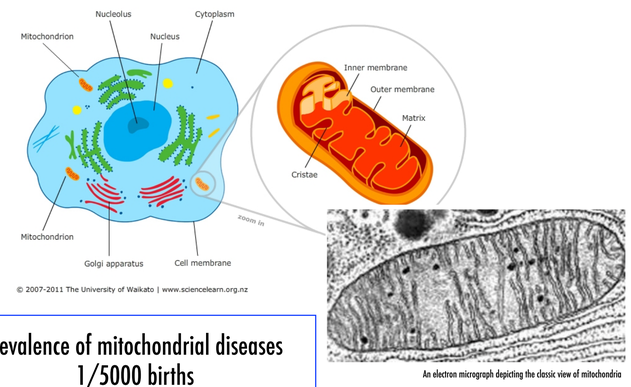
Introduction. Genetic origin of mitochondrial respiratory chain protein



An electron micrograph depicting the classic view of mitochondria

Prevalence of mitochondrial diseases
1/5000 births

Wide and complex clinical presentation
Neuromuscular, cardiac, hepatic, renal ...

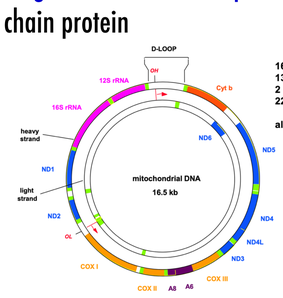
Mitochondrial diseases : respiratory chain defect

1) Respiratory chain enzyme deficiency

Deficiency of one specific complex
Combined deficiency

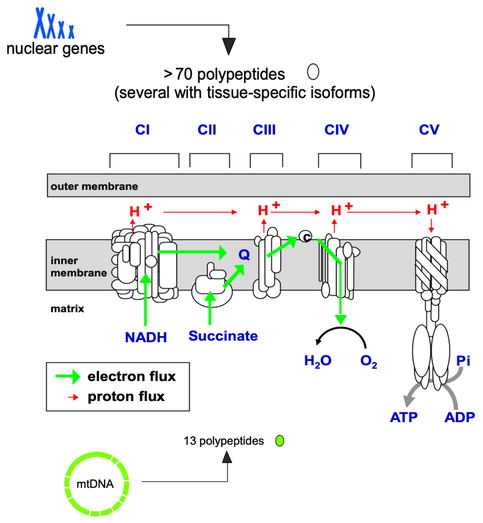
2) Mutation of genes involved in respiratory chain

Respiratory chain protein
Regulation



16.5 Kb
13 subunits of respiratory chain
2 ribosomal RNA
22 transfert RNA

all other proteins are encoded by nuclear genes



Genetic origin

		mtDNA	nuclear genes
C I	41 sub-units	7	34
C II	4 sub-units	0	4
C III	11 sub-units	1	10
C IV	13 sub-units	3	10
C V	14 sub-units	2	12
	Total	13	70

Several hundreds of nuclear genes are involved in
- mtDNA maintenance
- respiratory chain assembly

Transmission modes described

- Sporadic → mtDNA mutations or nuclear gene mutations
- Maternal → mtDNA mutations
- AD, AR, X linked → nuclear gene mutations

