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# Implication des mitochondriopathies dans les néphropathies de l'adulte

Hugo Bakis

► **To cite this version:**

Hugo Bakis. Implication des mitochondriopathies dans les néphropathies de l'adulte. Sciences du Vivant [q-bio]. 2020. dumas-02974889

**HAL Id: dumas-02974889**

**<https://dumas.ccsd.cnrs.fr/dumas-02974889>**

Submitted on 22 Oct 2020

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**U.F.R. DES SCIENCES MEDICALES**

Année 2020

Thèse n°3096

THESE POUR L'OBTENTION DU

**DIPLOME D'ETAT de DOCTEUR EN MEDECINE**

Présentée et soutenue publiquement

Par Hugo BAKIS

Né le 08/03/1991 à Pau

Le 29/09/2020

**Implication des mitochondriopathies dans les  
néphropathies de l'adulte**

Sous la direction de Madame Claire RIGOTHIER

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# Remerciements

**A Madame Claire Rigothier, directrice de thèse,** professeur des universités et praticienne hospitalier dans le service de néphrologie, transplantation dialyse et aphérèses du CHU de Bordeaux.

C'est un honneur et un plaisir de travailler avec toi, que ce soit pour ce projet, mais aussi pour l'année recherche et à l'hôpital. Tes qualités humaines et professionnelles sont énormes à mes yeux, et rendent ton encadrement exceptionnel. J'espère sincèrement que l'avenir nous réserve de nombreuses collaborations.

**A Monsieur Christian Combe, président de jury de thèse,** professeur des universités et praticien hospitalier dans le service de néphrologie, transplantation, dialyse et aphérèses du CHU de Bordeaux.

Merci d'avoir accepté de présider cette thèse et de m'avoir accompagné pendant ma formation médicale. Votre soutien est d'une grande importance et vos valeurs morales et médicales sont des exemples pour moi. C'est un honneur de travailler à vos côtés.

**A Monsieur Aurélien Trimouille, rapporteur et jury de thèse,** assistant universitaire dans le service de génétique médicale au CHU de Bordeaux.

Merci de m'avoir aidé lors de ma prise de contact avec le domaine complexe de la génétique médicale. Ton soutien, notamment lors de l'Assise de génétique et du 3<sup>ème</sup> jeudi de génétique médical consacré à la mitochondrie, m'a été d'une grande aide. Merci aussi pour ta disponibilité. J'espère que notre collaboration s'inscrira dans le temps.

**A Monsieur Lionel Couzi,** professeur des universités et praticien hospitalier dans le service de néphrologie, transplantation, dialyse et aphérèses du CHU de Bordeaux.

Merci de m'avoir accueilli lors de mon premier semestre en transplantation rénale, mes premiers pas hospitaliers. C'est un plaisir d'être au contact de tes connaissances et de ta positivité. Merci d'avoir accepté de juger mon travail de thèse.

**A Monsieur Cyril Goizet,** professeur des universités et praticien hospitalier dans le service de génétique médicale au CHU de Bordeaux et au Centre de référence pour les maladies mitochondriales de l'enfant à l'adulte (CARAMMEL).

Merci pour votre aide et votre support lors de nos premiers échanges collaboratifs. C'est un honneur que vous évaluiez mon travail de thèse.

**A Madame Marie-Laure Martin-Negrier,** professeur des universités et praticienne hospitalier dans le service d'anatomopathologie du CHU de Bordeaux et au Centre de référence pour les maladies mitochondriales de l'enfant à l'adulte (CARAMMEL).

Merci de m'avoir ouvert les portes des mitochondriopathies et de ces différents projets. De mes premiers cours en PACES à ma soutenance de thèse, c'est un honneur que mon travail soit évalué par vous, et c'est un plaisir de collaborer avec vous.

**A Monsieur Pierre Merville,** professeur des universités et praticien hospitalier dans le service de néphrologie, transplantation, dialyse et aphérèses du CHU de Bordeaux.

Merci de m'avoir accueilli et de m'avoir accordé votre confiance lors de mon passage en transplantation rénale. Au plaisir de travailler de nouveau avec vous à l'avenir.

**A Monsieur Guilhem Solé**, praticien hospitalier dans le service de Département de neurologie, unité nerf-muscle au CHU de Bordeaux.

Merci d'avoir accepté de juger mon travail de thèse. Un juste retour des choses, beaucoup des patients inclus dans ma cohorte étant des patients que vous avez brillamment diagnostiqués.

**A Ludivine, ma partenaire, mon amour.** Tu me connais comme personne, alors merci pour ton soutien et ta patience constante. Le bonheur se définit pour moi comme le quotidien à tes côtés. Ma plus grande chance est certainement d'être aimé par cette femme brillante, drôle et attentionnée que tu es. Mon avenir s'inscrit avec toi.

**A Marie-Pierre, ma mère.** Merci de m'avoir appris à vivre. La sérénité et les valeurs que tu m'as inculquées m'accompagneront bien au-delà de ce travail. Tu es toujours là pour moi, pour me soutenir et pour m'encourager, merci. Merci à Jean pour son espièglerie et ses débats qui amènent toujours à la réflexion.

**A Célestin, mon père.** Merci pour m'avoir élevé. Tu m'as beaucoup transmis, notamment le goût pour les sciences humaines et les études (longues). Merci pour m'avoir accompagné et soutenu pour surmonter les différentes étapes de la vie.

**A Marie-Laure, ma marraine.** Tu seras à jamais dans ma vie.

**Aux C&P, frères et sœurs de cœur.** Sans le savoir peut-être, vous m'avez offert une famille. C'est dans la liesse mais aussi dans l'adversité que se construisent les vraies amitiés. Miloud (Rouya) et Daz (le vieux Couz), tellement de moments partagés (éprouvants parfois) qui resteront gravés en moi. Raufaste, Moncla, Brioulet, Loiseau, Didi, Adrien, Deseg j'espère que les promesses du futur seront à la hauteur de notre passé. Heureux que la famille s'agrandisse (Géraldine, La douce, Méli, Mon Quentin, Marion, Chloé...).

**A Aurélien de Béchillon.** C'est une fierté d'avoir grandi et évolué à tes côtés. Réunis par nos histoires de cœur, tu es entré dans le mien pour ne pas en sortir. Tu fais partie de ces personnes qui trouveront leur place dans n'importe quel domaine appréhendé. Tu as toute ma confiance.

**Aux amitiés qui sont nées dans le travail.** Camille (on taira ton surnom), merci pour ta générosité et ton écoute. Je sais que je peux compter sur toi, tant tu l'as prouvé dans toutes les circonstances. La Tuj, merci tout simplement, ta gentillesse n'a aucune limite. Lacap pour avoir partagé les moments de « fissure », et d'autres « noirs ». Pfirmann, pour tes qualités de compagnonnage et de confident. Petitout parce que tu n'es pas grand. Magic pour ton humilité (non je rigole) et ta bonne humeur communicative. Aux autres non moins importants mais dont la décence m'oblige à résumer : Victor, Olivier, Emma, Alessandra, Taton, Arsène, Juliette, Lisa, Simon, Felix, Sébastien, Marion... Quelle chance que des connaissances franchissent la frontière de l'amitié.

**Aux amis de la plateforme.** Des situations les plus critiques peuvent ressortir de belles choses. Merci à Nabil (on s'en occupe), Yaniss (à qui je dois une fière chandelle) et Maëlys.

**A la belle famille.** Merci Isabelle pour ces moments de décompression autour de bons dînés. Merci à Pauline, Pascal, Magali et Léa.

**Merci à toutes les personnes qui font que le quotidien reste une partie de plaisir.**

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# Abréviations

<b>Acétyl-CoA</b>	Acétyl-Coenzyme A	<b>HSF</b>	Hyalinose segmentaire et focale
<b>Acyl-CoA</b>	Acyl-Coenzyme A	<b>IRA</b>	Insuffisance rénale aigüe
<b>ADNmt</b>	ADN mitochondrial	<b>MID</b>	Mitochondriopathie primitive
<b>ADNn</b>	ADN nucléaire	<b>MIDAN</b>	Néphropathie associée aux mitochondriopathies primitives
<b>ADP</b>	Adénosine di-phosphate	<b>Mfn</b>	Mitofusine
<b>AIF</b>	Apoptosis-inducible factor	<b>MRC</b>	Maladie rénale chronique
<b>AMP</b>	Adénosine mono-phosphate	<b>mTORC1</b>	Mammalian target of rapamycin complex 1
<b>AMPK</b>	AMP-activated protein kinase	<b>NADH</b>	Nicotinamide adénine dinucléotide
<b>ARNr</b>	ARN ribosomal	<b>NGS</b>	Next-generation sequencing
<b>ARNt</b>	ARN de transfert	<b>NRF2</b>	Nuclear-respiratory factors 2
<b>ATP</b>	Adénosine triphosphate	<b>O<sub>2</sub></b>	Oxygène
<b>CC</b>	Canal collecteur	<b>OXPHOS</b>	Oxydation phosphorylante
<b>CO<sub>2</sub></b>	Dioxyde de carbone	<b>OPA1</b>	Optic atrophy type 1
<b>CoQ</b>	Coenzyme Q 10	<b>PCT</b>	Tubule contourné proximal
<b>DCT</b>	Tubule contourné distal	<b>PGC-1<math>\alpha</math></b>	Peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$
<b>EROs</b>	Espèces réactives de l'oxygène	<b>PTP</b>	Pore de transition de perméabilité
<b>ETC</b>	Chaîne de transport d'électron	<b>SOD</b>	Superoxyde dismutase
<b>FADH<sub>2</sub></b>	Flavine adénine dinucléotide	<b>TAHL</b>	Branche ascendante large de Henlé
<b>GPx</b>	Glutathion peroxydase	<b>UCP</b>	Protéines non couplantes
<b>GSH</b>	Glutathion réduit	<b>WES</b>	Whole-exome sequencing
<b>GSSG</b>	Glutathion oxydé	<b>WGS</b>	Whole-genome sequencing
<b>HIF1<math>\alpha</math></b>	Hypoxia inducible factor 1 $\alpha$		



# Introduction

## I. Les mitochondries : historique et généralités

En 1890, Richard Altmann observe des granulations allongées intracellulaires cytoplasmiques ubiquitaires, semblables à des bactéries, indépendantes du cycle cellulaire. Il les suspecte d'être autonomes en terme de matériel génétique et de métabolisme<sup>1</sup>. En 1898, Carl Benda leur donne le nom de mitochondrie, du grec ancien mitos [fil] et chondros [granules])<sup>2</sup>. C'est le développement de la microscopie électronique qui permet en 1953 à Palade et Sjöstrand de révéler l'ultrastructure mitochondriale : une membrane externe perméable aux petits molécules (avec des pores de 5000 Daltons), et une membrane interne imperméable siège de replis formant des crêtes<sup>3-5</sup>. Cette double membrane définit 2 compartiments : l'espace intermembranaire (entre les membranes interne et externe) et la matrice mitochondriale (à l'intérieur de la membrane interne) (Figure 1).

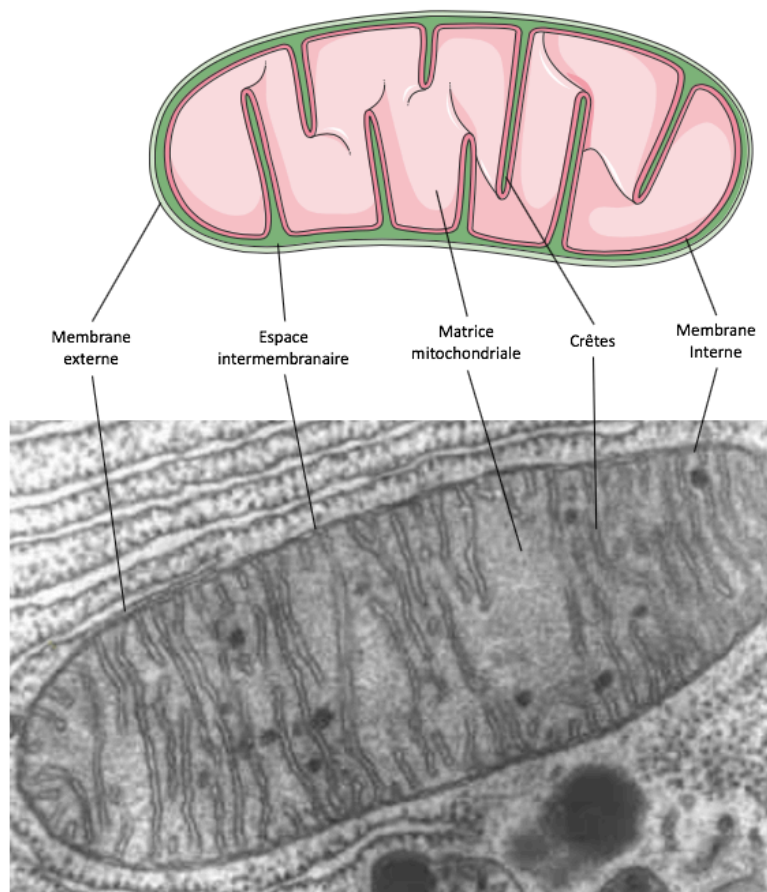


Figure 1 : Rappels morphologiques de la mitochondrie. Schéma d'une mitochondrie (en haut) et photographie en microscopie électronique d'une mitochondrie musculaire (en bas).

Les mitochondries résultent d'un processus endosymbiotique il y a 2 milliards d'années entre une bactérie (semblable à *Rickettsia prowazekii*<sup>6</sup>) et un précurseur de cellule eucaryote. Ce phénomène a conféré un avantage sélectif aux cellules eucaryotes dont le métabolisme était strictement anaérobie, les pourvoyant d'un métabolisme aérobie de meilleur rendement énergétique : la respiration cellulaire. Celle-ci, déjà décrite depuis la fin du XVIII<sup>ème</sup> siècle par le chimiste français Antoine Lavoisier comme étant « une lente combustion de carbone et d'hydrogène », est attribuée aux mitochondries depuis les années 1910. Entre les années 1920 et 1940, où ces organelles sont extraites de cellules hépatiques<sup>7,8</sup>, de nombreux travaux ont permis la description de la respiration mitochondriale (Prix Nobel de médecine ou physiologie en 1931 de Otto Warburg) (Figure 2) : les nutriments et l'oxygène (O<sub>2</sub>) sont utilisés par les mitochondries pour générer de l'énergie, du dioxyde de carbone (CO<sub>2</sub>) et de l'eau. Le pigment énergétique responsable de cette respiration est ensuite identifié comme étant des cytochromes<sup>9</sup> prenant part dans une chaîne de transport d'électron (ETC) responsable de phénomènes d'oxydations successives aboutissant à la phosphorylation de l'adénosine di-phosphate (ADP) en adénosine tri-phosphate (ATP) : l'oxydation phosphorylante (OXPHOS)<sup>10-12</sup>. C'est la théorie chimio-osmotique de Peter Mitchell dans les années 1960-1970 (récompensé du prix Nobel de chimie en 1978) (Figure 2) qui permet de relier la respiration cellulaire mettant en jeu l'OXPHOS et l'ETC, avec la formation d'ATP : des protons sont pompés vers l'espace transmembranaire, permettant la création d'un gradient transmembranaire électrochimique utilisé pour obtenir l'énergie nécessaire à la création d'adénosine triphosphate<sup>13</sup>. La synthèse d'ATP est ensuite détaillée par le travail de Paul Boyer en 1977 (récompensé du prix Nobel de chimie en 1997) (Figure 2).



Figure 2 : De la gauche vers la droite, et dans l'ordre chronologique d'obtention des prix Nobel, Otto Warburg pour son travail sur la chaîne respiratoire mitochondriale, Peter Mitchell pour sa théorie chimio-osmotique reliant l'ETC et l'OXPHOS à la synthèse d'ATP et Paul Boyer pour avoir décrypté la synthèse de l'ATP. Source : Nobel Foundation Archives.

C'est dans la matrice mitochondriale que se trouve le matériel génétique de l'organelle, autre témoin de l'endosymbiose originelle, l'ADN mitochondrial (ADNmt). Séquencé pour la première fois par des scientifiques de l'université de Cambridge en 1981<sup>14</sup>, il s'agit d'un ADN bicaténaire circulaire de 16569 paires de base et 37 gènes, sans intron, sans chromatine et avec un code génétique différent de l'ADN nucléaire (ADNn) (Figure 3). Pour autant, l'expression de l'ADNmt, ainsi que certaines fonctions mitochondriales, sont sous la dépendance de gènes de l'ADNn dont les transcrits sont importés dans les mitochondries. La respiration mitochondriale est donc soumise à une dualité de contrôle génétique rendant leur étude très intéressante. Les protéines de la chaîne respiratoire mitochondriale sont présentes sur la membrane interne mitochondriale et sont composées de 5 complexes multi-protéiques (complexes I à V), et de 2 transporteurs d'électrons [la coenzyme Q10 (CoQ) et le cytochrome c]. Ce système engendre un gradient de protons transmembranaire sous contrôle du complexe protéique V, ou F<sub>0</sub>F<sub>1</sub> ATP synthase, responsable de la production d'ATP<sup>13</sup> (Figure 3).

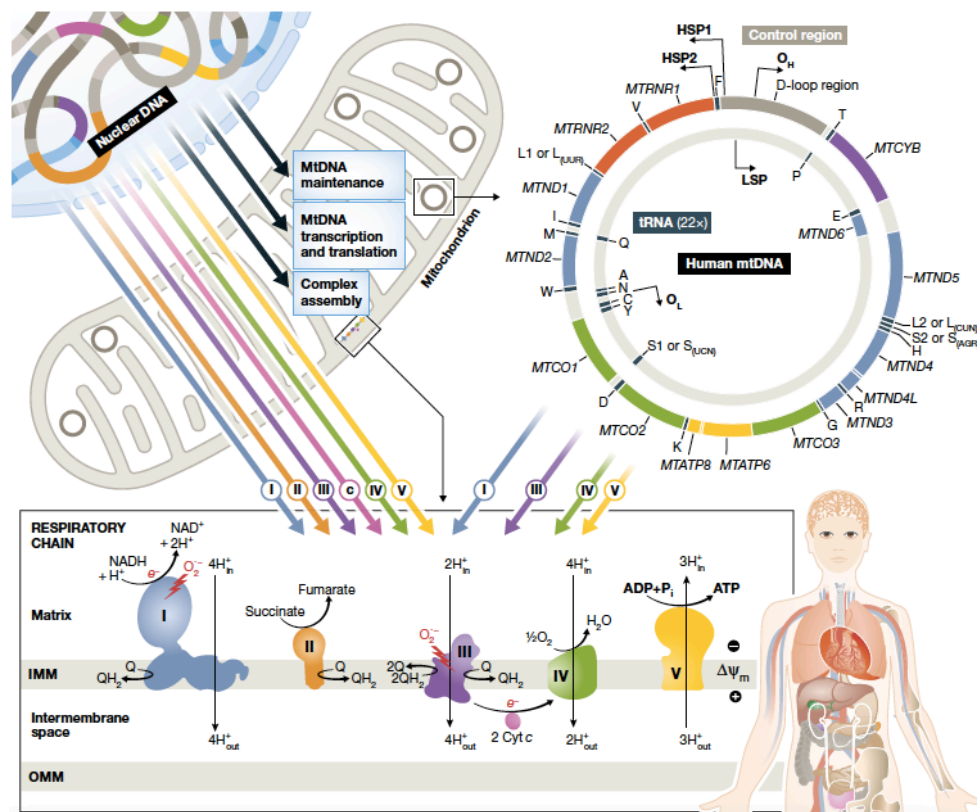


Figure 3 : La dualité du contrôle génétique des fonctions mitochondriales. Dans la partie supérieure du schéma, l'ADNmt (à droite) code pour des protéines de la chaîne respiratoire mitochondriale. L'ADNn (à gauche) est impliqué dans la régulation, la réparation et la transcription de l'ADNmt, mais code aussi pour des protéines de la chaîne respiratoire mitochondriale. Dans la partie inférieure, la chaîne respiratoire mitochondriale avec les 5 complexes protéiques permettant les réaction d'OXPHOS et l'ETC. D'après Chinnery P., EMBO, 2015<sup>15</sup>.

Cette organelle intracellulaire est depuis considérée comme la « centrale énergétique cellulaire » (« powerhouses of the cell »)<sup>16</sup>, étant responsable de la production de plus de 90% de l'énergie du corps humain à travers la production d'ATP<sup>17</sup>. Par la suite, d'autres fonctions ont été attribuées aux mitochondries, notamment la régulation d'espèces réactives de l'oxygène (EROs)<sup>18</sup> et la régulation des processus de survie et d'apoptose cellulaire<sup>19</sup>.

## II. Les fonctions mitochondriales

Les mitochondries sont présentes dans le cytoplasme de toutes les cellules humaines en dehors des érythrocytes matures. Elles forment un réseau plastique d'organelles garantissant des processus et des fonctions vitales aux cellules.

### 1. La respiration cellulaire

Les mitochondries sont les « centrales énergétiques cellulaires »<sup>16</sup>. En effet, une de leur principale fonction est la synthèse d'ATP et la régulation du métabolisme énergétique cellulaire. Cette production d'ATP est le garant de la rupture d'équilibre compatible avec la survie et les fonctions cellulaires. Cette synthèse dépend des nutriments catabolisés (glucose, acides aminés et acides gras) et implique la consommation d'O<sub>2</sub> avec production de CO<sub>2</sub> et d'eau à travers la respiration mitochondriale.

#### Métabolisme du glucose

Quel que soit le statut d'oxygénation de la cellule, la glycolyse aboutit à la formation de pyruvate. En condition d'aérobiose, ce dernier pénètre dans la matrice mitochondriale via un transporteur membranaire spécialisé<sup>20</sup> où il est transformé en acétyl-coenzyme A (acétyl-CoA) sous l'action du pyruvate déshydrogénase<sup>21</sup>. L'acétyl-coA entre ensuite dans le cycle de Krebs (cycle de l'acide citrique) (Figure 4). En condition d'anaérobiose, le produit de la glycolyse est orienté vers la production de lactate à partir du pyruvate sous l'effet du lactate déshydrogénase (LDH). Les molécules de lactate ainsi produites vont pouvoir être importées dans les mitochondries (rôle des monocarboxylates transporteurs) et être transformés en pyruvate par le LDH mitochondrial pour produire de l'ATP (en entrant dans le cycle de Krebs lorsque les apports en O<sub>2</sub> le permettent) et pour la gluconéogenèse (cycle de Cori)<sup>22</sup>.

En cas d'anomalie de la chaîne respiratoire mitochondriale, la glycolyse est déviée vers la production de lactate, qui va donc s'accumuler (hyperlactatémie), avec un taux de pyruvate normal ou bas et donc un ratio lactate/pyruvate augmenté. Les lactates peuvent aussi être augmentés lorsqu'ils sont dosés dans les urines (hyperlactaturie)<sup>23</sup>. De plus, le pyruvate formé

ne pouvant être consommé via sa transformation en acétyl-CoA, il va être utilisé pour produire de l'alanine dont le taux plasmatique peut être augmenté (Figure 4).

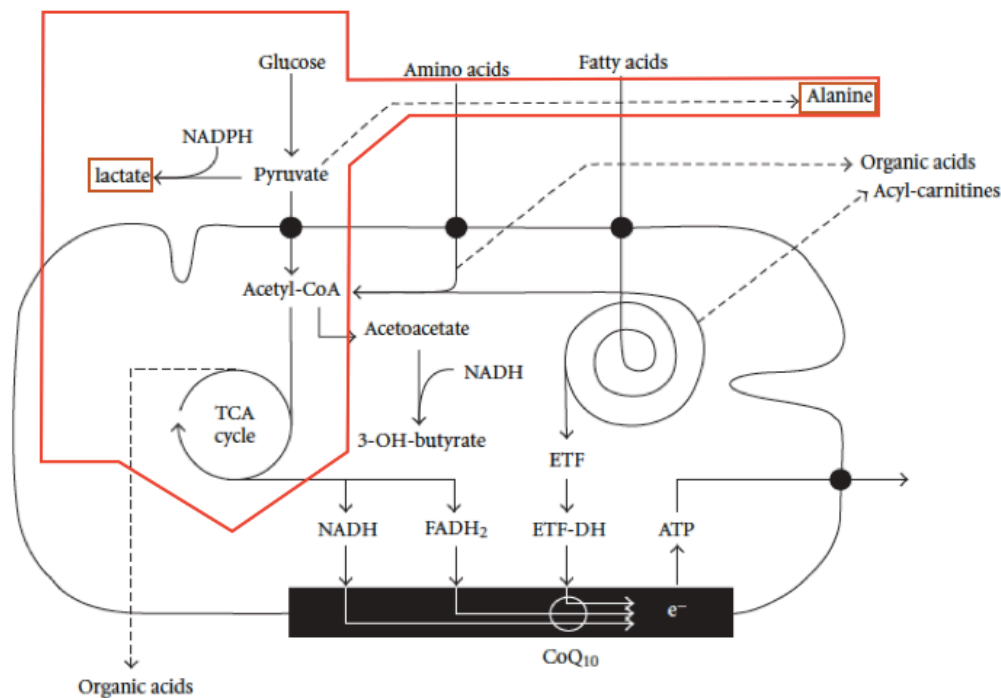


Figure 4 : La respiration cellulaire centrée sur le métabolisme du glucose (encadré rouge). Les anomalies de chaîne respiratoire mitochondriale peuvent s'accompagner de l'accumulation de produits de dégradation de la glycolyse (encadrés oranges). La chaîne respiratoire mitochondriale est schématisée en noir, avec les flèches blanches représentant le transport d'électrons. D'après Emma F. et al., *Int. J. Nephrol.*, 2012<sup>23</sup>.

### Métabolisme des acides gras et cétogenèse

Les acides gras vont, dans le cytosol, être activés par la coenzyme A pour former l'acyl-coenzyme A (acyl-CoA). Si l'acide gras est long (plus de 22 groupements carbone), il va être dirigé vers le peroxysome pour être oxydé sous forme d'acétyl-CoA qui pourra ensuite rentrer dans la mitochondrie grâce à la carnitine qui joue un rôle de navette<sup>24</sup>. L'acétyl-CoA pourra ensuite rentrer dans le cycle de Krebs. Si l'acide gras est plus court, il sera transporté directement dans la mitochondrie sous forme d'acyl-CoA par la carnitine. Dans la mitochondrie, va avoir lieu la bêta-oxydation de cet acide gras, initiée par la hydroxyacyl-CoA déshydrogénase, par tour d'hélice de Lynen. De chaque tour d'hélice résulte la formation d'une molécule de nicotinamide adénine dinucléotide (NADH), d'une molécule de flavine adénine dinucléotide (FADH<sub>2</sub>) et d'une molécule d'acétyl-CoA qui rentrera dans le cycle de Krebs<sup>25</sup> (Figure 5).

Lorsque le cycle de Krebs est à l'arrêt, dans des conditions physiologiques de carence en glucose entraînant un déficit d'oxaloacétate (alors utilisé pour la gluconéogenèse), l'acétyl-CoA peut être le substrat de la cétogenèse. Cette réaction peut avoir lieu dans les tissus spécialisés que sont le foie et le rein (au niveau des cellules du tubule contourné proximal) et aboutissent à la production de 3 produits exportés vers les tissus demandeurs d'énergie (*i.e.* le muscle) : l'acétone, l'acétoacétate et le  $\beta$ -hydroxybutyrate. Les 2 derniers sont reliés par une réaction réversible dépendant du ratio NADH/NAD, qui en diminuant favorise la transformation du  $\beta$ -hydroxybutyrate en acétoacétate qui va ensuite être transformé en acétyl-CoA<sup>25</sup>.

En conditions pathologiques d'anomalies de la chaîne respiratoire mitochondriale, l'acétyl-CoA peut aussi être un substrat de la cétogenèse aboutissant aux 3 mêmes produits. Seulement, le NADH étant en excès, le  $\beta$ -hydroxybutyrate est majoritaire sur l'acétoacétate et peut donc se retrouver en excès dans les urines (Figure 5).

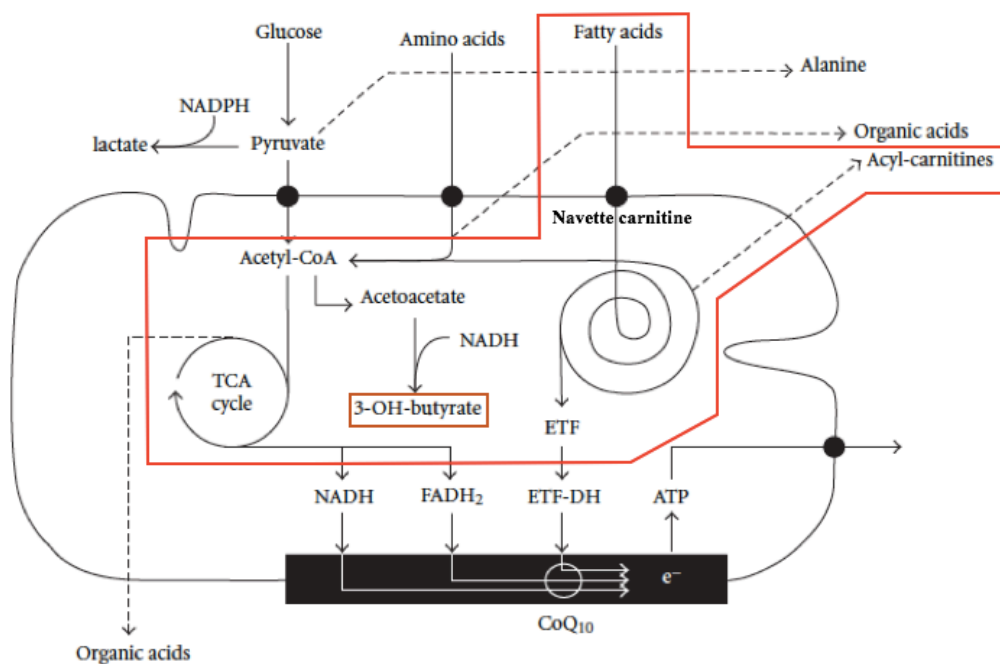


Figure 5 : La respiration cellulaire centrée sur le métabolisme des acides gras (encadré rouge). Les hélices de Lyden sont schématisées par la spirale dans la matrice mitochondriale. Les produits de dégradation pouvant être retrouvé en excès en cas d'anomalies de la chaîne respiratoire mitochondriale sont encadrés en orange. D'après Emma F. et al., *Int. J. Nephrol.*, 2012<sup>23</sup>.

## Métabolisme des acides aminés

Les acides aminés vont aussi avoir un rôle dans le métabolisme énergétique, à hauteur de 10% environ<sup>26</sup>. Après une étape de transamination hépatique via l' $\alpha$ -cétoglutarate, il est obtenu du glutamate et un  $\alpha$ -cétacide correspondant à l'acide aminé d'origine. Le glutamate est soit utilisé afin de réaliser l'excrétion azotée rénale, soit pour générer des composants du cycle de l'urée. L' $\alpha$ -cétacide donne des substrats du cycle de Krebs qui peuvent entrer dans la gluconéogenèse (oxaloacétate et pyruvate) ou dans la cétogenèse (acétyl-CoA, acétoacétate et  $\beta$ -hydroxybutyrate). Cela donne lieu à la classification en acides aminés cétoformateurs et glucoformateurs.

En cas d'altération de la chaîne respiratoire mitochondriale, on peut observer une accumulation d'acides organiques notamment dans les urines (*i.e.* l'excrétion urinaire de 5-oxoproline)<sup>23</sup>. De plus, l'accumulation de corps cétogénés avec un ratio NADH/NAD élevé va amplifier l'accumulation de  $\beta$ -hydroxybutyrate (Figure 6).

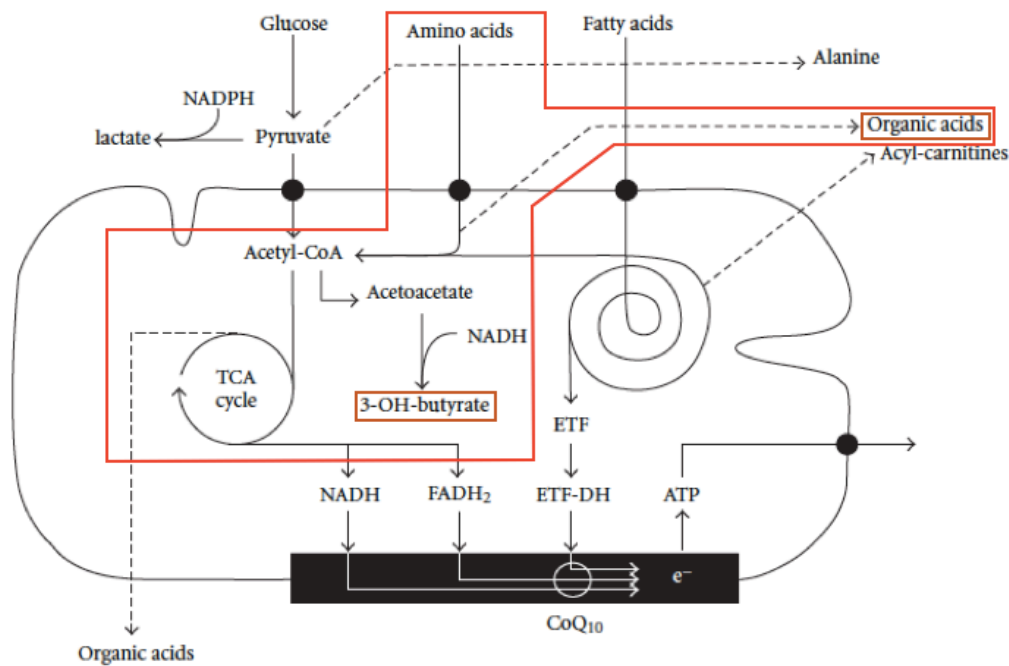


Figure 6 : La respiration cellulaire centrée sur le métabolisme des acides aminés (encadré rouge). Certains produits de dégradation peuvent être augmentés en cas d'anomalie de chaîne respiratoire mitochondriale (encadrés oranges). D'après Emma F. et al., *Int. J. Nephrol.*, 2012<sup>23</sup>.

## Cycle de Krebs (ou de l'acide citrique)

Même si de nombreux éléments ont été mis en évidence par le biologiste hongrois Albert Szent-Györgyi dans les années 1930 (prix Nobel de médecine en 1937), c'est le biochimiste allemand Hans Krebs qui met en évidence son caractère cyclique en 1937 (prix Nobel de médecine en 1953)<sup>27</sup>. C'est le point commun du catabolisme des acides gras, du glucose et des acides aminés, aboutissant tous à l'acétyl-CoA qui est une voie d'entrée dans ce cycle. Il est composé de 10 étapes métabolisées par 8 enzymes. Il a lieu entièrement dans la matrice mitochondriale. La première étape est le transfert du groupement acétyl de l'acétyl-CoA sur l'oxaloacétate pour l'obtention du citrate (Figure 7). Le bilan, à partir d'une molécule d'acétate, est de 3 NADH, 1 GTP et 1 CoQ. Le NADH est ensuite oxydé dans la chaîne respiratoire mitochondriale en condition physiologique.

