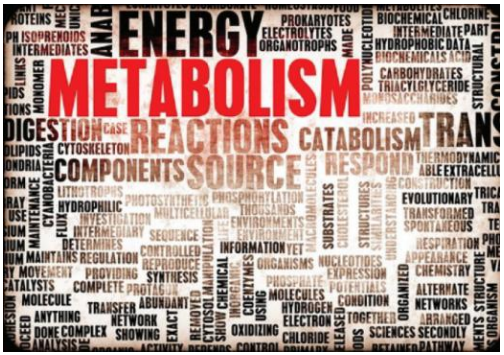


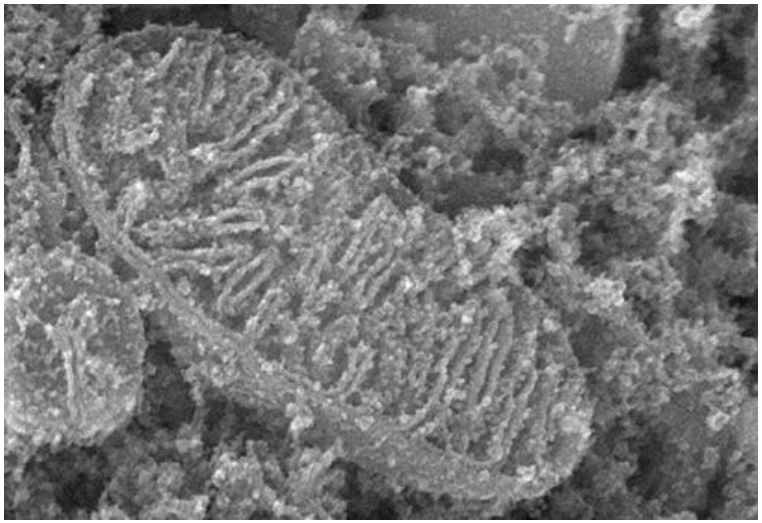
XIII Curso Básico de Doenças Hereditárias do Metabolismo

Hospital Pediátrico Carmona da Mota – CHUC.EPE

Centro de Congressos dos HUC - CHUC.EPE

21 a 23 de setembro de 2015

DOENÇAS MITOCONDRIAIS



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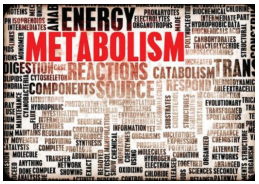
CHUC HOSPITAL PEDIÁTRICO



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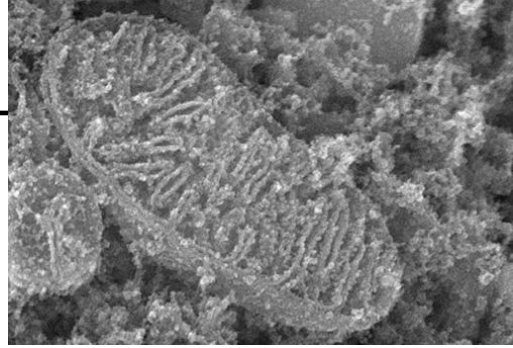

- **Introdução**
  - Estrutura e função da mitocôndria
  - Erros inatos do Metabolismo energético
  - A Cadeira Respiratória Mitocondrial (CRM)
  - A genética da Mitocôndria
    - dupla regulação genética
    - hereditariedade mitocondrial
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  - Definição
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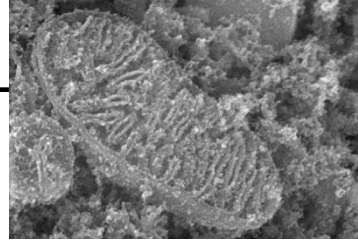
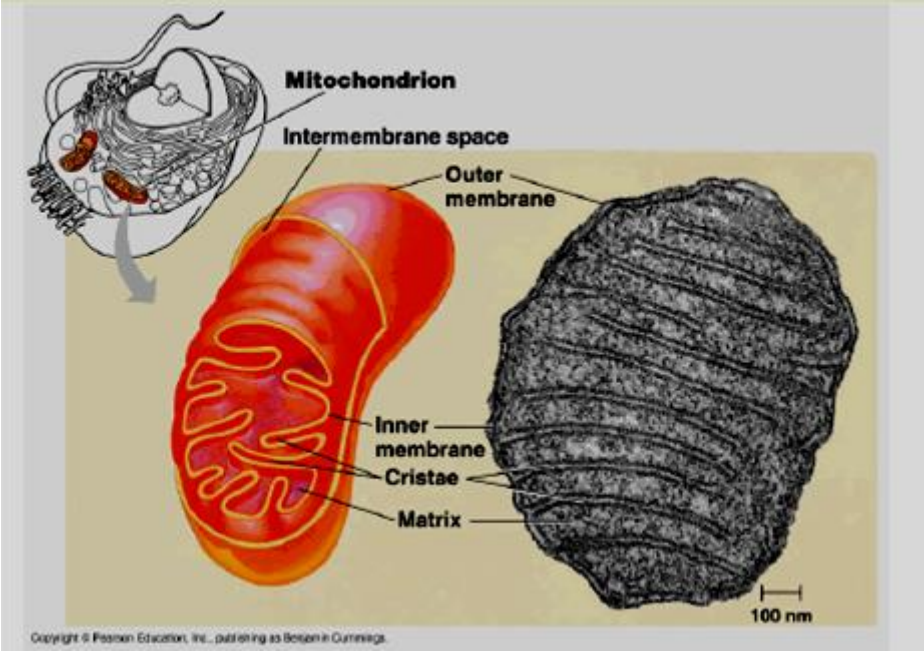


Mitocôndria – morfologia

***Mitos* = fio + *Condrion* = grânulo**

Organelos dupla mb ; ~2μ (~bactérias)

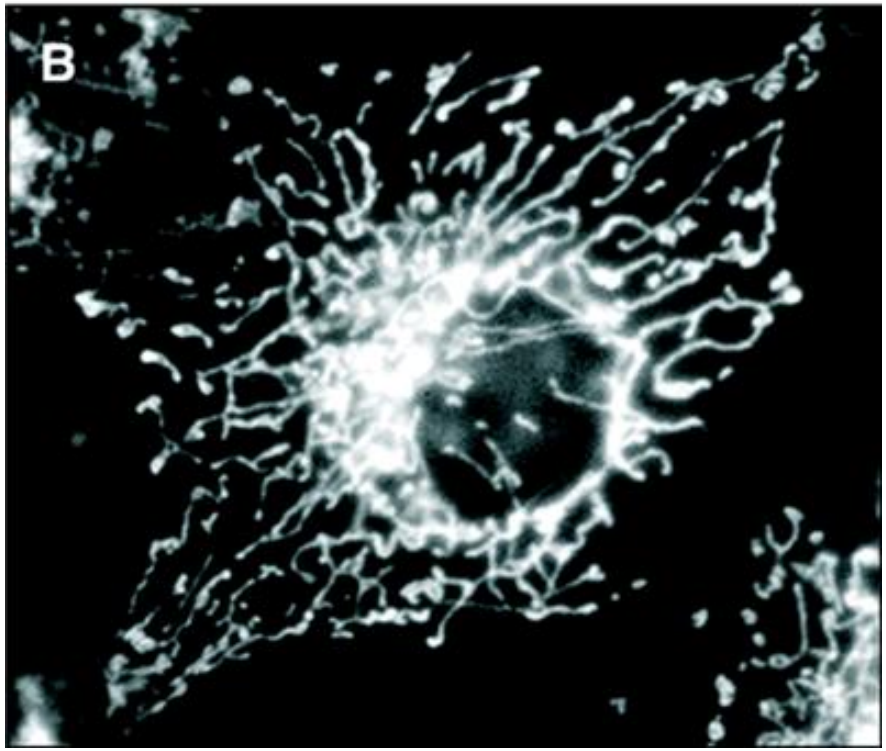
x100 a x1000 - citoplasma das células

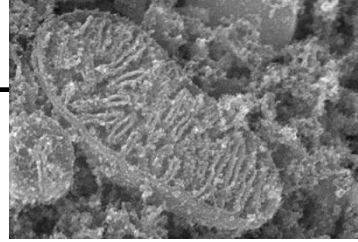


Mitocôndrias vs Rede mitocondrial

Fusão e fissão

Distribuição dinâmica, heterogénea na célula





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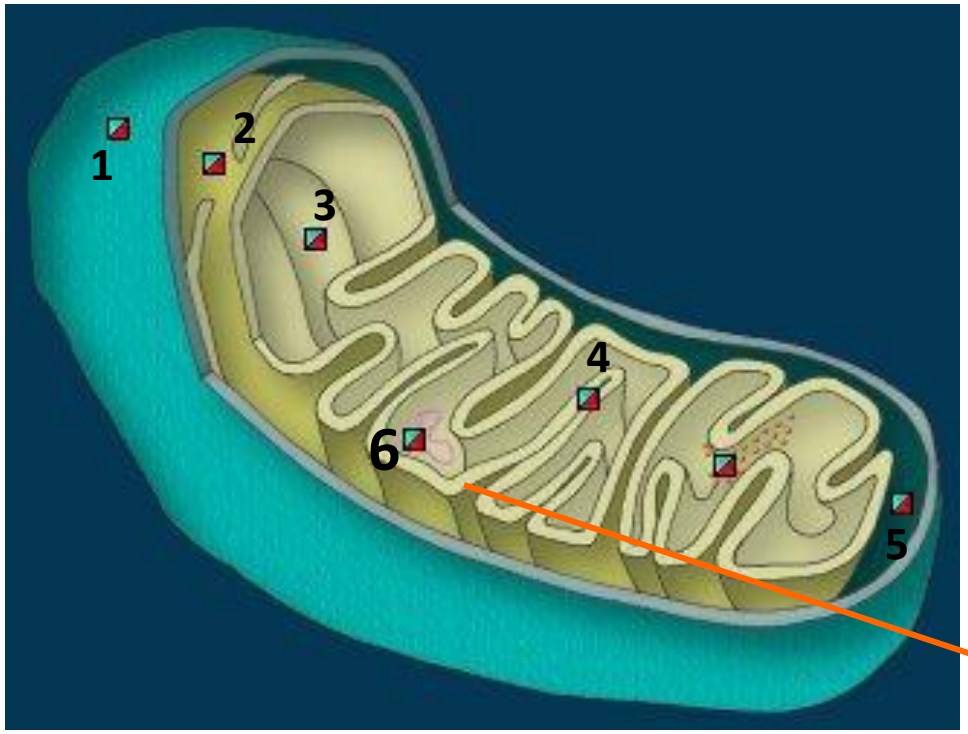
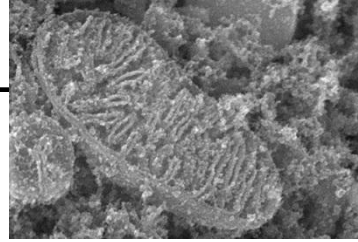
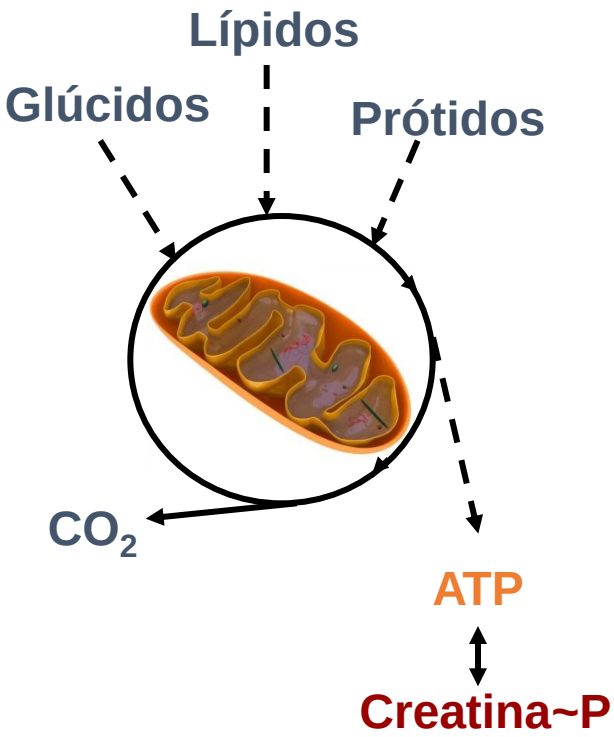


Mitocôndria:

1. Membrana externa
2. Membrana interna
3. Matriz
4. Crista
5. Espaço intermembranar
6. **DNA mitocondrial**



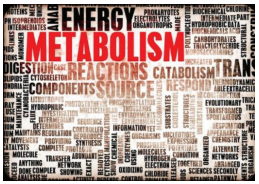
Erros inatos metabolismo energético



Défice da produção de energia

Cérebro, Fígado, Músculo Esquelético, Miocárdio, Fígado, Rim, Retina.