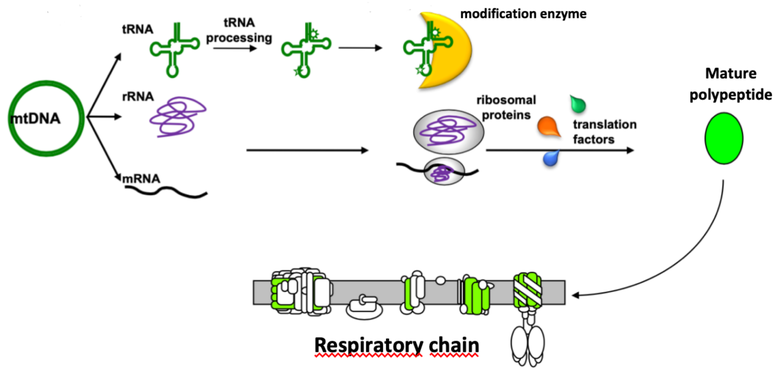
I. Abstract

Mitochondrial diseases are metabolic diseases due to the respiratory chain defect. Mutations responsible for mitochondrial diseases touch nuclear genes or mitochondrial DNA (mtDNA). In children, nuclear mutations are usually recessive. My laboratory at Imagine Institute has evidences suggesting in some families a complex heredity of a coexisting paternal mutation in a nuclear gene and maternal mutation in mtDNA. My M2 project will focus on two independent families in which a child has a heterozygous mutation in a nuclear gene coding for an enzyme modifying a mitochondrial transfer RNA (tRNA) and a mtDNA mutation in the genes TRIT1 and MTO1 coding for this mitochondrial tRNA. I will use fibroblasts of those patients with a respiratory chain defect to surexpress normal complementary DNA (cDNA). I will then use fibroblasts of their asymptomatic parents to surexpress mutated cDNA.
Biochemical analysis of the respiratory chain in different cell lines surexpressing cDNA will be performed to demonstrate that the nuclear mutations are indeed deleterious and therefore verify a digenism hypothesis.

Association of a nuclear gene mutation and a mtDNA mutation causing a disease, a new transmission mode ?

II. General presentation – Material and Methods

Protein synthesis coded by mtDNA needs transfert RNA (tRNA). To be functional, tRNA go through chemical modifications by enzymes encoded by nuclear genes. My laboratory has identified two patients from indepedant families with the same tRNA mutation and a heterozygous mutation of a nuclear gene coding for a tRNA modification enzyme.
tRNA mutation is located in tRNA Tryptophane Trp (MT-TW, m.5542C>T) (Fig. A) and is homoplasmic meaning that 100% of mtDNA is mutated in both patients and in their mothers.
In family 1, we found a heterozygous mutation fatherly inherited of TRIT1 (c.174+3A>G) coding a tRNA isopentenyltransferase adding an isopentenyl groupe in position 37 of MT-TW.
In family 2, we found a heterozygous mutation fatherly inherited of MTO1 (c.346C>T), nuclear gene known to be useful in modifications of tRNA.
TRIT1 et MTO1 are predicted to be deleterious. Recessive mutations of both genes have been reported in other patients.



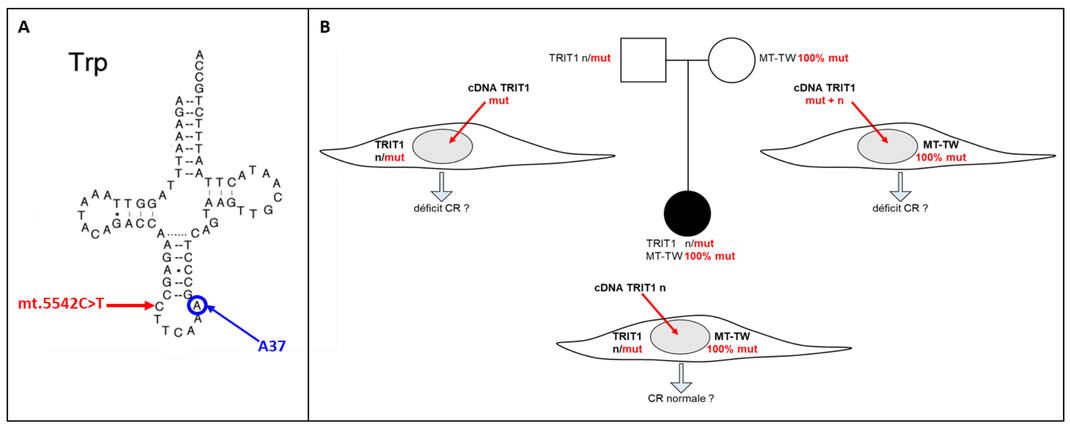


Figure A. Localization of tDNA Tryptophane mutation (MT-TW, mt.5542C>T) coded by mtDNA, identical in both families. A37 represents Adenine modified by the addition of an isopentenyl group by TRIT1. B. Validation strategy of TRIT1 or MTO1 mutations (only TRIT1 is represented on this figure). n : normal, mut : mutated, cDNA: complementary DNA, CR : respiratory chain.