En cas d'altération pathologique de la chaîne respiratoire mitochondriale, les substrats intermédiaires du cycle de Krebs se retrouvent en excès et peuvent être détectés en effectuant une spectrophotométrie des acides organiques urinaires (*i.e.* succinate, malate et fumarate)<sup>23</sup>.

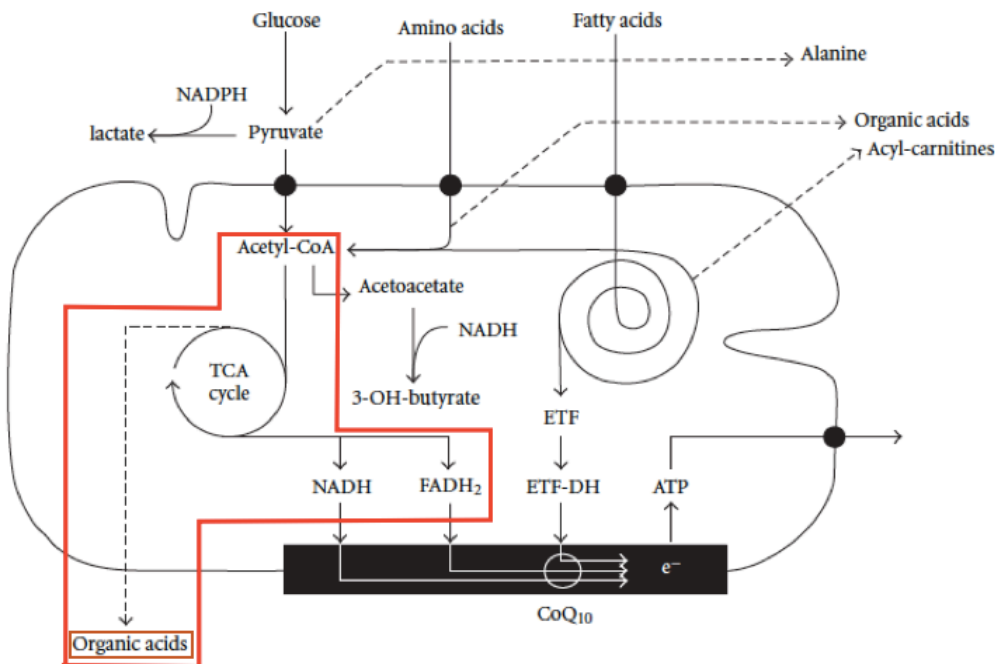


Figure 7 : Schéma de la respiration cellulaire centrée sur le cycle de Krebs (encadré rouge). Les anomalies de la chaîne respiratoire mitochondriale pouvant être à l'origine d'un excès d'intermédiaire du cycle de Krebs sous forme d'acides organiques (encadré orange). D'après Emma F. et al., *Int. J. Nephrol.*, 2012<sup>23</sup>.



## OXPHOS

Il s'agit de plusieurs étapes d'oxydation de substrats réduits, dans l'ETC de la membrane interne mitochondriale, aboutissant à la phosphorylation de l'ADP en ATP et se déroulant selon la théorie chimiosmotique de Peter Mitchell<sup>13,28</sup>. Cinq complexes protéiques enzymatiques et deux transporteurs d'électrons (CoQ et cytochrome c) sont sollicités (Figure 8) :

- Le complexe I, ou NADH ubiquinone déshydrogénase, est composé de 46 sous-unités. C'est la première enzyme de la chaîne, qui va oxyder le NADH. Cela permet le transport de 2 électrons jusqu'au CoQ qui est alors réduit (CoQH<sub>2</sub>). De plus, 4 protons sont pompés dans l'espace intermembranaire<sup>29</sup>.
- Le complexe II, ou succinate quinone oxydoreductase, est composé de 4 sous-unités. Cette enzyme participe d'une part au cycle de Krebs par l'oxydation du succinate en fumarate, et d'autre part à l'oxydation du FADH<sub>2</sub> avec don de 2 électrons au CoQ ainsi réduit en CoQH<sub>2</sub><sup>30</sup>.
- Le complexe III, ou coenzyme Q – cytochrome c réductase, est un dimère composé chacun de 11 sous-unités et de cytochromes<sup>31,32</sup>. Il opère l'oxydation d'une molécule de CoQH<sub>2</sub> et la réduction de 2 cytochromes c (portant chacun un seul électron). Cela est couplé au transport de 4 protons dans l'espace intermembranaire.
- Le complexe IV, ou cytochrome c oxydase, est composé de 13 sous-unités<sup>33</sup>. Cette enzyme va oxyder les cytochromes c, récupérant leurs électrons, et les utiliser pour catalyser 2 molécules d'H<sub>2</sub>O à partir de 4 protons matriciels et de 2 molécules d'O<sub>2</sub> (accepteurs d'électrons forts). Quatre protons sont transportés vers l'espace intermembranaire.
- Le résultat de ces réactions d'oxydoréduction est l'obtention d'un gradient électrochimique de proton de part et d'autre de la membrane interne mitochondriale. Ce gradient est utilisé pour que le complexe V, ou ATP synthase, qui est une F<sub>0</sub>F<sub>1</sub> ATPase, produise de l'ATP. Le gradient électrochimique de protons (énergie chimiosmotique) permet la rotation de la partie F<sub>0</sub>, avec changement de conformation de la partie F<sub>1</sub> (énergie mécanique) qui permet la phosphorylation de l'ADP en ATP en présence de phosphate matriciel<sup>13,34</sup> (Figure 8).

Ces complexes, initialement considérés comme mobiles et indépendants au sein de la membrane mitochondriale interne<sup>35</sup>, pourraient en fait s'assembler en supercomplexes (ou respirasomes) pour optimiser le rendement énergétique et diminuer la production d'EROs<sup>36,37</sup>.

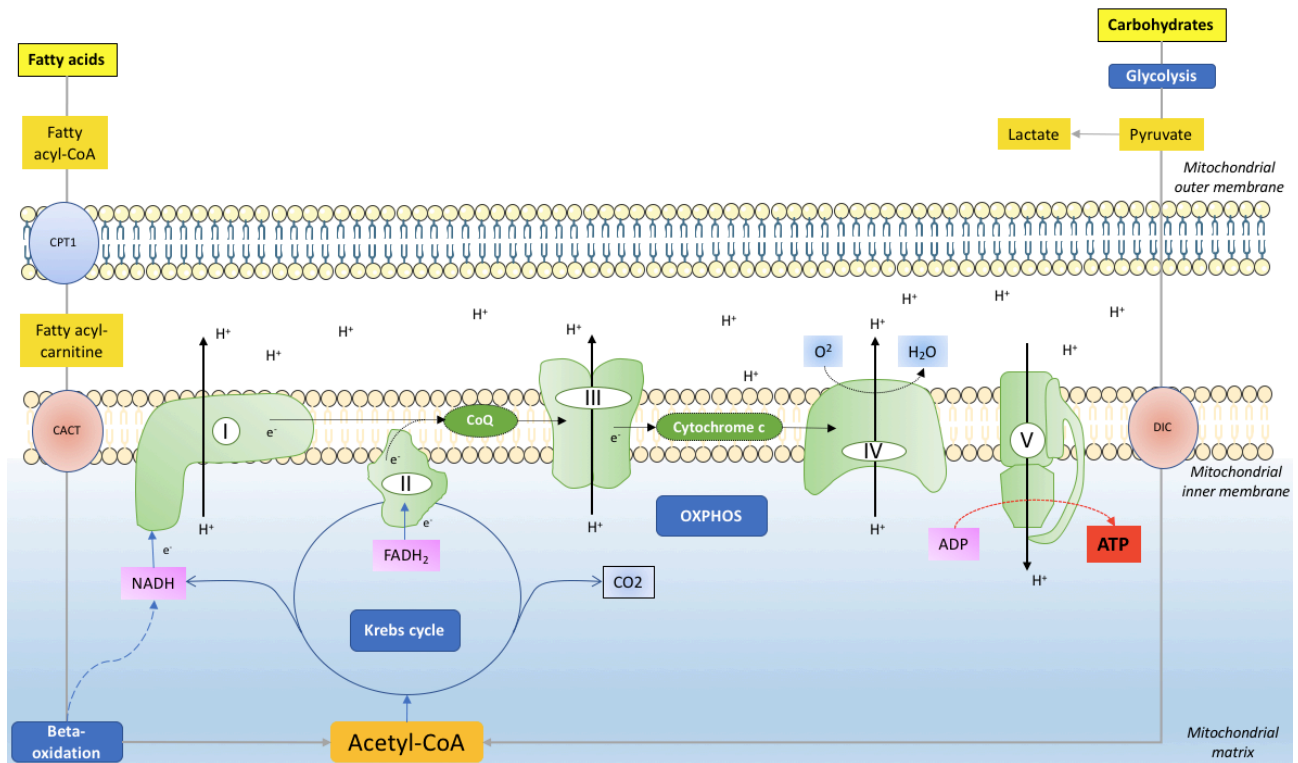


Figure 8 : La respiration cellulaire centrée sur l'OXPHOS via l'ETC, aboutissant à la synthèse d'ATP. CACT = carnitine acyl-carnitine translocase / CPT1 = carnitine palmitoyltransferase 1 / DIC = dicarboxylate carrier.

L'ATP est utilisé pour fournir l'énergie nécessaire à la quasi-totalité des fonctions cellulaires, et par extension de l'ensemble des organes. En effet, l'hydrolyse de l'ATP en ADP libère une variation d'enthalpie libre de -30,5 kJ/mol permettant de fournir l'énergie nécessaire à ces fonctions.

## 2. Stress oxydatif et rôle antioxydant

### Formation et conséquences des EROs

Les mitochondries produisent donc de l'ATP via l'ETC et l'OXPHOS. En situation physiologique, lors de la respiration mitochondriale, environ 0,4 à 4% de l'O<sub>2</sub> consommé est transformé en EROs sous forme d'anions superoxydes<sup>39</sup>, essentiellement au niveau des complexes I et III<sup>40</sup>. A des concentrations physiologiques, ces anions vont jouer un rôle de médiateur cellulaire<sup>41</sup>. Cependant, en cas de concentration d'EROs trop importante, cela va avoir des conséquences néfastes au niveau cellulaire, répondant au terme de stress oxydatif. Les conséquences au niveau mitochondrial sont les suivantes :

- des mutations de l'ADNmt, en lien avec le fait que l'ADNmt n'est pas protégé par des histones, est très compact et présente des capacités de réparation bien moindre que l'ADN<sup>42,43</sup> ;

- la peroxydation des lipides qui entraîne une altération de l'agencement membranaire, notamment de la membrane interne mitochondriale. Ces modifications peuvent entraîner des anomalies de fonctionnement des complexes de l'ETC<sup>44</sup> ;
- l'oxydation des protéines qui va induire des dysfonctions, notamment au niveau des complexes protéiques de l'ETC<sup>18</sup> ;
- les produits de la glycation avancée, en s'accumulant, vont entraver le fonctionnement du cycle de Krebs<sup>45</sup>, voire induire un signal pro-apoptotique cellulaire<sup>46</sup>.

Le stress oxydatif va donc entraîner une diminution du rendement énergétique de la respiration cellulaire mitochondriale et une augmentation de la formation d'EROs<sup>47</sup>. Ce cercle vicieux doit donc être contrecarré par une défense antioxydante, sans quoi ses conséquences ne sont pas compatibles avec la survie cellulaire (Figure 9).

### Rôle antioxydant

Pour éviter cette situation, les mitochondries sont munies de défenses antioxydantes. Il est à noter que les EROs vont stimuler, via le nuclear-respiratory factors 2 (NRF2), la transcription nucléaire de certains facteurs permettant ce rôle antioxydant<sup>46</sup>. Un antioxydant enzymatique mitochondrial, la superoxyde dismutase (SOD), va catalyser la réaction transformant l'anion superoxyde en H<sub>2</sub>O<sub>2</sub><sup>48</sup>. Une catalase opère ensuite la transformation de 2 molécules d'H<sub>2</sub>O<sub>2</sub> en 2 molécules d'eau et une molécule d'O<sub>2</sub><sup>49</sup>. Une autre enzyme antioxydante d'importance est le glutathion, un tripeptide nucléophile qui existe sous forme réduite (GSH) et sous forme oxydée (GSSG, glutathion di-sulfide). Les mitochondries possèdent leur propre pool de glutathion qui va interagir directement avec les anions superoxydes pour former du GSSG. Ce dernier ne peut pas sortir de la mitochondrie et sera soit éliminé, soit recyclé par la glutathion réductase<sup>50</sup>. Le GSSG est aussi le produit intermédiaire de la réduction du peroxyde d'hydrogène en eau par le glutathion peroxydase (GPx)<sup>51</sup>. Ces antioxydants enzymatiques sont les acteurs de première ligne de la défense antioxydante mitochondriale<sup>52</sup> (Figure 9).

Une défense antioxydante non enzymatique est représentée par les protéines non couplantes (UCP) de la membrane interne. Le sous-type UCP2 notamment est surexprimé en cas de stress oxydatif avec importation des protons intermembranaires vers la matrice mitochondriale à travers la membrane interne<sup>53</sup>. Cela a pour effet de diminuer le gradient électrochimique, et donc la respiration cellulaire mitochondriale, avec diminution de la production d'anions superoxyde<sup>54</sup>. En cas d'échappement à ces systèmes, le dernier mécanisme adaptatif est l'élimination des mitochondries compromises par la mitophagie<sup>55</sup>, ou des cellules compromises par l'apoptose, voire la nécrose<sup>56,57</sup>.

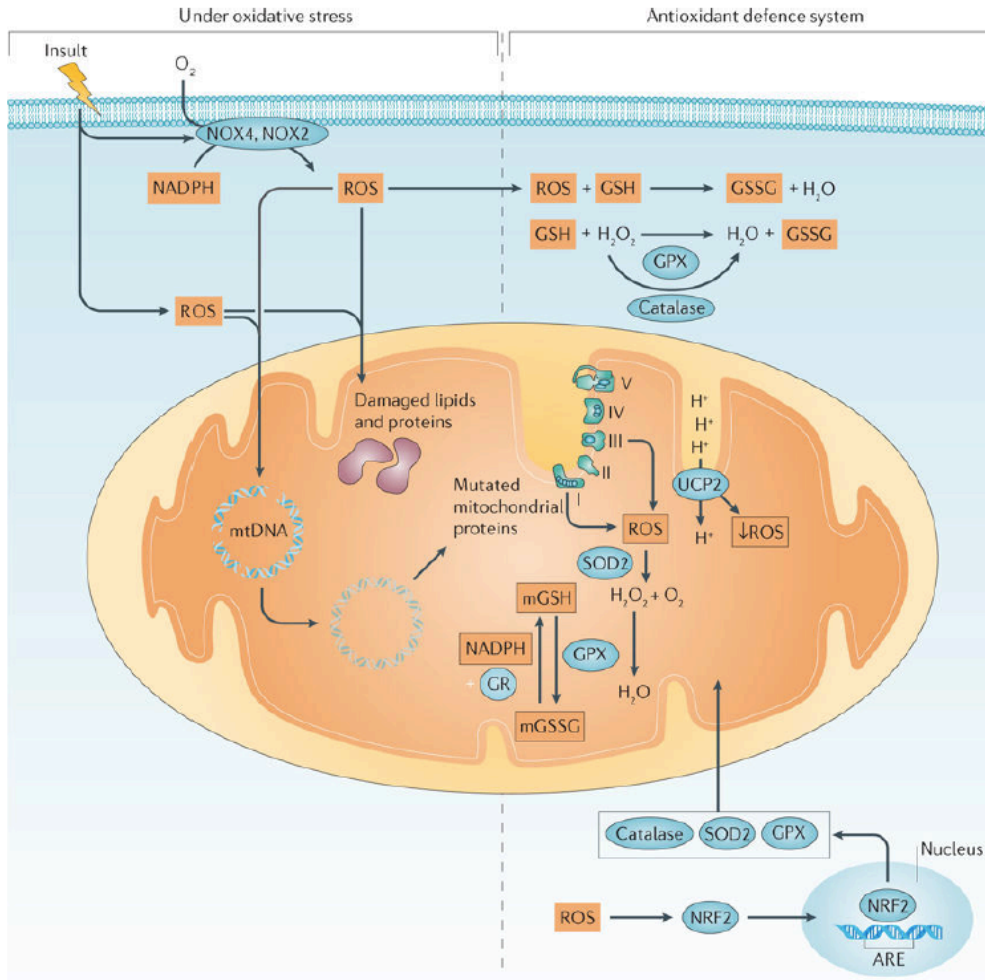


Figure 9 : Le stress oxydatif et le rôle antioxydant mitochondrial. A gauche, la formation d'EROs entraîne un stress oxydatif sur les complexes de l'ETC et sur l'ADNmt. A droite les réponses antioxydantes mises en œuvre par les mitochondries, centrées sur le glutathion (GSH et GSSG) recyclé par le couple catalase/glutathion peroxydase (GPX), la superoxyde dismutase (SOD2) et les protéines non couplantes (UCP2). D'après Bhargava P. and Schnellmann R., Nat. Rev. Nephrol. 2017<sup>58</sup>.

### 3. Apoptose et survie cellulaire

L'apoptose est un processus biologique physiologique définissant la mort cellulaire programmée<sup>59</sup>. Il existe différentes voies de signalisation de l'apoptose, parmi lesquelles 2 sont bien définies : la voie extrinsèque faisant appel aux récepteurs de la famille des TNF à la surface cellulaire, et la voie intrinsèque au cœur de laquelle se situe la mitochondrie<sup>60</sup>.

La cellule perçoit initialement des signaux apoptotiques (*i.e.* stress oxydatif important), qui se traduisent sur le plan moléculaire par une modulation des protéines de la famille Bcl-2<sup>61</sup>. Certaines sont pro-apoptotiques, et donc surexprimées (Bid, Bim, Bad, Bak, Bax), alors que d'autres, anti-apoptotiques, vont être diminuées (Bcl-2, Bcl-xL). Parallèlement, le réticulum

endoplasmique, interconnecté avec les mitochondries, percevant le signal apoptotique, va augmenter l'efflux calcique intramitochondrial<sup>62</sup>.

L'ensemble de ces phénomènes aboutit à la perméation de la membrane mitochondriale par un pore de transition de perméabilité (PTP)<sup>63</sup>. Celle-ci va s'accompagner d'une perte du gradient électrochimique avec disruption de l'ETC entraînant une diminution de la production d'ATP et une augmentation de celle d'EROs<sup>60</sup>. D'autre part, cette perméation va entraîner la libération, depuis la membrane mitochondriale interne, d'apoptosis-inducible factor (AIF)<sup>64</sup> et de cytochrome c<sup>65</sup> qui vont permettre la formation de l'apoptosome (caspase 9, cytochrome c et Apaf-1)<sup>66</sup>, activant la caspase 3 impliquée dans la voie terminale de l'apoptose avec la fragmentation nucléaire et l'activation d'une ADNase<sup>67</sup>. Cela s'accompagne d'une fragmentation mitochondriale par l'activation des protéines de la fission mitochondriale (Drp1, Fis1) en réponse à l'augmentation du volume mitochondrial secondaire à l'efflux calcique<sup>68</sup> (Figure 10).

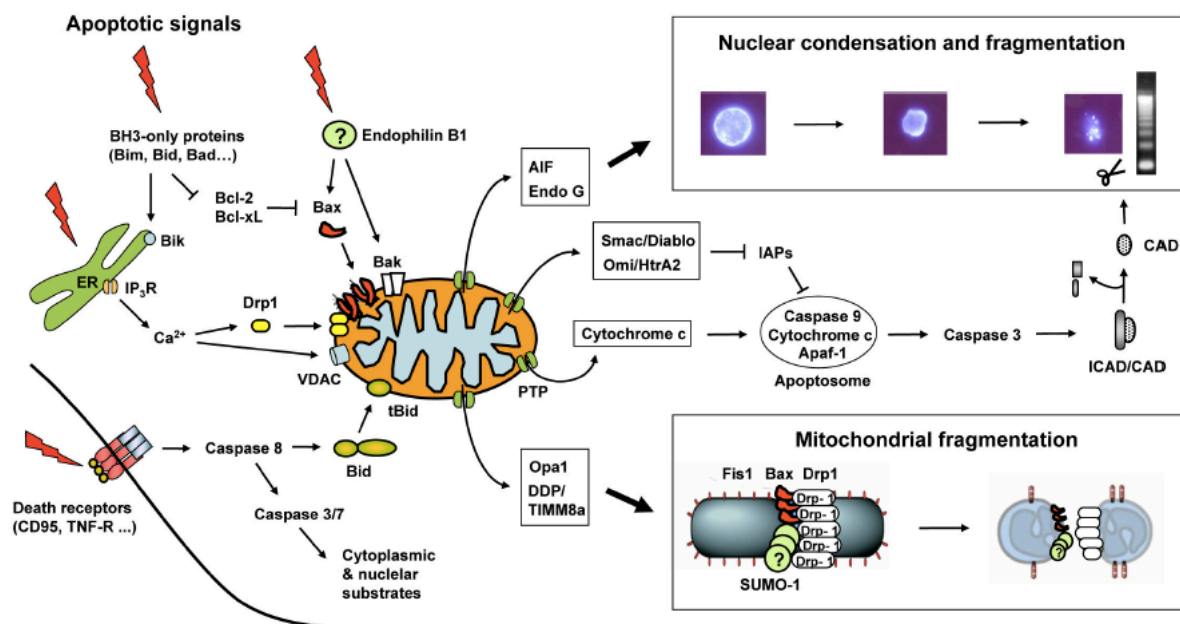


Figure 10 : Les voies de signalisation impliquées dans l'apoptose mitochondriale. D'après Jeong S-Y. and Seol D-W., *BMB reports.*, 2008<sup>68</sup>.

### III. Homéostasie mitochondriale

L'homéostasie mitochondriale dépend de l'équilibre entre la biogenèse, la fission, la fusion et la mitophagie. Ces processus sont coordonnés afin d'assurer un maintien en quantité suffisante de mitochondries fonctionnelles avec la bonne distribution cellulaire<sup>69</sup>, répondant ainsi aux besoins métaboliques des cellules.

## 1. Biogenèse mitochondriale

La biogenèse mitochondriale est la synthèse *de novo* de mitochondrie fonctionnelle. Cette biogenèse répond à une augmentation de la demande énergétique à l'échelle cellulaire qui se traduit par une augmentation du ratio adénosine mono-phosphate (AMP)/ATP ou NAD<sup>+</sup>/NADH, avec respectivement une surexpression d'AMP-activated protein kinase (AMPK) et de SIRT1/SIRT3<sup>70-72</sup>. Des médiateurs favorisant l'anabolisme, comme le mammalian target of rapamycin complex 1 (mTORC1), vont aussi être surexprimés<sup>73</sup>. Ces voies de signalisation aboutissent à l'expression de peroxysome proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC-1 $\alpha$ ), véritable chef d'orchestre de la biogenèse mitochondriale<sup>74</sup> (Figure 11). L'augmentation de son expression s'accompagne d'une part de l'augmentation de la synthèse mitochondriale (par activation de NRF1 et 2), et d'autre part d'une augmentation du rendement énergétique mitochondrial (par augmentation de l'OXPHOS et de la  $\beta$ -oxydation des acides gras)<sup>75-77</sup>.

## 2. Fusion et fission mitochondriale

La fusion mitochondriale résulte de la fusion de la membrane externe, puis de la membrane interne, de 2 mitochondries. C'est un processus qui dépend à l'échelle moléculaire de GTPases de la famille des dynamines : les mitofusines (Mfn) 1, 2 et optic atrophy type 1 (OPA1)<sup>78</sup> (Figure 11). Ce processus, aboutissant à une mitochondrie allongée, permet d'augmenter le rendement énergétique mitochondrial via l'OXPHOS<sup>79,80</sup>.

La fission, qui est le processus opposé par lequel 2 mitochondries sont individualisées à partir d'une seule mitochondrie, est sous la dépendance de Drp1 et Fis1<sup>81,82</sup> (Figure 11). L'accumulation de Drp1, autour de la membrane externe mitochondriale, sous l'action d'une GTPase, permet la scission membranaire<sup>83</sup>. Elle est déclenchée par une anomalie du potentiel transmembranaire mitochondrial pouvant être secondaire soit à une perméation mitochondriale (*i.e.* PTP), soit à un défaut de production de protons par mise à l'arrêt de la respiration mitochondriale (*i.e.* hypoxie, déprivation énergétique)<sup>84</sup>. Les médiateurs anaboliques de la voie mTORC1 sont à nouveau au cœur de cette régulation<sup>85</sup>. Les mitochondries résultantes sont ensuite orientées vers la mitophagie, si leur potentiel transmembranaire n'est pas rétabli<sup>84</sup>.

## 3. Mitophagie

La mitophagie est la mort programmée des mitochondries, faisant partie de l'autophagie des organelles cellulaires<sup>55</sup>. La régulation se fait autour d'une kinase : PINK1. Celle-ci est dégradée

dans la matrice mitochondriale en conditions physiologiques, évitant la mitophagie<sup>86</sup>. Dans des conditions de diminution du potentiel transmembranaire (déprivation énergétique, stress oxydatif, PTP), la voie AMPK va être sollicitée et va réprimer les médiateurs de mTORC1<sup>87,88</sup>. Cela va entraîner l'activation de PINK1 qui s'autophosphoryle en se fixant sur des protéines membranaires mitochondriales (dont Mfn1 et 2), recrutant le système PARKIN avec ubiquitination et destruction mitochondriale par l'autophagosome<sup>89-93</sup> (Figure 11). En condition d'hypoxie, une autre voie de régulation est sollicitée, avec l'activation de l'hypoxia inducible factor 1- $\alpha$  (HIF1 $\alpha$ ) qui va induire l'expression de protéines membranaires recrutant l'autophagosome<sup>94,95</sup>.

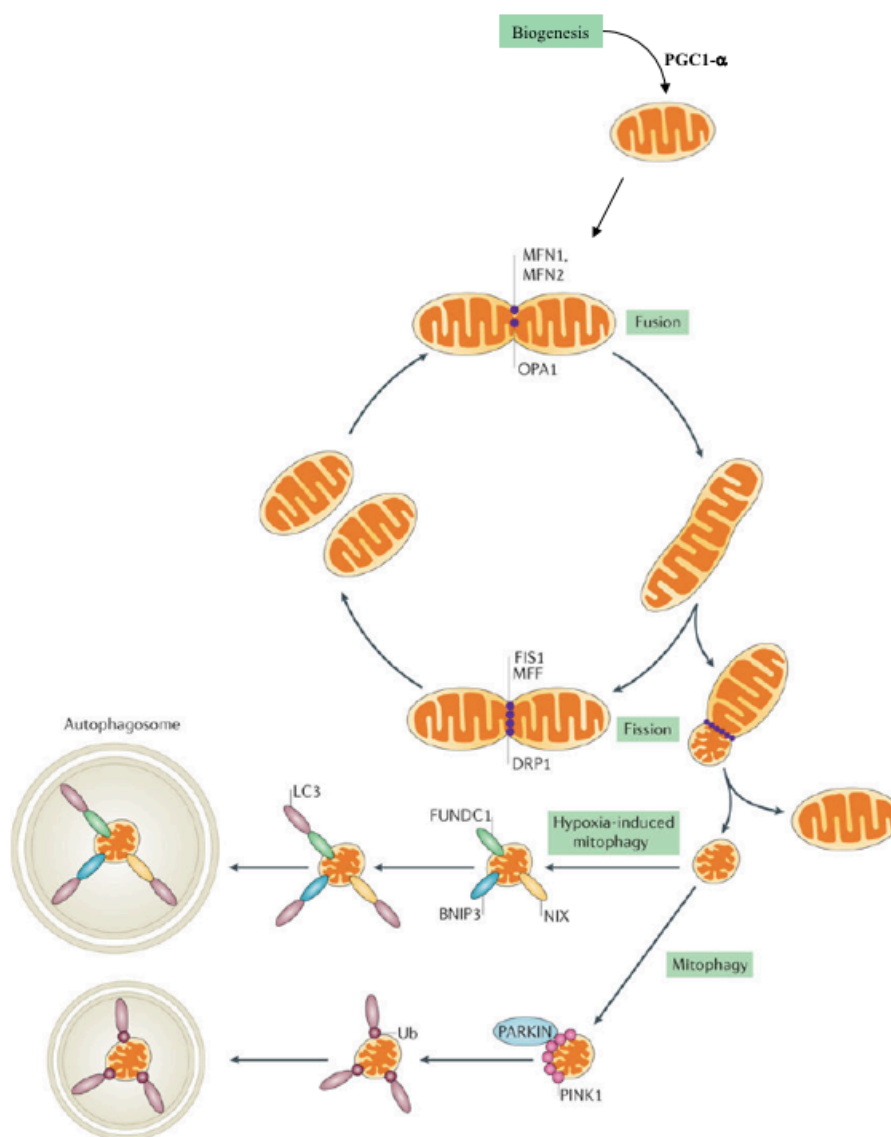


Figure 11 : Les mécanismes impliqués dans l'homéostasie mitochondriale résultent d'un équilibre entre la biogenèse mitochondriale, la fission, la fusion et la mitophagie. D'après Bhargava P. and Schnellmann R., Nat. Rev. Nephrol. 2017<sup>58</sup>.

## IV. Génétique mitochondriale

Les fonctions mitochondriales sont soumises à une dualité de contrôle par le matériel génétique mitochondrial et les gènes nucléaires impliqués dans la machinerie mitochondriale. Les variants pathogènes affectant l'un ou l'autre vont être susceptibles d'interférer avec les fonctions mitochondriales, et notamment la chaîne respiratoire mitochondriale<sup>96</sup>, responsable de mitochondriopathies primitives (MIDs).

### 1. ADN mitochondrial

#### Génomique mitochondriale

Les mitochondries sont les seules organelles ayant leur propre matériel génétique, l'ADNmt. Il est circulaire, bicaténaire et composé de 16569 paires de bases<sup>14</sup>. Il est très compact, ne présentant que des régions codantes ou impliquées dans la régulation de la transcription<sup>97</sup>. Les 2 brins diffèrent par leur composition en bases, avec un brin lourd (heavy ou H-strand) contenant la majorité des gènes et un brin léger (light ou L-strand). La transcription s'opère en permanence sur les deux brins, de manière indépendante du cycle cellulaire<sup>98</sup>.

L'ADNmt est composé de 37 gènes codant pour 22 ARN de transfert (ARNt), 2 ARN ribosomiaux (ARNr) et 13 protéines de l'ETC : 7 pour le complexe I (NADH, ND1 à 6), 1 pour le complexe III (cytochrome b), 3 pour le complexe IV (la cytochrome c oxydase, COX I à III) et 2 pour le complexe V (ATPase 6 et 8)<sup>99</sup> (Figure 12). Les mitochondries possèdent aussi leur propre machinerie cellulaire avec des ARNt et ARNr, directement impliqués dans la synthèse de protéines mitochondriales.

#### Variants pathogènes de l'ADNmt

Les variants pathogènes affectant l'ADNmt peuvent être des mutations ponctuelles, des délétions ou des duplications (<https://www.mitomap.org/MITOMAP>)<sup>100</sup>. Celles-ci ne suivent pas les règles de transmissions mendéliennes, mais des règles génétiques spécifiques résumées dans le Tableau 1.

Chaque tissu contient de nombreuses cellules, contenant environ une centaine de mitochondries, contenant chacune plusieurs molécules d'ADNmt. Si l'homoplasmie caractérise le fait que toutes les copies d'ADNmt sont identiques (état sauvage, polymorphismes mais aussi variants pathogènes<sup>101</sup>), l'hétéroplasmie caractérise le pourcentage d'ADNmt mutés coexistant avec l'ADNmt sauvage<sup>102</sup>. Lors de la division cellulaire, les organelles sont distribuées aléatoirement aux cellules filles, le taux d'hétéroplasmie cellulaire peut donc être modifié suite



aux mitoses : c'est la dérive neutre<sup>103</sup>. En fonction des tissus et de leur besoins énergétiques, du taux d'hétéroplasmie du variant pathogène et de l'altération de la fonction mitochondriale dont il est responsable, il y a un effet seuil à partir duquel le variant pathogène va s'exprimer phénotypiquement<sup>104</sup>. Le seuil d'hétéroplasmie nécessaire à l'expression phénotypique dans un tissu donné est lié à son besoin énergétique : plus les besoins sont grands, plus le seuil est faible<sup>105</sup>. Ainsi, le taux d'hétéroplasmie du variant pathogène mitochondrial est associé au pronostic de la MID<sup>106</sup>.

La transmission des variants pathogènes de l'ADNmt est exclusivement maternelle, en lien avec une exclusion active des mitochondries paternelles dans l'ovocyte<sup>107,108</sup>. La pénétrance est variable du fait d'un « bottleneck effect » (ou effet goulot) avec une diminution du matériel mitochondrial transmis au moment de l'ovogénèse, puis de l'embryogénèse, ce qui peut aggraver ou minimiser la transmission des copies de variants pathogènes de l'ADNmt<sup>109,110</sup>.

Les variants pathogènes de l'ADNmt surviennent fréquemment *de novo*<sup>111,112</sup>. Il est important de noter que cela peut être lié à une difficulté médicale à mettre en évidence la transmission, que ce soit par une faiblesse de l'interrogatoire pour dépister des cas pauci ou asymptomatiques, mais aussi par une difficulté à mettre en évidence la mutation chez les apparentés. En effet, le dépistage des apparentés se fait souvent sur prélèvement sanguin (leucocytes circulants), or on sait que le taux d'hétéroplasmie peut être plus bas dans ces cellules à fort taux de réplication, et qu'une analyse des cellules épithéliales urinaires, notamment, semble plus adaptée<sup>111</sup>. Du fait du l'effet goulot de l'ADNmt lors de l'ovogénèse et de l'embryogénèse, il est possible qu'un variant pathogène en très faible degré d'hétéroplasmie se retrouve au seuil nécessaire dans la descendance pour s'exprimer cliniquement. Enfin, il est aussi possible que le caractère *de novo* de ces mutations soit bien réel. Le génome mitochondrial étant très compact et dépourvu d'histones, il est 5 à 10 fois plus susceptible aux mutations que le génome nucléaire<sup>113</sup>.