- **Atraso de crescimento,**
- **Hipoglicémia, hiperlactacidémia,**
- **Hipotonia generalizada, miopatia,**
- **Cardiomiopatia, colapso circulatório,**
- **Morte súbita,**
- **Malformações**



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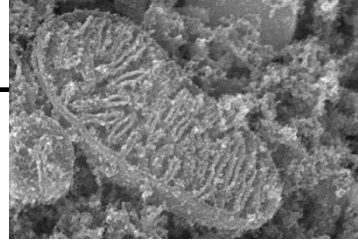


Glicogénio

Glicose

~ 2 ATP

Aminoácidos

Citosol

Alanina

Piruvato

Lactato

PDH

Ác. gordos

βOxid.AG

Mitocôndria

Acetil-CoA

C. cetónicos

Oxaloacetato

Ácido cítrico

C. Krebs

NADH

ADP

ATP

Creatina~P

$FADH_2$

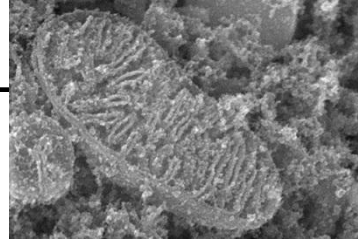
$2CO_2$

CRM

O_2

H_2O

~38 ATP



Metabolismo energético

Oxidação mitocondrial ➔ ATP (>90% energia celular)

Capacidade oxidativa

Oxigénio

Substratos energéticos

ATP/ADP

Homeostase energética

Mitocôndria

Transferência

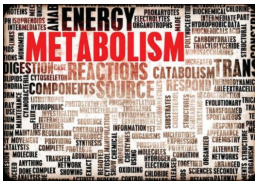
Feed-back

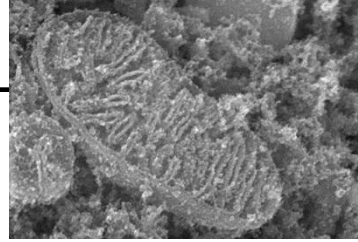
Creatina~P

Pi

Cálcio

Locais de utilização de energia

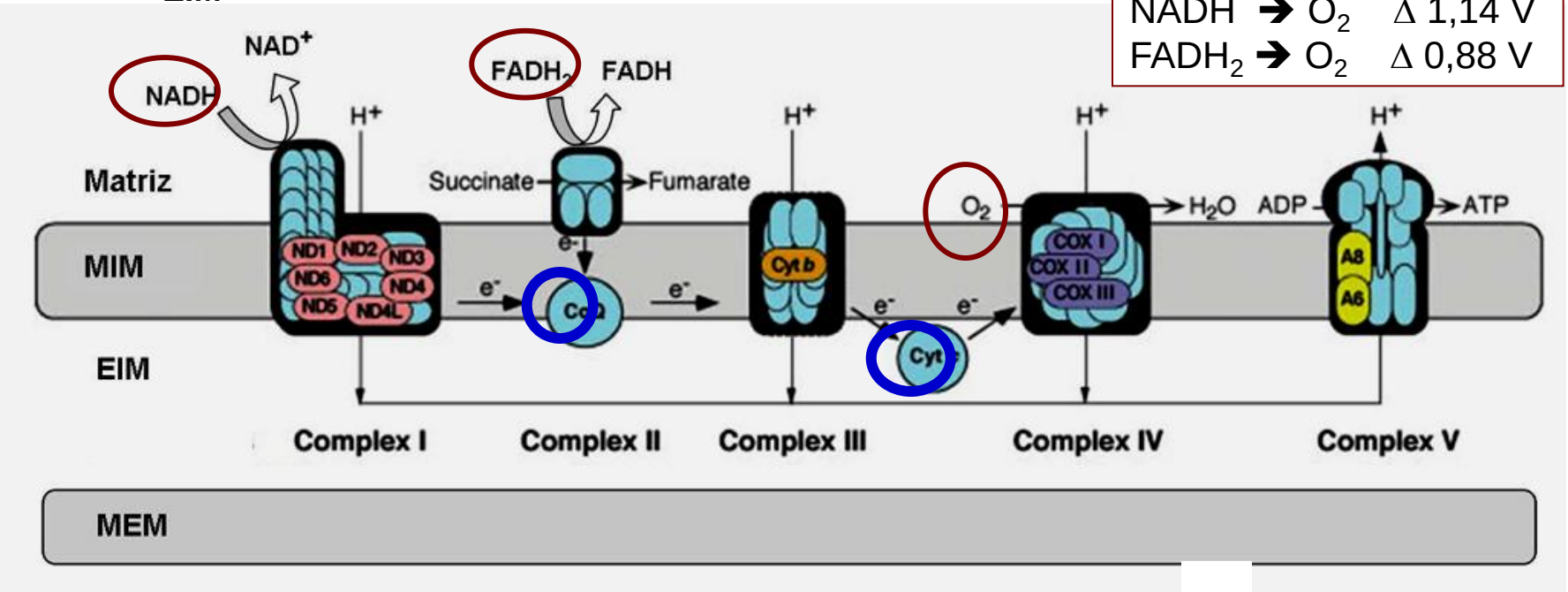




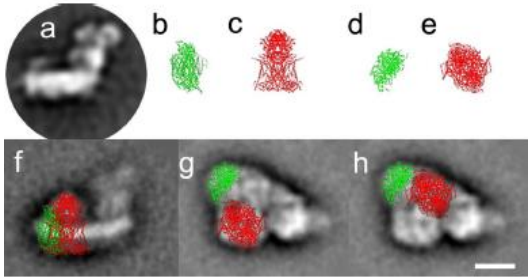
Cadeia respiratória mitocondrial

MIM
Matriz
MEM
EIM

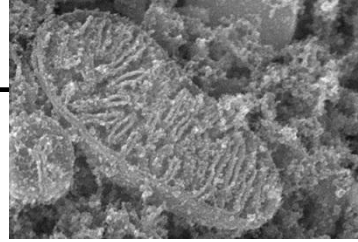
NADH ➔ O_2 Δ 1,14 V
$FADH_2$ ➔ O_2 Δ 0,88 V



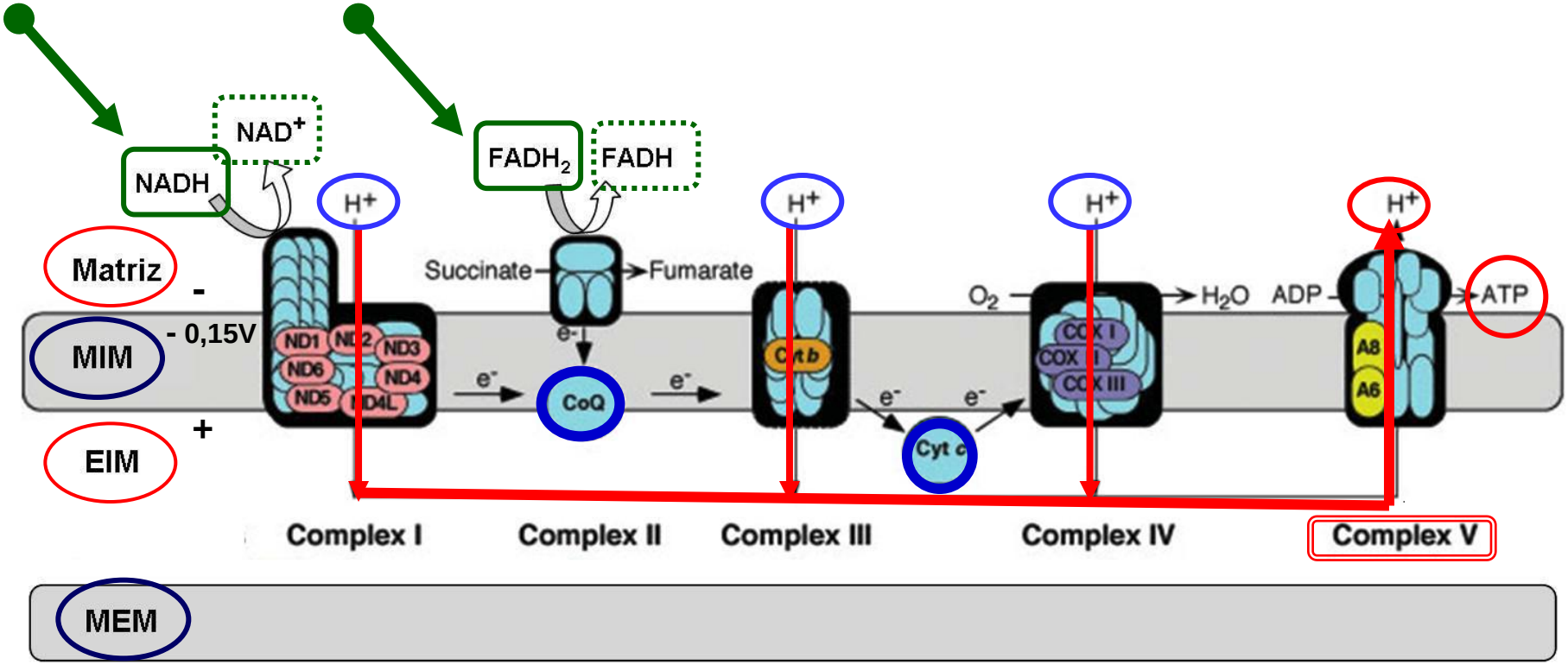
Supercomplexos
respirassomas



- ☑ Loc. membrana mitocondrial interna
- ☑ Constituída por
  - 2 transportadores e- coQ e Citocromo c
  - 5 complexos multi-enzimáticos I, II, III, IV, V
- ☑ ➔ mecanismos regulatórios complexos, pouco conhecidos



Cadeia respiratória mitocondrial



(adaptado de DiMauro and Schon, 2006)

Oxidação substratos energéticos → redução de coenzimas (NAD⁺ e FADH-flavoproteínas)

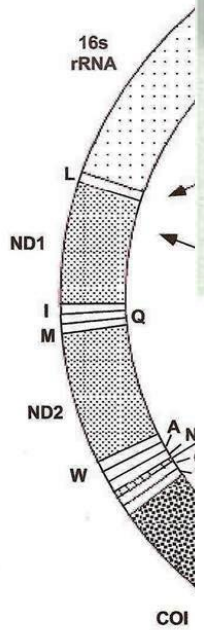
Reoxidação NADH e $FADH_2$ → transf. H^+ da matriz p/ espaço intermb →

Retorno H^+ p/ matriz → +++ ATP sintase (complexo V) → síntese de ATP

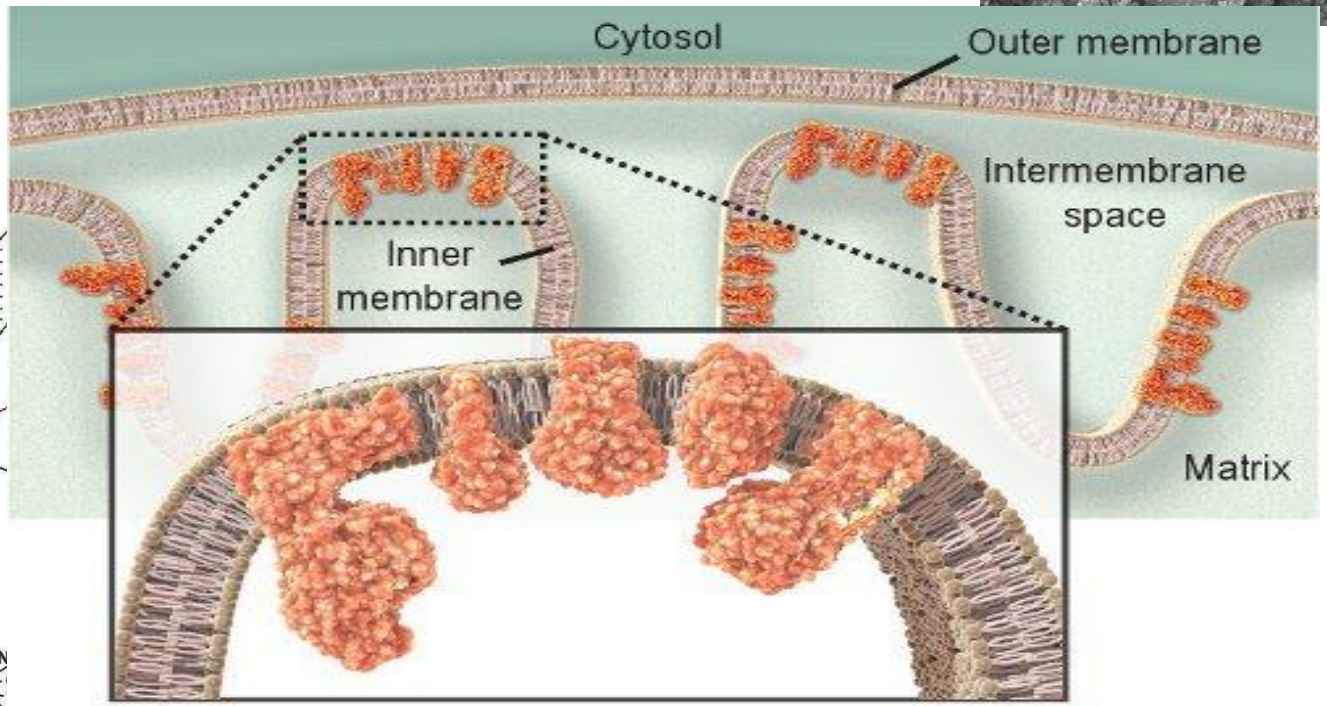
Mitocôndria - dupla regulação genética

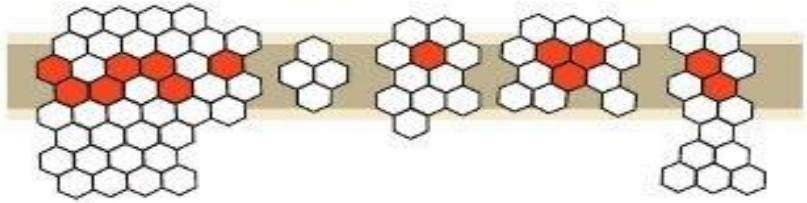
(adaptado de Mitomap, 2008
e de DiMauro e Schon, 2006)