Fibroblasts phenotyping of patients and of their parents

Fibroblasts of both patients have a respiratory chain (RC) deficit. We will use phenotyping and Western Blot of TRIT1 or MTO1 and other RC proteins coded by mtDNA. RC assemblage will be analyzed in BN-PAGE (Blue Native Poly Acrylamide Gel Electrophoresis). My laboratory has already found abnormal results that will be confirmed and completed during my internship. Fibroblasts of their asymptomatic parents are available. We will also analyze Western Blot and BN-PAGE. We hypothesized that their RC is functional.

Validation of nuclear mutation in TRIT1 in Family 1

In Family 1, the mutation (c.174+3A>G) in TRIT1 is predicted to modify splicing of the first exon. We will verify this hypothesis using patients' fibroblasts RNA extraction, reverse transcription, amplification and sequencing. Normal cDNA of TRIT1 will be cloned in a lentiviral vector (pD2109-CMV) and transducted in the patients' fibroblasts (Fig. B). Our hypothesis is that the heterozygous TRIT1 nuclear mutation is causing RC deficit only when combined to homoplasmic MT-TW mutation. Our hypothesis will be validated if RC functions normally after surexpression of normal cDNA TRIT1.
Simultaneously, we will use asymptomatic father's fibroblasts to surexpress mutated cDNA TRIT1. Our hypothesis is that TRIT1 nuclear mutation is deleterious but recessive ; pathogenic only when homozygous. Surexpression using transduction of mutated cDNA TRIT1 in father's fibroblasts will lead to RC deficit. After transduction, RC phenotyping will be evaluated using western blot and BN-PAGE.

Validation of mtDNA mutation in MT-WT in Family 1 and Family 2

Mothers' fibroblasts are homoplasmic for the mutation in MT-WT and have no mutation in TRIT1. Surexpression using transduction of mutated cDNA TRIT1 in mothers' fibroblasts will lead to RC deficit. To be in close conditions corresponding to heterozygotes, we will surexpress both mutated and normal cDNA TRIT1 (Fig. B). After transduction, RC phenotyping will be evaluated using western blot and BN-PAGE. Our hypothesis will be validated if there is a RC deficit, demonstrating that combination of mutation in MT-WT with mutation in TRIT1 is causing a RC defect.

Validation of nuclear mutation in MTO1 in Family 2

An identical approach will be used in Family 2 with maternal mtDNA mutation in MT-TW and paternal heterozygous mutation in MTO1.

III. Research team – Genetics of mitochondrial diseases, Institut Imagine

Organigramme de l'équipe

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Cérane CAFOURNET — Doctorant (oct 2018- oct 2022)

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Alessandra PENNISI — Doctorant (oct 2020 - oct 2022)
Manon MARCHAIS — Ingénieur (CDD)

Flux métaboliques, thérapie génique de la leucinose
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Clément PONTOIZEAU — AHU
Nolan TULAN — Technicien

Réplication de l'ADNmt dans le développement embryo-fétal
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Elisa TURCK — M2 (feb 2022-august 2022)
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Identification de gènes par génomique et transcriptomique
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Mitochondrial diseases are characterized by a huge clinical and genetic heterogeneity and the mitochondrial and nuclear disease causing genes have been identified in only 20% of cases.
Moreover, there is almost no therapy for these devastating diseases.

Therefore our objectives are :
1. to identify new nuclear genes responsible for mitochondrial dysfunction in human for a better understanding of its heterogeneity,
2. to decipher the physiopathology of these diseases,
3. to develop gene therapy for some of them in mouse models,
4. to improve our understanding on the replication of mitochondrial DNA during embryo-fetal development.

Project planning :

	months	1	2	3	4	5	6
Phenotyping of fibroblasts							
	Western blot						
	BN-PAGE						
Validation of mutations							
	Cloning normal and mutated cDNA						
	Transduction of fibroblasts						
	Analyse of phenotype						
Conclusion and writting							

IV. Bibliography

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