De l'ensemble de ces règles génétiques spécifiques découle une grande hétérogénéité clinique associée aux mutations de l'ADNmt responsables de MIDs, qui peuvent survenir à tous les âges de la vie et toucher n'importe quel organe<sup>114</sup>. Chez l'adulte notamment, la prévalence semblerait être de 1/4300<sup>115</sup>, chiffre qui pourrait être sous-estimé et qui sera possiblement corrigé par la découverte de nouvelles mutations par Next-Generation Sequencing (NGS) de l'ADNmt<sup>15</sup>.

Tableau 1 : Résumé des règles de génétique mitochondriale et de leurs implications cliniques.

Règles génétiques mitochondriale	Caractérisation	Implications cliniques
<b>Hétéroplasmie</b>	Coexistence d'ADNmt sauvage et muté au sein d'un même tissu.	Hétérogénéité clinique.
<b>Effet seuil</b>	Seuil d'hétéroplasmie au-dessus duquel le variant pathogène s'exprime cliniquement.	Les tissus les plus consommateurs d'énergie sont préférentiellement affectés.
<b>Dérive neutre</b>	Distribution aléatoire des organelles lors de la mitose.	Hétérogénéité clinique au sein d'une même famille. Peut survenir à tout âge.
<b>Transmission maternelle</b>	Élimination active des mitochondries paternelles dans l'ovocyte.	Conseil génétique. Orientation diagnostique.
<b>Mutations <i>de novo</i></b>	Absence de mise en évidence de transmission maternelle.	Histoire familiale non indispensable au diagnostic. Peut survenir à tout âge.

## 2. ADN nucléaire

### Génomique nucléaire

Les mitochondries sont composées de 1100 à 1400 protéines, dont 90 rentrent dans la composition de la chaîne respiratoire<sup>116</sup>. Le reste des protéines mitochondriales, des sous-unités de l'ETC et de leurs facteurs d'assemblage, des protéines nécessaires à l'expression et à la réparation de l'ADNmt (dont la polymérase  $\gamma$  et l'hélicase Twinkle), de la synthèse protéique intra-organelle ainsi que des protéines régulant la dynamique mitochondriale, sont codées par des gènes de l'ADNn, et ensuite transférées dans les mitochondries<sup>117</sup> (Figure 12).

### Variants pathogènes de l'ADNn

Les variants pathogènes impliquant ces gènes de l'ADNn vont être classés en fonction de l'altération de la fonction mitochondriale dont ils sont responsables :

- variants altérant la synthèse des sous-unités de la chaîne respiratoire mitochondriale (le plus souvent dans le complexe I) ;
- variants altérant les facteurs d'assemblage des complexes de la chaîne respiratoire (*i.e.* gènes contrôlant la biosynthèse de la chaîne respiratoire mitochondriale, comme *COX10*)<sup>118</sup> ;

- variants altérant la dynamique mitochondriale (gènes de OPA1 et de la Mfn2 notamment) ;
- variants altérant les transporteurs d'électrons (*i.e.* gènes impliqués dans la biosynthèse de CoQ tels que *COQ*)<sup>119</sup> ;
- variants altérant la synthèse de la machinerie protéique mitochondriale (*i.e.* gènes codant pour la traduction de l'ARNm, pour l'aminoacyl-ARNt, pour les protéines ribosomales mitochondriales) ;
- variants altérant la maintenance de l'ADNmt<sup>120</sup> (*i.e.* gènes codant pour la réplication, la transcription de l'ADNmt, par exemple *TWINKLE* et *POLG*, responsable de délétions étendues et/ou multiples de l'ADNmt)<sup>121</sup>.

Le mode de transmission suit une loi Mendélienne, avec une transmission le plus souvent autosomique récessive (notamment dans des familles consanguines)<sup>105</sup>. De fait, ces mutations, responsables de MIDs, sont bien plus fréquentes dans l'enfance avec une prévalence chez l'adulte de 2,9/100000<sup>115</sup>. Néanmoins, l'avancée des connaissances avec le développement de Whole Exome (WES) et du Whole Genome Sequencing (WGS) pourraient faire découvrir de nouveaux variants pathogènes de l'ADNn associées avec des pathologies à l'âge adulte, ce qui augmenterait cette prévalence<sup>15</sup>.

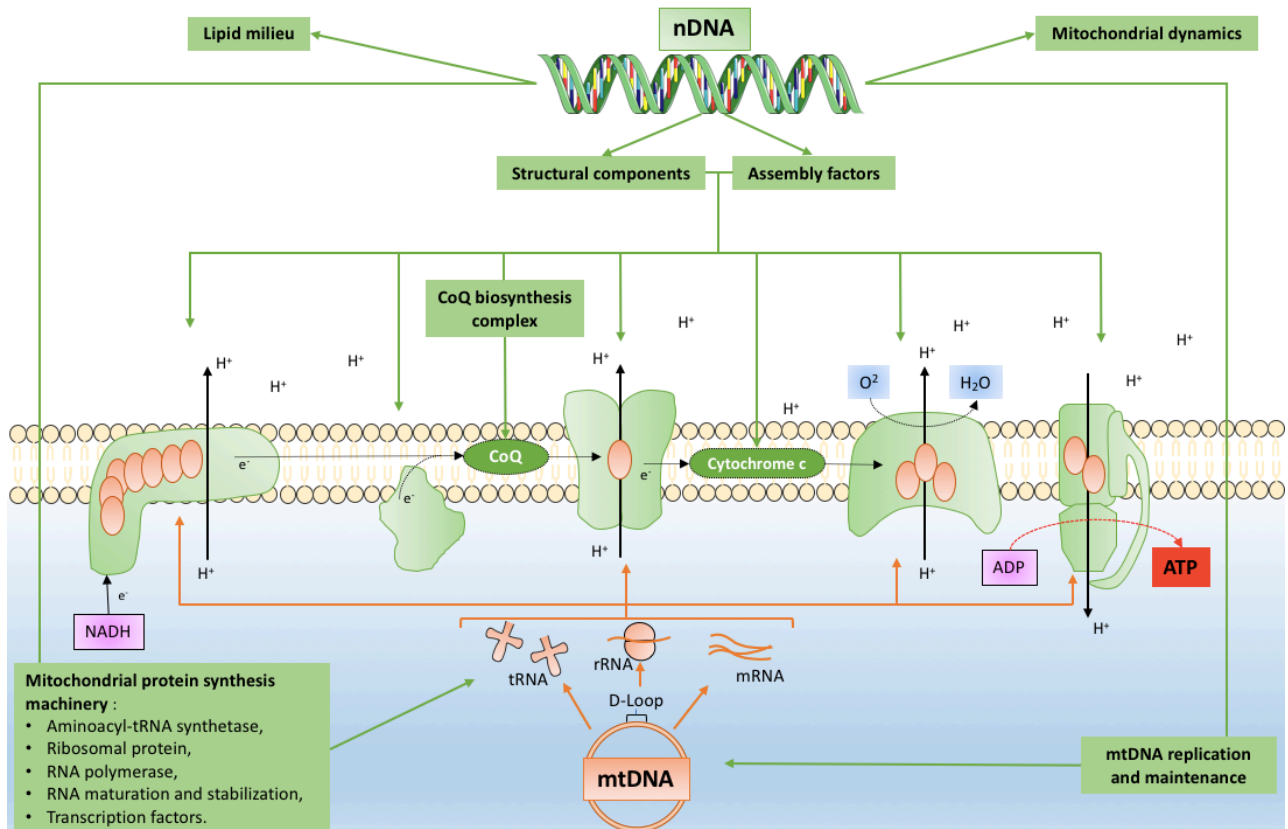


Figure 12 : Vision détaillée de la dualité génétique contrôlant les fonctions mitochondriales : dépendantes de gènes de l'ADNmt (en orange) et de l'ADNn (en vert).

## V. Le réseau mitochondrial au cœur du métabolisme rénal

Les reins sont les 2<sup>èmes</sup> principaux consommateurs d'O<sub>2</sub> et d'énergie une fois normalisé au poids de l'organe<sup>122,123</sup>. Ils reçoivent 20% du débit cardiaque (soit 1 L/min) pour assurer les apports en O<sub>2</sub> et en nutriments nécessaires à la haute consommation énergétique de cet organe. Le néphron est l'unité fonctionnelle rénale, particulièrement riche en mitochondries, dont la richesse varie selon les segments et leurs métabolismes<sup>124</sup>. On peut différencier le segment glomérulaire et le segment tubulaire, dans lesquels les anomalies du métabolisme mitochondrial sont impliquées dans des processus pathologiques.

### 1. Métabolisme mitochondrial glomérulaire : le podocyte

La barrière de filtration glomérulaire est composée de deux types cellulaires : les cellules endothéliales fenêtrées tapissant le capillaire glomérulaire, et les podocytes se prolongeant en processus puis en pédicelles reposant sur le versant externe de la membrane basale glomérulaire et connectés entre eux par le diaphragme de fente. Entre ces deux types cellulaires, se trouve la membrane basale glomérulaire. L'atteinte de ces structures s'accompagne d'un passage d'albumine dans les urines définissant la néphropathie glomérulaire.

Les podocytes ont une place prépondérante dans cette barrière de filtration, avec 4 fonctions essentielles, particulièrement consommatrices d'énergie<sup>125</sup> (Figure 13A):

- le soutien mécanique contrebalançant la pression hydrostatique des capillaires glomérulaires ;
- la synthèse de molécules essentielles à la communication intercellulaire (*i.e.* le VEGF sécrété par les podocytes agissant sur les récepteurs au VEGFR des cellules endothéliales) ;
- la synthèse de composants de la membrane basale glomérulaire ;
- la constitution ainsi que la modulation du diaphragme de fente. Ce diaphragme est essentiel à la barrière de filtration glomérulaire, par son intégrité structurale<sup>126,127</sup>, mais aussi par la formation d'un pore assurant des fonctions de filtration. En effet, les protéines constituant le diaphragme de fente (*i.e.* néphrine, podocine) sont directement ou indirectement ancrées à l'actine, dont la contraction peut assurer une modulation du diamètre d'ouverture (entre 5 et 15 nm)<sup>128</sup>.

La source d'énergie nécessaire aux podocytes pour l'ensemble de ces fonctions semble être assurée par les mitochondries. En microscopie électronique, de nombreuses mitochondries intra-podocytaires sont observées, que ce soit au niveau du corps cellulaire mais aussi au niveau des pieds podocytaires<sup>129</sup> (Figure 13B). De plus, les podocytes présentent sur leur membrane cellulaire les transporteurs nécessaires à l'importation du glucose, des acides aminés et des acides gras (GLUT<sup>130</sup>, LAT<sup>131</sup> et CD36<sup>132</sup>, respectivement). Or, ces nutriments sont impliqués dans la respiration mitochondriale.

Les études physiologiques réalisées sur des podocytes murins ont mis en évidence que l'OXPPOS est responsable de 75% de leur métabolisme énergétique à partir de divers substrats (pyruvate, lactate, palmitate et acides aminés) et que la glycolyse assure la formation de 25% de l'ATP cellulaire<sup>133</sup>. La glycolyse semble être prédominante dans les pieds podocytaires (dynamisme du cytosquelette d'actine), alors que les mitochondries, en plus grand nombre dans le corps cellulaire, seraient garantes de l'énergie nécessaire à la différenciation, à la communication intercellulaire et à la synthèse des protéines du diaphragme de fente<sup>134</sup>.

Les podocytes sont hautement dépendant de ces 2 mécanismes, l'inhibition sélective de l'OXPPOS ou de la glycolyse étant très mal compensée sur le plan de la production d'ATP<sup>135</sup>. Ces conditions s'accompagnent de lésions podocytaires avec une altération de la barrière de filtration glomérulaire pouvant donner lieu à une néphropathie glomérulaire. De plus, les podocytes étant des cellules extrêmement différenciées et sans capacité de renouvellement, les mécanismes d'homéostasie mitochondriales (fission, fusion, biogenèse et mitophagie) sont essentiels pour éviter l'accroissement de mitochondries de moindre qualité fonctionnelle avec un ADNmt accumulant des mutations<sup>129,136,137</sup>.

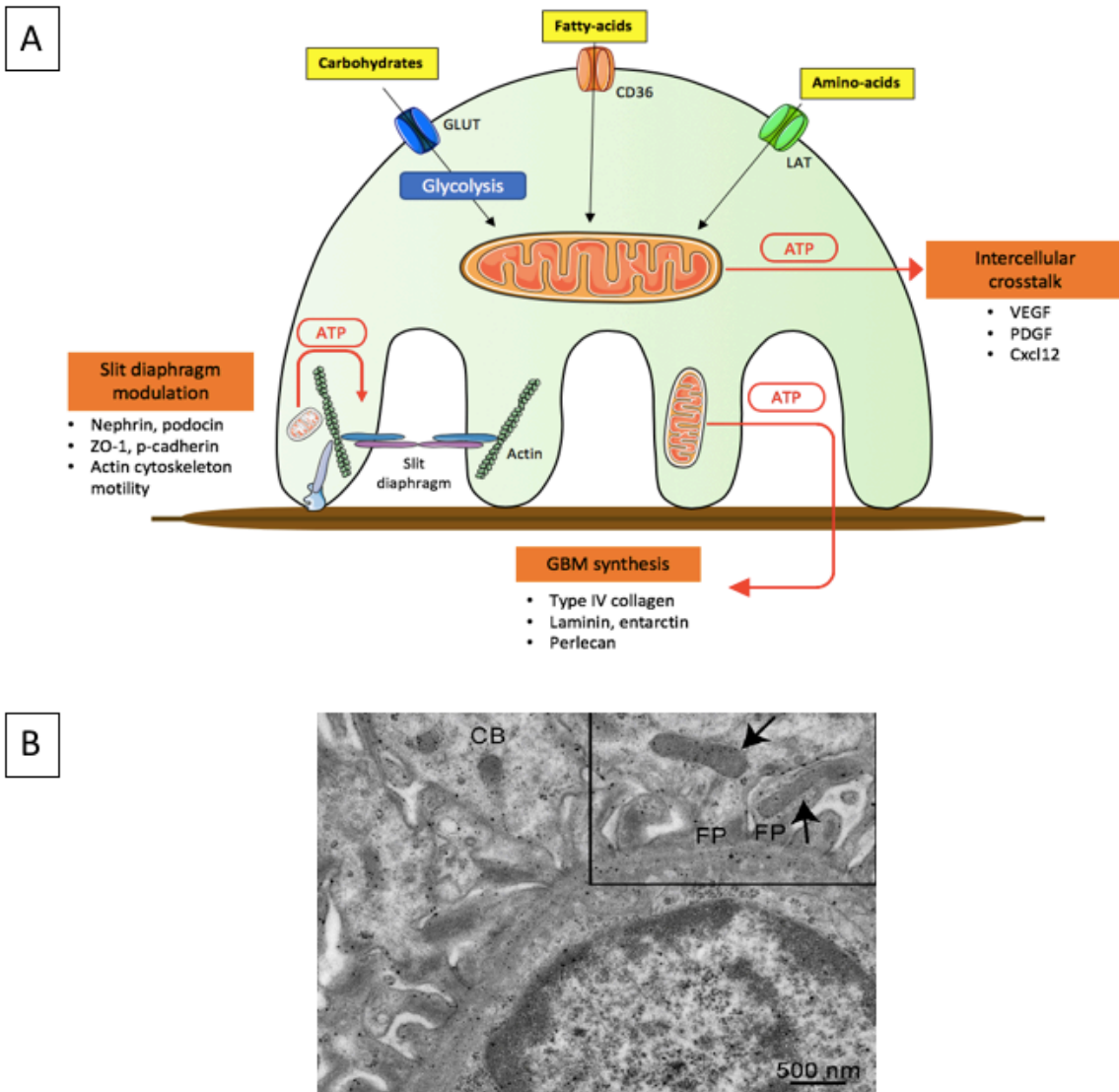


Figure 13 : Les mitochondries sont essentielles aux fonctions podocytaires. **A.** Un matériel mitochondrial fonctionnel et en bonne quantité est nécessaire pour assurer les fonctions podocytaires. **B.** Photographie d'une barrière de filtration glomérulaire en microscopie électronique, mettant en évidence des mitochondries dans les pieds podocytaires (FP) et dans le corps cellulaire (CB). D'après Imasawa T. et Rossignol R., *Int. J. Biochem. Cell Biol.*, 2013<sup>129</sup>.

L'urine primitive formée après la filtration glomérulaire, bénéficie ensuite des modifications de composition lors de son trajet le long des segments tubulaires du néphron pour aboutir à l'urine définitive.

## 2. Métabolisme mitochondrial tubulo-interstitiel

Ce sont les tubules rénaux qui sont les garants de l'homéostasie hydro-électrolytique par des phénomènes de réabsorption et de sécrétion *via* les cellules épithéliales tubulaires. L'ion réabsorbé en plus grande quantité est le sodium avec, pour un patient ayant une fonction rénale normale (DFG de 120 mL/min), une natrémie à 140 mmol/L et des apports sodés de 10 g/j, une

quantité de 24192 mmol/j de sodium réabsorbé et 170 mmol/j de sodium excrété, soit une réabsorption à plus de 99% du sodium filtré. Les membranes apicales des cellules épithéliales tubulaires sont imperméables aux ions mais possèdent des transporteurs et/ou canaux spécifiques le long des différents segments (Figure 14A). La réabsorption apicale n'est possible qu'avec l'obtention d'un gradient électrochimique favorable, garanti par les pompes  $\text{Na}^+/\text{K}^+$  ATPases présentes à la membrane basolatérale de l'ensemble des cellules épithéliales tubulaires. Elles permettent la sortie de la cellule de 3 molécules de sodium et l'entrée de 2 molécules de potassium contre le gradient électrochimique, grâce à l'énergie libérée par l'hydrolyse de l'ATP fourni par la respiration mitochondriale<sup>138</sup>. Les cellules épithéliales tubulaires expriment aussi à leur membrane basolatérale, les transporteurs des nutriments nécessaires à la respiration, à savoir GLUT<sup>139</sup>, CD36<sup>140</sup> et LAT<sup>141</sup>, pour importer respectivement du glucose, des acides gras et des acides aminés. On estime ainsi la consommation d' $\text{O}_2$  au niveau tubulaire pour permettre la réabsorption de sodium à environ 1250 mmol/j<sup>122,142</sup>.

Le métabolisme énergétique tubulaire dépend de nombreux facteurs, tels que le segment tubulaire impliqué, son oxygénation et les besoins de réabsorption électrolytique. Cela rend difficile la schématisation de la production de l'ATP le long du tubule rénal<sup>143</sup>. En effet, l'hétérogénéité anatomique entre la corticale, la médullaire externe et interne, s'accompagne de conditions d'oxygénation, et donc, de métabolisme énergétique différents<sup>144</sup>. Cependant, on peut simplifier la répartition du matériel mitochondrial en suivant la quantité de sodium réabsorbée le long du tubule, avec une forte densité de mitochondries dans le tubule proximal (PCT) et la branche ascendante large de Henlé (TAHL), et une densité s'amenuisant dans le tubule contourné distal (DCT) et le canal collecteur (CC) (Figure 14B)<sup>145,146</sup>.

Le PCT, de situation plutôt corticale, lieu d'environ 65% de la réabsorption sodée, bénéficie de cellules riches en mitochondries. Il n'y a pas de glycolyse dans ce segment tubulaire qui est très dépendant de l'OXPPOS avec la  $\beta$ -oxydation des acides gras et la cétogenèse<sup>143</sup>. Le métabolisme glucidique est orienté vers la gluconéogenèse, qui peut être à l'origine de plus de 50% des sources glucidiques en condition de jeûne prolongé.

L'anse de Henlé, descendant plus ou moins profondément dans la médulla, dont la TAHL, remontant vers la corticale, réabsorbe environ 25% du sodium filtré. Les conditions d'oxygénation de ce segment tubulaire sont très variables avec un métabolisme énergétique qui lui aussi sera très variable. En effet, la TAHL est très riche en mitochondries<sup>147</sup>, avec un métabolisme des acides gras et des corps cétoènes prépondérant<sup>148</sup>, mais aussi un catabolisme

glucidique (aérobie ou anaérobie)<sup>142</sup>, avec une oxygénation variable selon les segments<sup>122</sup>. Il est possible que cette pluralité métabolique réponde à une capacité d'adaptation pour la réabsorption du sodium *via* le rétrocontrôle tubulo-glomérulaire<sup>149</sup>.

Les cellules du DCT, réabsorbant 8% du sodium filtré, sont pauvres en mitochondries, avec un métabolisme énergétique reposant essentiellement sur la glycolyse aérobie grâce à une vascularisation adéquate, mais aussi en moindre proportion sur la  $\beta$ -oxydation des acides gras et les corps cétonés<sup>142,143</sup>.

Le métabolisme du CC, moins étudié, dépendrait essentiellement de la glycolyse (aérobie et anaérobie) le long de la médulla, ainsi que de la  $\beta$ -oxydation des acides gras en moindre proportion<sup>143</sup>.

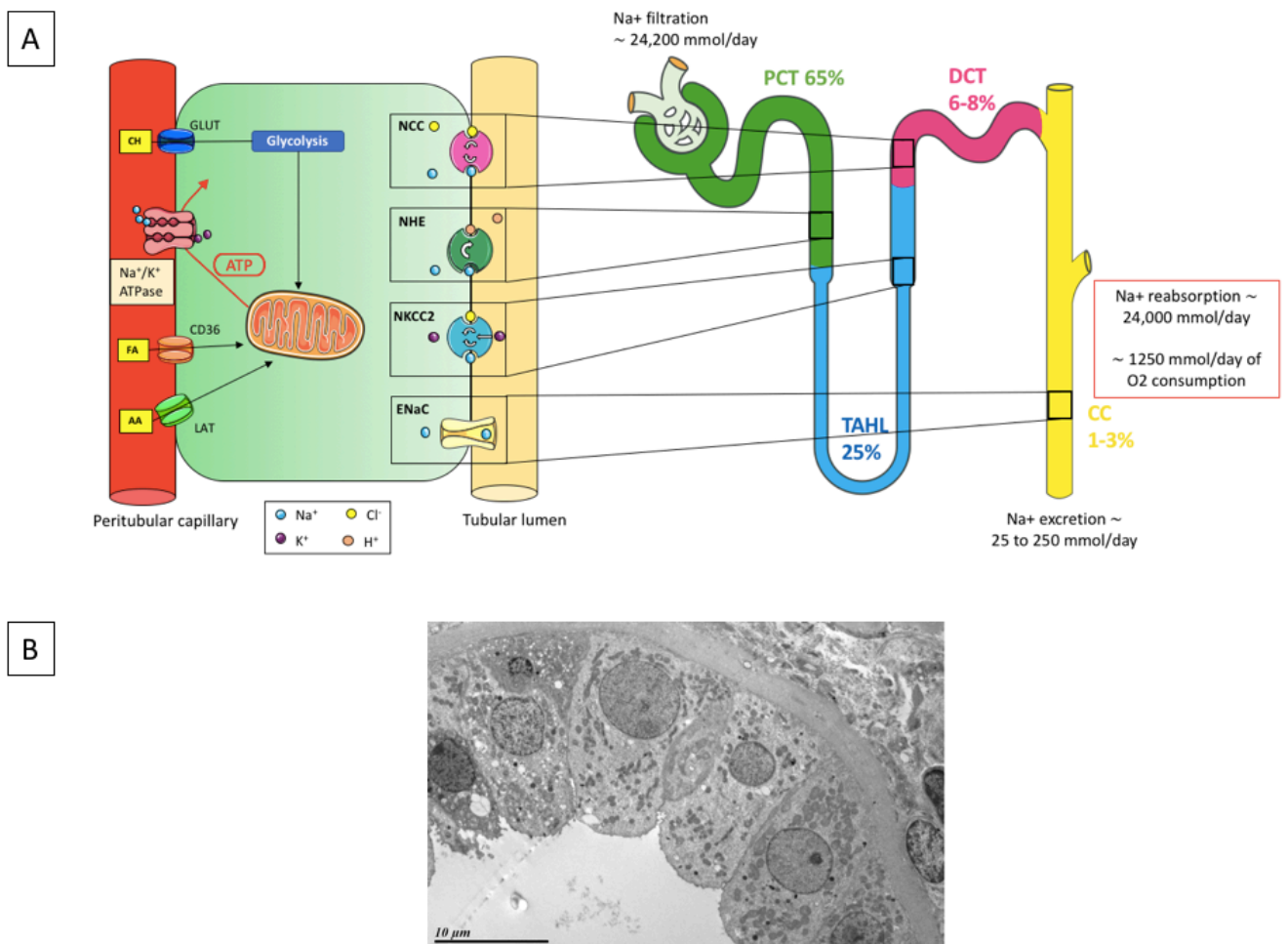


Figure 14 : Les mitochondries sont essentielles aux fonctions des cellules tubulaires rénales. **A.** Les mitochondries sont nécessaires à la réabsorption sodée par les cellules épithéliales le long de l'ensemble des différents segments tubulaires. **B.** Photographie d'une de cellules épithéliales tubulaires en microscopie électronique, montrant la richesse en mitochondries de ces cellules. D'après Bakis H. et al., *Clin. Genet.*, 2020<sup>150</sup>. CH : glucose / FA : acides gras / AA : acides aminés.



## VI. Les mitochondriopathies secondaires et les maladies rénales

Les mitochondriopathies secondaires sont définies par des dysfonctions mitochondriales acquises suites à des affections systémiques (*i.e.* l'hypertension artérielle, le diabète), par opposition aux MIDs liées à des variants pathogènes de l'ADNmt ou nucléaire.

### 1. Implications dans le développement des glomérulopathies

#### La hyalinose segmentaire et focale

Le métabolisme énergétique mitochondrial est prépondérant et indispensable aux fonctions podocytaires. Les atteintes podocytaires sont considérées comme l'élément central des lésions de hyalinose segmentaire et focale (HSF)<sup>127,151</sup>. Il a été observé sur modèle murin de HSF des anomalies morphologiques mitochondriales<sup>152</sup>. Les mécanismes reliant l'atteinte podocytaire et l'atteinte mitochondriale sont pour l'heure peu connus. Cependant, Yamagata *et al.* mettent en évidence des délétions communes de l'ADNmt par PCR *in situ* dans les podocytes de 59% des biopsies rénales de HSF primitive<sup>153</sup>. Kawakami *et al.* montrent, sur un modèle murin *Atg5<sup>-/-</sup>* conditionnel (indispensable à la mitophagie), uniquement sur l'épithélium rénal, que le défaut de mitophagie des podocytes entraîne l'apparition de lésions de HSF avec effacement des pieds podocytaires<sup>154</sup>. Les lésions de l'ADNmt observées et résultantes des lésions de HSF pourraient donc venir de l'accumulation de mitochondries qualitativement altérées, dont le rendement en ATP est moindre et la production EROs augmenté.

#### La néphropathie diabétique

La néphropathie glomérulaire diabétique est la deuxième cause de maladie rénale chronique (MRC) stade 5 en France<sup>155</sup>. Elle est définie par la présence d'un diabète déséquilibré avec une atteinte microvasculaire rénale caractérisée par une albuminurie, un déclin du DFG faisant suite à une phase d'hyperfiltration, et une hypertrophie glomérulaire et tubulaire sur l'analyse de la biopsie rénale. Le métabolisme mitochondrial est altéré en réponse à l'hyperglycémie chronique avec une augmentation de la production d'EROs entraînant un stress oxydatif important avec l'accumulation de mutations de l'ADNmt et l'altération de l'OXPPOS<sup>156,157</sup>. Ce phénomène est potentialisé par l'augmentation de produits de la glycation avancée<sup>158</sup>. De plus, l'hyperglycémie entraîne des anomalies de l'homéostasie mitochondriale avec une augmentation de la fission mitochondriale via l'activation de Drp1<sup>159,160</sup> et une diminution de la mitophagie par activation de la voie impliquant mTORC1 associée à une diminution d'AMPK<sup>161,162</sup>. Il existe aussi une diminution de la biogenèse mitochondriale par diminution

de PGC-1 $\alpha$ <sup>163</sup>, et de la fusion mitochondriale par diminution de Mfn2<sup>164</sup>. Il en résulte une inadéquation entre la demande et les besoins énergétiques glomérulaire, notamment à l'échelle podocytaire<sup>135</sup> avec un effacement des pieds podocytaires en étude microscopique, et une diminution du nombre de podocytes par autophagie (Figure 15)<sup>165,166</sup>.

### La néphropathie hypertensive

L'altération du métabolisme mitochondrial podocytaire est aussi impliquée dans le développement de la glomérulopathie hypertensive, première cause de MRC en France<sup>155</sup>. En effet, des études murines ont montré que l'aldostérone entraîne une diminution de l'OXPPOS précédant l'altération des podocytes avec l'effacement des pieds podocytaires et la diminution du nombre de podocytes<sup>167,168</sup>. Les podocytes déplétés, n'ayant pas de capacité de renouvellement, laissent place à un processus cicatriciel responsable de lésions de glomérulosclérose. Il existe aussi une atteinte des mitochondries endothéliales avec des segments artériels à destination rénale qui perdent leur capacité de relaxation. Ce phénomène participe aussi aux lésions glomérulaires.

## 2. Implications dans les atteintes tubulaires rénales

### L'insuffisance rénale aigüe

L'insuffisance rénale aigüe (IRA) est définie par une altération brutale du DFG se traduisant par une élévation de l'urée, de la créatininémie, voire même d'une diminution du volume urinaire<sup>169</sup>. Elle peut être la conséquence de mécanismes pré-, post- ou intra-rénaux, et le plus souvent de mécanismes hypoxiques (ischémie-reperfusion). Un des principaux sites lésionnels de l'IRA est le PCT<sup>170</sup>, qui est le segment tubulaire dont les cellules contiennent un grand nombre de mitochondries. Au cours de l'IRA, les mitochondries tubulaires apparaissent microscopiquement altérées, gonflées, avec perte de la structure en crête<sup>121</sup>. Plusieurs études se sont alors intéressées à l'implication du matériel mitochondrial dans le développement des lésions responsables de l'IRA (Figure 15).

Une altération de l'OXPPOS à la phase précoce de l'IRA est objectivée dans le PCT induisant une carence en production d'ATP et une augmentation des EROs<sup>58,171</sup>. Le stress oxydatif s'accompagne d'une altération des cellules endothéliales qui vont aggraver l'hypoxie rénale. A cela s'ajoute une altération de la  $\beta$ -oxydation<sup>172,173</sup>, en partie par diminution de la navette carnitine<sup>174</sup>, empêchant l'entrée mitochondriale des acides gras qui s'accumulent dans le cytoplasme cellulaire<sup>175,176</sup>. La disruption du potentiel membranaire mitochondrial entraîne

alors la formation d'un PTP avec libération de cytochromes c pro-apoptotiques pour les cellules tubulaires<sup>177,178</sup>.

La dynamique mitochondriale est perturbée, avec une augmentation de la fission mitochondriale par augmentation très précoce de Drp1<sup>179,180</sup>. La mitophagie est promue dans les modèles d'IRA ischémique, par activation de la voie HIF1 $\alpha$ , et semble avoir un rôle direct dans la constitution des lésions<sup>181,182</sup>. La biogenèse mitochondriale ainsi que la transcription de protéines mitochondriales sont fortement diminuées, résultant d'une diminution de PGC-1 $\alpha$ <sup>171</sup>. De plus, le retour à la normale de l'expression de PGC-1 $\alpha$  s'accompagne d'une récupération de la fonction rénale<sup>183,184</sup>. L'ensemble de ces découvertes physiopathologiques récentes impliquant le matériel mitochondrial dans la physiopathologie de l'IRA sont prometteuses en terme de thérapies émergentes<sup>185</sup>.

#### La néphropathie tubulo-interstitielle chronique

Les anomalies du métabolisme mitochondrial sont engagés dans des processus pro-fibrosant<sup>186</sup>. La transition épithélio-mésenchymateuse et endothélio-mésenchymateuse, jouant un rôle central dans les processus de fibrose rénale<sup>187</sup>, est stimulée par la production d'EROs (Figure 15)<sup>188</sup>. De plus, l'expression de TGF- $\beta$  va être stimulée par les EROs<sup>189</sup>, augmentant la transition épithélio- et endothélio-mésenchymateuse, le stress oxydatif<sup>190,191</sup>, et donc la fibrose rénale. Les mitochondriopathies secondaires semblent donc aussi impliquées dans le développement de lésions chroniques de fibrose interstitielle et d'atrophie tubulaires, définissant les néphropathies tubulo-interstitielles chroniques lorsqu'elles sont prépondérantes<sup>192</sup>, mais accompagnant aussi les lésions rénales évoluant vers la MRC et le vieillissement rénal.

### 3. Implications dans l'initiation et la progression de la MRC

La MRC est définie par l'altération de la fonction ou de la structure rénale pendant plus de 3 mois<sup>193</sup>. La physiopathologie de sa progression pourrait impliquer le réseau mitochondrial<sup>194</sup>. Les lésions glomérulaires, dont la physiopathologie est associée à des dysfonctions mitochondriales (glomérulopathies diabétiques<sup>195</sup>, hypertensives<sup>196</sup> et lésions de HSF<sup>153</sup>), sont responsables d'une albuminurie. Cette dernière altère le métabolisme mitochondrial au niveau tubulaire, et notamment au niveau du PCT, avec une augmentation de la production d'EROs<sup>197</sup> et de l'apoptose cellulaire<sup>198</sup>. De plus, sans considérer la protéinurie, les dysfonctions mitochondriales sont impliquées dans la physiopathologie des IRA<sup>121</sup> qui peuvent évoluer vers

la MRC<sup>199</sup>. Cette évolution peut se faire, notamment, par l'engagement de processus pro-fibrosant aboutissant à des lésions d'atrophie tubulaire et de fibrose interstitielle<sup>186</sup>.

La baisse de DFG s'accompagne de l'accumulation de toxines urémiques, lesquelles peuvent être produites par les mitochondries, mais aussi les cibler<sup>200</sup>. En effet, le stress oxydatif est augmenté chez les patients avec une MRC<sup>201</sup>. L'hyperuricémie, par exemple, va augmenter la formation d'EROs dans les cellules endothéliales rénales, entraînant une apoptose et nécrose de ces dernières par activation de calpaïnes engendrant un efflux calcique intracellulaire<sup>202</sup>. De plus, le nombre de copies d'ADNmt circulant, utilisé comme marqueur de l'altération des fonctions mitochondriales<sup>203</sup>, est plus bas dans la population avec une MRC incidente<sup>204</sup>.

Il est intéressant de noter que les manifestations attribuées aux toxines urémiques, telles que la myopathie<sup>205</sup> et la neuropathie urémique<sup>206</sup>, pourraient être la conséquence de dysfonctions mitochondriales médiées par ces toxines.