mtDNA



Mitomap, 2002





Jaime M. Ross, J Vis Exp. 2011

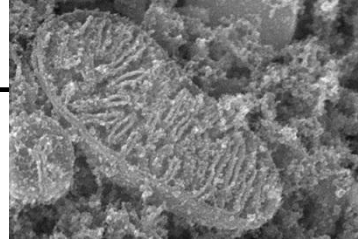
nDNA

~1500 proteínas mitocondriais

Complex I II III IV V

KSS

Sub-unidades	Complexo I	Complexo II	Complexo III	Complexo IV	Complexo V
mtDNA	7	0	1	3	2
nDNA	~39	4	10	10	~14



Genoma mitocondrial (mtDNA)

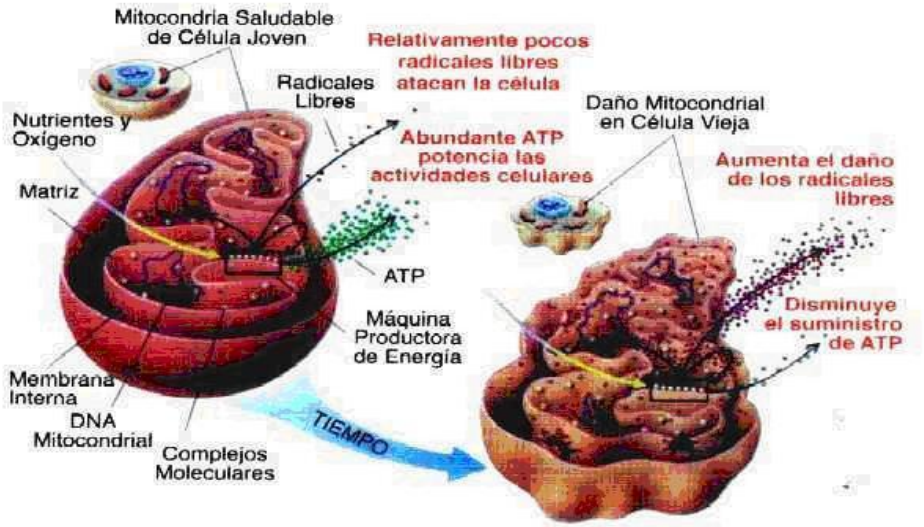
Circular, 16569 pb, monocatenar

Codifica: 2 RNAr; 22 RNAt; 13 peptídeos (CRM)

Muitas cópias/célula (poliplasmia)

5-10/ mit. e até 1000 mit/célula

➔ até 10 000 mtDNA/ cél.



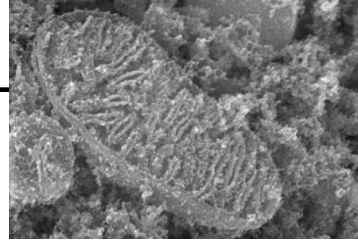
Ovócito maduro: 100 000 cópias

Espermatozóide: 100 cópias

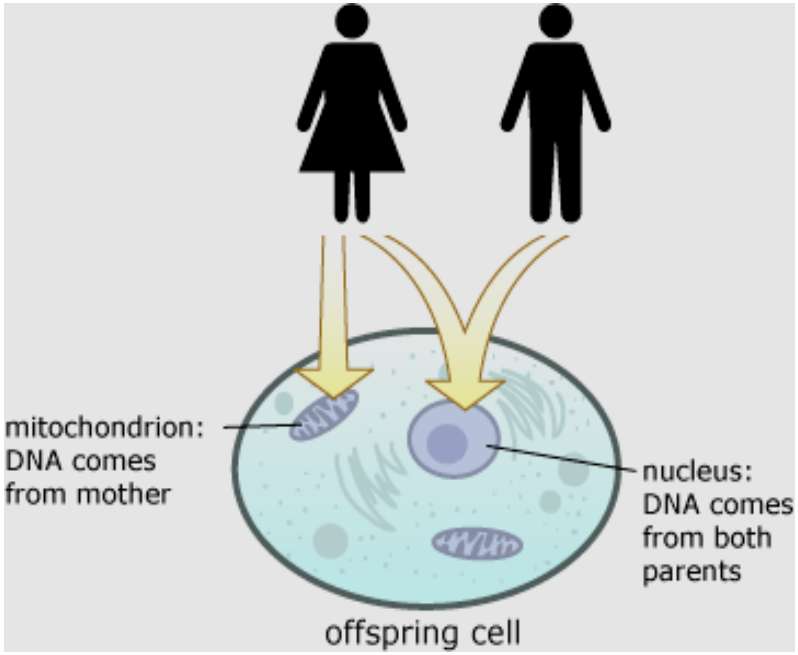
> exposição vs < protecção

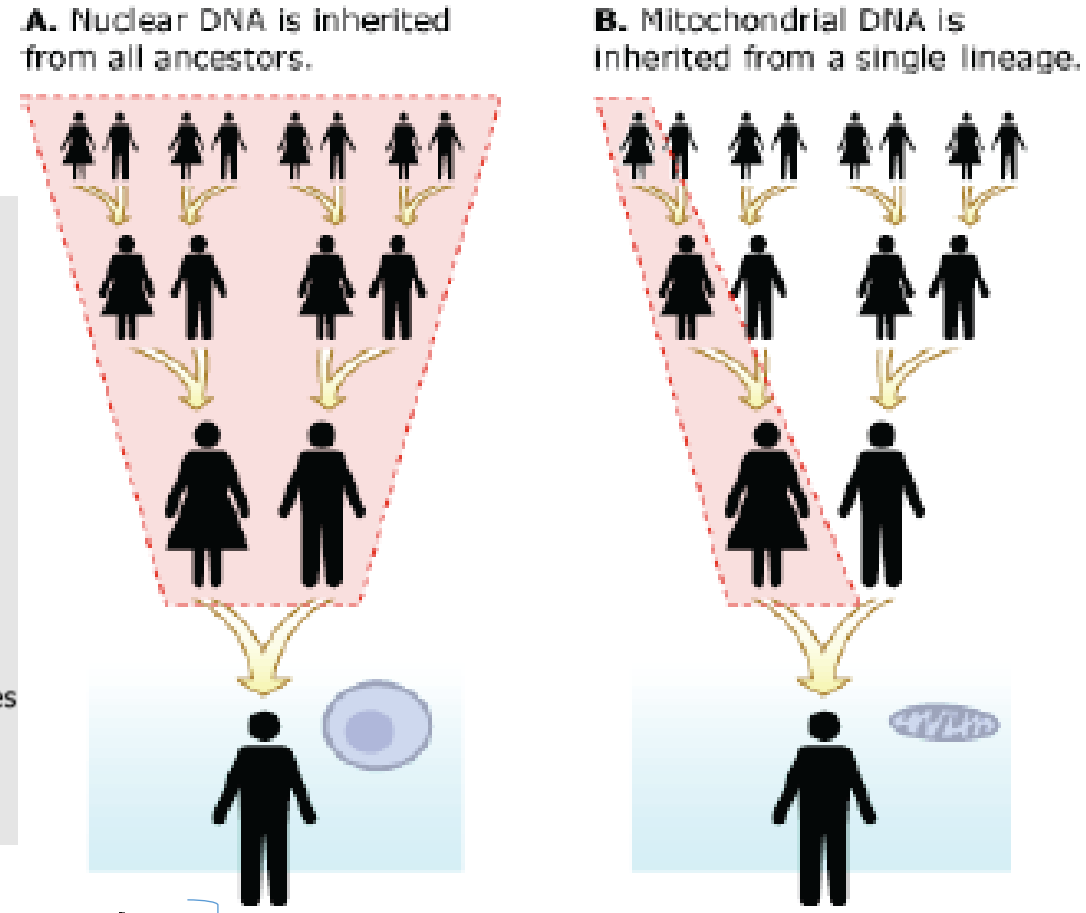
< capacidade de reparação

→ **alta tx de mutação**



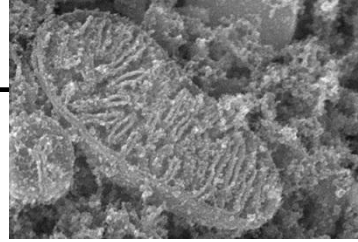
Hereditariedade – nDNA *vs* mtDNA





Mut. pontuais → Esporádico
Sind. Depleção → AR
Del. Múltiplas → AR

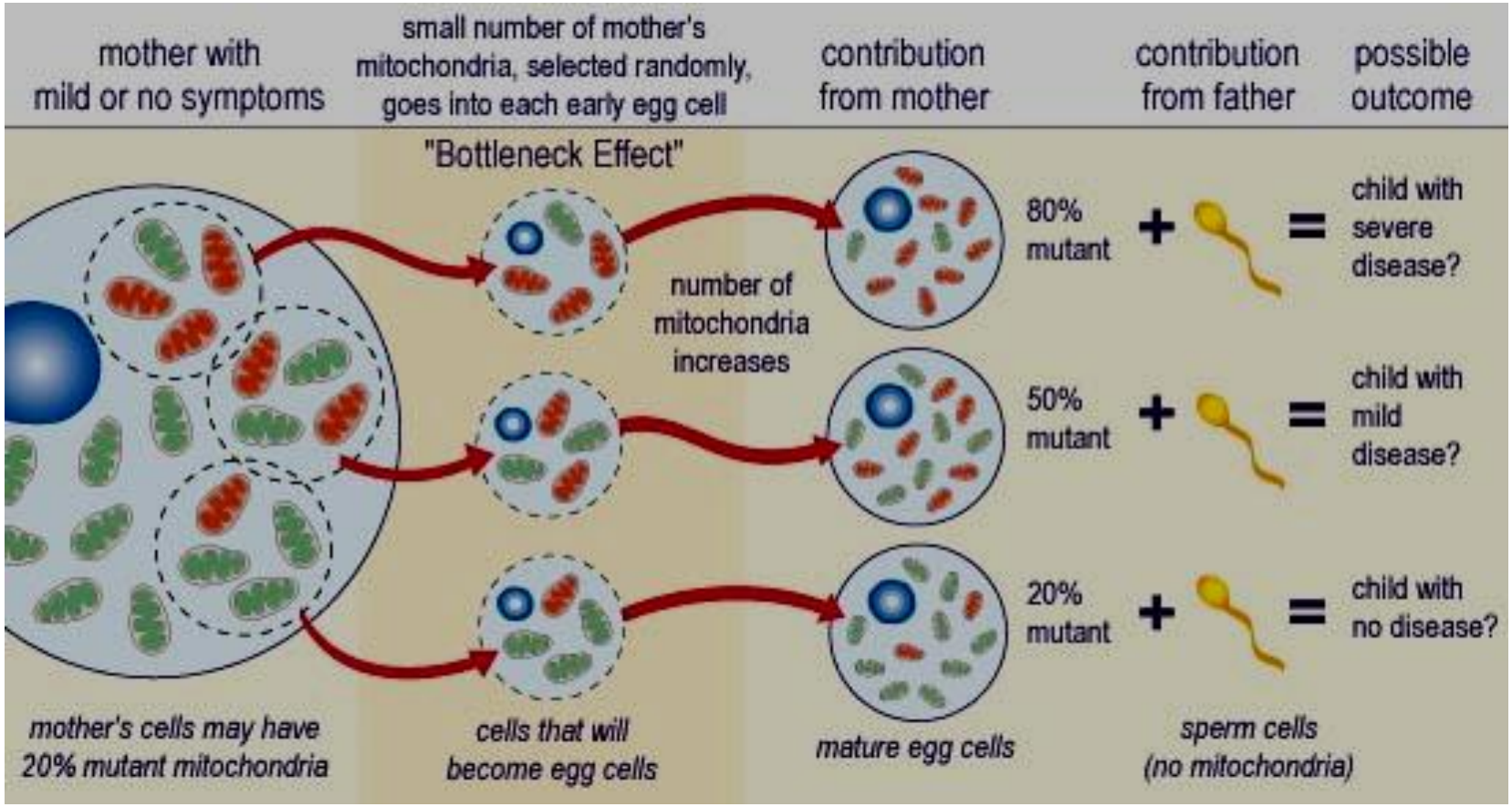
Hereditariedade Mendeliana

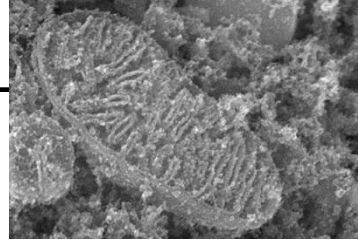


Hereditariedade Mitocondrial

Efeito de "gargalo" (*bottleneck*) na oogénese.