L'ensemble de ces données sont en faveur d'un cercle vicieux entre l'apparition de lésions rénales (*i.e.* glomérulaires, tubulo-interstitielles), la progression de la MRC, l'accumulation de toxines urémiques et les dysfonctions mitochondriales.

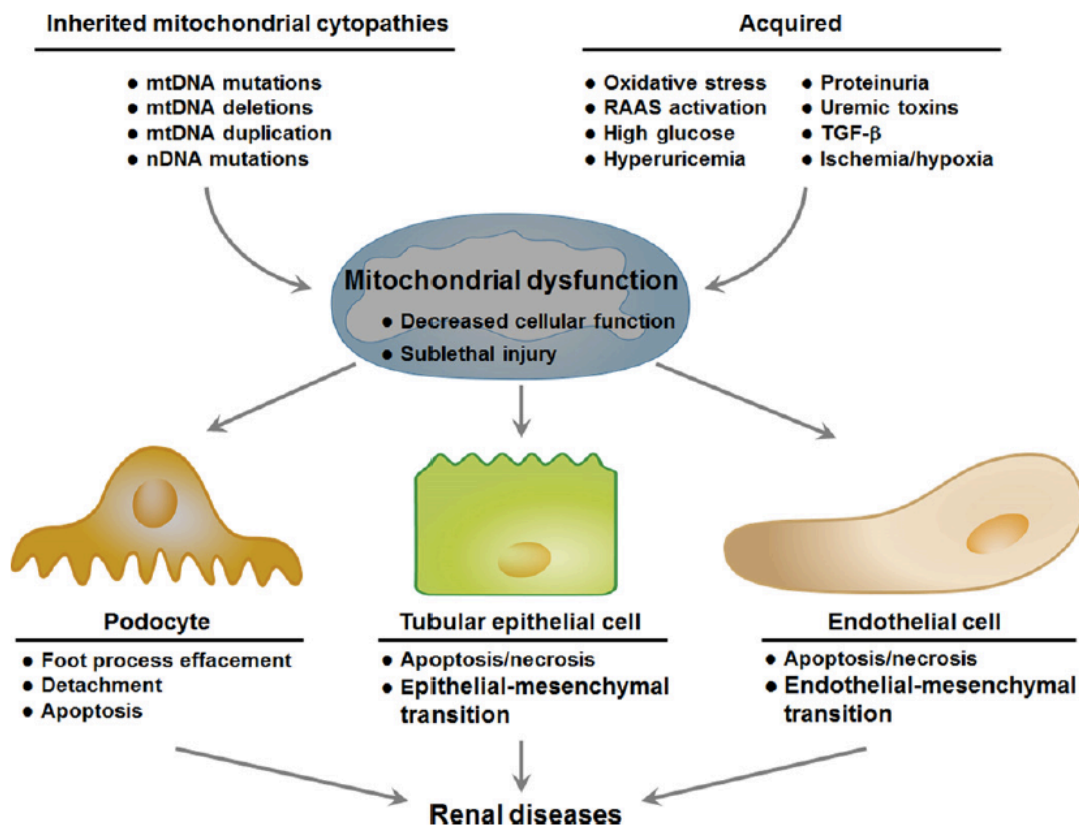


Figure 15 : Les mitochondriopathies tiennent une place centrale dans le développement des néphropathies. Ces mitochondriopathies peuvent être secondaires à des situations pathologiques (*i.e.* diabète, IRA et MRC), ou peuvent être primitives, liées à des variants pathogènes de l'ADNn ou de l'ADNmt. D'après Che R. et al., *Am. J. Physiol. Renal. Physiol.*, 2014<sup>194</sup>.

Les mitochondries ont une place centrale dans le développement et la progression des néphropathies. En effet, nous venons de voir que les dysfonctions mitochondriales pouvaient être secondaires à des situations pathologiques, et viennent déclencher, entretenir et/ou aggraver des maladies rénales.

Les variants pathogènes affectant les gènes codant pour les fonctions mitochondriales sont responsables de MIDs qui sont aussi responsables de néphropathies auxquelles nous allons nous intéresser, notamment chez l'adulte, à travers une revue de la littérature. Ensuite, nous rapporterons la première description de 3 patients adultes associant une néphropathie tubulointerstielle à des variants pathogènes de *MT-ND 5*. Pour finir, nous analyserons une cohorte de patients ayant eu un diagnostic de MID à l'âge adulte et dans laquelle nous déterminerons la prévalence de l'atteinte rénale. Les atteintes rénales seront détaillées afin d'étayer ce champ peu exploré des néphropathies associées aux MIDs (MIDANs).

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## **Article 1 : Revue de la littérature**



# Primary mitochondrial-disorders-associated nephropathy in adulthood

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**Keywords:** mitochondrial disorders, adult, kidney disease, tubulo-interstitial nephritis, glomerular disease.

**Authors contribution :** H.B., A.T., M.L.M.N. and C.R. designed the study; H.B. collected and analyzed the data; H.B. wrote the manuscript; A.T., C.C., M.L.M.N. and C.R were involved in the critical revision of the manuscript; all authors approved the final version of the manuscript.

## Disclosures:

The authors of this manuscript have no conflict of interest to disclose

## **Abstract**

Oxidative phosphorylation (OXPHOS) by mitochondria ensures the production of ATP, the major source of energy for cells. This function is the product of proteins encoded by both mitochondrial and nuclear DNA genes. Pathogenic variants affecting this genomic duality are evidenced by primary mitochondrial disorders (MIDs). Initially considered as a childhood affliction, MIDs have also been diagnosed in adults. The heterogeneous manifestations of MIDs in adults, a product of the complexity of mitochondrial genetics rules, requires the involvement of various medical specialties.

As mitochondria are present in nearly all cells, MIDs can affect every organ depending on its energy reliance. Kidneys are second to the heart in terms of energy consumption. Tubular epithelial cells are highly dependent on OXPHOS to ensure sodium reabsorption. The glomerular filtration barrier and, above all podocytes, require ATP to maintain plasma filtration. Reports of primary mitochondrial-disorders-associated nephropathy (MIDANs) in the adult population are scarce, and limited to case reports. Nevertheless, a systematic literature review revealed that MIDANs in adults seemed to be a frequent but underestimated entity. Kidney involvement is represented by a large contingent of glomerular diseases and tubulo-interstitial nephritis (TIN). The former consists mainly of focal segmental glomerulosclerosis, frequently reported with diabetes mellitus and hearing impairment. TIN is associated with extrarenal manifestations ranging from an isolated pathology to multisystemic syndrome.

Next-generation sequencing as well as exome and whole-genome sequencing are expected to pave the way for a new era of diagnostics in patients with MIDs and MIDANs. Current treatments have yet to be tested in randomized control trials but emerging therapies aimed at modulating mitochondrial functions hold promise. Studies evaluating MIDAN diagnosis among adults with MID and in patients with poorly characterized kidney disease are mandatory for a comprehensive approach to MIDANs.

## Introduction

Sometime during evolution, an aerobic bacterium formed an endosymbiotic relationship with an eukaryote precursor lacking aerobic metabolism. This bacteria (trusted to be *Rickettsia prowazekii*<sup>1</sup>) eventually became a mitochondrion, an intracellular organelle allowing aerobic metabolism and conferring survival benefits to eukaryote cells. Mitochondria were first observed by Richard Altmann in 1890, with a description of their ultrastructure reported during the 1950s, facilitated by the development of electron microscopy. Mitochondria are composed of a permeable external membrane (5000-Dalton pores), an intermembrane space, an impermeable inner membrane characterized by folds responsible for the structure of mitochondrial cristae, and the mitochondrial matrix (contained by the inner membrane)<sup>2,3</sup>.

Mitochondria are a plastic network of organelles that perform essential functions for cells, including the regulation of reactive oxygen species (ROS)<sup>4</sup>, of cell survival, as well as the of apoptosis<sup>5</sup>, the maintenance of calcium homeostasis and, in humans, the generation of 90% of the body's energy in the form of adenosine triphosphate (ATP)<sup>6</sup>. The latter is achieved by the electron transport chain (ETC) and oxidative phosphorylation reactions (OXPHOS) through the mitochondrial respiratory chain, located in the inner mitochondrial membrane (Figure 1). The multimeric protein complexes that make up the mitochondrial respiratory chain are nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase (complex I, 46 subunits), succinate quinone oxidoreductase (complex II, 4 subunits), ubiquinone-cytochrome c reductase (complex III, 11 subunits), and cytochrome C oxidase (complex IV, 1c3 subunits). Two small electron carriers, ubiquinone [coenzyme Q10 (CoQ10)] and cytochrome c, are also involved in the respiratory chain. The final component of the respiratory chain is ATP synthase, a F<sub>0</sub>F<sub>1</sub> ATPase whose 16 subunits make up complex V. Although the complexes were initially thought to be independent and freely moving<sup>7</sup>, their assembly into supercomplexes (association of complexes I, III and V) that enhance energy production and diminish ROS production was later demonstrated<sup>8,9</sup>.

ATP synthesis relies on the chemiosmotic theory, proposed by Peter Mitchell, who was awarded the 1978 Nobel Prize in chemistry for this work<sup>10</sup>. Electrons from NADH and flavin adenine dinucleotide (FADH<sub>2</sub>) are transported to oxygen through ETC complexes, resulting in the formation of water. At the same time, protons are pumped into the intermembrane space by complexes I, III, and IV, resulting in an electrochemical proton gradient across the inner membrane. ATP synthase uses the force of this gradient to phosphorylate adenosine-diphosphate (ADP) to generate ATP<sup>10,11</sup> (Figure 1).

The mitochondrion is the only organelle with its own genetic material, mitochondrial DNA (mtDNA), and its own RNA and protein synthesis machineries<sup>12</sup>. However, mtDNA and mitochondrial functions are under the control of nuclear DNA (nDNA) genes that are transcribed in the cytoplasm, with their protein products then transferred into the mitochondrial matrix. This duality is unique and accounts for the complexity of primary mitochondrial disorders (MIDs), which can involve defects in nDNA or mtDNA<sup>13</sup>. MIDS were thought to be limited to young children<sup>14</sup>, but it is now clear that they can cause disease in adults<sup>15</sup>. In this review, we focus on MIDs-associated nephropathy (MIDANs) in the adult population ( $\geq 16$  years-old at diagnosis).

## **The genetics of MIDs**

### Mitochondrial genetics

MtDNA is a double-stranded, circular molecule composed of 16,569 base-pairs and 37 genes<sup>12</sup>. Unusually, it is intron-free, with the only non-encoding region being the displacement loop (D-loop), which regulates the replication, transcription, and translation of mtDNA<sup>16</sup>. Thirteen of the 37 genes encode subunit proteins belonging to the complexes of the ETC: seven for complex I (NADH, ND1 to ND6), one for complex III (cytochrome b), three for complex IV (cytochrome c oxidase, COXI to III), and two for complex V (ATPase 6 and 8)<sup>17</sup>. Other genes encode two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) involved in mitochondrial translation (Figure 2). Variations that may occur in mtDNA include single-base substitutions, deletions, and duplications (<https://www.mitomap.org/MITOMAP>)<sup>18</sup>.

Cells contain hundreds of mitochondria, with each mitochondrion containing up to ten mtDNA copies. The term homoplasmy refers to mtDNA copies that are all the same (including wild-type, polymorphisms, and even pathogenic variants) in an individual, whereas heteroplasmy indicates the coexistence of two or more different mtDNA populations<sup>19</sup>. MtDNA replication is independent of the cell cycle and, during cell division, intracellular organelles are randomly split. This random drift can modify the level of heteroplasmy in daughter cells<sup>20</sup>. There is a heteroplasmic threshold above which a mitochondrial defect becomes clinically relevant, depending on the extent to which the affected tissue relies on OXPHOS, but also on the function altered by the mtDNA pathogenic variant<sup>21</sup>.

Due to the active destruction of paternal mitochondria in the oocytes, mtDNA is strictly maternally inherited<sup>22</sup>. Penetrance is highly variable, reflecting bottleneck effects during oogenesis and embryogenesis<sup>23</sup>. In addition to maternal transmission, mtDNA pathogenic

variants frequently occur *de novo*<sup>24</sup>. This may be a consequence of a misdiagnosis, given that (1) oligosymptomatic carriers who are maternal relatives or a sibling's proband may be misleading; (2) mtDNA pathogenic variants are usually identified from blood samples but the analysis of non-replicating cell tissues (e.g., muscle, urinary epithelial cells) may provide more accurate information<sup>25</sup>. However, a high frequency of *de novo* mtDNA pathogenic variants is also possible, such as due to the compaction of mtDNA, the absence of protective histone, and exposure to noxious external and internal agents, which in mtDNA induce a mutation rate 5- to-10 fold higher than in nDNA<sup>26</sup>.

Regardless of the cause, clinical heterogeneity is the hallmark of mtDNA pathogenic variants, *de novo* mutations could be frequent and mutations do not systematically correlate with clinical manifestations. Nonetheless, mtDNA pathogenic variants are considered responsible for ~75% of MIDs in the adult population, with an estimated prevalence of 1 in 4300<sup>27</sup>.

### Nuclear genetics

Mitochondria are composed of 1,100 to 1,400 proteins, of which 90 are directly involved in OXPHOS and thus mostly encoded by nDNA<sup>28</sup>. Since the first report in 1995<sup>29</sup> and with the introduction of molecular techniques, there has been growing interest in nDNA pathogenic variants affecting the mitochondrial respiratory chain. These variants, classified according to the affected mitochondrial function, can directly alter ETC structural components or assembly factors, electron carrier biosynthesis, mitochondrial dynamics, mitochondrial protein synthesis machinery, and the replication and maintenance of mtDNA<sup>30</sup> (Figure 2). The inheritance of nuclear pathogenic variants follows Mendelian rules and is frequently autosomal recessive. *De facto*, these pathogenic variants are less frequent in the adult population, with a prevalence 2.9 per 100,000 and accounting for ~23% of adult MIDs diagnosed (notably, mutations in *SPG7*, *PEO1*, *OPA1*, *RRM2B* and *POLG*)<sup>27</sup>. However, with the development of exome (WES) and whole-genome sequencing (WGS), novel nDNA pathogenic variants have been identified as a cause of adult MIDs<sup>15</sup>.

## **Extra-renal manifestations**

Organ function is altered as a consequence of OXPHOS alteration (increased production of ROS and a decrease in ATP production) secondary to defects in nDNA or mtDNA genes. As mitochondria are present in every human cell (except mature erythrocytes), the clinical manifestations of their impairment may be multiple and heterogeneous, and dependent on the amount of energy needed to sustain the involved organ. An important feature of MIDs is a trend

toward an increasing number of tissues that become affected during the natural clinical course of the disease<sup>14</sup>.

Aggregations of the myriad of symptoms can result in a diverse range of syndromes. One of the most frequently described is MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome, which is typically associated with the m.3243A>G pathogenic variant of *MT-TL1*<sup>31,32</sup>. However, the same mutation can cause MIDD (maternally inherited diabetes and deafness)<sup>33</sup> and is probably underestimated within the type 2 diabetes mellitus population (1% of this population have this mutation)<sup>34</sup>. MERRF (myoclonic epilepsy with red-ragged fibers) syndrome is associated with mtDNA pathogenic variants of *tRNA<sup>Lys</sup>*<sup>35</sup>. Chronic progressive external ophthalmoplegia, Kearns-Sayre syndrome (progressive external ophthalmoplegia, pigmentary degeneration in the retina, and cardiac conduction defect) and Pearson syndrome (sideroblastic anemia and exocrine pancreatic insufficiency) are the most common manifestations of large-scale mtDNA deletions<sup>36</sup>.

Beyond these well-characterized syndromes, clinical symptoms may be isolated or associated with other multisystemic manifestations. According to resting metabolic rate, heart is the first energy consumer<sup>37</sup>. Cardiac involvement among MID patients is estimated at 31% and is associated with a poor long-term prognosis<sup>38</sup>. Manifestations include arterial hypertension, cardiac conduction abnormalities, Wolff-Parkinson-White syndrome, and hypertrophic or dilated cardiomyopathy<sup>38</sup>. The organs with the third highest energy consumption (after the heart and kidneys) due to their strong dependence on OXPHOS are those of the neuromuscular and sensory systems<sup>37</sup>. Skeletal muscle is often affected, with symptoms including muscle weakness, exercise intolerance<sup>39</sup>, creatine phosphokinase (CPK) elevation, and electromyographic abnormalities<sup>40</sup>. Central neurologic involvement results in movement disorders (seizure, myoclonus, cerebellar ataxia, Parkinsonism, dystonia)<sup>41</sup> and mitochondrial encephalopathies (stroke-like, leukoencephalopathy, developmental delay, intellectual disabilities)<sup>42</sup>. Sensory system involvement typically consists of conductive hearing impairments<sup>43</sup> and ophthalmological pathologies [retinitis pigmentary abnormalities, macular dystrophy, ophthalmoplegia, ptosis, optic nerve atrophy and Leber's hereditary optic neuropathy (LHON)]<sup>44</sup>. The liver and digestive tract have the fourth highest rates of resting metabolism<sup>37</sup>, with MIDs frequently inducing hepatopathy, enteropathy (pseudo-obstruction, diarrhea, recurrent vomiting and dysphagia), and pancreatic disorders (exocrine insufficiency and diabetes mellitus)<sup>45,46</sup>.

Organs that are less dependent on aerobic metabolism may nonetheless be affected, especially the skin and hair (alopecia, hypertrichosis, skin pigmentation disorders in UV-exposed areas)<sup>47</sup>. Pulmonary hypertension<sup>48</sup>, hematological manifestations<sup>49</sup>, and endocrine (thyroid, parathyroid, adrenal, pituitary glands, and gonads) dysfunctions have also been described<sup>50</sup>. In general, any organ can be involved, at any age and associated with any mutation<sup>14</sup>.

## **MIDANs**

The kidneys receive 20% of cardiac blood flow and are second to the heart in terms resting metabolic rate per dry weight of tissue<sup>37,51</sup>. MIDANs are the consequence of both nDNA or mtDNA defects<sup>52</sup>. The kidney disease may be isolated at diagnosis, with or without progression toward multisystemic manifestations<sup>53-56</sup>, or it may from the onset be associated with multisystemic manifestations (mostly MELAS, MIDD, Pearson, and Kearns-Sayre syndromes)<sup>53,57-60</sup>. Because MIDANs can be mild rather than presenting with florid clinical manifestations<sup>58-60</sup>, they tend to be underestimated<sup>59</sup>. In fact, proteomic and metabonomic urinalyses of 117 adult patients with MIDs found that more than a third had tubular (39%) and/or glomerular (31%) abnormalities<sup>61</sup>.

### Tubular and tubulo-interstitial manifestations

The metabolism of the renal tubular segments is highly dependent on OXPHOS to ensure ion homeostasis<sup>62</sup>. Because the apical membrane of epithelial cells is impermeable to ions, basolateral Na<sup>+</sup>/K<sup>+</sup> ATPases use the energy from ATP hydrolysis to create an electrochemical gradient allowing apical reabsorption and secretion through specific channels and transporters. Sodium is the most strongly reabsorbed ion during tubular transit, powered by an estimated average of 1255 mmol/day of consumed oxygen<sup>63,64</sup>. The number of mitochondria per epithelial cell along different kidney tubular segments is proportional to the amount of sodium reabsorption<sup>65</sup> and is thus very high in the proximal convoluted tubules (PCTs) and thick ascendant Henle's limb (TAHL) and lower in the distal convoluted tubules (DCTs) and collecting canals (CCs) (Figure 3). TAHL metabolism highly depends on the inner vs. outer medullary position, because of differences in oxygen delivery, and on tubuloglomerular balance<sup>51,66</sup>. Tubular epithelial cells oxidize glucose, fatty acids, and amino acids to satisfy this constant metabolic demand, thus maintaining respectively GLUT, CD36, and LAT expression on the basolateral membrane<sup>62</sup>.

PCT is the tubular segment most frequently affected by MIDANs (Table 1), and its involvement results in DeToni-Fanconi-Debré syndrome, characterized by a more or less complete urinary loss of amino acids, glucose, low-molecular-weight proteins, phosphate, uric acid, bicarbonate, potassium, and sodium<sup>67</sup>. This MIDAN is above all diagnosed in the pediatric population, mostly with large-scale mtDNA deletions that result in multisystemic manifestations (Pearson and Kearns-Sayre syndromes), but also with nDNA defects<sup>56,68-72</sup>. Disease attributable to PCT involvement has also been described in adults, such as isolated amino aciduria associated with the *MT-TK* m.8344A>G pathogenic variant and manifesting as MERRF and MIDD syndromes<sup>73</sup>. The defect in mitochondrial dynamics that occurs with the *GATM* nDNA pathogenic variant results in a proximal tubulopathy that leads to end-stage renal disease (ESRD) during the second to third decade of life<sup>74</sup>. In a pediatric study of 42 patients with MIDs, 21 had MIDAN and only 8 had considered kidney disease reflecting mild tubular involvement<sup>59</sup>. Thus, it may be the case that proximal tubulopathy in the adult population, even if less frequent, is underestimated because of its mild manifestations.

Other tubular segments can be affected as well, but with a lower frequency. These MIDANs, are more often reported in pediatric population<sup>75-79</sup>. In adults, renal tubular acidosis secondary to a m.5656A>G point mutation in a non-coding region between tRNA<sup>Ala</sup> and tRNA<sup>Asn</sup> has been described and is associated with retinitis pigmentary abnormalities and hypertrophic cardiomyopathy<sup>80</sup>. Familial MIDAN, with hypomagnesemia secondary to DCT involvement, dyslipidemia, and hypertension, was diagnosed in a large kindred adult population affected with *MT-TI* m.4291T>C pathogenic variant<sup>77</sup> (Table 1).

Besides these specific tubular defects, tubulo-interstitial nephritis (TIN) are described, and are most likely to manifest during adulthood (Table 1). We recently described *MT-ND5* m.13513G>A and m.13514A>G pathogenic variants in adults with TIN, either isolated or associated with multisystemic manifestations (MELAS, LHON, Wolf-Parkinson-White syndrome, hearing impairment)<sup>53</sup>. Conor *et al.* identified *MT-CR* m.547A>T and *MT-TF* m.616T>C pathogenic variants in English families with isolated TIN diagnosed during adulthood<sup>81</sup>. Guéry *et al.* evaluated 9 adult patients with renal manifestations associated with *MT-TL1* m.3243A>G pathogenic variants; 3 of them (33%) had TIN, and one was isolated at diagnosis but later progressed to include extrarenal manifestations (diabetes mellitus, hearing impairment, central neurologic abnormalities)<sup>57</sup>. To emphasize clinical heterogeneity of MIDANs, TIN has been reported with *MT-COIII* m.9267G>C pathogenic variant associated with MIDD, while this manifestation is commonly reported with glomerular disease<sup>82</sup>. Some



mtDNA deletions have also been associated with TIN<sup>73</sup>. Even if diagnosed in young children, *GATM* nDNA pathogenic variant has been reported with MIDAN evolving from proximal tubulopathy to TIN then to ESRD<sup>74</sup>.

### Glomerular manifestations

Glomerular diseases are linked to alterations of the glomerular filtration barrier<sup>83</sup>. Podocytes are highly differentiated cells with limited replicative capacity and a central role in glomerular barrier function, including structural support, the maintenance of glomerular capillary hydrostatic pressure, glomerular basement membrane synthesis, intercellular crosstalk (e.g., VEGF secretion that acts on the VEGF receptors of endothelial cells), slit diaphragm protein synthesis (nephrin and podocin), and permselectivity modulation through actin cytoskeleton interactions with slit diaphragm proteins<sup>84</sup>. These functions require ATP synthesis by the mitochondrial respiratory chain and, thus, the intracellular importation and oxidation of carbohydrates, fatty acids, and amino acids (Figure 4)<sup>85</sup>. MIDANs often feature glomerular disease, manifesting as focal segmental glomerulosclerosis (FSGS), the consequence of podocyte dysfunction<sup>86</sup>.

Pathogenic variants affecting nDNA genes mostly alter electron carriers and CoQ10 biosynthesis (*COQ2*, *COQ6*, *PDSS1*, *PDSS2* and *ADCK4*)<sup>54,87-93</sup> (Table 2). A commonly described MIDAN is pediatric steroid-resistant nephrotic syndrome (usually appearing before 2 years of age) accompanied by FSGS lesions<sup>94</sup>. Neuromuscular involvement, including cerebellar ataxia, mitochondrial myopathy, and optic atrophy, is frequently associated with this MIDAN<sup>54,94</sup>. However, *ADCK4* nDNA pathogenic variants also occur in the adult population. *ADCK4* is normally expressed in podocytes and interacts with CoQ10 biosynthesis in the foot processes of these cells. Defects in this gene similarly lead to a steroid-resistant nephrotic syndrome seen in young adults, but goiter, hypertension and dilated cardiomyopathy may develop as well<sup>93</sup>. Since genetic causes of adult idiopathic nephrotic syndromes are of increasing interest<sup>95,96</sup>, nDNA pathogenic variants resulting in MIDAN during adulthood may be underestimated for the moment.

A large mtDNA deletion associated with FSGS and TIN, without nephrotic syndrome markers, has been described<sup>97</sup>. However, point mutations are the most frequently reported mtDNA pathogenic variants associated with glomerular diseases<sup>55-57,98-100</sup> (Table 2). Among these, *MT-TL1* m.3243A>G pathogenic variants are often seen in the adult population with glomerular

disease (22% to 31% of cases) and result in FSGS during the second to third decade of life that typically leads to ESRD within 10 years<sup>57,61</sup>. Women are 4-fold more likely to be affected<sup>100,101</sup> and nephrotic syndrome is present in only about one-third of all patients<sup>60,98,100,102–104</sup>. Glomerular diseases can be isolated, at least at diagnosis, preceding systemic manifestations<sup>99,100</sup>. An association with hearing impairment seems frequent, which may lead to a misdiagnosis of Alport's syndrome even in the absence of hematuria detection<sup>98,103,105</sup>. Diabetes mellitus (and MIDD), induced in some cases by high-dose prednisolone therapy (e.g., kidney transplant induction), are frequently associated with glomerular disease in the context of *MT-TL1* defects<sup>98,106</sup>. Indeed, renal impairment during MIDD is frequent (28–53%)<sup>60,107</sup> whereas diabetic retinopathy is rare (8%). In this situation, kidney disease is likely to be a MID manifestation with FSGS rather than a diabetic microvascular complication<sup>60,61,98</sup>. Other mtDNA point mutations responsible for glomerular disease in adulthood and associated with diabetes mellitus<sup>108,109</sup> and hearing impairment<sup>110–114</sup> have also been identified and are presented in Table 2. These observations highlight the strong association of hearing impairment, diabetes mellitus and glomerular disease with MIDs.

## **Diagnosis of MIDAN**

The diagnosis of MIDAN may be challenging as it requires knowledge drawn from various medical specialties as well as the use of biochemical, histological, imaging, and genetic techniques. The Mitochondrial Medicine Society has published recommendations for the diagnosis of MIDs<sup>115</sup>. Bedside criteria for the diagnosis have been proposed, but these have been validated only for pediatric MID assessment<sup>116</sup>.

### Clinical management

For nephrologists, every TIN or glomerular disease of undetermined origin should trigger mitochondrial investigations, regardless of whether there are associated multisystemic manifestations or a history indicating maternal inheritance. Indeed, MIDANs with mild manifestations appear to be frequent but underestimated because the absence of kidney disease identification. Every MID diagnosis must be followed by a consultation with a nephrologist with expertise in this field for MIDAN screening and follow-up.

Two situations must be underscored. First, in a patient with a familial glomerular disease, the mode of transmission and hematuria have to be carefully assessed to avoid a diagnosis of Alport's syndrome rather than MIDAN<sup>98,103</sup>. In a patient with kidney disease associated with diabetes mellitus characterized by an atypical clinical course (young adult with normal BMI,

absence of diabetic retinopathy and/or glycated hemoglobin), especially if maternal relatives are affected and/or macular dystrophy is observed in the fundus, MID should be investigated<sup>60,107</sup>.

### Biochemical investigations

To evaluate the presence of kidney disease in the context of suspected MID, blood analyses of urea, creatinine, uric acid, glycemia, proteinemia, albuminemia, and electrolytes (sodium, potassium, phosphorus, calcium, bicarbonate) must be systematically performed. Considering the high frequency of low muscle mass in patients suffering from neuromuscular manifestations, the glomerular filtration rate estimated using the CKD-EPI equation may fail to assess renal function correctly<sup>117,118</sup>. The urinary clearance of exogenous markers (<sup>51</sup>Cr-EDTA) may be informative in these cases. Systematic urinalyses (e.g., using dipsticks) of proteinuria and its composition as well as leukocyturia and hematuria should be performed together with determinations of urinary excretion sodium, potassium, phosphorus, calcium, uric acid, amino acids, and glucose. These analyses will enable MIDAN ascertainment and characterization (glomerular or tubular affection)<sup>73</sup> as well as the staging of kidney disease<sup>118</sup>.

Initial diagnostic investigations of MID include measurements of the levels of blood lactate, pyruvate, CPK, amino acids, albumin, uric acid, and urine organic acids<sup>115</sup>. OXPHOS defects should be suspected in patients with an increased lactate level (in blood and cerebrospinal fluid). Elevated lactate-to-pyruvate and 3-hydroxybutyrate-to-acetoacetate ratios can distinguish MID from other defects with a specificity of 100%. Urine organic acids analysis can detect lactic and/or dicarboxylic aciduria<sup>119</sup>. However, neither serum lactate elevation nor lactaciduria are specific for MID, and MIDAN (e.g., tubular impairment) may interfere with determinations of these parameters<sup>73,120</sup>.

### Renal and cerebral imaging

Renal imagery is required to investigate renal and urinary tract malformations, cysts, and/or increased kidney echogenicity<sup>53,57</sup>. The latter finding is frequently associated with tubulo-interstitial disease<sup>75,78,121,122</sup>.

Cerebral imaging by magnetic resonance imaging is of great interest in the adult population with MIDs. Morphological abnormalities are frequent and include white and gray matter lesions, cerebral and optic nerve atrophies, stroke-like lesions, and calcifications<sup>123</sup>. Magnetic resonance spectroscopy enables estimates of the brain lactate level<sup>124</sup>.

### Histopathology

Open muscle biopsy remains the gold standard for the diagnosis of OXPHOS defects. Histochemical staining can reveal cyclooxygenase-deficient fibers and the accumulation of mitochondria, resulting in ragged-red fibers seen using modified Gomori trichome staining, and an increase in lipid droplets in muscle fibers stained with oil-red. Electron microscopy can show the accumulation and swelling of mitochondria and abnormal mitochondrial inclusions<sup>125</sup>. Spectrophotometric measurements of enzymatic activities can be performed on muscle biopsy tissue to detect individual or combined defects in the various mitochondrial complexes<sup>126</sup>. The results of a kidney biopsy may support a diagnosis of MIDAN. Light microscopy might reveal granular swollen epithelial cells<sup>127</sup> and podocyte hypertrophy, indirect and non-specific features of mitochondrial proliferation and alteration<sup>128</sup>. Electron microscopy will show mitochondrial abnormalities (within tubular epithelial cells and/or podocytes) including swelling, with loss of cristae structure and an increased number of altered mitochondria<sup>53,54,57,75,100,129,130</sup>.

### Genetic approach

Next-generation sequencing (NGS) has initiated a new era in the molecular diagnosis of MIDs. Genome sequencing of mtDNA can be performed rapidly and at low cost<sup>131</sup> and offers an accurate single test that can demonstrate both pathogenic variants, such as point mutations, even at low levels of heteroplasmy, and deletions. Mitochondrial genome NGS as a first-line test for the diagnosis of MIDs may represent a new approach<sup>132</sup>, especially in patients presenting with kidney disease that raises suspicion of MIDANs. Moreover, NGS can be performed from urine samples and kidney biopsy tissue and is likely to be an important contribution to the diagnosis of MIDs<sup>53,133</sup>. NGS-based panels of multiple nDNA genes are available for diagnostic use in MIDs, and WES and WGS have already been implicated in a large number of nDNA pathogenic variants, notably in diseases associated with multiple deletions in mtDNA<sup>134</sup>. The widespread availability of these molecular techniques will enable a comprehensive diagnostic approach to MIDs.

## **Therapeutic approach**

The confirmation of a MID is a strong diagnostic leading to genetic counseling and potential treatment.

Genetic counseling may be straightforward in a patient with a nDNA defect, but more difficult if a mtDNA pathogenic variant has been detected. Indeed, the risk that a woman with a mtDNA deletion will transmit the defect to offspring is lower than if she has a mtDNA point mutation<sup>135</sup>. *De novo* mtDNA mutations also have a lower rate of transmission to offspring<sup>25</sup>. Amniocentesis and chorionic villi samples are not informative because the mutant load can differ in fetal tissue vs. at birth (due to mitotic segregation)<sup>136</sup>.

Few randomized double-blinded clinical trials have evaluated the efficacy of pharmacological treatment for MIDs<sup>137</sup>. This may be related to the lack of studies tracking the clinical course of MIDs and to the enormous heterogeneity in the clinical presentation and affected tissues<sup>138</sup>. Specific therapies beneficial for specific MIDANs include prompt CoQ10 therapy for patients with nDNA defects that lead to alterations in electron carriers and CoQ10 synthesis<sup>89</sup>. In patients harboring mtDNA pathogenic variants, clinical trials examining CoQ10 supplementation, dichloroacetate, and creatine supplementation have not shown any benefit<sup>138</sup>. Aerobic exercise may have a positive effect by promoting mitochondrial biogenesis<sup>139</sup> and should be systematically recommended in association with symptomatic supportive care.

Emerging therapies aimed at enhancing mitochondrial biogenesis, regulating mitochondrial dynamics, and bypassing OXPHOS blocks have generated interest<sup>137</sup>. Inhibitors of mTOR (e.g., rapamycin) were shown to confer survival benefits in mouse models of MIDs, of both the nDNA defect<sup>140</sup> and mtDNA depletion<sup>141</sup> type. This approach is particularly interesting to nephrologists because mTOR inhibitors are used as an immunosuppressive therapy after kidney transplantation. Johnson *et al.* reported that mTOR inhibitor therapy (as a calcineurin inhibitor replacement) improved extra-renal manifestations in four kidney transplant recipients (with MIDAN corresponding to FSGS as the initial kidney disease) diagnosed with MELAS syndrome<sup>142</sup>. This result remains to be confirmed in a randomized clinical trial, but it may be relevant to the overall MID population, regardless of the transplantation status. Furthermore, gene therapy offers new perspectives and may be applicable for both nDNA mutations (adeno-associated viruses as a vector or based on CRISPR/Cas9 endonuclease) and mtDNA defects, which are more challenging. Novel approaches include the use of recombinant-mtDNA-encoded proteins containing a mitochondrial target sequence in the nucleus<sup>143</sup>, shifting the mtDNA pathogenic variant heteroplasmy level using nucleases<sup>144</sup>, and stabilizing mitochondrial tRNA<sup>145</sup>.