A transmissão de uma alteração => geração seguinte é imprevisível.





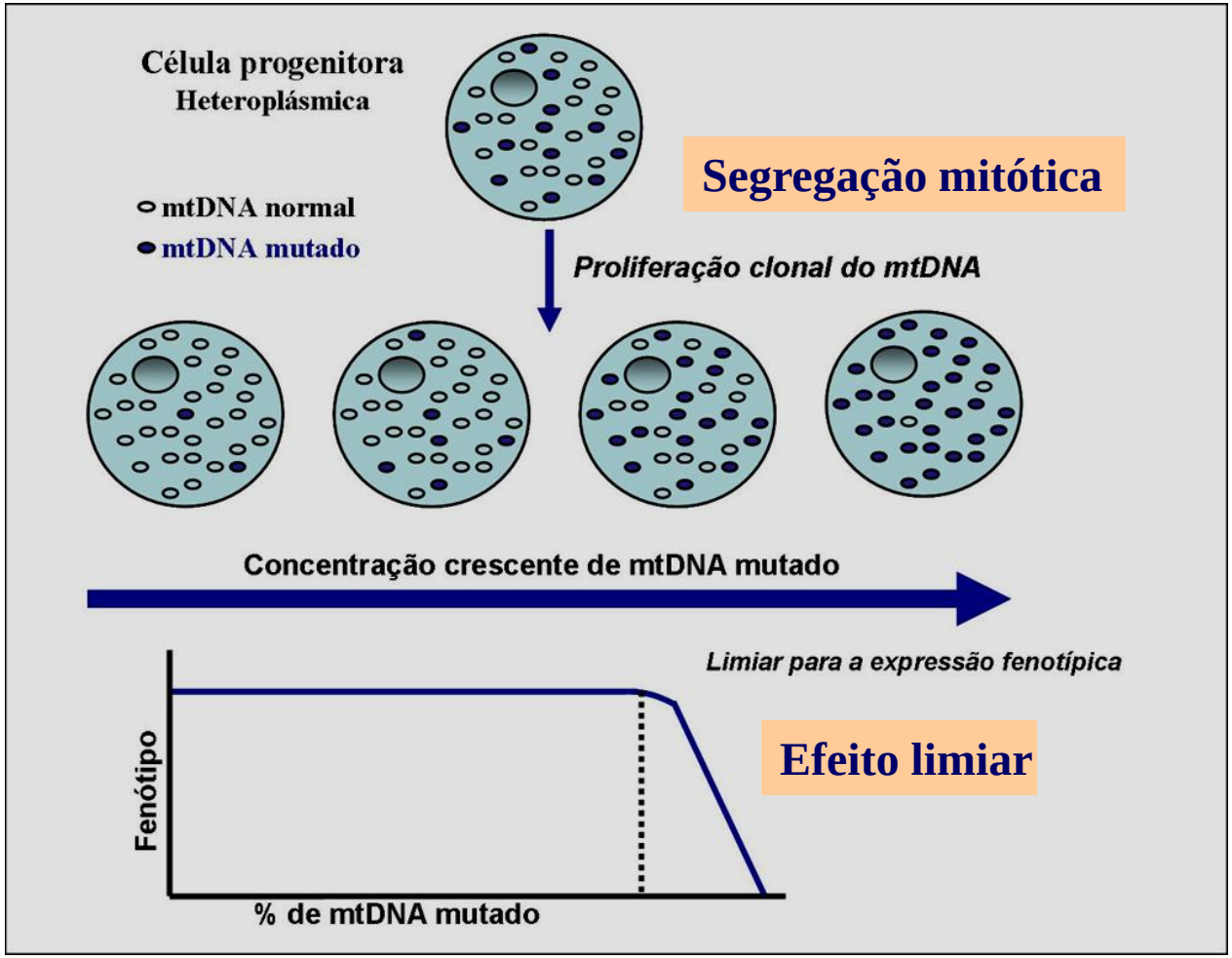
Hereditariedade Mitocondrial

Segregação mitótica
➔ *heteroplasmia*

fenótipo ←➔ limiar

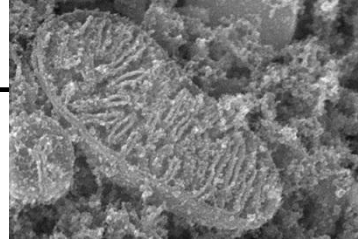
função de:

- **% DNA mutado**
- **necessidades energéticas da célula**
- **outros fatores ...**

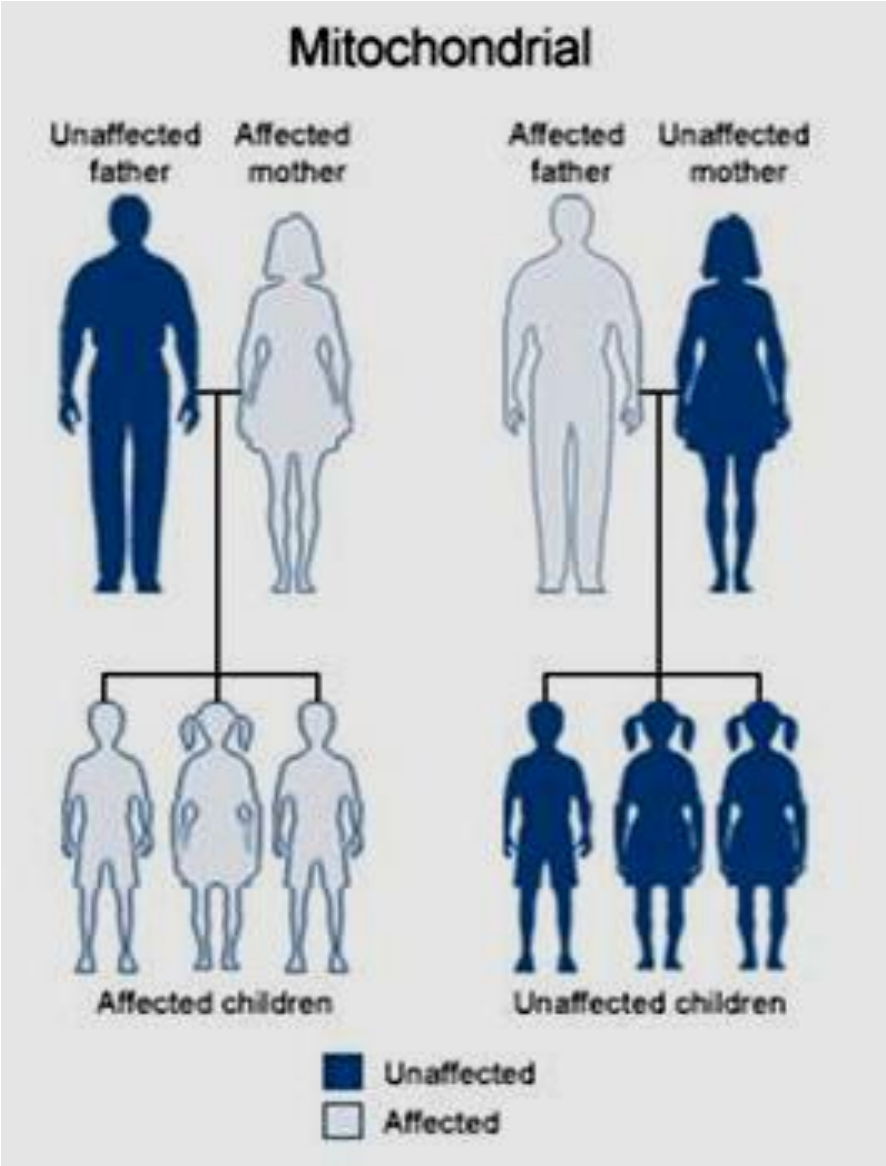


(adaptado de Shoffner e Wallace, 1995)

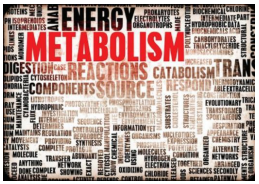
A <u>% mtDNA mutado</u> varia na ovogénese e ➔➔ pode <u>variar</u> de tecido p/ tecido e ao longo da vida.



Hereditariedade Mitocondrial



- **Transmissão materna**
- **Segregação mitótica**
- **Heteroplasmia**
- **Efeito limiar**



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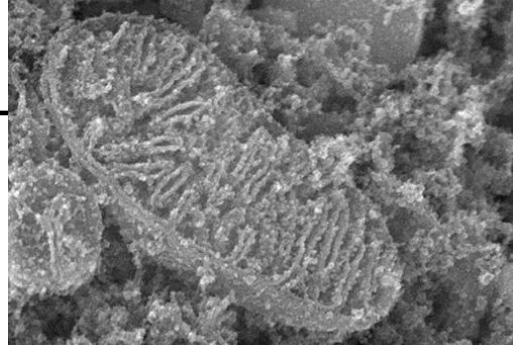

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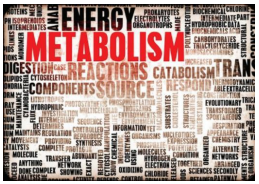
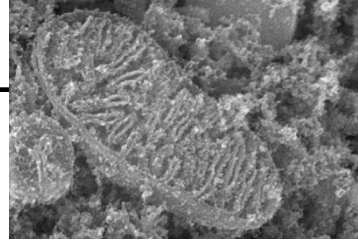


DOENÇAS MITOCONDRIAIS



- D^as^ da cadeia respiratória mitocondrial (DCRM)
- Citopatias mitocondriais ou
- Doenças da fosforilação oxidativa

➔ doenças primárias da fosforilação oxidativa

= cadeia respiratória + síntese de ATP

(Naviaux, 2004).