## **Conclusion**

The potential development of kidney disease in MID-affected adults should be borne in mind by nephrologists, triggering an investigation of possible MIDAN in every patient with TIN and/or glomerular disease of undetermined etiology. A patient diagnosed with a MID should then be seen by a nephrologist for MIDAN screening and long-term follow-up. The clinical heterogeneity and genetic duality of MIDs pose a diagnostic challenge. The prevalence, characteristics, and management of MIDANs during adulthood remain to be elucidated in a large cohort of MID patients. The widespread availability of NGS and WES/WGS are expected to pave the way for new approaches to the diagnosis of undetermined nephropathy.

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## Tables

**Table 1:** MIDANs in adulthood characterized by tubular defects and tubulo-interstitial nephritis

	<b>Point mutation</b>	<b>Gene</b>	<b>Kidney affection</b>	<b>Mitochondria abnormalities within kidney tissue (EM)</b>	<b>Morphological kidney abnormalities</b>	<b>Extra-renal manifestations</b>	<b>Age at diagnosis</b>	<b>Ref.</b>
<b>mtDNA point mutations</b>	m.547A>T	MT-CR	TIN	/	/	Isolated at diagnosis	26-40	81
	m.616T>C	MT-TF	TIN	/	/	Epilepsy	26-40	81
	m.3243	MT-TL1	TIN	Yes	Absence	HI	27	129
			TIN / Cysts	Yes	Absence / Cysts	MELAS / HI / LVH / DM	5-47	57
	m.4291T>C	MT-TI	DCT (low Mg)	/	/	Dyslipidemia / Hypertension	18-60	77
	m.5656A>G	non coding	RTA / TIN	Yes	/	Retinopathy / LVH	11-17	80
	m.8344A>G	MT-TK	PT	/	/	MERRF / MIDD	23-61	73
	m.9267G>C	MT-COIII	TIN	/	/	MIDD	2 - 57	82
	m.13513G>A	MT-ND5	TIN	Yes	Cysts	Isolated / MELAS / WPW / HI	29-34	53
	m.13514A>G	MT-ND5	TIN	Yes	Absence	LHON	53	53
<b>mtDNA deletions</b>	<b>Size</b>	<b>Location</b>						
	5000	8469_13446	TIN	/	/	Kearns-Sayre / Myopathy / LVH	43	73
	2905	11520_14426	TIN	/	/	Kearns-Sayre / Pearson / LVH	16	97
<b>nDNA mutations</b>	<b>Altered function</b>	<b>Gene</b>						
	Mitochondrial dynamics	GATM	PT / TIN	Yes	Echogenicity	Isolated at diagnostic (ESRD 2nd/3rd decade)	<2	74

DCT: distal convoluted tubule / EM: electron microscopy / ESRD: end-stage renal disease / HI: hearing impairment / LVH: left ventricular hypertrophy / Mg: magnesium / mtDNA: mitochondrial DNA / nDNA: nuclear DNA / PT: proximal tubulopathy / RTA: renal tubular acidosis / TIN: tubulo-interstitial nephritis / WPW: Wolf-Parkinson-White.



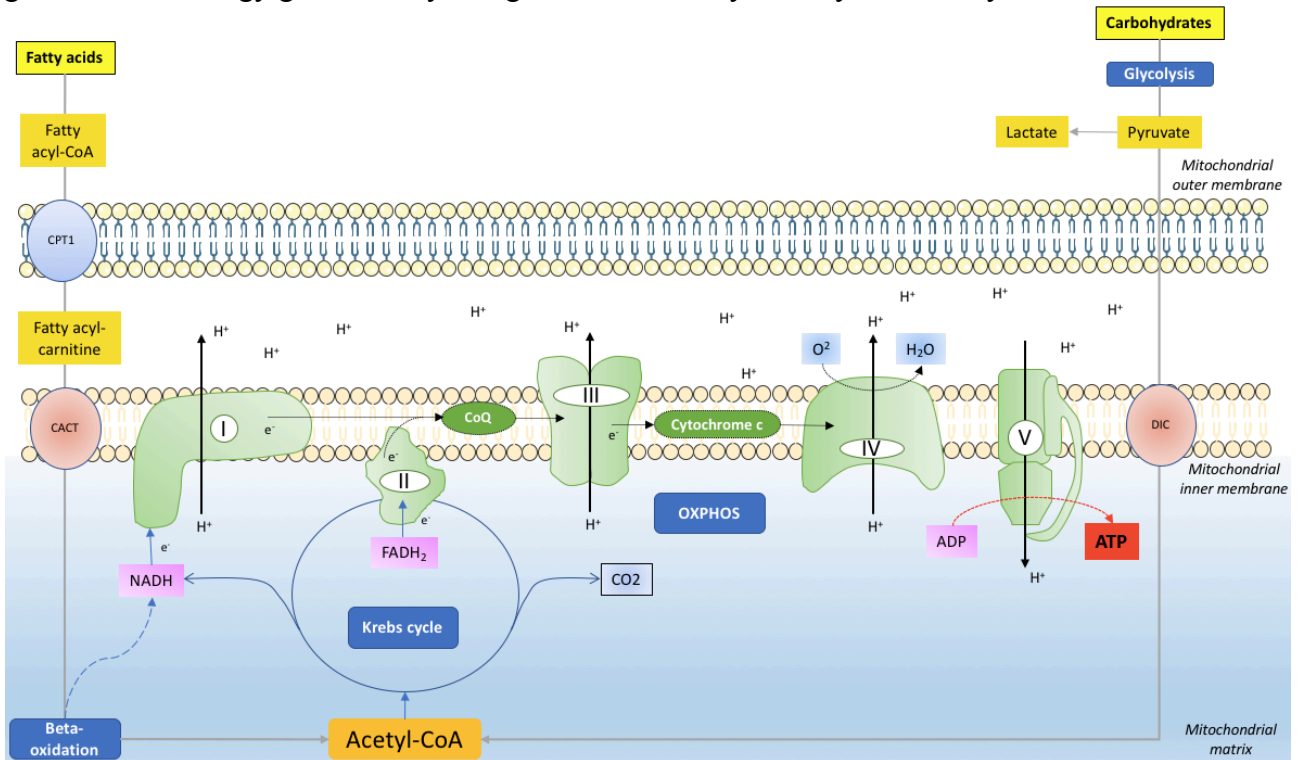
**Table 2:** MIDANs in adulthood characterized by glomerular disease

	<b>Point mutation</b>	<b>Gene</b>	<b>Kidney affection</b>	<b>Mitochondria abnormalities within kidney tissue (EM)</b>	<b>Morphological kidney abnormalities</b>	<b>Extra-renal manifestations</b>	<b>Age at diagnosis</b>	<b>Ref.</b>
<b>mtDNA point mutations</b>	m.3243	MT-TL1	FSGS / Cysts	Yes	Cysts	MELAS / HI / LVH / DM	5-47	57
			FSGS	Yes	Absence	Isolated / MELAS / Kearns Sayre	11-14	56
			FSGS	Yes	/	MELAS / MIDD	17-35	98
			FSGS	Yes	/	Isolated / MIDD	10-17	55
			FSGS	/	/	DM	25	109
			FSGS	/	/	MELAS / MIDD	15-21	99
			FSGS	Yes	Absence	HI / LVH	27	102
			FSGS	Yes	/	HI / LVH	31	111
			FSGS	/	/	MIDD / Retinopathy / Encephalomyopathy	18-32	100
			FSGS	Yes	/	HI	32	112
			FSGS	Yes	/	CPEO / WPW / HI	20	113
			FSGS	/	/	MIDD / MELAS	>16	114
			FSGS	Yes	/	HI	38	103
			FSGS	Yes	/	MIDD	33	104
	m.7501T>A	MT-TS1	FSGS	Yes	Absence	Thyroiditis / DM	54	108
	m.13513G>A	MT-ND5	FSGS	Yes	/	MELAS / HI	26	110
<b>mtDNA deletions</b>	<b>Size</b>	<b>Location</b>						
	2905	11520_14426	FSGS + TIN	/	/	Kearns-Sayre / Pearson / LVH	16	97
<b>nDNA mutations</b>	<b>Altered function</b>	<b>Gene</b>						
	Electron carriers	ADCK4	FSGS	Yes	/	Isolated / LVH / Encephalopathy	Birth - 21	93

CPEO: chronic progressive external ophthalmoplegia / DM: diabetes mellitus / EM: electron microscopy / FSGS: focal segmental glomerulosclerosis / HI: hearing impairment / LVH: left ventricular hypertrophy / mtDNA: mitochondrial DNA / nDNA: nuclear DNA / WPW: Wolf-Parkinson-White.

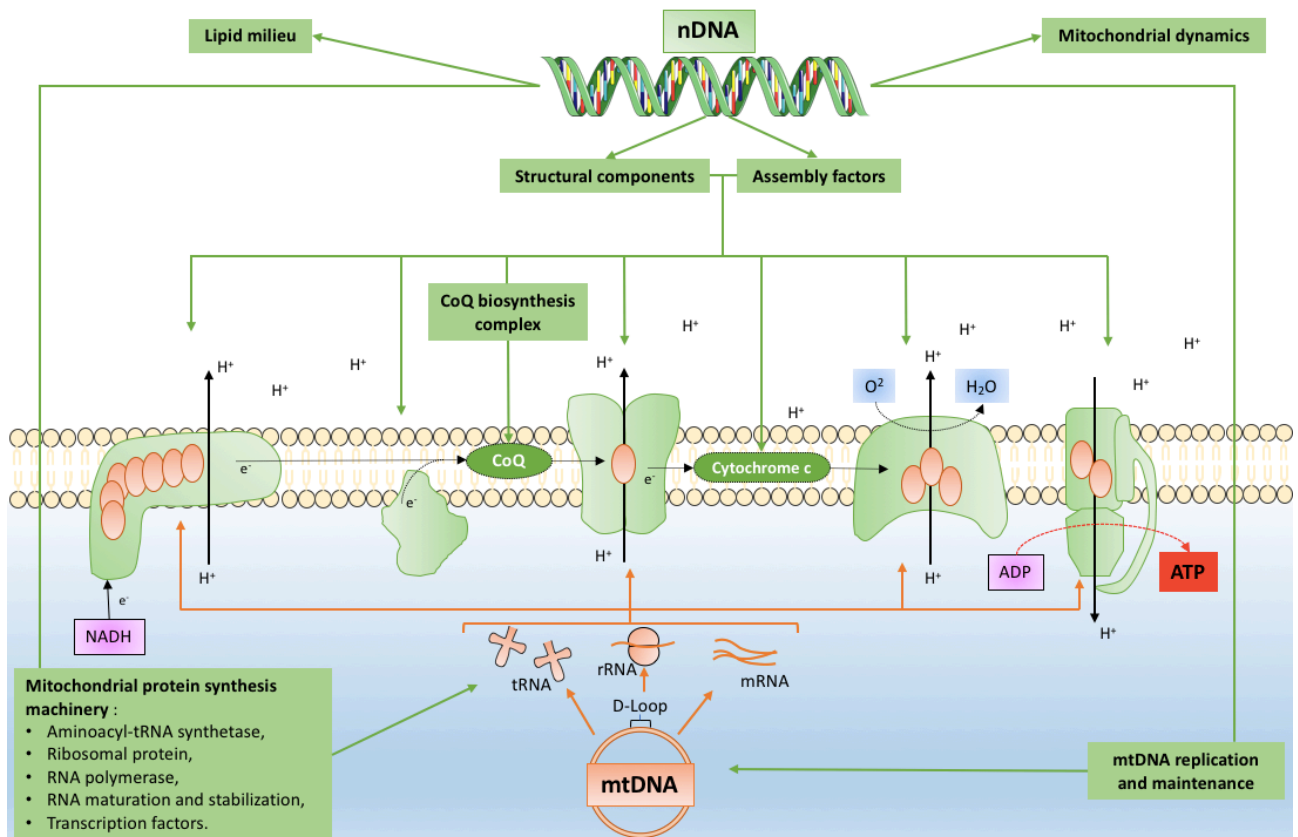
# Figures

**Figure 1: The mitochondrial respiratory chain.** Mitochondrial respiration uses cell nutrients (carbohydrates, fatty acids) that cross the outer (porous) and inner membranes via specific transporters. Fatty acids and carbohydrates enter the mitochondrial matrix via the transporters CPT1/CACT and DIC, respectively. Both are transformed to acetyl-CoA, one of the main substrates of the Krebs cycle. NADH and FADH<sub>2</sub> enter ETC and OXPHOS reactions and allow electron transport to oxygen, resulting in the formation of water. At the same time, protons are pumped into the intermembrane space (complexes I, III, and IV), resulting in an electrochemical gradient. The energy generated by this gradient is used by ATP synthase to synthesize ATP.



*CACT: carnitine acyl carnitine translocase / CoA: coenzyme A / CoQ: coenzyme Q10 / CPT1: carnitine palmitoyl transferase 1 / DIC: dicarboxylate carrier / ETC: electron transport chain / FADH<sub>2</sub>: flavin adenine dinucleotide / NADH: nicotinamide adenine dinucleotide / OXPHOS: oxidative phosphorylation*

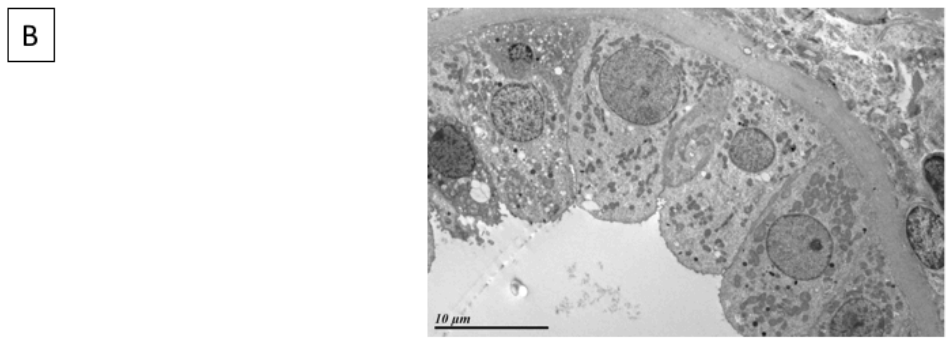
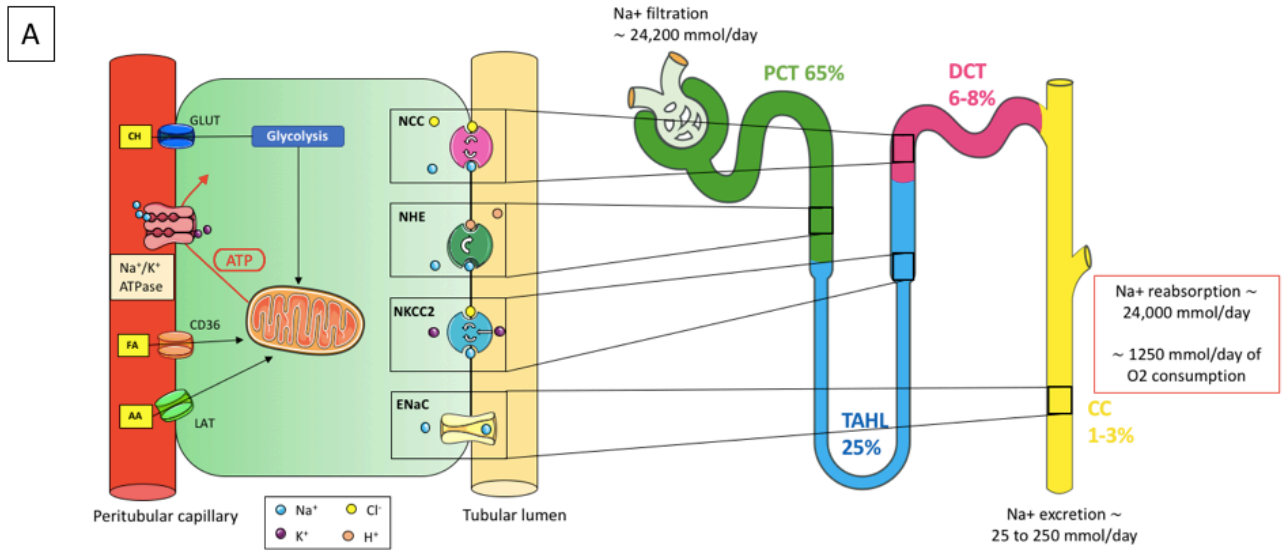
**Figure 2: Genetic duality of the mitochondrial machinery.** Mitochondria possess their own mtDNA, encoding for subunit proteins that form the different complexes, tRNA, and rRNA (orange structures and arrows). MtDNA replication, transcription, and maintenance, as well as other mitochondrial functions are dependent on nDNA (green structures and arrows).



*ADP: adenosine diphosphate / ATP: adenosine triphosphate / CoQ: coenzyme Q 10 / mtDNA: mitochondrial DNA / NADH: nicotinamide adenine dinucleotide / nDNA: nuclear DNA / tRNA: transfer RNA / rRNA: ribosomal RNA.*

**Figure 3: Tubular epithelial cells are highly dependent on mitochondrial respiration.**

- A.** Schematic representation of a tubular epithelial cell. Mitochondria are of paramount importance for all tubule segments. Nutrients (fatty acids, carbohydrates, and amino acids) are carried from the blood into tubular cells via specific transporters located on the basolateral membrane. Mitochondrial respiration provides energy for cell functions, notably sodium reabsorption. Basolateral  $\text{Na}^+/\text{K}^+$  ATPase uses the energy generated by ATP hydrolysis to create an electrochemical gradient that favors sodium reabsorption through specific apical transporters along the tubules' segments: NHE in PCT, NKCC2 in TALH, NCC in DCT and ENaC in CC.
- B.** Electron microscopy of tubular epithelial cells shows a large number of mitochondria.

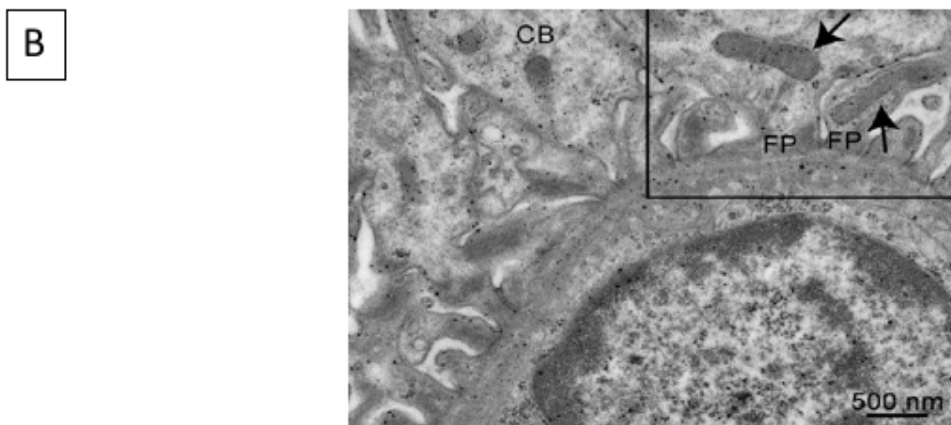
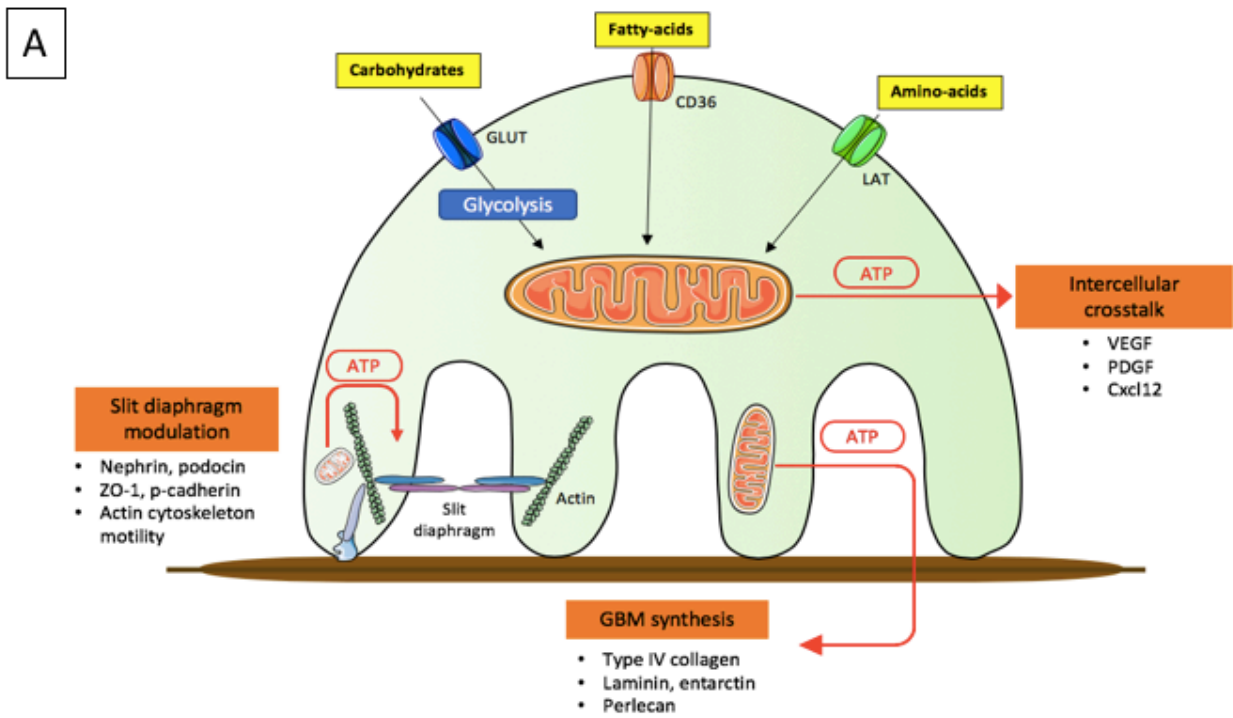


AA: amino-acids / CA: carbohydrates / CC: collector canal / DCT: distal convoluted tubule / FA: fatty-acids / PCT: proximal convoluted tubule / TALH: thick ascendant Henle's limb.

**Figure 4: Podocyte functions are essential for mitochondrial respiration.**

**A.** Schematic representation of a podocyte and its main functions within the glomerular filtration barrier. Podocyte slit diaphragm modulation, glomerular basement membrane synthesis and intercellular cross-talk depend on mitochondrial ATP synthesis. Nutrient transporters on the podocyte membrane enable mitochondrial respiration (fatty acids, carbohydrates, and amino acids).

**B.** Electron microscopy of the glomerular filtration barrier, centered on a podocyte (black arrow). Note the mitochondria in cell bodies (CB) and in foot processes (FP). From Imasawa T and Rossignol R, *Int. J. Biochem. Cell Biol.*, 2013<sup>85</sup>.



AA: amino-acids / CA: carbohydrate / CB: cell body / FA: fatty acids / FP: foot process / GBM: glomerular basement membrane.

Les dysfonctions mitochondriales tiennent une place centrale dans la physiologie et la physiopathologie rénale. En effet, de nombreuses affections systémiques vont engendrer des mitochondriopathies secondaires (*i.e.* le diabète, l'hypertension artérielle, l'ischémie-reperfusion), allant initier et/ou favoriser l'apparition de lésions rénales évoluant vers la MRC. De plus, nous venons de souligner, à travers cette revue de littérature, que les MIDs sont responsables de maladies rénales chez l'adulte. Les cas rapportés décrivent essentiellement des néphropathies tubulointerstitielles et des néphropathies glomérulaires avec des lésions de HSF. Cela diffère fortement des cas pédiatriques qui relatent essentiellement des tubulopathies proximales et des syndromes néphrotiques corticorésistants.

Ce diagnostic de MIDAN à l'âge adulte pourrait être fréquent, mais sous-estimé pour plusieurs raisons : du fait de présentations clinique hétérogènes ne faisant pas évoquer ce diagnostic en première intention (*i.e.* en cas d'atteinte rénale modérée isolée) ; du fait de variants pathogènes pour l'instant inconnus responsables de MIDAN à l'âge adulte.

C'est pourquoi la réalisation d'un NGS de l'ADNmt sur sang, urine, voire sur la biopsie rénale peuvent s'avérer d'une grande aide pour ce diagnostic complexe. Nous allons décrire ici 3 cas de MIDAN diagnostiqués à l'âge adulte grâce à cette méthode de diagnostic génétique moléculaire. Il s'agit de 2 variants pathogènes de *MT-ND5* que nous décrivons pour la première fois comme étant associés à des MIDANs de l'adulte avec des TIN.

## **Article 2 : Série de cas cliniques**



SHORT REPORT

CLINICAL GENETICS WILEY

# Adult onset tubulo-interstitial nephropathy in MT-ND5-related phenotypes

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## Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/cge.13670>.

## Abstract

Kidney is a highly adenosine triphosphate dependent organ in human body. Healthy and functional mitochondria are essential for normal kidney function. Clinical and genetic variability are the hallmarks of mitochondrial disorders. We report here the involvement of two *MT-ND5* pathogenic variants encoding for ND5 subunit of respiratory chain complex I, the m.13513G>A and the m.13514A>G, in adult-onset kidney disease in three unrelated patients. The first patient had myopathy encephalopathy lactic acidosis and stroke syndrome, left ventricular hypertrophy with Wolff-Parkinson-White syndrome and tubulo-interstitial kidney disease. The second presented Leber hereditary optic neuropathy associated with tubulo-interstitial kidney disease. The third presented with an isolated chronic tubulo-interstitial kidney disease. These mutations have never been associated with adulthood mitochondrial nephropathy. These case reports highlight the importance to consider mitochondrial dysfunction in tubulo-interstitial kidney disease.

## KEYWORDS

adult, complex I, mitochondrial disorder, MT-ND5, tubulo-interstitial nephropathy

The kidneys are highly energy-consuming organs which require a normal mitochondrial respiratory chain function.<sup>1,2</sup> Although mitochondrial diseases most often have a muscular or nervous phenotype, they can affect any organ. Primary renal manifestations in mitochondrial disorders include tubulopathy, interstitial nephritis, glomerular pathology and cystic disease.<sup>3,4</sup> Kidney pathology is more frequently reported in children than in adult.<sup>3</sup> The most common mitochondrial DNA (mtDNA)

defects reported with a kidney phenotype during adulthood are the mutation m.3243A>G in the *tRNA-leu* gene associated to myopathy encephalopathy lactic acidosis and stroke (MELAS) syndrome<sup>5</sup> and mtDNA large scale deletions associated to Kearns-Sayre Syndrome.<sup>4</sup> Rare single case reports have described supplemental point mutations, most of them involving tRNA genes.<sup>5,6</sup> Complex I is the most frequent mitochondrial respiratory chain complex involved in mitochondrial



diseases and is associated with a wide range of clinical presentations in children and young adults.<sup>7,8</sup> It is a multi-subunit complex consisting of 44 subunits of which 7 are encoded by the mtDNA. Mutations in the mitochondrial *ND5* gene are associated with LHON (Leber hereditary optic neuropathy), MELAS and Leigh syndrome.<sup>9-14</sup>

We report here the cases of three patients displaying adult tubulo-interstitial renal disease, all carrying pathogenic variants in the mitochondrial *ND5* gene, two at position m.13513G>A and one at position m.13514 A>G.

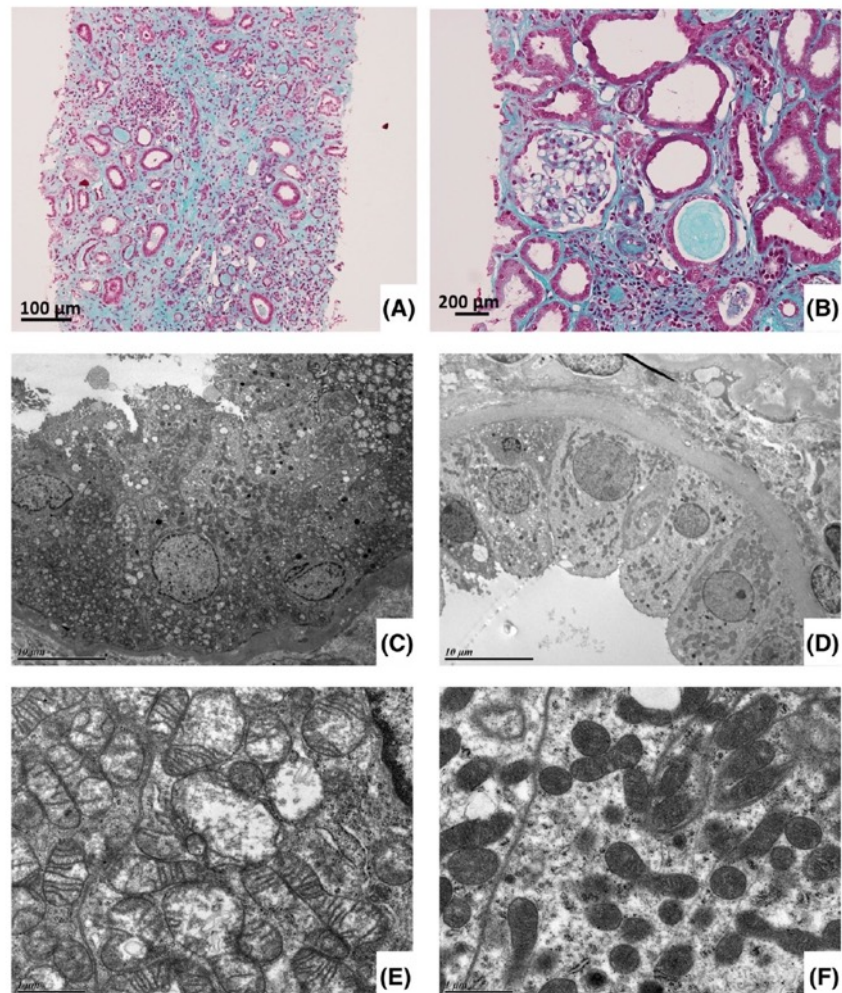
## 1 | PATIENT 1

A 29-year-old man was admitted to the intensive care unit for acute pulmonary edema with anuric acute kidney injury following an epstein-barr virus infection. He presented a severe hyperlactatemia with metabolic acidosis. Respiratory situation improved with hemofiltration and the patient was transferred to our nephrology unit.

The patient was short in stature (1.60 m,  $-2.5$  SD) and weighed 49 kg ( $<-2$  SD). Neuromuscular phenotype was characterized by developmental delay and exercise intolerance. Cerebral imaging showed stroke-like lesion in occipital region with cortical atrophy for his age.

He had a history of unexplored chronic kidney disease (CKD) stage 2, with a baseline Glomerular filtration rate (GFR) at 64 mL/min/1.73 m<sup>2</sup> and a familial history of kidney cysts without kidney impairment. Exploring his worsening kidney function, abdominal computerized tomography (CT) showed multi-cystic kidneys without hepatic cyst. Urine analysis showed hematuria and glomerular proteinuria (3.75 g/24 hours). Immunological and virological analyses were negative. Histological renal biopsy showed severe interstitial fibrosis with severe tubular atrophy in optic microscopy (Figure 1). No glomerular or vascular lesion was observed. Immunofluorescence was negative. In electron microscopy, tubular mitochondria were swollen and cristae were lost or simplified (Figure 1). Renal situation did not recover leading to chronic intermittent hemodialysis.

An electrocardiogram showed a short period in electrocardiography (PR) interval with delta wave in relation with a Wolff-Parkinson-White



**FIGURE 1** Pathology studies in optic microscopy.  $\times 20$  magnification, masson's trichrome staining. A, Global aspect of severe interstitial fibrosis and tubular atrophy. B, Dissociation between the severe tubular atrophy and the normal glomerular aspect. Pathology studies in electron microscopy detected abnormal tubular mitochondria, of increased size with a loss and/or disorganization of mitochondrial ridges in our patient (C-E) compared to healthy patient (D-F) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

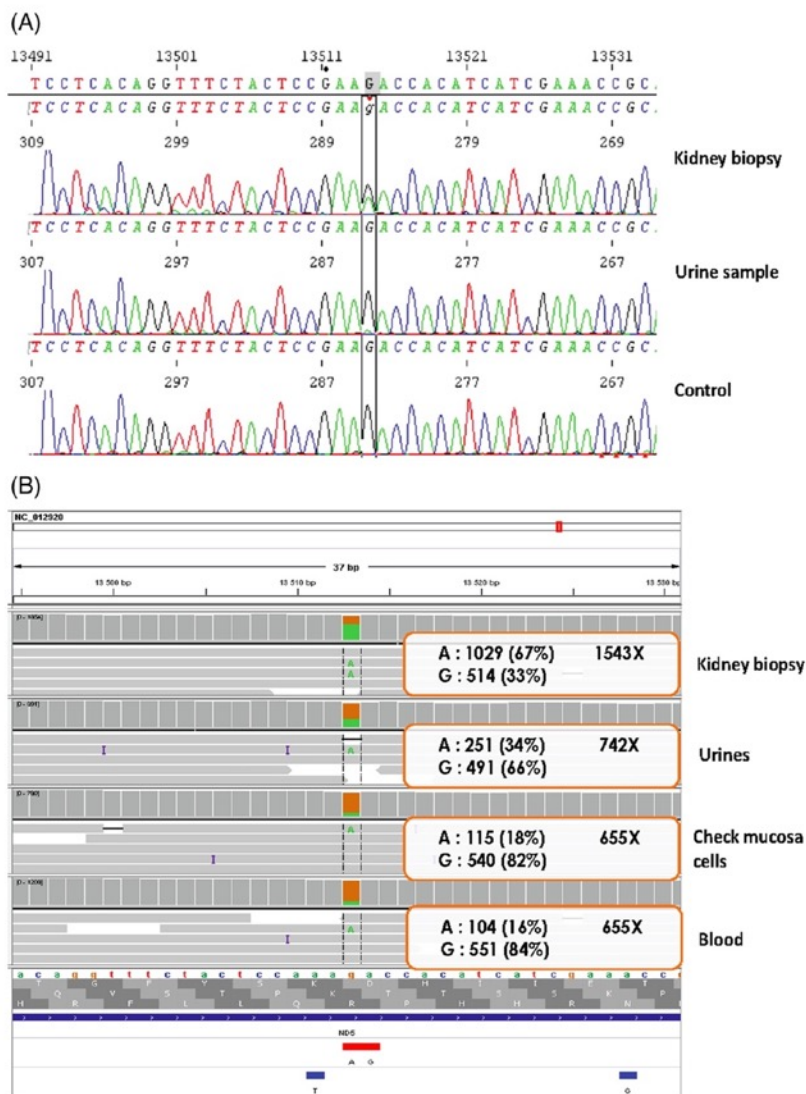
syndrome. Electrophysiological explorations found 4 accessory pathways (3 of which were ablated). Echocardiography detected severe hypertrophic cardiomyopathy with altered systolic function.