1962 Primeiro caso (Luft)

1963 *Ragged red fibers* (Engel & Cunning)

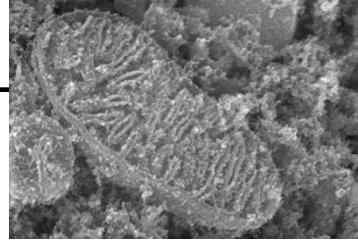
1977 Encefalomiopatia mitocondrial (Shapira)

1988 Primeira del. (Holt; Lestionne & Ponsot)

«« mut. (Wallace) mtDNA

1995 Primeira mut. nDNA (Bourgeron)

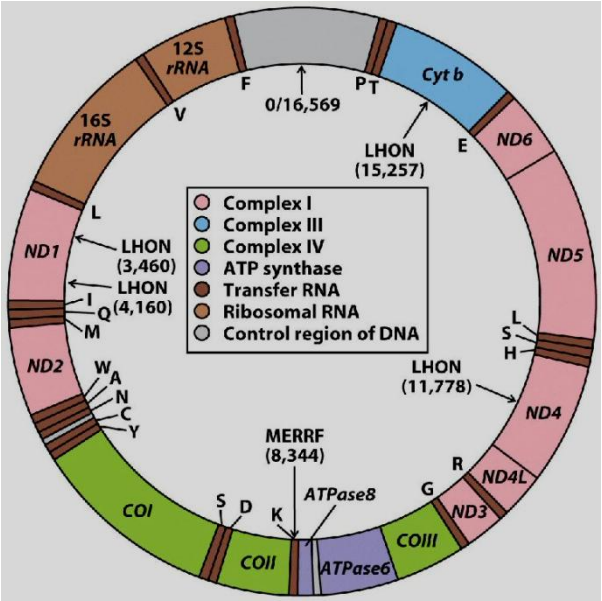
2013: > 250 genes associados a dª dos >1200 codificantes para proteínas mitocondriais



DCRM – classificação genética

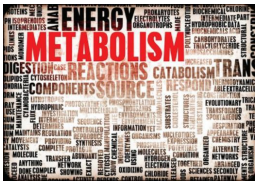
mtDNA

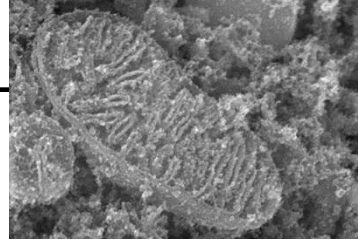
- ✓ genes de síntese proteica (rRNAs, tRNAs)
- ✓ genes codif. de subunidades da CRM
- ✓ grandes deleções



nDNA

- ✓ Defeitos das subunidades da cadeia respiratória
- ✓ Defeitos na biogénese da mit
- ✓ Defeitos da membrana mt (ex.: cardiolipinas)
- ✓ Defeitos de proteinas "auxiliadoras" ("assembly")
- ✓ Defeitos da sinalização intergenómica (manutenção mtDNA)
- ✓ Defeitos do ambiente lipídico
- ✓ Defeitos da homeostase da mitocôndria





DCRM - incidência & prevalência

Incidência - 1/ 2.000 a 1/ 7.600 nadovivos (Naviaux,2004; Thorburn, 2004)

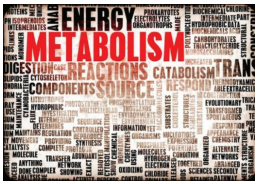
Prevalência

<16 A: 4,7 - 8,7 / 100.000 (Darin et al., 2001; Skladal et al., 2002)

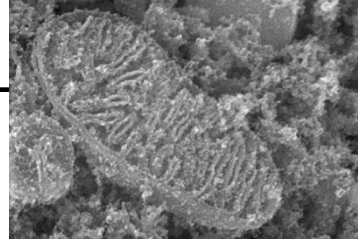
Dª mtDNA *adultos em idade activa*: 6,6 / 100.000 (Chinnery e Turnbull, 2001)

Prevalência dª mtDNA – 1/5000

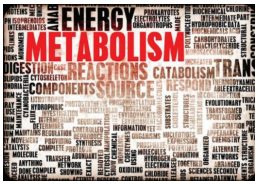
Schaefer et al. 2007



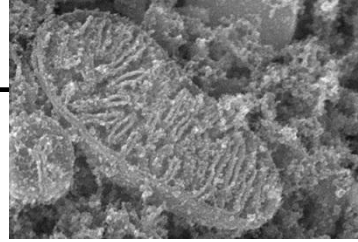
DOENÇAS MITOCONDRIAIS

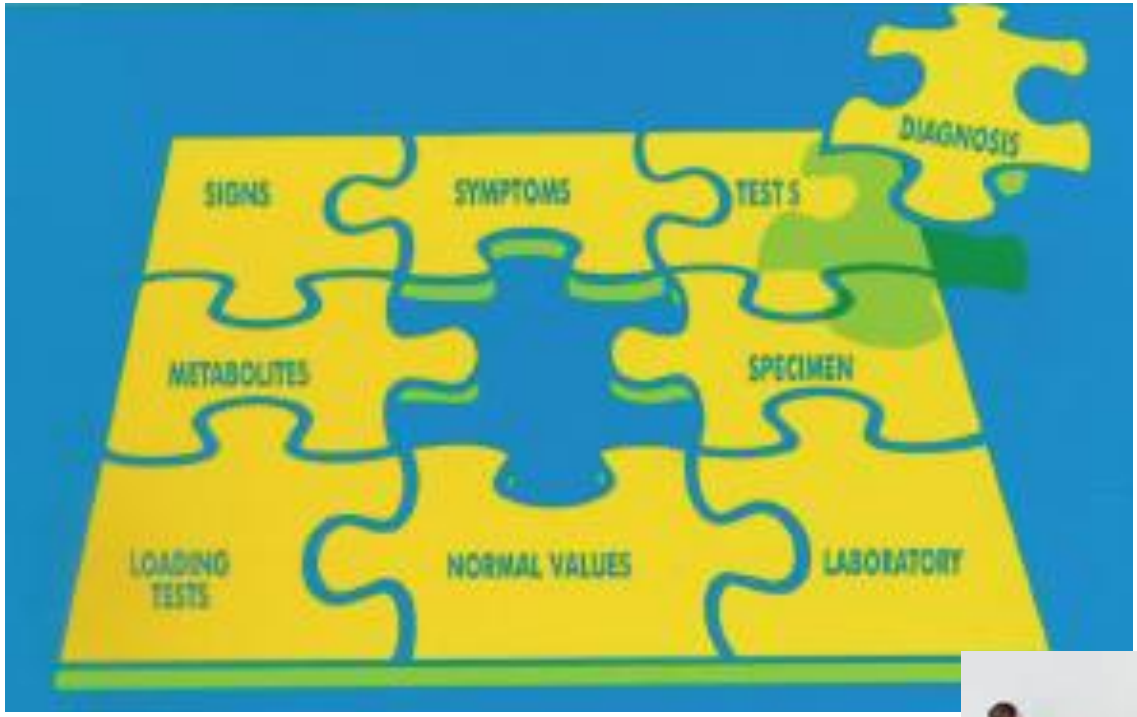


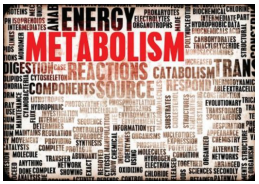
- **Diagnóstico diferencial vasto**
- **Variação do quadro clínico com a evolução**
- **Critérios de diagnóstico diversos**
- **Défices secundários da CRM frequentes**
- **Dificuldade interpretação resultados testes genéticos**

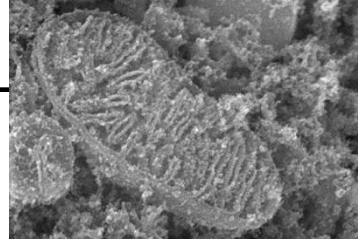


DOENÇAS MITOCONDRIAIS









DCRM – como chegar ao diagnóstico?

Manifestações clínicas + História Familiar

Rastreio laboratorial – 1ª linha

basal – estado *redox* plasma & LCR, AA, Ácidos orgânicos urina

post prandial / TTGO/ exercício

Rastreio de envolvimento multi-orgânico – 2ª linha

(músculo, SNC, SN periférico, cardíaco, endócrino, hepático, pâncreas exoc., intestino, medula óssea, olho, audição, cutâneo)

RMS ^{1}H: cérebro > lactato; ^{31}P: músculo e cérebro> Pi/Pcr

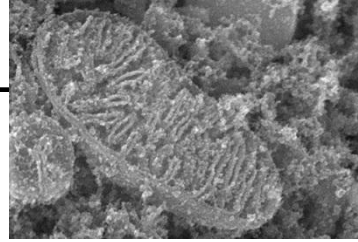
Histologia músculo, outros tecidos

Estudos funcionais

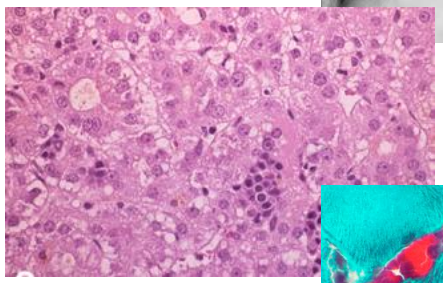
Polarografia (O_2), oxidação ^{14}C metabolitos, ATP, Pcr, enzimas CRM

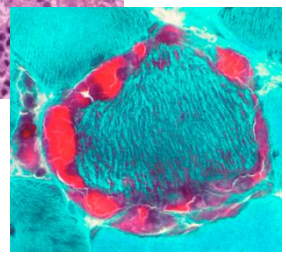
Estudos genéticos (mtDNA, nDNA)

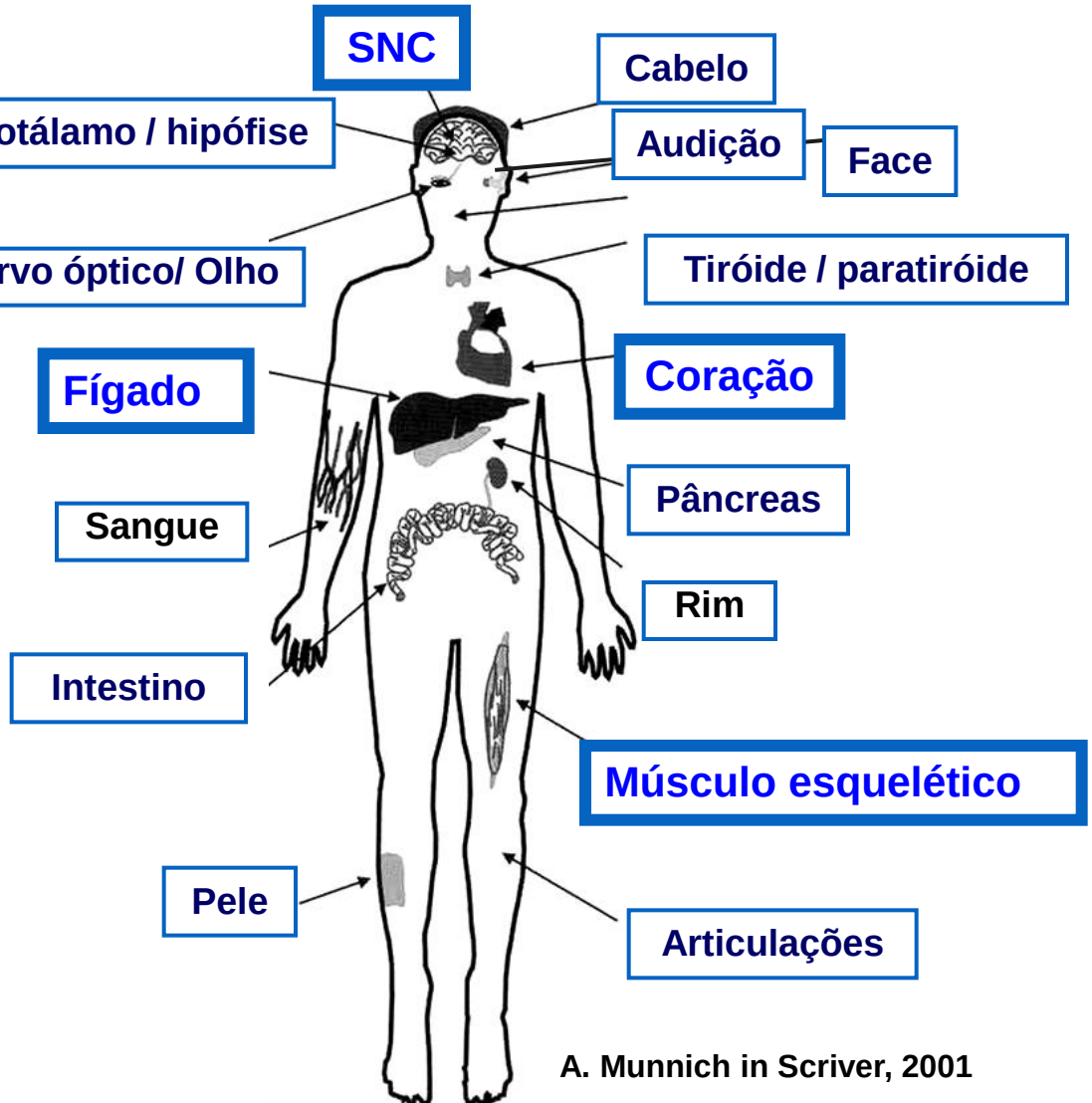
Outros (proteínas CRM, RNA ...) investigação



DCRM – manifestações clínicas





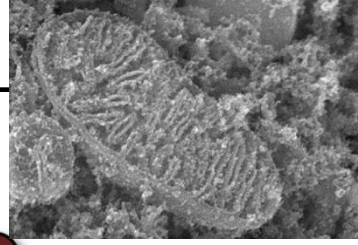


A. Munnich in Scriver, 2001

~50%: início idade pediátrica

< 6 A (Naviaux, 2004)

< 16 A (Skladal et al., 2002)



DCRM – manifestações clínicas

Neurologic

1. Cerebral stroke-like lesions in a nonvascular pattern
2. Basal ganglia disease
3. Encephalopathy, recurrent or with receiving valproate
4. Neurodegeneration
5. Epilepsia Partialis Continua
6. Myoclonus
7. Ataxia
8. MRI findings consistent with Leigh disease
9. Characteristic MRS peaks
   a. Lactate peak at 1.3 ppm TE at 35 and 135
   b. Succinate peak at 2.4 ppm

Ophthalmologic

1. Retinal degeneration
2. Ophthalmoplegia/paresis
3. Fluctuating, dysconjugate eye movements
4. Ptosis
5. Sudden- or insidious-onset optic neuropathy/ atrophy

Gastroenterologic

1. Unexplained or valproate induced liver failure
2. Severe dysmotility
3. Pseudo-obstructive episodes

Cardiovascular

1. Hypertrophic cardiomyopathy with rhythm disturbance
2. Unexplained heart block in a child
3. Cardiomyopathy with lactic acidosis (> 5 mM)
4. Dilated cardiomyopathy with muscle weakness
5. Wolff-Parkinson-White arrhythmia

Other

1. Exercise intolerance out-of-proportion to weakness
2. Delayed waking from general anesthesia
3. Episodes of acute rhabdomyolysis
4. Unexplained hypotonia, failure-to-thrive, and acidosis

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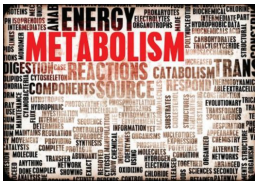
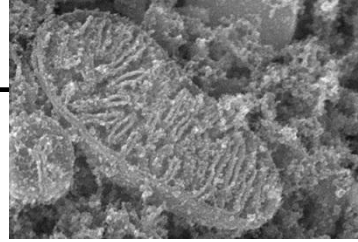
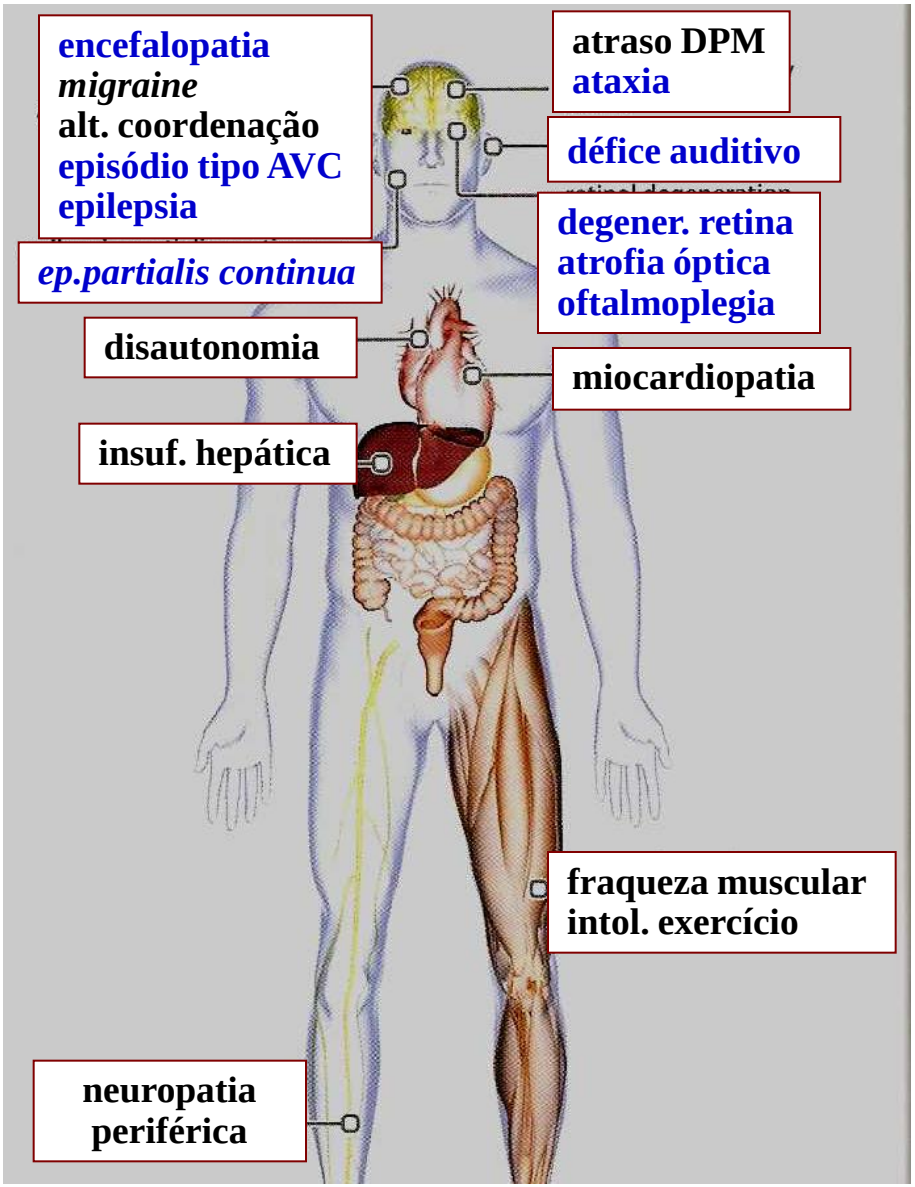




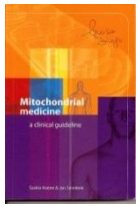
DCRM - Pistas para o "diagnóstico clínico"

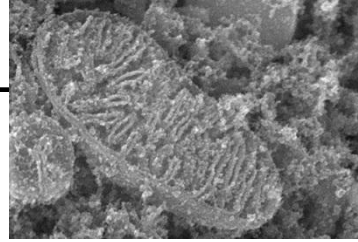


- Envolvimento ≥ 3 orgãos / sistemas
- Hist. familiar➔ hereditariedade materna
- ≥ 1 sintomas altamente suspeitos
- ≥ 2 sintomas suspeitos

Suspeita elevada de doença mitocondrial

Mitochondrial medicine
- a clinical guideline - 2011-
S.Koene & J.Smeitink





DCRM – manifestações clínicas

Sintomático ←→ Subclínico

Isolado ←→ Multi-sistémico

nDNA

Idade de início

gravidade

prognóstico

variabilidade intrafamiliar

mtDNA

Crianças +++

Início mais precoce
1os M ←→ infância

Curso progressivo, grave

Mais uniforme

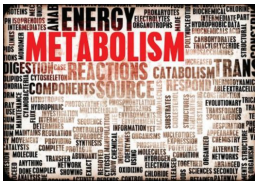
Adultos +++

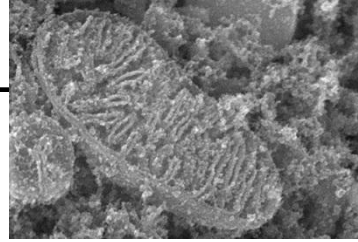
Espectro clínico mais amplo em
Idade de início e gravidade

Mais gradual,

Menos grave

Rubio-Gozalbo et al. Hum Mutat 2000;15:522-32

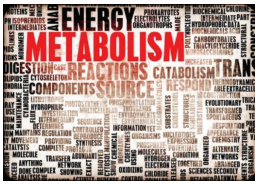


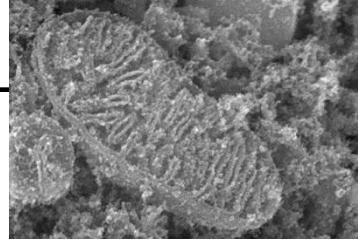


DCRM – manifestações clínicas

➔ ➔ Evolução

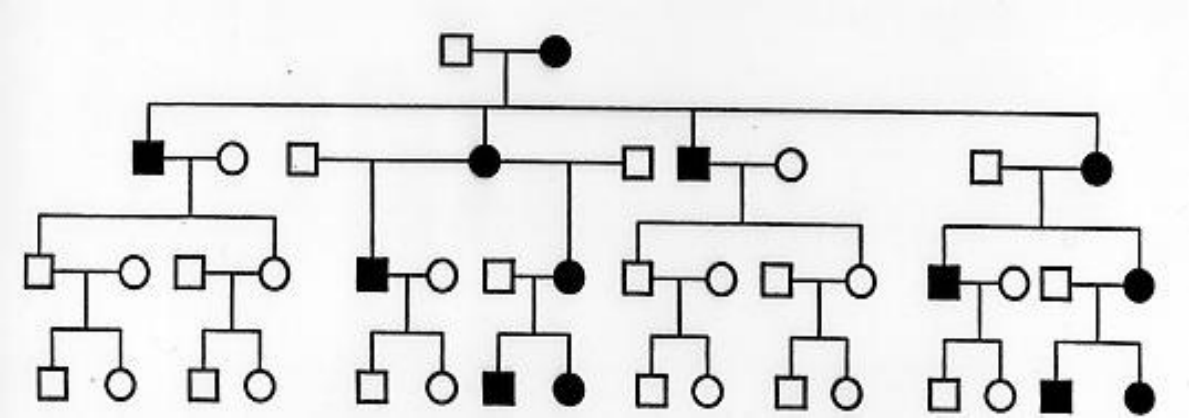
Síndrome de Leigh. Défice complexo IV. Deleção *SURF1*





DCRM – Doenças Hereditárias

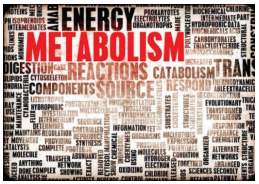
História familiar…



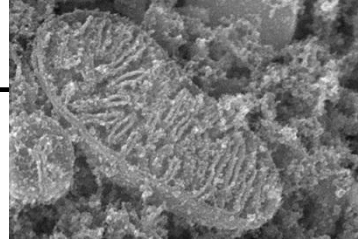
140.116.225.137/site2/ocwCoursePPT/73Determin...

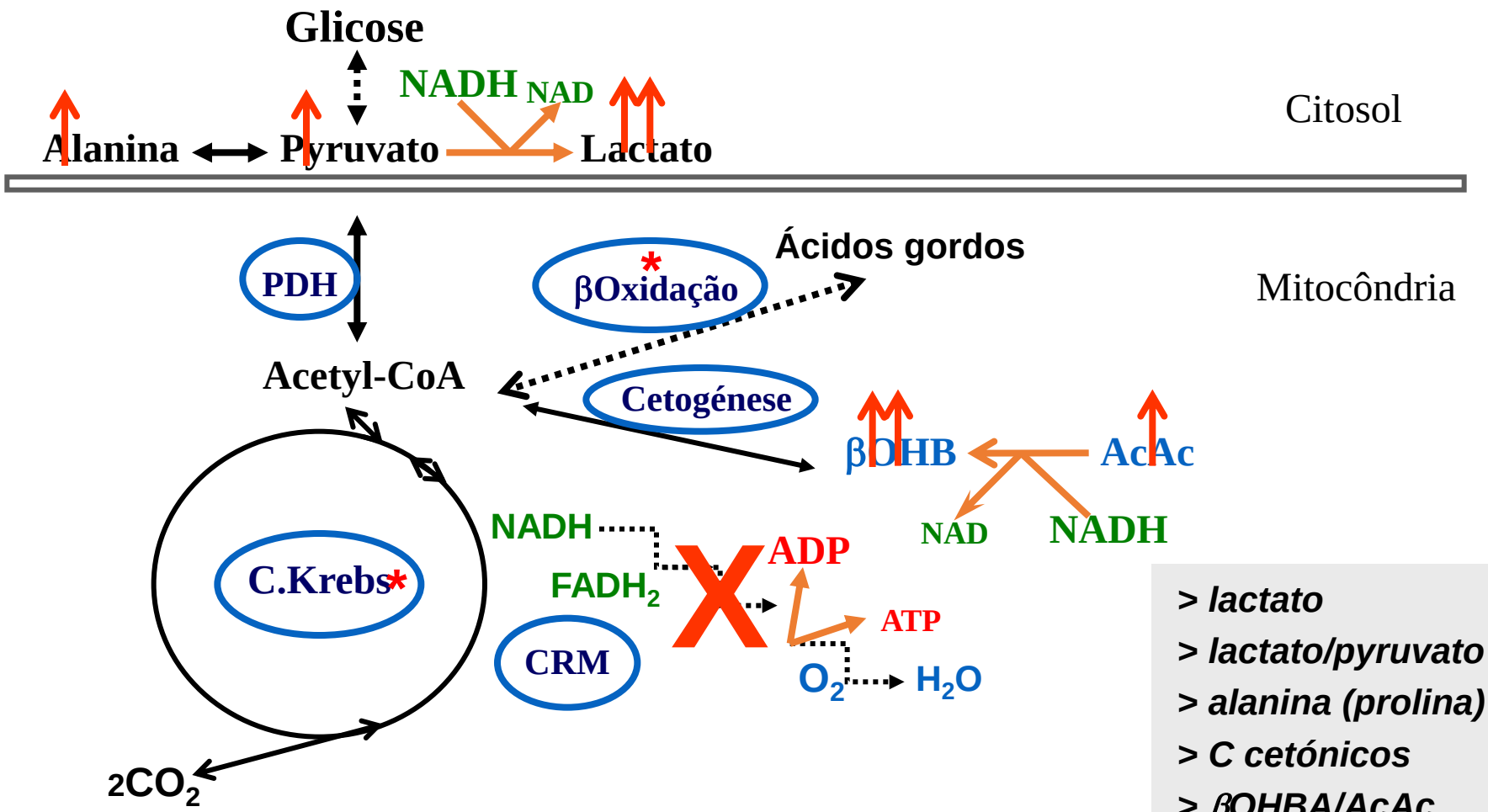
Miopatia, miocardiopatia
mtDNA 3302A>G

… pode ser não informativa.