Optic fundus showed an optic atrophy. OCT analyses found atrophy predominant on the external nuclear layer. The lesions were bilateral but mainly affected the left eye. Finally, an audiogram exhibited bilateral perception deafness about 50%.

Interestingly, a renal gene panel testing (Supplemental materials) identified a pathogenic variant of *PKD2* (NM\_000297.2: c.2182\_2183del p.Leu729Alafs\*11). This pathogenic variant was also present in his mother (mother's abdominal CT scan showing multiple bilateral kidney cysts without kidney dysfunction at 67 years old) and sister.

Functional mitochondrial analysis showed a hyperlactacidemia with an increase of lactate/pyruvate ratio and acetoacetate level, suggesting oxidative phosphorylation defect. Analyses of organic acid elimination in urine did not show dicarboxylic aciduria or lactaturia, with the major limitation of kidney failure.

Sequencing of the total mtDNA by next-generation sequencing (NGS) (Supplementary Materials, Method and Supplementary Materials, Table 1) revealed the pathogenic variant MT-ND5 m.13513G>A (NC\_012920.1: c.1177G>A, p.(Asp393Asn)) with a variable heteroplasmic rate in the different tissues studied: 34% in urinary sediment, 18% in blood and 67% in kidney biopsy (Figure 2 and Supplementary Materials, Table 2). Mother blood and urine screening led to the diagnosis of de novo mutation.



**FIGURE 2** Genetic analysis of patient 1. MtDNA sequencing using Sanger (A) and NGS (B) found the MT-ND5 m.13513G>A variant in a heteroplasmic state: 34% in urinary sediment, 18% in epithelial cheek mucosa, 16% in blood and 67% in kidney biopsy [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## 2 | PATIENT 2

A 53-year-old female presented stage 3a CKD (GFR 52 mL/min/1.73 m<sup>2</sup>). Renal echography was normal. Proteinuria was physiological without hematuria or leukocyturia.

Kidney function progressively worsened and at the age of 56, renal biopsy was performed with no specific vascular or glomerular lesion. Interstitial fibrosis (20%) was noted associated with interstitial mononuclear cell infiltration (10%) and severe tubule atrophy. Immunofluorescence microscopy detected IgM and C3 deposits in sclerotic glomeruli. The final diagnosis was a tubulo-interstitial nephropathy.

At the same period, she presented a sudden loss of vision in the right eye followed few weeks later by left one. Visual field detected temporal and peri-foveal defects. Fundus and optical coherence tomography (OCT) showed bilateral inter-papillo-macular atrophy. Visual evoked potentials were decreased. Retinal angiography was normal. Cerebral and medullary magnetic resonance imaging were performed, showing inflammation lesions with bilateral optic nerve and chiasma T2 hypersignal taking gadolinium contrast. Neuromyelitis optica (NMO) and aquaporin-4 autoantibodies were negative. The diagnosis of Leber optic neuropathy was suspected.

Analysis of the oxidative phosphorylation function revealed hyperlactatemia with a lactate/pyruvate ratio at the upper limit. Urinary amino-acid chromatography was normal.

Whole MtDNA sequencing using NGS detected the heteroplasmic m.13514A>G (NC\_012920.1:c.1178A>G, p.(Asp393Gly)) pathogenic variant in the *MT-ND5* gene at 5% in blood and 50% in urine (Supplementary Materials, Figure 1 and Supplementary Materials, Table 2). We found the same pathogenic variant in her mother (2% in urine) confirming the maternal inheritance.

## 3 | PATIENT 3

A 34-year-old male patient with high blood pressure consulted for renal impairment. He presented in 2013 a serum creatinine at 133 and 240 μmol/L in 2017. Serum creatinine increased to 440 μmol/L in 2018. He had a glomerular proteinuria at 3 g/24 hours without nephrotic syndrome, hematuria or leukocyturia. Renal echography did not show any particularity. Immunological and infectious diseases were ruled out.

Kidney biopsy showed 4 hypertrophic glomeruli and 10 sclerotic glomeruli. Arterioles were sinuous with hyperplastic media. Interstitial fibrosis and tubular atrophy interested 25% of biopsy material. Immunofluorescence analyses were positive in IgM and C3 in hypertrophic glomeruli. We concluded at a tubulo-interstitial chronic nephropathy with secondary focal segmental glomerulosclerosis.

Ear, nose and throat (ENT) analyses did not evidence any hearing or ophthalmological abnormalities. Echocardiography was normal without left ventricular hypertrophy.

MtDNA sequencing using NGS detected the heteroplasmic m.13513G>A (NC\_012920.1:c.1177G>A, p.(Asp393Asn)) pathogenic variant in the *MT-ND5* gene at 66% in urine sample (Supplementary

Materials, Figure 2 and Supplementary Materials, Table 2), with a de novo mutation after family screening.

## 4 | DISCUSSION

We report three cases of adult chronic tubulointerstitial kidney diseases associated with pathogenic variants in the *MT-ND5* gene. Two cases were related to the pathogenic variant m.13513G>A and one caused by the pathogenic variant m.13514A>G. The two variants m.13513G>A (p.D393G) and 13514A>G (p.D393N) hits the same codon and converts the evolutionary conserved and negatively charged aspartic acid to uncharged amino acids. The report of many cases in the literature (<https://www.mitomap.org/MITOMAP>) of these two heteroplasmic mutations establishes their pathogenicity and shows that the D393 is crucial for the function of ND5 and complex I.

Renal phenotype in mitochondrial cytopathy classically includes glomerular disease as focal segmental glomerulosclerosis or proximal tubular disease.<sup>14</sup> Renal involvement is commonly associated with syndromic mitochondrial disorders.<sup>5</sup> In patient 1, renal disease was associated with a MELAS syndrome and in patient 2 to a Leber neuropathy. In contrast the patient 3 presented only kidney manifestation.

Tubulo-interstitial kidney diseases associated with m.13513G>A and m.13514A>G variants in the *MT-ND5* gene have never been previously described in adult population.<sup>8-13</sup> *MT-ND5* pathogenic variants have only been described in cases of segmental and focal glomerulosclerosis.<sup>15,16</sup> Heteroplasmy levels in the different tissue samples are not always correlated to clinical significance.<sup>8,10</sup> Higher level of heteroplasmy was found in urine samples compared to blood. Urinary sediment should be preferred to blood for the analysis of mitochondrial disorder when muscle or specific tissue biopsy is not available.<sup>17,18</sup> For the patient 1, the high level of heteroplasmy for m.13513G>A in kidney biopsy (67%) brought us a crucial argument for the involvement of this pathogenic variant in the kidney and systemic phenotype of the patient.<sup>3</sup> Indeed, we found also in this patient a *PKD2* pathogenic variant in front of family history of kidney cysts. This *PKD2* variant is pathogenic according to Mayo Clinic database.<sup>19</sup> Nonetheless, considering the absence of kidney dysfunction in his grand-mother and 67 years-old mother (identical *PKD2* mutation) and the clinical spectrum of *PKD2* mutation,<sup>20</sup> we concluded that the most likely cause of our patient end-stage renal disease was mtDNA pathogenic variant. Presence of the two mutations may lead to a modulation of renal phenotype.

In the case of tubulointerstitial kidney disease of unknown origin, the possibility of mitochondrial disease must be evoked. mtDNA NGS from urine sample is non-invasive and profitable<sup>17</sup> and if necessary, it can be completed by the analysis of the kidney biopsy.<sup>21</sup> Mitochondrial disorder is probably an underdiagnosed cause of kidney disease due to variable clinical manifestations extending from systemic symptoms with neurologic, ophthalmologic or cardiologic features (patient 1 and 2) to isolated tubulo-interstitial kidney disease (patient 3).<sup>22</sup> It

may occur in adult population with de novo or inherited pathogenic variant. The whole mtDNA analysis by NGS offers the possibility of identifying rare pathogenic variants even at very low rate of heteroplasmy, in kidney diseases such as tubulo-interstitial nephropathy. The whole mtDNA analysis by NGS of urine, muscle or kidney biopsies offers the possibility of identifying with certainty rare pathogenic variants even at very low rate of heteroplasmy, whereas negative mtDNA analysis from the whole exome sequencing data does not exclude the possibility of mtDNA mutation.

## 5 | CONCLUSION

Our case series is the first description of adulthood onset nephropathies in relation to the pathogenic variants m.13513G>A and m.13514A>G in the *MT-ND5* gene. The clinical presentations were highly variable ranging from isolated renal failure to syndromic mitochondrial disorders such as MELAS syndrome and Leber optic neuropathy. Mitochondrial disorders must be evoked in front of undiagnosed kidney disease to prevent missing a diagnosis.

## ACKNOWLEDGEMENTS

We thank Dr Jean-Philippe Bourdenx for his help transferring data for the second patient. We thank patients for the participation to this report.

## ETHICAL APPROVAL

The study follows the principles outlined in the Helsinki Declaration and patients gave written informed consent for molecular study and publication.

## CONFLICT OF INTEREST

None declared. No support or funding.

## AUTHOR CONTRIBUTIONS

H.B. managed clinical follow-up, collected data and wrote the article, A.T. performed and interpreted genetic analysis, A.V. performed and interpreted histological data, I.R. performed biochemical investigations, R.B. performed and interpreted cardiological investigations, D.L. and C.G. reviewed the article, C.C. managed clinical follow-up, reviewed the article, M.L.M.N. performed and interpreted genetic analysis and histological data, C.R. managed clinical follow-up, collected data and wrote the article.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Bakis H, Trimouille A, Vermorel A, et al. Adult onset tubulo-interstitial nephropathy in MT-ND5-related phenotypes. *Clin Genet*. 2020;97:628–633. <https://doi.org/10.1111/cge.13670>

### ***Renal panel of genes***

Renal panel used in the genetic study concerned the following genes: PKD1, PKD2, HNF1 $\beta$ , SEC63, PRKCSH, UMOD, LRP5, GANAB. Renal panel of genes was only performed for the patient 1 in front his clinical presentation. Based on their clinical and biological phenotype, the panel didn't conduct for the patient 2 (Leber retinopathy with tubulo-interstitial nephropathy) and 3 (tubulo-interstitial chronic nephropathy with severe glomerulosclerosis in a context of glomerular proteinuria).

### ***Method***

#### Molecular analysis of mitochondrial DNA

Total genomic DNA was extracted from peripheral blood using Tecan Freedom EVO<sup>®</sup> workstation (Promega<sup>®</sup>) and the Wizard<sup>®</sup> kit has been used for manual DNA extraction from cells and tissue samples following the manufacturer's instructions. Whole mtDNA was amplified from 50 ng of genomic DNA, using the TaKaRa Kit (Ozyme<sup>®</sup>), in two overlapping long-range (LR)-PCR amplicons according to the manual's instructions. The two fragments were respectively 15.5 and 16 kb (Primers pair 1: Fw: 446- 473/Rv: 16220-16193; Primers pair 2: Fw: 9391-9411/Rv: 9027-9008). Library preparations were performed per patient, following the manufacturer's instructions (Ion Xpress plus Fragment Library Kit; Life Technologies) for 200 bp single-end reads. It consists of three main steps: enzymatic shearing, ligation of the adapters and size selection. Library material was purified using AMPure beads, and concentration and length were determined using the Qubit 2.0 fluorimeter (Invitrogen, Merelbeke, Belgium). Both PCR fragments were normalized to a quantity of 100 ng and then pooled. Size and quality of fragmented DNA were determined with the Tape Station system (Agilent<sup>®</sup> 2200 TapeStation System) following the manufacturer's recommendations. Emulsion PCR was performed on pooled libraries (Ion One Touch 200 Template Kit v2 DL Kit;

Life Technologies), and template-positive Ion Sphere particles were enriched using Dynabeads Myone streptavidin C1 beads. Samples were prepared according to the instructions provided by Ion PGM 200 Sequencing Kit (Life Technologies) protocol. For each run, ten pooled samples were loaded at a final concentration of about 32 pM per target on an Ion 316 v2 chip.

Data analysis

Reads were generated using the Ion Torrent platform pipeline software v.3.6 (Life Technologies). High-quality reads were aligned to the reference sequence (GenBank NC\_012920.1) using TMAP (Torrent Mapping Alignment Program). For each sample, the coverage per base was obtained from the BAM (Binary Alignment Map) files data. Sequence variants were detected using the Variantcaller v4.4.2.1 plugin of the Torrent Suite (Life Technologies), run with default somatic-low stringency settings. For Data coverage and depth see supplemental data 2. The classe 5 and 4 variants identified by NGS were confirmed by Sanger technique.

There was an average of 198K mapped reads per DNA sample (range 64K–372K reads) with 95% of 179 bases reads aligned to the mitochondrial genome. There was 100% of coverage of mtDNA at 200X minimum, with average coverage depth of 2178X.

*Table 1: Quality metrics*

		>=Q20 Bases	Mean Read Length (bp)	Mapped Reads	On target	Mean Depth
Patient 1	Blood	17449221	167	117875	94,46	1134
	Urine	11885942	164	81616	92,74	754,6
	Cheek cells	10941545	158	78074	94,14	709,5
	Kidney	9270197	162	64300	92,48	580,1
Patient 2	Blood	40485583	171	254003	95,23	2503
Patient 2 mother	Blood	68769677	195	372953	96,7	4242
	Urine	50682699	201	270143	96,72	3162
Patient 3	Urine	68454474	212	347051	97,36	4335

**Table 2: Variants**

Patient 1											
Blood			Urine			Cheek cells			Kidney		
Variant	Heteroplasmy	Depth	Variant	Heteroplasmy	Depth	Variant	Heteroplasmy	Depth	Variant	Heteroplasmy	Depth
A263G	100	640	A263G	100	312	A263G	99,6	255	A263G	100	676
A750G	100	1665	A750G	100	1319	A750G	100	1002	A750G	100	1991
A1438G	100	1803	A1438G	100	1343	A1438G	100	1119	A1438G	100	1996
A2581G	100	1998	A2581G	100	1362	A2581G	100	1389	A2581G	100	1990
A4769G	100	344	A4769G	98	201	TC3109CA	4,4	1148	A4769G	100	474
C5911T	100	1074	C5911T	99,1	686	A4769G	100	192	C5911T	100	1031
T6776C	100	1027	T6776C	100	611	C5281-	2,6	195	T6776C	100	1149
A8860G	98,5	131	C10812T	5,4	616	C5911T	100	596	A8860G	99,1	220
C10812T	5,5	912	-T2311AT	2,7	791	T6776C	100	536	G13513A	68,5	1613
G13513A	19	1054	G13513A	37,8	770	C9678A	3,2	759	T14502A	3,7	860
A15326G	100	779	T14502A	8,3	229	C10812T	4,7	577	A15326G	100	1281
G16129A	100	872	A15326G	100	638	-T2311AT	2,2	695	G16129A	100	1221
T16519C	100	558	G16129A	98,9	640	G13513A	21	656	T16519C	100	483
			T16519C	100	298	A13773T	3,4	291			
						A15326G	100	589			
						G16129A	100	572			
						T16519C	98,9	272			

Patient 2				Patient 2 mother				
Blood			Blood			Urine		
Variant	Heteroplasmy	Depth	Variant	Heteroplasmy	Depth	Variant	Heteroplasmy	Depth
T239C	100	1520	T239C	100	1999	T239C	100	1259
A263G	100	1527	A263G	100	2000	A263G	100	1243
A750G	100	1996	CA514-	2,9	1951	A750G	100	1996
A1438G	100	1999	A750G	100	1995	A1438G	100	1998
G3915A	97,8	1953	A1438G	100	1999	G3915A	100	1952
A4727G	98,4	827	G3915A	98,9	1969	A4727G	100	961
A4769G	100	1059	C4604A	2,7	147	A4769G	100	1237
-5282A	1,8	1320	A4727G	100	1204	A8860G	100	671
A8860G	100	381	A4769G	100	1606	G9380A	98,7	1927
G9380A	97,8	1116	-8497A	2,6	1963	T11253C	100	1991
T11253C	99,9	1988	A8860G	100	694	A13231-	2	1968
-13046C	3,8	1154	G9380A	96,9	1549	A13514G	2	1994
A13514G	4,8	1994	T11253C	100	1983	A15326G	100	1995
A15326G	100	1997	A15326G	100	1994	A16037G	100	1949
T15831C	3	1989	-16033G	100	1974	T16362C	100	1641
T16362C	100	1197	A16037G	100	1970	A16482G	100	1209
A16482G	100	1240	T16362C	100	1988			
			A16482G	99	2000			

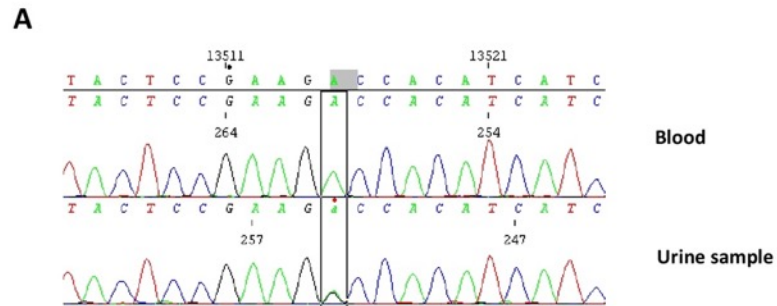
Patient 3		
Urine		
Variant	Heteroplasmy	Depth
A263G	99,9	1650
CA514-	2,7	1838
A750G	99,9	5163
A1438G	100	5959
T4550C	98,9	762
A4769G	99,8	1627
T6776C	99,9	3930
A8860G	99,9	867
G9053A	99,9	1898
A10496G	100	5037
G13513A	66,1	3900
A15326G	99,7	4978
T16519C	99,6	1218



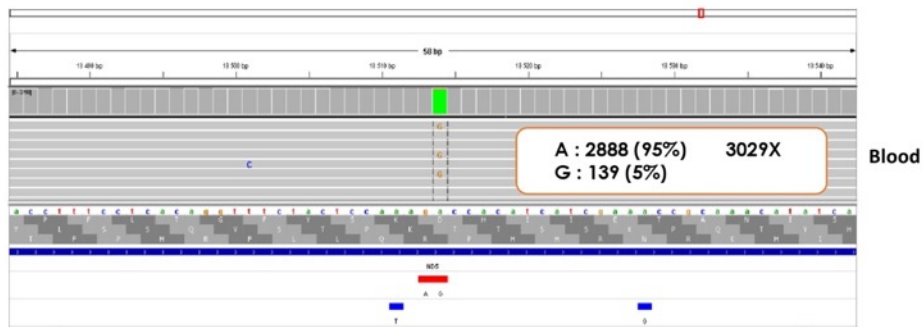
**Figure 1**

Genetic analysis of patient 2

MtDNA sequencing using Sanger (A) and NGS (B) found the *MT-ND5* m.13514G>A variant in a heteroplasmic state: 5% in blood and 50% in urine.



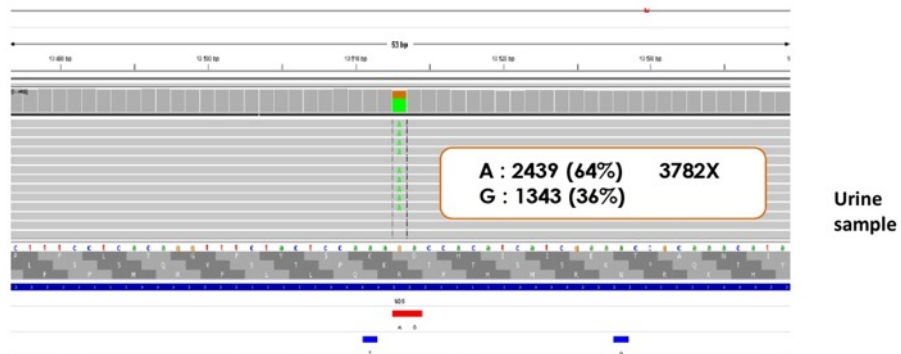
**B**



**Figure 2**

Genetic analysis of patient 3

MtDNA sequencing using NGS found the *MT-ND5* m.13513G>A variant in a heteroplasmic state: 64 % in urine.



Nous avons souligné le fait que les mitochondriopathies tiennent une place centrale dans la physiopathologie de l'IRA et de MRC d'étiologies fréquentes (*i.e.* diabétique, hypertensive). De plus, des affections rénales accompagnent les MIDs à l'âge pédiatrique, mais aussi à l'âge adulte. Ces MIDANs sont des diagnostics complexes, et les avancées en techniques de diagnostic génétique (NGS, WES/WGS) sont des aides incommensurables.

En effet, nous rapportons pour la première fois 3 adultes dont l'enquête étiologique autour de néphropathies tubulointerstitielles retrouve des variants pathogènes de *MT-ND5* (2 m.13513G>A et 1 m.13514A>G). Ces diagnostics mettent en avant l'utilité, rapidement et pour un faible coût, du séquençage de l'ADNmt urinaire par NGS. Ce séquençage, pouvant être aussi facilement réalisé sur les biopsies rénales, ouvre une porte sur le diagnostic de MIDAN.

Pour aller plus loin, nous émettons l'hypothèse, à la lumière de la revue de la littérature sur les MIDANs à l'âge adulte et de la série de cas cliniques publiée, que les MIDANs sont fréquentes et sous-estimées dans la population adulte. Nous avons donc réalisé une étude observationnelle rétrospective monocentrique, en étudiant une population de patients adultes avec un diagnostic génétique de MID et des données permettant d'affirmer la présence ou non d'une maladie rénale. Ainsi, l'objectif est double : confirmer la fréquence importante et la sous-estimation des MIDANs chez l'adulte ; détailler les caractéristiques rénales et extra-rénales (phénotypiques, biologiques, histologiques, génotypiques) des MIDs, associées ou non à des MRC, afin de rechercher une association entre ces caractéristiques et la survenue de MIDANs.

## **Article 3 : Article original**

# **Renal involvement is frequent and underestimated during primary mitochondrial disorders in adulthood**

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## **Running title:**

Kidney disease during adulthood MID

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**Key words:** mitochondrial disorders, adult, kidney disease, tubulo-interstitial nephritis, glomerular disease.

## **Abstract:**

**Background:** Mitochondrial functions integrate the regulation of reactive oxygen species, cell survival, apoptosis, and energy production. These functions are controlled by genes of both mitochondrial and nuclear DNA. Pathogenic variants affecting any of these are responsible for primary mitochondrial disorders (MIDs), that can be diagnosed during adulthood. The kidneys' functions are highly dependent on mitochondrial respiration. However, the prevalence of MID-associated nephropathies (MIDANs) is unknown in the adult population.

**Methods:** Adults ( $\geq 16$ -years-old) with genetically diagnosed MID between 2000 and 2020 in our tertiary care academic center, were included in a retrospective observational study when they had a kidney disease (KD) evaluation. MIDANs were ascertained by KD occurring in MIDs. The phenotypic, biological, histopathological, and genotypic characteristics were recorded from the medical charts.

**Results:** We included 80 MID-affected adults and ascertained MIDANs in 28/80 (35%). KDs were identified before the study for 14/18 (50%) of the adults with MIDAN. MIDANs were mostly tubulointerstitial nephritis (TIN) for 14/28 (50%), and glomerular disease (GD) for 9/28 (32.1%). MIDAN was independently associated with higher albumin level [odds ratio (OR), 0.79; 95% confidence interval (95%CI), 0.67–0.95], hypertension (OR, 4.23; 95%CI, 1.04-17.17), and vision abnormalities (OR, 0.17; 95%CI, 0.03–0.94). In contrast, the histopathological and genotypic characteristics were not associated with MIDANs.

**Conclusion:** MIDANs are frequent and underestimated among adult MIDs. They are mostly represented by TIN or GD. Vision abnormalities, hypertension and albumin levels were independently associated with MIDANs. Our results pave the way for larger prospective studies investigating MIDANs prevalence among undetermined KD populations.

## Introduction

Mitochondria are intracellular organelles resulting from an endosymbiotic process that occurred billions of years ago between bacteria and an eukaryotic cell precursor<sup>1</sup>. Mitochondria are essential for cell survival, as they perform multiple functions, such as regulation of reactive oxygen species, apoptosis signaling, and energy production<sup>2-4</sup>. Mitochondrial respiration consists of nutrient oxidation (carbohydrates, amino acids, and fatty acids) resulting in the formation of water and carbon dioxide, and phosphorylation of adenosine diphosphate to adenosine triphosphate (ATP) as a source of energy for cells<sup>5</sup>. ATP synthesis is secondary to the oxidative phosphorylation reaction (OXPHOS) through the electron transport chain (ETC) on the mitochondrial inner membrane<sup>6</sup>.

The mitochondrion is the only organelle that possesses genetic material called mitochondrial DNA (mtDNA). mtDNA is a circular double-stranded 16,569 bp with 37 genes encoding 13 ETC proteins, 2 ribosomal RNAs, and 22 transfer RNAs<sup>7</sup>. The remainder of the 1,400 mitochondrial proteins are encoded by nuclear DNA (nDNA), which is also responsible for mtDNA replication, maintenance, and transcription<sup>8,9</sup>. This duality is unique because both defects in nDNA or mtDNA can lead to primary mitochondrial disorders (MIDs)<sup>10</sup>. Initially considered to be detected only in young children, MIDs are now also considered an adulthood disease<sup>12,13</sup>. Mitochondrial genetics rules include maternal inheritance, but *de novo* mtDNA pathogenic variants appear to be common, even if under-reported in adult population<sup>12,14</sup>. The estimated prevalence of MIDs in the adult population is 1 in 4,300 with a preponderance of the mtDNA pathogenic variants accounting for 75%<sup>15</sup>. Pathogenic nDNA variants are estimated to be less frequent in the adult population and account for overt disease in 2.9 per 100,000 of the adult population<sup>15</sup>. Nevertheless, new molecular techniques, such as next-generation sequencing (NGS) of mtDNA and whole-exome sequencing (WES) democratization could increase MID diagnoses and prevalence in adulthood<sup>16-18</sup>.

Mitochondria are present in every cell (mature erythrocytes excepted), with each mitochondrion containing up to ten mtDNA copies. The coexistence of two or more different mtDNA populations (e.g., wild-type and pathogenic variant) in an individual defines heteroplasmy. There is a heteroplasmic threshold above which a mitochondrial defect becomes clinically relevant, depending on the extent to which the affected tissue relies on OXPHOS, but also on the function altered by the mtDNA pathogenic variant<sup>19</sup>. In line with this, all organs can be affected by MIDs, with susceptibility depending on energy consumption<sup>20</sup>. The kidneys are second to the heart in terms of oxygen consumption and resting metabolic rate per dry weight of tissue<sup>21,22</sup>. The MID-associated nephropathies (MIDANs) affecting the adult population might be underestimated<sup>23</sup>. Nevertheless, the incidence and prevalence of MIDANs among the adult population are not known, because most of the literature refers to case reports<sup>17,24</sup>. MIDANs in this population are

likely to be tubulointerstitial nephritis (TIN) and glomerular diseases (GD) with focal segmental glomerulosclerosis (FSGS)<sup>25–31</sup>.

Clinical presentations are often multisystemic and refer to specific syndromes, such as mitochondrial encephalopathy, lactic acidosis, stroke-like episode syndrome (MELAS)<sup>25,27</sup> and maternal inherited diabetes and deafness syndrome (MIDD)<sup>28</sup> with the *MT-TL1* m.3243A>G pathogenic variant, or Kearns–Sayre syndrome (ophthalmoplegia, ptosis, and pigmentary retinitis) with mtDNA single deletions<sup>32,33</sup>. However, a significant number of MIDANs have been isolated at diagnosis, sometimes preceding other organ manifestations<sup>26,29,34</sup>. This heterogeneity, considered the hallmark of MID because of its genetics rules, makes MIDAN a strong diagnosis with medical implications. Indeed, it provides possibilities for genetic counseling and potential therapeutic approaches<sup>35</sup>. Moreover, it offers the opportunity to increase the ratio of diagnosed nephropathy before kidney transplantation, as one-sixth of KD are undiagnosed<sup>36</sup>.

This study aimed to establish the prevalence of MIDANs among a MID-affected adult population diagnosed in different departments of our academic tertiary care hospital. Furthermore, we provide a full characterization of the observed KD with clinical, biological, and histopathological descriptions. Then, we investigated the association between MIDAN outcome and phenotypic, biological, and/or genotypic characteristics of MID-affected adults.

## **Methods**

### **Study population**

Adult patients ( $\geq 16$ -years-old) with a genetically confirmed MID diagnosis made at Bordeaux University Hospital between January 2000 and January 2020 were screened. We included patients with a KD evaluation comprised of at least creatinine dosage for estimated glomerular filtration rate (eGFR), and urinalysis. Exclusion criteria were evidence for a KD with another etiology, could not consult the medical records, and the patient's refusal to participate. The follow-up period included the period from the MID diagnosis to the last KD assessment. Patients were censored if they died or were lost to follow-up (defined as the absence of a KD evaluation for more than 1 year). Our study population consisted in a majority of unrelated patients, as our inclusion criteria brought us to study only 2 couples of siblings, and 2 mother-child couples of adults.

This study was approved by the local institutional review board (number CE-GP-2020-32). Informed consent was obtained from each patient for retrospective use of their clinical samples before inclusion in the study. This study followed the guidelines outlined in the Declaration of Helsinki and the Declaration of Istanbul.

### **MID ascertainment**

Our criteria included adults exhibiting compatible MID symptoms and confirmation of underlying mutation known to cause MID by genetic analysis. Molecular genetic testing of mtDNA was performed on muscle biopsy when available, on urine and/or blood samples. Individuals with mtDNA pathogenic variants (point mutations, duplications, or single deletions) were included. Moreover, we encompassed those with multiple mtDNA deletions in muscle when there was no evidence of other muscle pathology and when the clinical and biochemical [ $>3\%$  of cyclooxygenase (COX)-deficient fibers] features were consistent with MID. Molecular genetic testing of nDNA pathogenic variants in genes coding for mitochondrial functions was based on target genes analysis (performed at Angers University Hospital or Paris Necker University Hospital) at the behest of the physician.

### **MIDAN definition**

MIDAN was defined as a KD occurring in a MID-affected patient with no other cause of KD. KD was ascertained according to the KDIGO guidelines<sup>37</sup> and was based on abdominal imagery (echography and/or abdominal CT) and biochemical data. eGFR was estimated from creatinine serum dosages and the 2009 Chronic Kidney Disease–Epidemiology Collaboration formula<sup>38</sup>. Pathological proteinuria was considered  $>0.3$  g/g of creatinine. Leukocyturia and hematuria were assessed by either a dipstick or cytobacteriological examination of the urine with a cut-off of



10,000/mL. Nephrotic syndrome was defined by the association between  $\geq 3$  g/g proteinuria and serum albumin  $< 30$  g/L. We defined as “identified KD”, the KD that have been previously diagnosed and under the care of a nephrologist.

We distinguished different KD: (1) proximal tubulopathy was defined by the more or less complete association of tubular proteinuria (low-molecular-weight proteinuria with albuminuria  $< 50\%$ ), normoglycemic glycosuria, amino-aciduria, hypophosphatemia with increased phosphate in urine samples, hypokalemia with increased kaliuresis, proximal acidosis, and hypouricemia with increased uric acid in urine samples; (2) distal tubulopathy was considered as hypokalemia with an abnormal increase of urine potassium, hypochloremia, metabolic alkalosis, and/or hypomagnesemia; (3) TIN was defined as a KD with tubular proteinuria and/or persistent aseptic leukocyturia and/or the predominance of tubular atrophy and interstitial fibrosis lesions in kidney biopsies<sup>39</sup>; (4) GD was defined as a KD with glomerular proteinuria (albuminuria  $\geq 50\%$ ) and then classified as FSGS or other diagnosis regarding histological lesions in kidney biopsies; (5) CAKUT accounted for kidney cysts and/or urinary tract malformations.

Renal biopsy tissues were fixed in formaldehyde solution, embedded in paraffin, and sectioned at  $2 \mu\text{m}$  for the optical microscopy (OM) study. The sections were stained with Masson’s trichrome and were reviewed by the same nephropathologist (A.V.). Tubulointerstitial changes and arteriolar hyaline thickening were scored following the Banff lesion score 2017<sup>40</sup>. Mitochondrial morphology was examined by electron microscopy (EM) when glutaraldehyde-fixed renal biopsy was available.

### **Clinical, biological, and histopathological assessments**

Clinical and biological data from the medical charts were reviewed. Hypertension was defined as office systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg and/or use of anti-hypertensive drugs. Extra-renal manifestations were screened as follows: (1) growth abnormality was evaluated as  $-2$  SD of height for age; (2) a stroke-like episode was diagnosed as an acute neurological alteration associated with magnetic resonance imaging that show a non-vascular distribution; (3) muscular affliction was defined as subjective exercise intolerance and/or an objective muscular change and/or an electromyogram myogenic abnormality and/or histopathological abnormalities (*i.e.* ragged-red fibers); (4) hypertrophic cardiomyopathy was assessed by echocardiography as a left ventricular (LV) wall thickness  $> 13$  mm in one or more myocardial segments that was not explained solely by the loading condition<sup>41</sup>; (5) dilated cardiomyopathy was diagnosed by echocardiography as LV dilatation  $> 32$  mm/m<sup>2</sup> body surface-indexed end-diastolic diameter for women and  $> 31$  mm/m<sup>2</sup> for men<sup>42</sup>; (6) Wolff–Parkinson–White syndrome, heart block, and other arrhythmias were screened by electrocardiogram; (7) retinal

pigmentary abnormalities, optic nerve atrophy accounting for Leber's optic neuropathy, and macular dystrophy were assessed by fundus, optical coherence tomography and by fluorescein angiography; (8) conductive hearing impairment was diagnosed from audiogram evaluations; (9) diabetes mellitus was considered a fasting plasma glucose  $>7$  mmol/L on two occasions; (10) we checked for age, sex, height, weight, seizures, ataxia, encephalopathy, vision abnormalities, ophthalmoparesis, ptosis, liver, and gastrointestinal afflictions in the medical records. Serum lactate was high when  $\geq 3$  mmol/L, uric acid  $>300$   $\mu\text{mol/L}$  for females and  $>360$   $\mu\text{mol}$  for males and creatinine phosphokinase (CPK) level when  $>150$  U/L<sup>17</sup>. The lactate-to-pyruvate ratio was considered an increase when  $\geq 25$ <sup>43</sup>.

Muscular biopsies were performed and interpreted by the same pathologist (M.L.M.N.). All were open biopsies routinely analyzed for mitochondrial dysfunction with systematic hematoxylin and eosin, modified Gomori trichrome (for ragged-red fibers), SDH, NADH-TR, COX (for COX-negative fibers), and combined SDH/COX staining.

Phenotypes were assessed in PhenoTips® (Gene42 Inc., Toronto, ONT, Canada) using Human Phenotype Ontology (HPO) terms. HPO terms relative to clinical data appear in different tables. A ">" following the HPO term accounts for all phenotypes in the subtree of the corresponding HPO term.

### **Statistical analyses**

Quantitative values are presented as median [1<sup>st</sup>–3<sup>rd</sup> quartile (IQR)]. Qualitative data are presented as numbers (percentages). Quantitative variables between groups were compared with the Wilcoxon test, and qualitative variables were compared with the chi-squared test. A p-value  $<0.05$  was considered significant. Univariate and multivariate logistic regression analyses were used to estimate odds ratios (OR) for independent variables associated with the occurrence of MIDAN. Variables with  $p < 0.25$  in the univariate analysis were considered in the multivariate analysis using a backward selection procedure. The best-fitting model was defined according to the Akaike information criterion (AIC). The significance of the parameters was evaluated with the Wald test and the likelihood test. Factors displaying a p-value  $<0.05$  in the multivariate analysis were considered independently associated with the tested outcome. Results are presented with their 95% confidence intervals (95%CI). Statistical analyses were performed with R version 4.0.1 (The R Foundation for Statistical Computing, Vienna, Austria).

## Results

### MID-affected adults phenotypic, biological, and genotypic characteristics

Among the 189 adults with genetically confirmed MID screened, 80 met all of the inclusion criteria (Figure 1). The median follow-up was 26 months (12–36); 12/80 patients died (15.0%) and 25/80 (31.2%) were lost to follow-up.

The baseline clinical and biological characteristics of the study population at the MID diagnosis are presented in Table 1. The median age at diagnosis was 52 years (34–64) with the majority males [50/80 (62.5%)]. Nervous system afflictions were described in most cases [61/80 (76.2%)], followed by ophthalmological [57/80 (71.2%)], cardiovascular [44/80 (55%)], and muscular [40/80 (50%)]. MIDAN accounted for 28/80 (35%) of MID-affected adults, immediately before conductive hearing impairment [27/80 (33.7%)] and endocrinological afflictions [26/80 (32.5%)]. The serum lactate level and the lactate-to-pyruvate ratio were elevated in 11/80 (13.7%) (e.g.,  $\geq 3$  mmol/L and  $\geq 25$ , respectively, see corresponding methods section) of MID-affected adults. CPK were increased (e.g.,  $>150$  U/L) in 19/80 (23.7%) patients, and uric acid was above the upper range (e.g.,  $>300$   $\mu\text{mol/L}$  for females and  $>360$   $\mu\text{mol}$  for males) in 14/80 (17.5%).

The MID genotypic features are presented in Table 2. A massive proportion of MID was due to mtDNA pathogenic variants [56/80 (70%)]. These defects were mostly a consequence of point mutations [43/80 (50.6%)], but also of single deletions, and duplications [11/80 (13.7%) and 2 (2.5%) of MID, respectively]. The predominant point mutations were *MT-TL1* m.3243A>G, *MT-ND4* m.11778G>A, and *MT-ND5* m.13513G>A [17/43 (39.5%), 7/43 (16.2%), and 6/43 (13.9%) of mtDNA point mutations, respectively] (Table 2). Multiple deletions accounted for 20/80 (25%) of the study population. Pathogenic variants affecting nDNA were responsible for 4/80 (5.0%) MID-affected adults, accounting for two *OPAI*, one *POLG*, and one *RMM2B* pathogenic variants.

### MIDANs are prevalent and with a majority of TIN or GD, while largely underestimated during adulthood

MIDANs were ascertained for 28/80 (35%) of MID-affected adults and their characteristics are listed in Table 3. The KD of adults with MIDAN were TIN, GD, CAKUT, proximal tubulopathy, and distal tubulopathy [14/28 (50%), 9/28 (32.1%), 4/27 (14.38%), 2/27 (7.4%) and 2/27 (7.4%), respectively].

We noted that 14/28 (50%) of patients with MIDAN had an identified KD (e.g., previously diagnosed and under the care of a nephrologist, see corresponding methods section). We also observed that 9/28 (32.1%) were in end-stage renal disease (ESRD), among whom 5/9 (55.6%) received a MID diagnosis after initiating dialysis and 3/9 (33.3%) were on dialysis before 1-year

post-diagnosis. The median eGFR at diagnosis for MIDAN-affected patients with preserved kidney function after a 1-year follow-up was 60 ml/min/1.73m<sup>2</sup> (27–84) and the median eGFR slope was -3.0 ml/min/year (-6.0–0). Kidney transplantations were performed in 4/9 (44.4%) of patients, three of whom (75%) were suspected of a MIDAN recurrence: one patient experienced recurrence of FSGS and the other two had an unexplained TIN (Table 3).

We subsequently focused on describing the leading causes of KD among patients with MIDAN, which were TIN and GD.

TIN accounted for 14/28 (50%) of patients who presented with MIDAN, as confirmed by kidney biopsy in 6/14 (42.9%). Four of these biopsies were reviewed and none exhibited granular swollen epithelial cells (GSEC) or hypertrophied podocytes (Figure 2A and Table 4). An EM analysis was performed on one patient and showed an increase in the number of swollen mitochondria with loss of the cristae structure inside the kidney tubular epithelial cells (Figure 2B and C).

GD represented 9/28 (32.1%) of patients with MIDAN, as confirmed by kidney biopsy in 3/9 (33.3%). The OM histopathological findings were FSGS for all three patients (Figure 3 and Table 4). One kidney specimen exhibited hypertrophied podocytes, with no possibility to perform EM because of the absence of a glomerular section on the frozen kidney biopsy specimen. Of note, none of the patients who experienced GD had hematuria, and 3/9 (33.3%) suffered from nephrotic syndrome (Table 3). The MID-affected adults with GD were more likely to suffer from endocrine afflictions and *MT-TL1* m.3243A>G pathogenic variants than patients presenting with TIN (Table 5, p=0.038 and p=0.01, respectively). Those two groups were comparable in terms of eGFR at diagnosis, number of patients with identified KD and with ESRD (p=0.508, p=0.985, and p=1, respectively). The slope of GFR decline over time was nearly flat in patients with GD [0.0 ml/min/year (-1.3–0.1)] while it was non-significantly steeper in patients with TIN [-4.0 ml/min/year (-6.0– -3.0), p=0.082].

### **MID-affected adults who displayed lower serum albumin levels, hypertension, and no abnormality of vision are more likely to have MIDAN**

The baseline phenotypic, biological, and genotypic characteristics of the study population according to the occurrence of MIDAN are presented in Table 6 (Supplemental Tables 1 and 2 for complete biological and genotypic analyses). In our cohort, MID-affected patients who suffered from myocardial afflictions, such as hypertrophic cardiomyopathy, dilated cardiomyopathy, and arterial hypertension were more likely to have MIDANs (p=0.008, p=0.018, p=0.001, and p=0.001, respectively). The same associations were observed for patients with endocrine afflictions and diabetes mellitus (p=0.014 and p=0.038). Conversely, patients with MIDAN

seemed less affected by visual impairments, ophthalmoparesis, and palpebral ptosis (Table 6,  $p=0.037$ ,  $p=0.007$  and  $p=0.024$ , respectively).

Biological analyses highlighted lower calcium and albumin concentrations in patients with MIDAN ( $p=0.04$  and  $p=0.004$ ) and a larger proportion of patients with increased uric acid (*i.e.*  $>300\mu\text{mol/L}$  for females and  $>360\mu\text{mol/L}$  for males, see corresponding method section) ( $p<0.001$ ). None of the other biological parameters differed between the groups (Supplemental Table 1). Genetic analyses underscored that the MIDAN population experienced more frequent mtDNA point mutations, notably *MT-ND5* m.13513G>A ( $p=0.020$  and  $p=0.018$ , respectively).

The multivariate model with the best AIC for MIDAN amongst MID-affected patients integrated the following variables: hypertension, gender, abnormality of vision and albuminemia (Table 7). After adjustment on the other variables, multivariate analysis with logistic regression identified abnormality of vision (OR, 0.17; 95%CI, 0.03–0.94;  $p=0.042$ ) and arterial hypertension (OR, 4.23; 95%CI, 1.04-17.17;  $p=0.044$ , respectively) as independently associated with MIDAN. Among the biological characteristics, higher serum albumin level was independently associated with MIDANs (OR, 0.79; 95%CI, 0.67–0.95;  $p=0.01$ ).

## Discussion

This is the first study to evaluate the prevalence of MIDAN in a large population of MID-affected adults. We showed that patients presenting with MIDAN accounted for 35% of this population. This cohort is one of the largest MID-affected adult populations analyzed, providing robustness to our findings. Furthermore, we did not restrict our study population to certain pathogenic variants or syndromic manifestations to benefit from a heterogeneous, representative adult population of MIDs based on a molecular genetic diagnosis. Nevertheless, the same proportions have been reported in more restricted populations, such as in MIDD (28–46%)<sup>28,44</sup> and MELAS syndrome cohorts (39%)<sup>31</sup>. Pediatric MID studies have shown an even higher rate of MIDAN of 5% to 50%<sup>45–47</sup>.

Another strength of our study is in the standardization of the patients' phenotypic characteristics using HPO terms. KD was in the fourth position among the most frequent phenotypes encountered in our study, immediately after cardiovascular afflictions. The kidneys are second to the heart in terms of resting metabolic rate<sup>21</sup>. The two first positions were occupied by neuromuscular and ophthalmological afflictions. Of note, these manifestations are accompanied by substantial symptoms, leading patients to seek medical attention. A large cohort of MID-affected patients emphasized that the adults' most frequent complaint was neuromuscular afflictions with exercise intolerance<sup>48</sup>. Moreover, the first descriptions of MIDs in the literature were encephalomyopathies and vision alterations associated with Leber's optic neuropathy<sup>49,50</sup>. This familiarity among ophthalmologists, neurologists, and MIDs could be a consequence of the precocity of these manifestations. Nevertheless, we cannot exclude recruitment bias. Longitudinal studies systematically evaluating the natural clinical course of MID are needed.

We have underscored that MIDANs were largely underestimated, as only 50% had identified KD that have been previously diagnosed and under the care of a nephrologist. Martín-Hernandez *et al.* screened for KD in 42 MID pediatric patients and found that 13/21 (63%) with MIDAN had mild tubular impairment; thus, they were undiagnosed and not under the care of a nephrologist<sup>45</sup>. Kidney involvement is not in the foreground of multisystemic manifestations, so it might go unnoticed<sup>26</sup>. Even if these data must be confirmed by larger prospective studies, we encourage clinicians to refer MID-affected patients systematically to nephrologists for MIDAN screening and follow-up. Indeed, MIDANs are a frequent but underestimated manifestation of MID.

Delay in the diagnosis and referral could have serious consequences. We observed that 32.1% of patients with MIDAN reached ESRD and 89% developed ESRD before or during the first year following the MID diagnosis. Even if we lacked longitudinal data (median follow-up of 26

months), the median eGFR slope in our study [-3.0 ml/min/year (-6.0–0)] was under the definition of KD rapid progression<sup>51</sup>. Prompt appreciation of the MIDAN diagnosis is mandatory to initiate supportive care and aerobic exercise conditioning to slow down the progression of KD and MID<sup>52</sup>. In a study evaluating 35 solid organ transplant recipients, MID was diagnosed after transplantation for 50% of them<sup>53</sup>. Of note, the MID population generally tolerates solid organ transplants. Nevertheless, Parikh *et al.* underscored that MID-affected kidney transplant recipients suffered from disease worsening (*i.e.* lactate acidosis and diabetes onset) for 4/12 (33%)<sup>53</sup>. Such complications have not been observed in our kidney transplanted recipients, but we noticed 3/4 (75%) of them with a suspected recurrence of MIDAN: two with undetermined TIN and one with FSGS recurrence. These lesions are not specific to MIDAN and could also be the consequence of another cause, such as calcineurin inhibitors toxicity or immunological effects. The mechanism of a potential MIDAN recurrence after kidney transplantation remains unclear and should be elucidated. Some new therapeutic approaches such as mTOR inhibitors for maintenance therapy in the solid organ transplant population is of paramount interest, as they at least improve extra-renal manifestations<sup>54</sup>.

A strong and complete characterization of MIDAN may allow clinicians to make this diagnosis without delay. Thus, we provided a very detailed description of MIDANs in our study. Pediatric MIDANs are better characterized and are dominated by proximal or distal tubular dysfunction and steroid-resistant nephrotic syndromes<sup>55</sup>. In our cohort, we observed major differences, as MIDANs were predominantly represented by TIN and GD (accounting for 50% and 32.1% of KD, respectively), which is consistent with reported cases<sup>25–27,29,31</sup>. Hall *et al.* screened 117 MID-affected adults using proteomic and metabolomic urinalyses and observed tubular afflictions in 39% and glomerular afflictions in 31% of patients (using retinol-binding protein and albumin as markers, respectively)<sup>23</sup>.

The most frequently described MIDAN in our study was TIN. Our team recently reported three cases of adult TIN leading to a MID diagnosis with *MT-ND5* pathogenic variants (two with m.13513G>A and one with m.13514A>G)<sup>26</sup>. This is of great interest regarding the complexity of the etiology before undetermined TIN. We reviewed kidney biopsy specimens (OM analyses) in light of the MIDAN diagnosis. We have been unable to describe some specific histopathological characteristics of the MIDANs. Notably, GSEC was absent in our study and may lack sensitivity and specificity<sup>56</sup>. In contrast, EM analyses could be of great interest, putting in evidence altered mitochondria into tubular and/or podocyte cells, even if improvements are mandatory to differentiate primary from secondary mitochondrial dysfunction.

We observed that only one-third of patients harbored a nephrotic syndrome. We emphasize that none of the patients presented with hematuria, ruling out Alport syndrome as a differential diagnosis<sup>27,57,58</sup>. Patients experiencing GD were more likely to suffer from endocrine afflictions than patients with TIN. It has been reported that MIDD patients rarely experience diabetic retinopathy, are younger, achieve better glycemic control, and suffer from more severe KD (lower GFR and higher urine albumin excretion). These observations support the fact that patients with KD are more likely to develop a consequence of MID than diabetes mellitus complications<sup>28,44</sup>. Furthermore, all patients with GD who benefited from kidney biopsy presented with FSGS lesions. Patients experiencing GD had a higher frequency of the *MT-TL1* m.3243A>G pathogenic variants, which is consistent with prior studies<sup>25,27,59</sup>. We did not perform a multivariate analysis to compare TIN and GD characteristics as it would have been underpowered.

Following the best AIC multivariate model after adjusting for various phenotypic, biological, and genotypic characteristics, we demonstrated that abnormalities of vision were negatively associated with the absence of MIDAN at the MID diagnosis. This could be a consequence of ophthalmological or neurological (*i.e.*, Leber's optic neuropathy) manifestations preceding MIDAN. We hypothesized that it was linked to recruitment bias of more neurological and ophthalmological manifestations. Lower albuminemia was positively associated with MIDAN, probably as a consequence of KD with albumin loss during GD.

More interestingly, hypertension was also positively associated with MIDANs. It is well established that kidney is both a cause and victim of elevated blood pressure<sup>60</sup>. More than 80% of the patients suffering from KD present hypertension<sup>61</sup>. Moreover, hypertension is the major risk factor for development and progression of KD. This could be the consequence of a mitochondrial dysfunction. Indeed, hypertension is responsible of an altered OXPHOS (e.g., ATP reduction and ROS increase) and mitochondrial homeostasis that leads to endothelial dysfunction in renal artery segments<sup>62</sup>. Furthermore, enhanced renin-angiotensin-aldosterone secretion has been demonstrated to affect podocytes OXPHOS, preceding podocytes injury and glomerulosclerosis<sup>63</sup>. Proximal tubule epithelial cells are also affected by mitochondrial dysfunction secondary to elevated blood pressure and might favor TIN development<sup>64</sup>. Mitochondrial dysfunctions are also related to promote and maintain hypertension through endothelial dysfunction (e.g., impairment of vasculature relaxation) and renal involvement with a rise in proximal tubule sodium reabsorption (apical Na<sup>+</sup>/H<sup>+</sup> exchanger 3) secondary to angiotensin II effect on mitochondria angiotensin receptor<sup>65</sup>. There are also numerous evidences for MID causative effect on arterial hypertension<sup>66</sup>. To note, extended genotypes of 32 participants of Framingham Heart Study highlighted 40 mtDNA single nucleotide polymorphisms associated with essential hypertension<sup>67</sup>.



Thus, the association observed in our study could be the consequence of a vicious circle between mitochondrial dysfunctions, hypertension and MIDANs, thus worsening each other. Interpreting such intricate findings is complex and requires a specially designed prospective evaluation that compares renal involvement in a hypertensive population without MID, to a hypertensive population with MID. However, this finding enhances the importance of the prompt initiation of supportive care such as antihypertensive drugs (e.g., angiotensin-converting enzyme inhibitor) to prevent KD worsening and cardiovascular events.

We noted, in univariate analysis, a strong association between elevated acid uric and MIDANs, then stepped out from AIC multivariable model as a consequence of a lack of measurement for 32.5% of the population. However, this is an interesting association as uric acid elevation is commonly associated with MID with musculature affliction secondary to nucleic acid and nucleotide catabolism<sup>68</sup>. Uric acid also increases as KD progresses because of filtration and reabsorption impairment<sup>69</sup>. HUPRA syndrome (hyperuricemia, pulmonary hypertension, renal failure and alkalosis) is evident in pediatric MIDAN with the nDNA pathogenic variant (*SARS2*), and hyperuricemia as a consequence of tubulopathy and decreased urinary excretion<sup>70</sup>. Furthermore, hyperuricemia is associated with mitochondrial calcium efflux, increased ROS production, lower ATP synthesis and secondary mitochondrial dysfunction<sup>71,72</sup>. Of note, hyperuricemia increased the risk of KD in a large cohort of healthy patients, even after adjusting for demographic factors<sup>73</sup>. The mechanism may be activation of inflammasome secondary to mitochondrial dysfunction, leading to KD *via* tubulointerstitial, glomerular, and endothelial lesions<sup>74</sup>. We can carefully speculate that uric acid might be the cornerstone of MIDANs. It would be of great interest to provide a prospective study that investigate the association between systematically measured uric acid increase and MIDAN to help for interpretation of such intricated data.

Despite the genetic molecular analyses in all patients, we failed to find an independent association between some pathogenic variants and MIDANs. Nevertheless, mtDNA NGS has paved the way for rapid and low-cost MID diagnoses<sup>75</sup>. It would be of tremendous interest to conceptualize a study where mtDNA NGS is systematically performed on urine samples and kidney biopsies in patients presenting with TIN or GD of undetermined cause<sup>26,76</sup>. Coupled with systematic blood lactate, uric acid, pyruvate, CPK, albumin, and urine organic acids, it could provide some markers of interest to guide clinicians to a MIDAN diagnosis and, eventually, create a diagnostic score.

Our study was an observational retrospective study based on an examination of medical files, so it has some limitations. Moreover, 33/113 (29%) lacked a KD assessment. This may have underpowered the current study. The other limitation point was the absence of longitudinal data

allowing us to characterize the clinical course of MID and MIDAN more fully. Even if genetic diagnoses were available for every patient, numerous patients had multiple deletions without nDNA analyses. The proportion of nDNA pathogenic variants, and the association with MIDANs, has probably been underestimated. Unfortunately, mtDNA pathogenic variant heteroplasmy levels were not determined. First, the evolution of molecular techniques during the inclusion period made it unmatchable. Second, the samples varied too much among patients (check mucosa, urine, blood, and specific tissue). Thus, we were unable to evaluate the impact of heteroplasmy level on the occurrence of MIDANs, which would have been of great interest<sup>77</sup>.

Overall, our results pave the way for further larger prospective studies as that aim: (1) to evaluate KD among a MID adult population with systematic and longitudinal clinical, biological and pathological evaluations, to develop a predictive score for the occurrence of MIDAN; (2) to screen undetermined KD (such as TIN and GD) for a MIDAN diagnosis using mtDNA NGS in urinalyses to assess MIDAN epidemiology, and clinical, biological, and pathological markers to establish diagnostic scores to mentor clinicians; (3) to develop routine mitochondrial functional analyses for kidney biopsies to achieve a certain diagnosis of MIDAN. We must keep in mind that MIDAN is a frequently underestimated etiology of KD in the adult population.

**Authors contribution:**

H.B., A.T., M.L.M.N. and C.R. designed the study; A.T., A.V. and M.L.M.N. carried out the experiments; H.B., A.T and M.L.M.N. collected the data; H.B., A.T, Y.B., M.L.M.N. and C.R. analyzed the data; H.B., A.T, M.L.M.N. and C.R. wrote the manuscript; A.V., G.S., C.C. and C.G. were involved in the critical revision of the manuscript; all authors approved the final version of the manuscript.

**Acknowledgements:**

We acknowledge archives and medical secretaries, for their valued assistance. We are grateful to the technicians of the pathology and genetic laboratories, for their technical expertise.

**Disclosures:**

The authors of this manuscript have no conflict of interest to disclose.

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## Tables

**Table 1: Baseline phenotypic and biological characteristics of MID-affected adults**

	MID-affected adults (n=80)
<b>Phenotypic characteristics</b>	
Age at diagnosis (years) (median, IQR)	52 (34 - 64)
Sex (M/F)	50/30
BMI (kg/cm <sup>2</sup> ) (median, IQR)	22 (18 - 26)
<b>Growth abnormality</b> [n,(%)] (0001507>)	31 (38.7)
<b>Abnormalities of the nervous system</b> [n,(%)] (0000707>)	61 (76.2)
Morphological abnormality of the central nervous system [n,(%)] (002011>)	32 (53.3)
Stroke-like episodes [n,(%)] (0002401)	12 (15)
Abnormality of nervous system physiology [n,(%)] (0012638>)	55 (68.7)
Seizures [n,(%)] (0001250>)	17 (21.2)
Ataxia [n,(%)] (0001251>)	19 (23.7)
Encephalopathy [n,(%)] (0001298>)	16 (20)
MELAS [n,(%)] (0002401 and 0001298 or 001250 and 0003737 and 0002151)	2 (2.5)
<b>Mitochondrial myopathy</b> [n,(%)] (0003737> and/or 0003546)	40 (50)
Ragged-red fibers [n,(%)] (0003200)	32 (40)
MERRF (0001250 and 0003200)	4 (5)
<b>Abnormality of the cardiovascular system</b> [n,(%)] (0001626)	44 (55)
Abnormality of the myocardium [n,(%)] (0001637>)	25 (31.2)
Hypertrophic cardiomyopathy [n,(%)] (0001639> and 0001714>)	15 (18.7)
Dilated cardiomyopathy [n,(%)] (001644)	6 (7.5)
Arrhythmia [n,(%)] (0011675>)	16 (20)
Wolff Parkinson White [n,(%)] (0001716)	6 (7.5)
Hypertension [n,(%)] (0000822>)	27 (33.7)
<b>Abnormality of the eye</b> [n,(%)] (000478>)	57 (71.2)
Abnormality of vision [n,(%)] (0000504>)	34 (42.5)
Ophthalmoparesis [n,(%)] (0000597>)	16 (20)
Ptosis [n,(%)] (0000508>)	13 (16.2)
Abnormal eye morphology [n,(%)] (12372>)	40 (50)
Abnormality of retinal pigmentation [n,(%)] (0007703>)	12 (15)
Leber's optic neuropathy [n,(%)] (0000587> and 000112)	25 (31.2)
Kearns-Sayre syndrome [n,(%)] (000597> and 0007703> and 0000508)	1 (1.2)
<b>Conductive hearing impairment</b> [n,(%)] (0000405>)	27 (33.7)
<b>Abnormality of the abdominal organs</b> [n,(%)] (0002012>)	18 (22.5)
Abnormality of the liver [n,(%)] (0001392>)	8 (10)
Gastrointestinal tract abnormalities [n,(%)] (0011024>)	12 (15)
<b>Abnormality of endocrine system</b> [n,(%)] (0000818>)	26 (32.5)
Diabetes mellitus [n,(%)] (0000819>)	18 (22.5)
<b>MIDAN</b> [n,(%)]	28 (35)
<b>Biological characteristics</b>	
Increased serum lactate ( $\geq 3$ mmol/L) [n,(%)] (0002151)	11 (13.7)
Increased lactate-to-pyruvate ratio ( $\geq 25$ ) [n,(%)]	11 (13.7)
Increased CPK ( $> 150$ U/L) [n,(%)]	19 (23.7)
Increased uric acid (F $> 300$ $\mu$ mol/L; M $> 360$ $\mu$ mol/L) [n,(%)]	14 (17.5)

HPO terms are written in brackets. The ">" refers to the preceding HPO term associated with the subtree HPO terms. BMI: body mass index / IQR: interquartile range / MID: primary mitochondrial disorder / MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes / MERRF: myoclonic epilepsy and red-ragged fibers / MIDAN: primary mitochondrial disorder-associated nephropathies.

**Table 2: Baseline genotypic features of MID-affected adults**

	<b>MID-affected adults</b> (n=80)
<b>mtDNA pathogenic variants [n,(%)]</b>	56 (70)
Point mutations [n,(%)]	43 (53.7)
616T>C, <i>MT-TF</i> [n,(%)]	2 (2.5)
3243A>G, <i>MT-TL1</i> [n,(%)]	17 (21.2)
3252G>A, <i>MT-TL1</i> [n,(%)]	1 (1.2)
3460G>A, <i>MT-ND1</i> [n,(%)]	3 (3.7)
3902_3908inv, <i>MT-ND1</i> [n,(%)]	1 (1.2)
8344A>G, <i>MT-TK</i> [n,(%)]	1 (1.2)
8993T>C, <i>MT-ATP6</i> [n,(%)]	1 (1.2)
11778G>A, <i>MT-ND4</i> [n,(%)]	7 (8.7)
13513G>A, <i>MT-ND5</i> [n,(%)]	6 (7.5)
13514A>G, <i>MT-ND5</i> [n,(%)]	1 (1.2)
14484T>C, <i>MT-ND6</i> [n,(%)]	1 (1.2)
14709T>C, <i>MT-TE</i> [n,(%)]	1 (1.2)
15257A>G, <i>MT-CYB</i> [n,(%)]	1 (1.2)
Single deletions [n,(%)]	11 (13.7)
Duplications [n,(%)]	2 (2.5)
<b>Multiple deletions [n,(%)]</b>	20 (25)
<b>nDNA pathogenic variants [n,(%)]</b>	4 (5)
<i>OPA1</i> [n,(%)]	2 (2.5)
<i>RMM2B</i> [n,(%)]	1 (1.2)
<i>POLG</i> [n,(%)]	1 (1.2)

MID: primary mitochondrial disorder / mtDNA: mitochondrial DNA / nDNA: nuclear DNA.

**Table 3: Description of patients presenting with MIDAN**

	MIDANs (n=28)
<b>Demography</b>	
Age at diagnosis (years) (median, IQR)	52 (34 - 64)
Sex (M/F)	19 / 9
BMI (kg/cm <sup>2</sup> ) (median, IQR)	24 (20 - 27)
<b>Kidney disease data</b>	
<b>Kidney disease</b>	
Tubulo-interstitial nephritis [n,(%)]	14 (50)
Kidney biopsy [n,(%)]	6 (25)
Glomerular disease [n,(%)]	9 (32.1)
Focal segmental glomerulosclerosis [n,(%)]	3 (10.7)
Other [n,(%)]	6 (21.4)
Nephrotic syndrome [n,(%)]	3 (10.7)
Kidney biopsy [n,(%)]	3 (10.7)
CAKUT [n,(%)]	4 (14.3)
Kidney cysts [n,(%)]	2 (7.1)
Urinary tracts abnormalities [n,(%)]	2 (7.1)
Proximal tubulopathy [n,(%)]	2 (7.1)
Distal tubulopathy [n,(%)]	2 (7.1)
<b>Identified kidney disease [n,(%)]</b>	<b>14 (50)</b>
<b>ESRD [n,(%)]</b>	<b>9 (32.1)</b>
<b>Transplantation [n,(%)]</b>	<b>4 (14.3)</b>
Recurrence suspicion [n,(%)]	3 (10.7)
Post-transplant complication [n,(%)]	0 (0)
<b>Biological characteristics</b>	
Increased serum lactate ( $\geq 3$ mmol/L) [n,(%)]	4 (14.3)
Increased lactate-to-pyruvate ratio ( $\geq 25$ ) [n,(%)]	5 (17.9)
Increased CPK ( $>150$ U/L) [n,(%)]	6 (21.4)
Increased uric acid (F $>300$ $\mu$ mol/L; M $>360$ $\mu$ mol/L) [n,(%)]	11 (39.3)
<b>Urinalysis</b>	
Proteinuria (median, IQR)	0.6 (0.36 - 2.07)
Albuminuria (median, IQR)	0.37 (0 - 1.82)
Hematuria [n,(%)]	1 (3.6)
Leukocyturia [n,(%)]	12 (42.9)
Glycosuria [n,(%)]	1 (3.6)
<b>Blood ionogram (median, IQR)</b>	
Calcium (mmol/L)	2.32 (2.2-2.41)
Phosphate (mmol/L)	1.2 (1.1-1.4)
Sodium (mmol/L)	139 (137-141)
Chloride (mmol/L)	103.5 (101-106)
Glycemia (mmol/L)	5.47 (4.9-6.0)
Potassium (mmol/L)	4.41 (3.9-4.7)
Proteins (g/L)	70.5 (64.75-74)
Albumin (g/L)	38.1 (34.7-42.5)
GFR at diagnosis (ml/min/1.73m <sup>2</sup> ) (median, IQR)	60 (27-84)
GFR slope (ml/min/year) (median, IQR)	-3 (-6-0)

BMI: body mass index / CAKUT: congenital abnormalities of kidney and urinary tracts / CPK: creatine phosphor-kinase / ESRD: end stage renal disease / GFR: glomerular filtration rate / IQR: interquartile range / MIDAN: primary mitochondrial disorder-associated nephropathies.

**Table 4: Histopathological characteristics of reviewed kidney biopsies**

<b>MIDAN</b>	<b>Number of glomeruli</b>	<b>Global sclerosis</b>	<b>Segmental sclerosis</b>	<b>Tubulo-interstitial changes</b>	<b>AH</b>	<b>GSEC</b>	<b>PH</b>	<b>Swollen mitochondria</b>
TIN	12	0 (0)	-	3	1	-	-	+
TIN	11	5 (37)	-	3	0	-	-	-
TIN	5	3 (60)	-	3	0	-	-	-
TIN	15	9 (60)	-	3	2	-	-	-
FSGS	10	10 (1)	+	0	0	-	+	-
FSGS	14	5 (36)	+	2	3	-	-	-
FSGS	2	1 (50)	+	3	0	-	-	-

AH: arteriolar hyaline thickening / FSGS: focal segmental glomerulosclerosis / GSEC: granular swollen epithelial cells / MIDAN: primary mitochondrial disorder-associated nephropathies / PH: podocyte hypertrophy / TIN: tubulointerstitial nephritis. Grading of severity for tubulointerstitial changes and arteriolar hyaline thickening followed the 2017 Banff Lesion Score<sup>40</sup>.

**Table 5: Evaluation of patients experiencing TIN and GD as MIDAN manifestations**

	TIN (n=14)	GD (n=9)	p-value*
<b>Phenotypic characteristics</b>			
Age at diagnosis (years) (median, IQR)	52 (32 - 60)	50 (41 - 61)	.825
Sex (M/F)	11 / 3	5 / 4	.480
BMI (kg/cm <sup>2</sup> ) (median, IQR)	24.2 (19.3 - 24.9)	24.8 (21.8 - 31.2)	.224
<b>Growth abnormality</b> [n,(%)] (0001507>)	5 (35.7)	6 (66.7)	.307
<b>Abnormalities of the nervous system</b> [n,(%)] (0000707>)	11 (78.6)	7 (77.8)	1
Morphological abnormality of the central nervous system [n,(%)] (002011>)	5 (35.7)	4 (44.4)	1
Abnormality of nervous system physiology [n,(%)] (0012638>)	11 (78.6)	6 (66.7)	.643
Seizures [n,(%)] (0001250>)	5 (35.7)	0 (0)	.116
Encephalopathy [n,(%)] (0001298>)	5 (35.7)	0 (0)	.116
<b>Mitochondrial myopathy</b> [n,(%)] (0003737> and/or 0003546)	5 (35.7)	4 (44.4)	1
Ragged-red fibers [n,(%)] (0003200)	2 (14.3)	3 (33.3)	1
<b>Abnormality of the cardiovascular system</b> [n,(%)] (0001626)	9 (64.3)	8 (88.9)	.409
Abnormality of the myocardium [n,(%)] (0001637>)	6 (42.9)	8 (88.9)	.077
Hypertrophic cardiomyopathy [n,(%)] (0001639> and 0001714>)	4 (28.6)	5 (55.6)	.392
Dilated cardiomyopathy [n,(%)] (001644)	3 (21.4)	2 (22.2)	1
Arrhythmia [n,(%)] (0011675>)	3 (21.4)	3 (33.3)	.643
Hypertension [n,(%)] (0000822>)	9 (64.3)	4 (44.4)	.613
<b>Abnormality of the eye</b> [n,(%)] (000478>)	9 (64.3)	7 (77.8)	.824
Abnormality of vision [n,(%)] (0000504>)	3 (21.4)	4 (44.4)	.480
Abnormal eye morphology [n,(%)] (12372>)	7 (50.0)	7 (77.8)	.371
Abnormality of retinal pigmentation [n,(%)] (0007703>)	1 (7.1)	4 (44.4)	.056
Leber's optic neuropathy [n,(%)] (0000587> and 000112)	7 (50.0)	2 (22.2)	.371
<b>Conductive hearing impairment</b> [n,(%)] (0000405>)	5 (35.7)	5 (55.6)	.613
<b>Abnormality of endocrine system</b> [n,(%)] (0000818>)	5 (35.7)	8 (88.9)	<b>.038</b>
Diabetes mellitus [n,(%)] (0000819>)	3 (21.4)	6 (66.7)	.083
<b>Kidney disease data</b>			
Identified kidney disease [n,(%)]	8 (57.1)	6 (66.7)	.985
ESRD [n,(%)]	5 (35.7)	4 (44.4)	1
GFR at diagnosis (ml/min/1.73m <sup>2</sup> ) (median, IQR)	49 (17.5 - 63.5)	47.0 (24.3 - 89.0)	.508
GFR slope (ml/min/year) (median, IQR)	-4.0 (-6.0 - -3.0)	0.0 (-1.3 - 0.1)	.082
<b>Biological characteristics</b>			
Increased serum lactate ( $\geq 3$ mmol/L) [n,(%)]	3 (21.4)	1 (11.1)	1
Increased lactate-to-pyruvate ratio ( $\geq 25$ ) [n,(%)]	4 (28.6)	0 (0)	.127
Increased CPK ( $>150$ U/L) [n,(%)]	3 (21.4)	2 (22.2)	1
Increased uric acid (F $>300$ $\mu$ mol/L; M $>360$ $\mu$ mol/L) [n,(%)]	7 (50.0)	3 (33.3)	1
<b>Genotypic characteristics</b>			
<b>mtDNA pathogenic variants</b> [n,(%)]	12 (85.7)	7 (77.8)	1
Point mutations [n,(%)]	11 (78.6)	7 (77.8)	1
3243A>G, <i>MT-TL1</i>	1 (7.1)	6 (66.7)	<b>.010</b>
13513G>A, <i>MT-ND5</i>	4 (28.6)	0 (0)	.127
<b>Multiple deletions</b> [n,(%)]	2 (14.3)	2 (22.2)	1

HPO terms are written in brackets. The ">" refers to the preceding HPO term associated with the subtree HPO terms. BMI: body mass index / CPK: creatine phosphokinase / ESRD: end-stage renal disease / GD: glomerular disease / GFR: glomerular filtration rate / IQR: interquartile range / MID: primary mitochondrial disorder / MELAS: mitochondrial encephalopathy, lactic acidosis and stroke-like episodes / MERRF: myoclonic epilepsy and red-ragged fibers / MIDAN: primary mitochondrial disorder-associated nephropathies / mtDNA: mitochondrial DNA / TIN: tubulointerstitial nephritis. \*Chi-square or Wilcoxon test, when appropriate.