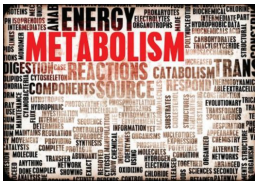
DCRM – rastreio laboratorial – 1ª linha

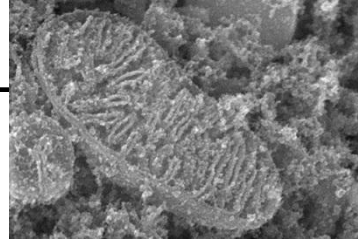




* aciduria orgânica

> *lactato*
> *lactato/pyruvato*
> *alanina (prolina)*
> *C cetónicos*
> *βOHBA/AcAc*
Cetonémia Px
Aciduria orgânica





DCRM – 2ª linha de avaliação

Rastreio de envolvimento multi-orgânico

- Ressonância Magnética com espectroscopia
- EEG
- Avaliação Oftalmológica
- Avaliação Audiológica
- Avaliação Cardíaca (Ecocardiograma)
- PL (se indicado)
- EMG (se indicado)

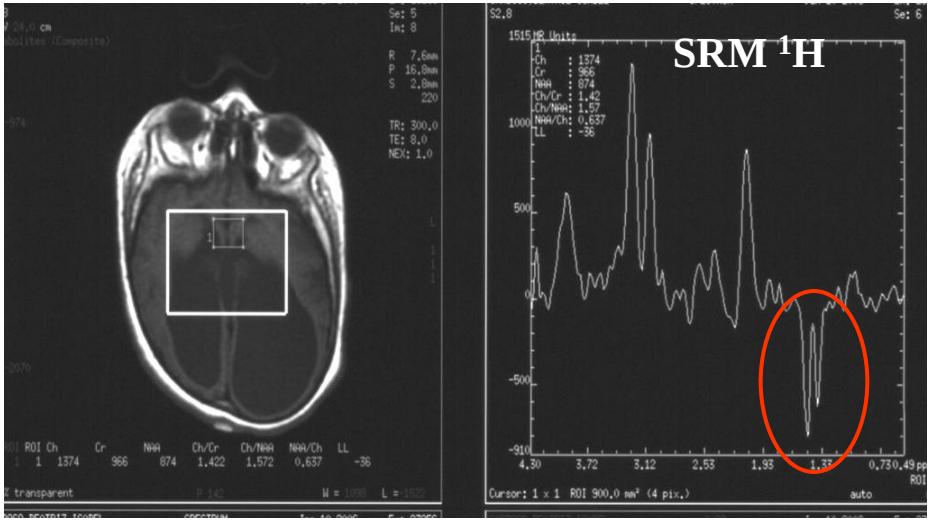
DCRM – 2ª linha de avaliação

MRS ^{1}H

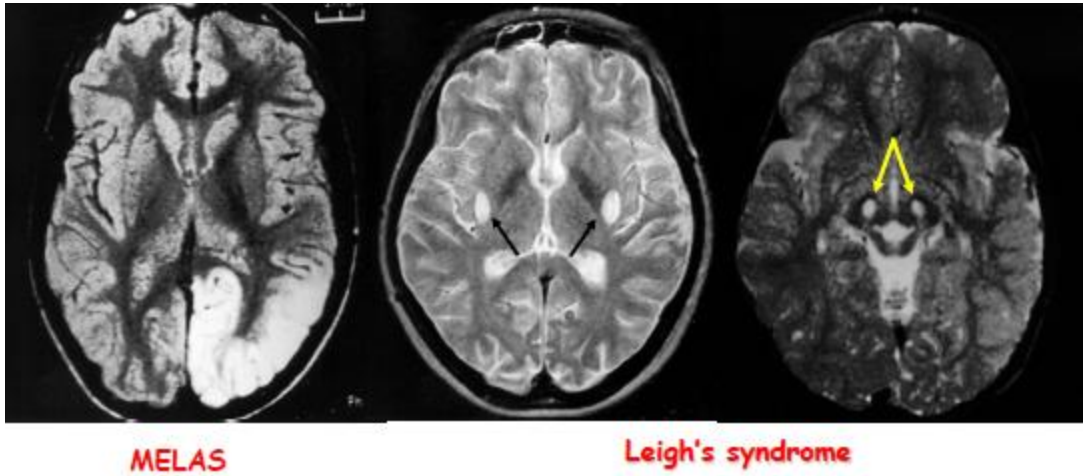
Cérebro (lesões; gânglios base)

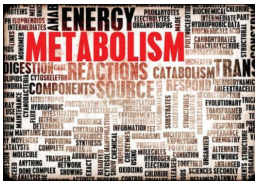
> lactato

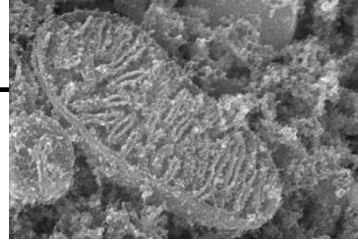
Não invasivo: metabolismo energético *in vivo*



RMN cerebral



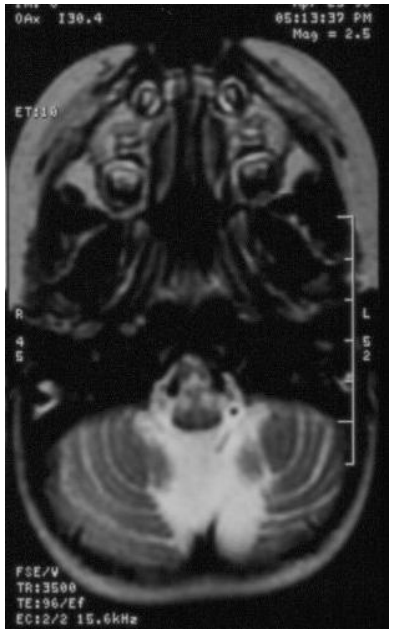


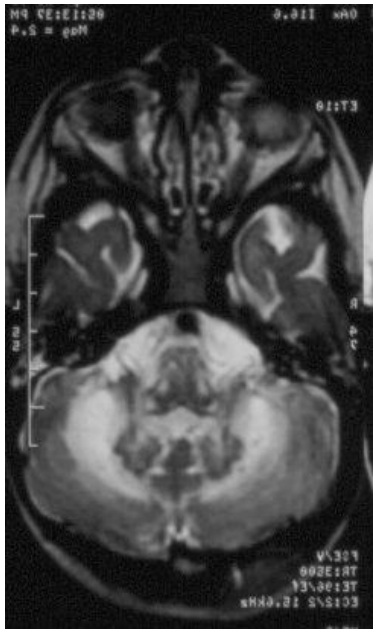


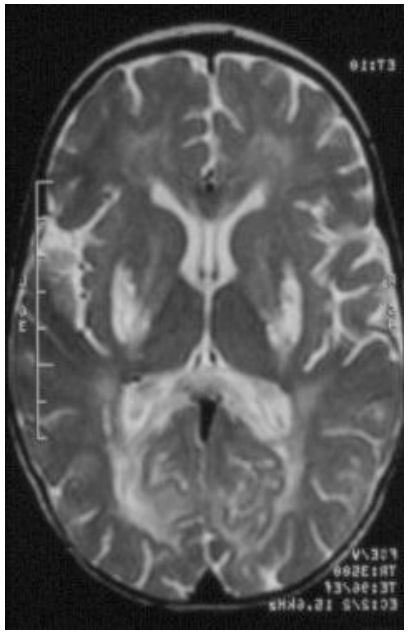
Exemplo: Síndrome de Leigh

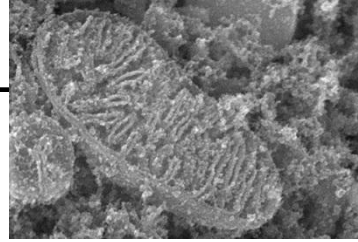
RMN cerebral

Atraso de crescimento
Hipotonia generalizada
Hiperlactacidémia
Défice do complexo IV









DCRM – meios de diagnóstico específico

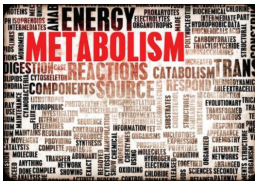
Tecido:

- ✓ **Leucócitos**
- ✓ **Músculo**
- ✓ **Fígado**
- ✓ **Fibroblastos**
- ✓ **…**

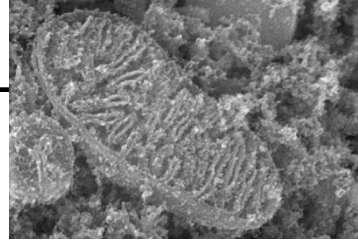
Estudos histológicos

Estudo funcional da CRM

Estudos genéticos (mtDNA e nDNA)



DCRM – meios de diagnóstico específico



Tecido:

- ✓ Leucócitos
- ✓ Músculo
- ✓ Fígado
- ✓ Fibroblastos
- ✓ ...

Estudos histológicos

Estudo funcional da CRM

Estudos genéticos (mtDNA e nDNA)

Histologia

- ➢ ***Microscopia clássica***
- ➢ **Histoenzimologia**

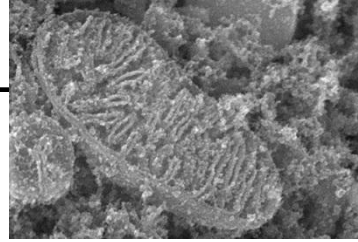
  ATPase

  fibras tipo I - *oxidativas*

  fibras tipo II - *glicolíticas*

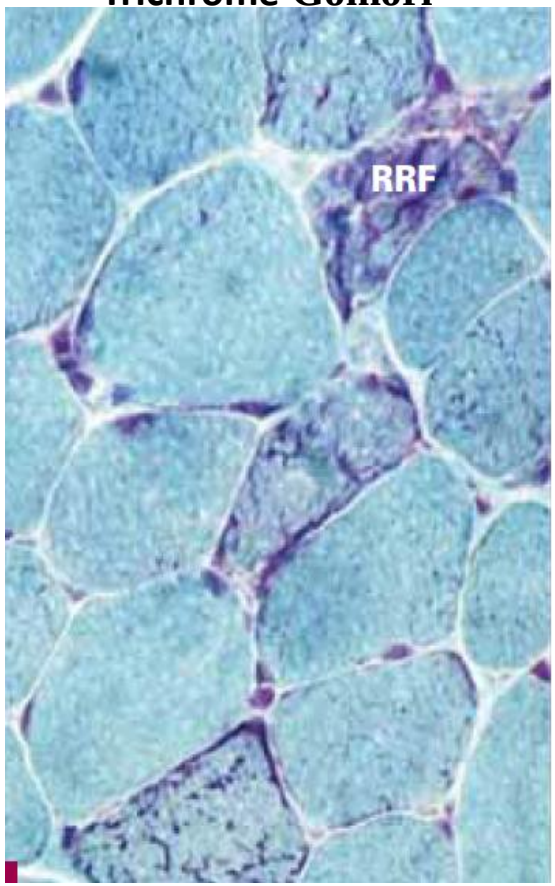
  COX (complexo IV da CRM)

  SDH (complexo II da CRM)
- ➢ **Imunocitoquímica**
- ➢ **Microscopia electrónica**