**Table 6: Characteristics of MID-affected patients according to the occurrence of MIDAN**

	MIDANs (n=28)	Non-renal MIDs (n=52)	p-value*
<b>Phenotypic characteristics</b>			
Age at diagnosis (years) (median, IQR)	52 (34 - 64)	52 (36 - 63)	.908
Sex (M/F)	19 / 9	31 / 21	.468
BMI (kg/cm <sup>2</sup> ) (median, IQR)	24 (20 - 27)	21 (18 - 25)	.231
<b>Growth abnormality</b> [n,(%)] (0001507>)	13 (46.4)	18 (34.6)	.301
<b>Abnormalities of the nervous system</b> [n,(%)] (0000707>)	23 (82.1)	38 (73.1)	.363
Morphological abnormality of the central nervous system [n,(%)] (002011>)	11 (39.3)	21 (40.4)	.924
Stroke-like episodes [n,(%)] (0002401)	3 (10.7)	9 (17.3)	.646
Abnormality of nervous system physiology [n,(%)] (0012638>)	22 (78.6)	33 (63.5)	.164
Seizures [n,(%)] (0001250>)	7 (25.0)	10 (19.2)	.547
Ataxia [n,(%)] (0001251>)	6 (21.4)	13 (25.0)	.720
Encephalopathy [n,(%)] (0001298>)	7 (25.0)	9 (17.3)	.412
<b>Mitochondrial myopathy</b> [n,(%)] (0003737> and/or 0003546)	13 (46.4)	27 (51.9)	.639
Ragged-red fibers [n,(%)] (0003200)	8 (28.6)	24 (46.2)	.190
<b>Abnormality of the cardiovascular system</b> [n,(%)] (0001626)	20 (71.4)	24 (46.2)	<b>.030</b>
Abnormality of the myocardium [n,(%)] (0001637>)	14 (50.0)	11 (21.2)	<b>.008</b>
Hypertrophic cardiomyopathy [n,(%)] (0001639> and 0001714>)	9 (32.1)	6 (11.5)	<b>.024</b>
Dilated cardiomyopathy [n,(%)] (001644)	5 (17.9)	1 (1.9)	<b>.018</b>
Arrhythmia [n,(%)] (0011675>)	6 (21.4)	10 (19.2)	.815
Wolff Parkinson White [n,(%)] (0001716)	1 (3.6)	5 (9.6)	.659
Hypertension [n,(%)] (0000822>)	16 (57.1)	11 (21.2)	<b>.001</b>
<b>Abnormality of the eye</b> [n,(%)] (000478>)	18 (64.3)	39 (75.0)	.453
Abnormality of vision [n,(%)] (0000504>)	7 (25.0)	27 (51.9)	<b>.037</b>
Ophthalmoparesis [n,(%)] (0000597>)	1 (3.6)	15 (28.8)	<b>.007</b>
Ptosis [n,(%)] (0000508>)	1 (3.6)	12 (23.1)	<b>.024</b>
Abnormal eye morphology [n,(%)] (12372>)	16 (57.1)	24 (46.2)	.348
Abnormality of retinal pigmentation [n,(%)] (0007703>)	5 (17.9)	7 (13.5)	.599
Leber's optic neuropathy [n,(%)] (0000587> and 000112)	10 (35.7)	15 (28.8)	.527
<b>Conductive hearing impairment</b> [n,(%)] (0000405>)	10 (35.7)	17 (32.7)	.785
<b>Abnormality of the abdominal organs</b> [n,(%)] (0002012>)	5 (17.9)	13 (25.0)	.466
<b>Abnormality of endocrine system</b> [n,(%)] (0000818>)	14 (50.0)	12 (23.1)	<b>.014</b>
Diabetes mellitus [n,(%)] (0000819>)	10 (35.7)	8 (15.4)	<b>.038</b>
<b>Biological and genotypic characteristics</b>			
Increased uric acid (F>300 μmol/L and M>360 μmol/L) [n,(%)]	11 (39.3)	3 (5.8)	<b>&lt;.001</b>
Calcium (mmol/L)	2.32 (2.2 - 2.41)	2.4 (2.29 - 2.46)	<b>.040</b>
Albumin (g/L)	38.1 (34.7 - 42.5)	43 (40 - 44.2)	<b>.004</b>
<b>mtDNA pathogenic variants</b> [n,(%)]	21 (75)	35 (67.3)	.132
Point mutations [n,(%)]	20 (71.4)	23 (44.2)	<b>.020</b>
3243A>G, <i>MT-TL1</i>	7 (25.0)	10 (19.2)	.547
13513G>A, <i>MT-ND5</i>	5 (17.9)	1 (1.9)	<b>.018</b>
Single deletions [n,(%)]	1 (3.6)	10 (19.2)	.110
<b>Multiple deletions</b> [n,(%)]	7 (25.0)	13 (25.0)	1

HPO terms are written in brackets. The ">" refers to the preceding HPO term associated with the subtree HPO terms. BMI: body mass index / IQR: interquartile range / MID: primary mitochondrial disorder / MELAS: mitochondrial encephalopathy, lactic acidosis and stroke-like episodes / MIDAN: primary mitochondrial disorder-associated nephropathies / mtDNA: mitochondrial DNA. \*: Chi-square or Wilcoxon test, when appropriate.

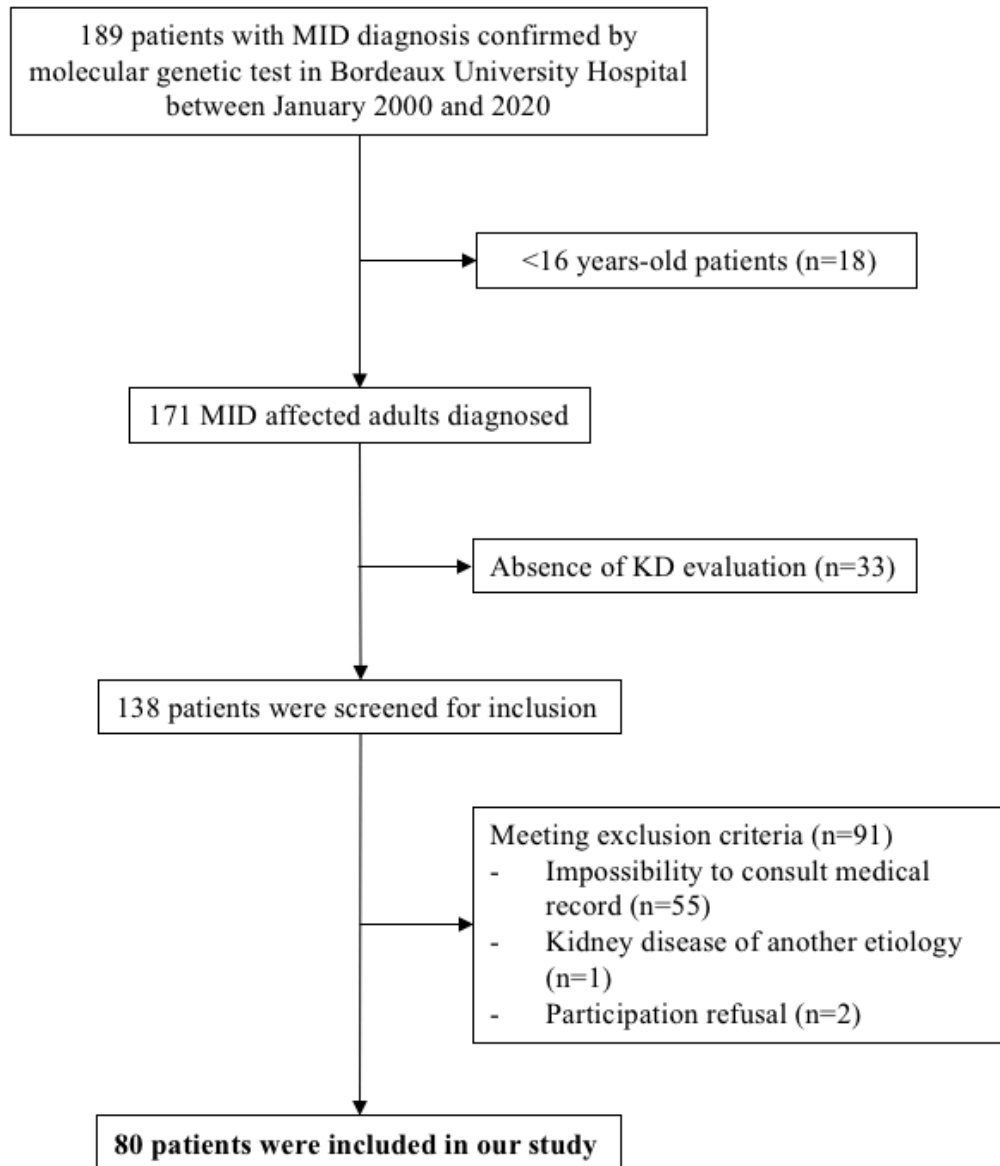
**Table 7: Factors associated with MIDAN**

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value*	OR	95% CI	p-value**
<b><i>Phenotypic characteristics</i></b>						
Age (years)	1	0.98-1.03	.836			
Sex (women)	1.43	0.54-3.76	.469	0.47	0.1-2.15	.332
<b>Abnormality of the myocardium (0001637&gt;)</b>	3.73	1.38-10.1	<b>.010</b>			
<b>Hypertrophic cardiomyopathy (0001639&gt; and 0001714&gt;)</b>	3.63	1.13-11.62	<b>.030</b>			
<b>Dilated cardiomyopathy (001644)</b>	11.09	1.23-100.34	<b>.032</b>			
<b>Hypertension (0000822&gt;)</b>	4.97	1.83-13.53	<b>.002</b>	<b>4.23</b>	<b>1.04-17.17</b>	<b>.044</b>
<b>Abnormality of vision (0000504&gt;)</b>	0.31	0.11-0.85	<b>.023</b>	<b>0.17</b>	<b>0.03-0.94</b>	<b>.042</b>
<b>Ophthalmoparesis (0000597&gt;)</b>	0.09	0.01-0.73	<b>.024</b>			
<b>Ptosis (0000508&gt;)</b>	0.12	0.02-1.01	<b>.051</b>			
<b>Diabetes mellitus (0000819&gt;)</b>	3.06	1.04-8.99	<b>.043</b>			
<b><i>Biological characteristics</i></b>						
Calcium (mmol/L)	0.02	0-0.73	<b>.033</b>			
Albuminemia (g/L)	0.82	0.71-0.93	<b>.003</b>	<b>0.79</b>	<b>0.67-0.95</b>	<b>.01</b>
<b>Increased uric acid (F&gt;300 µmol/L; M&gt;360 µmol/L)</b>	14.67	3.29-65.29	<b>&lt;.001</b>			
<b><i>Genotypic characteristics</i></b>						
<b>Point mutations</b>	3.15	1.18-8.45	<b>.022</b>			
<b>Single deletions</b>	0.16	0.02-1.28	<b>.084</b>			

OR: odds ratio. \*Logistic regression and \*\*backward multivariate analysis including factors with  $p < 0.2$  in the univariate analysis.

## Figures

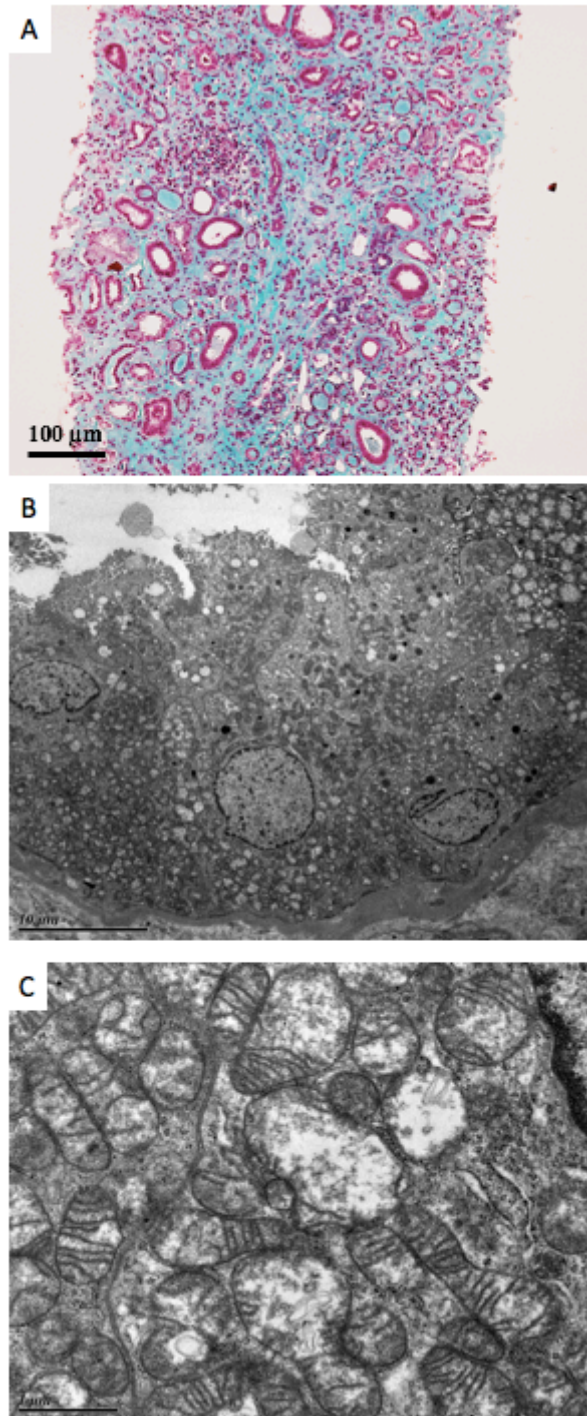
Figure 1: Flow chart of the studied MID adult population.





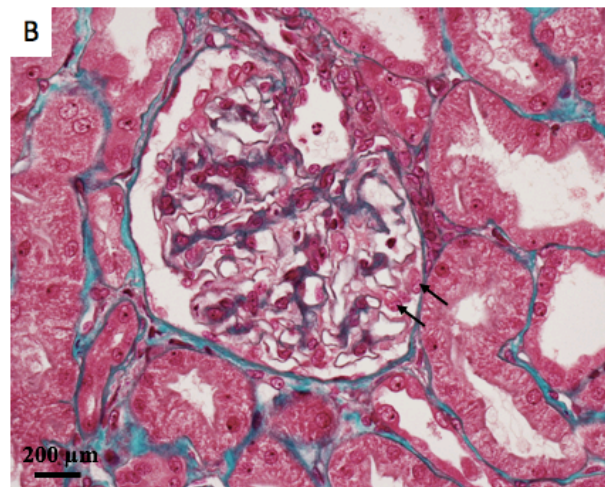
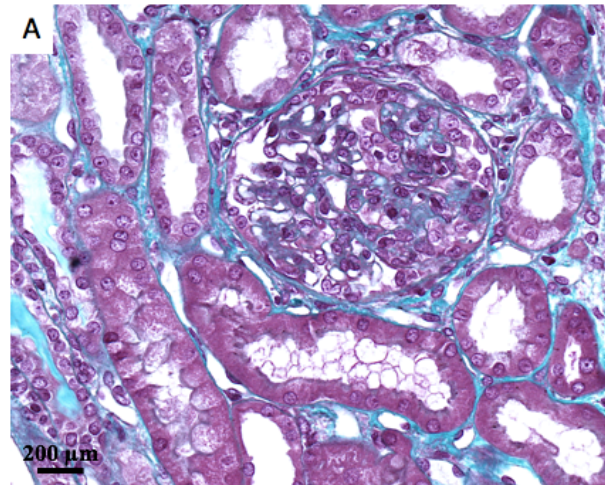
**Figure 2: TIN histopathological manifestations related to MIDAN**

- A. Pathology study by optical microscopy.  $\times 10$  magnification, Masson's trichrome staining. Global aspect of severe interstitial fibrosis and tubular atrophy.
- B. and C. Pathological electron microscopic studies detected abnormal tubular mitochondria that were swollen, with a loss of cristae structures.



**Figure 3: GD histopathological manifestations related to MIDAN.**

- A. Pathology study by optical microscopy. ×20 magnification, Masson's trichrome staining. Glomerulus presented segmental lesions (*i.e.* affecting only a portion of the glomerular tuft), that associated proliferation and hypertrophy of visceral epithelial cells and synechiae between the Bowman's capsule and the tuft. These lesions are compatible with FSGS.
- B. Pathology study by optical microscopy. ×20 magnification, Masson's trichrome staining. Glomerulus presented with hypertrophied podocyte lesions (black arrows), but without FSGS lesion.



## Supplemental tables:

**Supplemental Table 1: Biological characteristics of MID-affected adults according to the occurrence of MIDAN**

	MIDANs (n=28)	Non-renal MIDs (n=52)	p-value*
Increased serum lactate ( $\geq 3$ mmol/L) [n,(%)]	4 (14.3)	7 (13.5)	.919
Increased lactate-to-pyruvate ratio ( $\geq 25$ ) [n,(%)]	5 (17.9)	6 (11.5)	.434
Increased CPK ( $>150$ mmol/L) [n,(%)]	6 (21.4)	13 (25.0)	.720
Increased uric acid (F $>300$ $\mu$ mol/L; M $>360$ $\mu$ mol/L) [n,(%)]	11 (39.3)	3 (5.8)	<b>&lt;.001</b>
<b>Blood ionogram (median, IQR)</b>			
Calcium (mmol/L)	2.32 (2.2 - 2.41)	2.4 (2.29 - 2.46)	<b>.040</b>
Phosphate (mmol/L)	1.2 (1.1 - 1.4)	1.15 (1.04 - 1.25)	.175
Sodium (mmol/L)	139 (137 - 141)	139 (137 - 141)	.922
Chloride (mmol/L)	103.5 (101 - 106)	104 (101.5 - 105)	.942
Glycemia (mmol/L)	5.47 (4.9 - 6.0)	5.15 (4.80 - 5.98)	.413
Potassium (mmol/L)	4.41 (3.9-4.7)	4.11 (4.01 - 4.33)	.358
Proteins (g/L)	70.5 (64.75 - 74)	71 (67.5 - 75.5)	.246
Albumin (g/L)	38.1 (34.7 - 42.5)	43 (40 - 44.2)	<b>.004</b>

CPK: creatine phosphor-kinase / GFR: glomerular filtration rate / IQR: interquartile range. \*:Chi-2 or Wilcoxon test, when appropriated.

**Supplemental Table 2: Genotypic characteristics of MID-affected adults according to the occurrence of MIDAN**

	MIDANs (n=28)	Non-renal MIDs (n=52)	p-value*
<b>mtDNA pathogenic variants [n,(%)]</b>	21 (75)	35 (67.3)	.132
Point mutations [n,(%)]	20 (71.4)	23 (44.2)	<b>.020</b>
616T>C, <i>MT-TF</i>	2 (7.1)	0 (0)	.120
3243A>G, <i>MT-TL1</i>	7 (25.0)	10 (19.2)	.547
3252G>A, <i>MT-TL1</i>	1 (3.6)	0 (0)	.350
3460G>A, <i>MT-ND1</i>	0 (0)	3 (5.8)	.548
3902_3908inv, <i>MT-ND1</i>	1 (3.6)	0 (0)	.350
8344A>G, <i>MT-TK</i>	0 (0)	1 (1.9)	1
8993T>C, <i>MT-ATP6</i>	0 (0)	1 (1.9)	1
11778G>A, <i>MT-ND4</i>	2 (7.1)	5 (9.6)	1
13513G>A, <i>MT-ND5</i>	5 (17.9)	1 (1.9)	<b>.018</b>
13514A>G, <i>MT-ND5</i>	1 (3.6)	0 (0)	.350
14484T>C, <i>MT-ND6</i>	0 (0)	1 (1.9)	1
14709T>C, <i>MT-TE</i>	1 (3.6)	0 (0)	.350
15257A>G, <i>MT-CYB</i>	0 (0)	1 (1.9)	1
Single deletions [n,(%)]	1 (3.6)	10 (19.2)	.110
Duplications [n,(%)]	0 (0)	2 (3.8)	.539
<b>Multiple deletions [n,(%)]</b>	7 (25.0)	13 (25.0)	1
<b>nDNA pathogenic variant [n,(%)]</b>	0 (0)	4 (7.7)	.292
<i>OPA1 [n,(%)]</i>	0 (0)	2 (3.8)	.539
<i>RMM2B [n,(%)]</i>	0 (0)	1 (1.9)	1
<i>POLG [n,(%)]</i>	0 (0)	1 (1.9)	1

MID: primary mitochondrial disorder / MIDAN: mitochondrial disorder associated nephropathies / mtDNA: mitochondrial DNA / nDNA: nuclear DNA. \*:Chi-2 or Wilcoxon test, when appropriated.

## Discussion et perspectives

Les mitochondries, issues d'un phénomène endosymbiotique initial, ont permis aux organismes eucaryotes d'acquérir un avantage sélectif indéniable. Un matériel mitochondrial intact est en effet indispensable pour assurer les fonctions des organes dépendant de l'énergie fournie par la respiration mitochondriale.

Les reins sont des organes parmi les plus consommateurs d'énergie afin d'assurer leurs fonctions. Ainsi, les dysfonctions mitochondriales sont au centre de la physiopathologie des maladies rénales. En effet, que ces mitochondriopathies soient secondaires à des affections exogènes (*i.e.* toxines urémiques, ischémie-reperfusion, protéinurie), ou primitives à des variants pathogènes de l'ADNmt ou nucléaire, elles vont initier et/ou aggraver des lésions responsables de maladies rénales.

Dans ce travail, nous nous sommes essentiellement intéressés aux MIDs. Ces pathologies sont récentes (première description en 1988) et les avancées techniques en termes de diagnostic moléculaire ne cessent d'étendre leur compréhension. Il est intéressant de noter que cette évolution des connaissances sur les MIDs ne cesse d'accroître le spectre de leurs manifestations. Initialement considérée comme une maladie pédiatrique, de récentes études ont montré qu'elle affecte aussi les adultes, avec une fréquence importante de mutation *de novo*. De même, les manifestations cliniques s'enrichissent notamment des MIDANs.

A travers une revue de la littérature médicale, nous avons mis en évidence de nombreux cas cliniques et séries de cas cliniques rapportant des maladies rénales chez des adultes bénéficiant d'un diagnostic de MID. Il semble que ces néphropathies diffèrent largement de celles décrites dans la population pédiatrique. En effet, dans la population pédiatrique, les principales MIDAN sont les tubulopathies proximales et les syndrome néphrotiques corticorésistants. Dans la population adulte, il semble que ce soit des atteintes glomérulaires (avec des lésions de HSF) et des néphropathies tubulointerstitielles qui dominent les MIDAN.

Ce diagnostic difficile est d'une grande importance : il permet de mettre fin à une certaine errance diagnostique, de proposer un conseil génétique et d'organiser un suivi rapproché avec des traitements innovants à l'essai. Les techniques de diagnostic moléculaire, telles que le NGS de l'ADNmt, pouvant être réalisés rapidement, à un coût raisonnable et dans les urines (avec une performance parfois supérieure au sang), voire directement sur les biopsies rénales, nous semblent être d'un intérêt incommensurable pour le diagnostic de MIDAN.

Il est actuellement à déplorer qu'aucun traitement n'ait apporté ses preuves dans des essais contrôlés randomisés. Cependant, des thérapies novatrices (*i.e.* la thérapie génique, inhibiteur des mTor) pourraient révolutionner la prise en charge des MIDANs.

Nous avons, par ailleurs, rapporté 3 présentations cliniques. Il s'agit de 3 variants pathogènes de l'ADNmt affectant le gène *MT-ND5* (2 mutations m.13513G>A et 1 mutation m.13514A>G), diagnostiqués à l'âge adulte et associés à des néphropathies tubulo-interstitielles. Certains patients se présentent avec une atteinte rénale isolée, d'autres avec des manifestations multisystémiques (syndrome MELAS). Il s'agit de la première description de ces mutations associées à des néphropathies tubulo-interstitielles à l'âge adulte.

Les affections rénales semblent fréquentes au cours des MIDs, notamment à l'âge adulte. Pourtant, aucune étude ne s'est intéressée à la prévalence de ces MIDANs, ni à leurs caractéristiques phénotypiques, biologiques, histologiques et génotypiques.

Nous avons donc réalisé une étude rétrospective monocentrique qui s'intéresse à une cohorte de 80 patients ayant bénéficié d'un diagnostic génétique de MID à l'âge adulte et présentant des données permettant d'affirmer la présence ou non d'une maladie rénale. Plusieurs résultats d'intérêts majeurs en ressortent : (1) les MIDAN sont fréquentes dans cette population (35%) ; (2) elles sont sous-estimées puisque 50% des atteintes rénales n'avaient pas été identifiées avant notre étude ; (3) le diagnostic est souvent tardif, survenant volontiers lors d'une maladie rénale déjà avancée, voire même après l'initiation d'un traitement de suppléance (*i.e.* transplantation rénale, dialyse) ; (4) les maladies rénales décrites sont pour la moitié des cas des néphropathies tubulointerstitielles, et pour un tiers des glomérulopathies avec des lésions de HSF sur les biopsies rénales analysées ; (5) certains facteurs tels que les anomalies de la vision, le taux d'albumine et une hypertension artérielle sont indépendamment associés aux MIDAN.

Nos principales limites étaient d'avoir écarté un tiers de la population initiale n'ayant pas de données permettant d'affirmer la présence ou non d'une maladie rénale, l'absence de données longitudinales uniformément exploitables (*i.e.* dosage du lactate, pyruvate, protéinurie, taux d'hétéroplasmie), le faible nombre de variants pathogènes de l'ADNn diagnostiqués, et l'absence de taux d'hétéroplasmie exploitables pour les variants pathogènes de l'ADNmt.

Nous espérons que nos résultats ouvriront la voie à d'autres études. En effet, une étude qui analyserait une cohorte de patients atteinte de MIDs de plus large effectif, avec des données cliniques, biologiques, histologiques et génétiques systématiques et longitudinales pourraient non seulement confirmer nos résultats, mais aussi établir un score permettant de prédire la probabilité

de survenue de MIDAN au cours de l'histoire naturelle de la maladie mitochondriale. Ce travail doit se poursuivre avec une étude multicentrique, favorisée par une incrémentation des données phénotypiques dans le logiciel PhenoTips® (Gene42 Inc., Toronto, Canada), accessible au niveau national dans les CHU de France.

Ce travail a permis de prendre conscience que, devant tout diagnostic de MID, un dépistage de MIDAN ainsi qu'un suivi par un néphrologue devait être initié. Cela pourra permettre un dépistage minutieux d'atteintes rénales, notamment lorsqu'elles sont modérées, mais surtout de mettre en place des mesures de néphroprotection.

De plus, une étude s'intéressant aux patients ayant des MRC d'étiologies indéterminées (notamment glomérulaires avec les HSF et tubulo-interstitielles) pour établir la prévalence des MIDAN dans cette population serait souhaitable. La réalisation de séquençage de l'ADNmt (par NGS) dans les urines des patients et/ou dans leurs biopsies rénales permettrait d'y parvenir. On pourrait ainsi établir la prévalence des MIDAN dans cette population de MRC grâce à des diagnostics génétiques. D'autre part, en comparant les patients affectés avec les patients non affectés, nous pourrions établir un score diagnostic orientant les cliniciens vers les MIDANs devant une néphropathie d'étiologie indéterminée.

Il est important de prendre conscience que cette entité, probablement sous-estimée, peut et doit être investiguée devant des situations complexes de diagnostics étiologiques de MRC. La large proportion de patients se présentant à un bilan pré-transplantation rénale sans diagnostic étiologique rénal établi pourrait alors se voir sensiblement diminuée.

Un objectif pour mettre en œuvre ces projets serait la constitution d'un hôpital de jour dédié à l'évaluation initiale et au suivi des MIDs. En effet, cette organisation permettrait aux différents spécialistes d'organe impliqués dans le parcours de soin du patient affecté (*i.e.* néphrologue, neurologue, généticien, ophtalmologue, anatomopathologiste) de réaliser une évaluation approfondie. De plus, un suivi annuel centralisé de ces patients serait probablement bénéfique et permettrait de recueillir les données longitudinales nécessaires afin de préciser l'évolution naturelle de ces maladies. Mais surtout, la coopération des différents médecins experts amènerait à des réflexions sur les cas complexes (réunions de concertation multidisciplinaire) et guiderait les explorations diagnostique complémentaires (*i.e.* WES et WGS, biopsie musculaire). Cette organisation favoriserait l'inclusion des patients dans des essais cliniques, notamment thérapeutiques. Enfin, les maladies mitochondriales, aussi complexes que passionnantes, seraient mises en valeur par une telle structure de soin.

# Mitochondrial disorders-associated nephropathies in adulthood

## **Abstract:**

**Background:** Mitochondrial functions integrate the regulation of reactive oxygen species, cell survival, apoptosis, and energy production. These functions are controlled by genes of both mitochondrial and nuclear DNA. Pathogenic variants affecting any of these are responsible for primary mitochondrial disorders (MIDs), that can be diagnosed during adulthood. The kidneys' functions are highly dependent on mitochondrial respiration. However, the prevalence of MID-associated nephropathies (MIDANs) is unknown in the adult population.

**Methods:** Adults ( $\geq 16$ -years-old) with genetically diagnosed MID between 2000 and 2020 in our tertiary care academic center, were included in a retrospective observational study when they had a kidney disease (KD) evaluation. MIDANs were ascertained by KD occurring in MIDs. The phenotypic, biological, histopathological, and genotypic characteristics were recorded from the medical charts.

**Results:** We included 80 MID-affected adults and ascertained MIDANs in 28/80 (35%). KDs were identified before the study for 14/18 (50%) of the adults with MIDAN. MIDANs were mostly tubulointerstitial nephritis (TIN) for 14/28 (50%), and glomerular disease (GD) for 9/28 (32.1%). MIDAN was independently associated with higher albumin level [odds ratio (OR), 0.79; 95% confidence interval (95%CI), 0.67–0.95], hypertension (OR, 4.23; 95%CI, 1.04-17.17), and vision abnormalities (OR, 0.17; 95%CI, 0.03–0.94). In contrast, the histopathological and genotypic characteristics were not associated with MIDANs.

**Conclusion:** MIDANs are frequent and underestimated among adult MIDs. They are mostly represented by TIN or GD. Vision abnormalities, hypertension and albumin levels were independently associated with MIDANs. Our results pave the way for larger prospective studies investigating MIDANs prevalence among undetermined KD populations.

**Keywords:** mitochondrial disorders, kidney disease, adult, tubulointerstitial nephritis, glomerular disease.

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## Implication des mitochondriopathies dans les néphropathies de l'adulte

### **Résumé :**

**Contexte :** Les mitochondries assurent la régulation des espèces réactives de l'oxygène, la survie cellulaire, l'apoptose et la production d'énergie. Ces fonctions sont contrôlées par des gènes de l'ADN mitochondrial et nucléaire. Les variants pathogènes affectant ceux-ci vont donc être responsables de mitochondriopathies primitives (MIDs), qui peuvent être diagnostiquées à l'âge adulte. Les fonctions rénales dépendent de la respiration mitochondriale. Cependant, la prévalence des néphropathies associées aux MIDs (MIDANs) est inconnue dans la population adulte.

**Méthodologie :** Les adultes ( $> 16$  ans) avec un diagnostic moléculaire de MID entre 2000 et 2020, et avec une évaluation de maladie rénale (MR), étaient inclus dans l'étude rétrospective. Les MIDANs étaient définies par l'association d'une MID et d'une MR. Les caractéristiques phénotypiques, biologiques, histologiques et génotypiques étaient recueillies à partir des dossiers médicaux.

**Résultats :** Nous avons inclus 80 adultes avec MID et confirmé la présence de MIDAN chez 28/80 (35%). Dans cette population, 14/28 (50%) des MR étaient identifiées avant l'étude. Les MIDANs étaient essentiellement des néphropathies tubulo-interstitielles (TIN) avec 14/28 (50%) et des néphropathies glomérulaires (GD) avec 9/28 (32,1%) des MIDANs. Les MIDANs étaient indépendamment associées avec une albumine élevée [odds ratio (OR), 0.79; 95% intervalle de confiance (95%IC), 0.67–0.95], l'hypertension artérielle (OR, 4.23; 95%IC, 1.04-17.17) et les anomalies de la vision (OR, 0.17; 95%IC, 0.03–0.94). Nous n'avons pas mis en évidence d'association entre les MIDANs, les données histologiques et génotypiques.

**Conclusion :** Les MIDANs sont fréquentes et sous-estimées chez les adultes avec MIDs. Ce sont essentiellement des TIN et des GD. Les anomalies de la vision, l'hypertension artérielle et l'albuminémie étaient indépendamment associés avec les MIDANs. Notre étude ouvre la voie à des études prospectives, évaluant notamment la prévalence des MIDANs au sein d'une population de MR indéterminées.

**Mots-clés :** mitochondriopathie, maladie rénale, adultes, néphropathie tubulo-interstitielle, néphropathie glomérulaire.

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**Discipline :** Thèse de médecine spécialisée de néphrologie.

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