DCRM – meios de diagnóstico específico

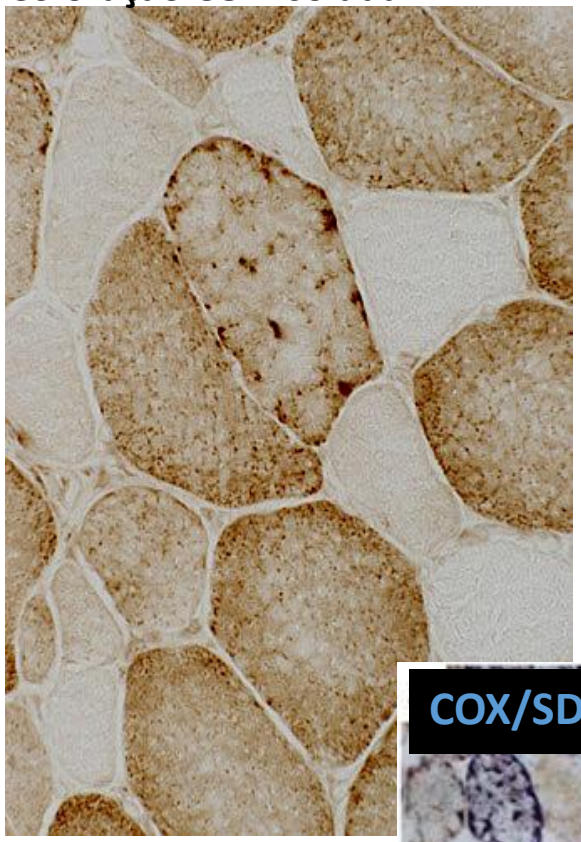
Trichrome Gömöri



Fibras Rotas Vemelhas - Ragged Red Fibers (RRF)

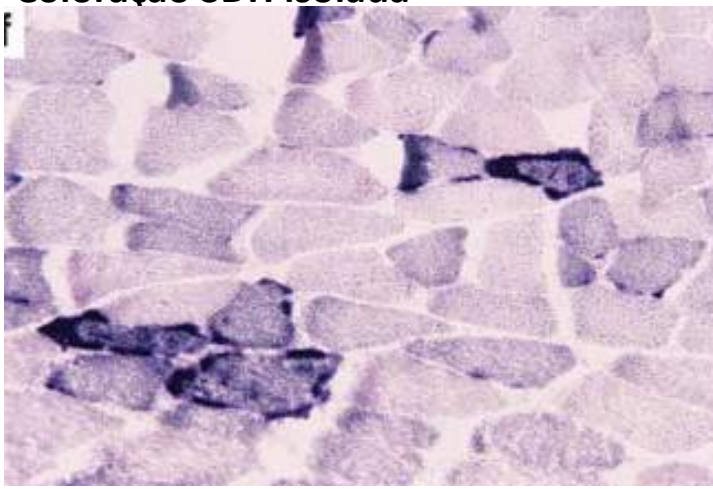
Rara na criança, mais frequente no adulto. **< 5%** (Smeitink)

Coloração COX isolada



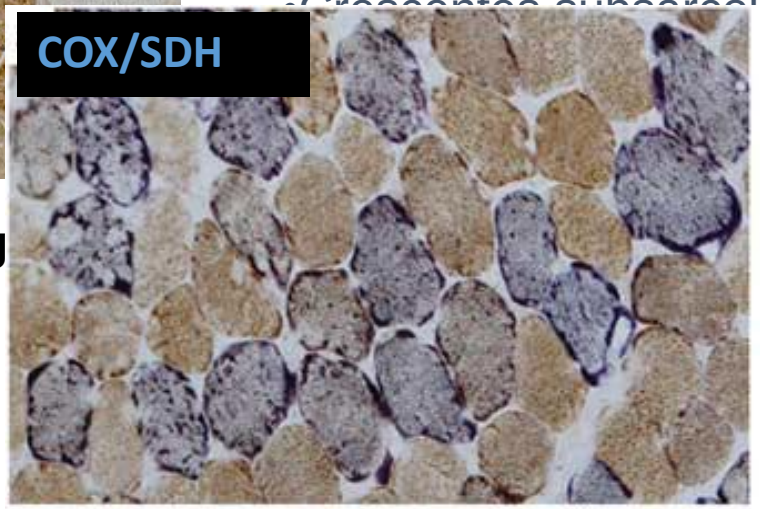
Fibras COX neg

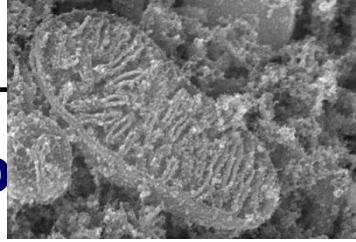
Coloração SDH isolada



- **➔ aumento da actividade oxidativa subsarcolémica**
- Crescentes subsarcolémicos

ianças

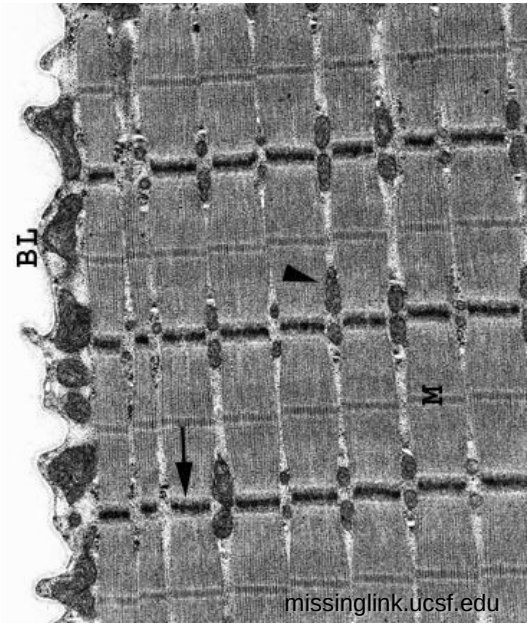




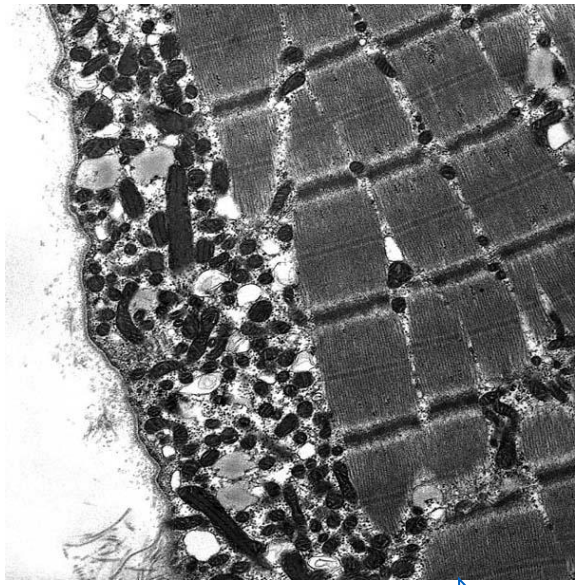
DCRM – meios de diagnóstico específico

Microscopia electrónica

Músculo normal

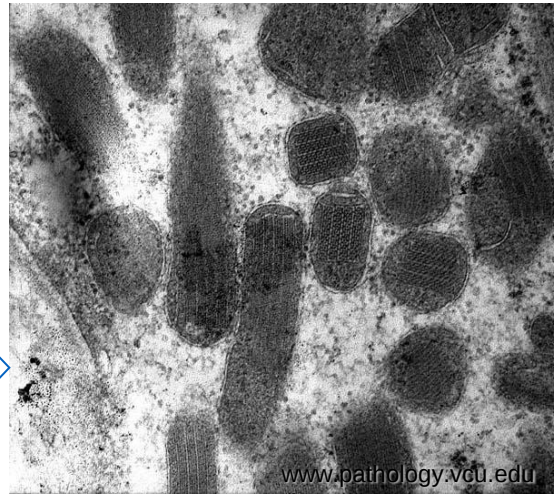


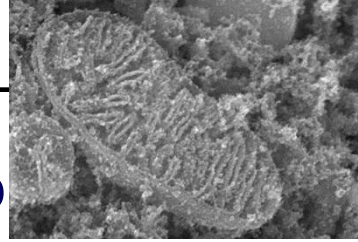
Miopatia mitocondrial



>> mitocôndrias no espaço subsarcolémico

inclusões paracristalinas aspecto "parking lot"





DCRM – meios de diagnóstico específico

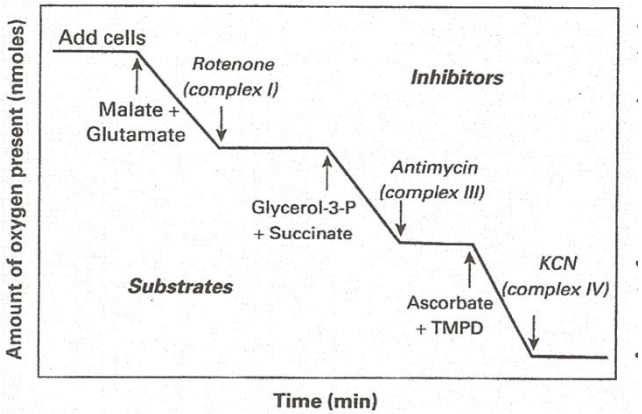
Tecido:

- ✓ Leucócitos
- ✓ Músculo
- ✓ Fígado
- ✓ Fibroblastos
- ✓ …

Estudos histológicos

Estudo funcional da CRM

Estudos genéticos (mtDNA e nDNA)

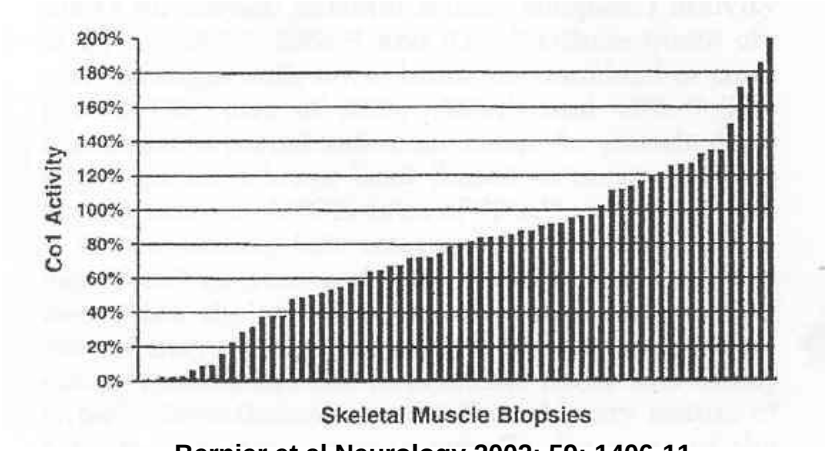


Hofhaus et al Methods Enzymol 1996;264:476-83

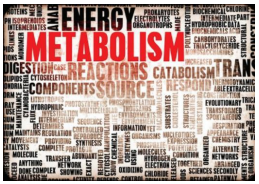
- ➢ **Polarografia (O_2)**
- ➢ **Oxidação metabolitos ^{14}C**
- ➢ **Síntese de ATP e Creatina~P**
- ➢ **Actividade enzimática da CRM**

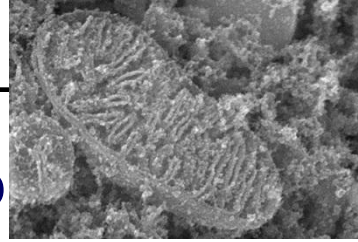
20-30% média/CS

<20% média/CS



Bernier et al.Neurology 2002; 59: 1406-11





DCRM – meios de diagnóstico específico

Tecido:

- ✓ Leucócitos
- ✓ Músculo
- ✓ Fígado
- ✓ Fibroblastos
- ✓ …

Estudos histológicos

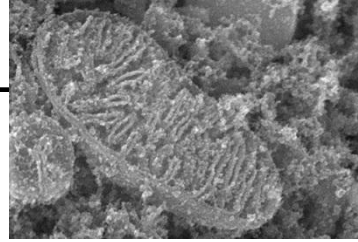
Estudo funcional da CRM

Estudos genéticos (mtDNA e nDNA)

Rastreio de mutações e deleções mtDNA

Quantificação do número de cópias do mtDNA

Pesquisa de mutações/sequenciação de genes no nDNA



DCRM – Doenças *vs* Síndromes Mitocondriais

- Leigh
  (other IEM of energy metabolism …)
- Pearson
- Alpers
- mtDNA depletion
- Barth
- Sengers
- GRACILE
- CoQ Deficiency

…

- MELAS
- MERRF
- MNGIE
- Kearns-Sayre
- NARP
- LHON
- CPEO

…

Síndromes típicas

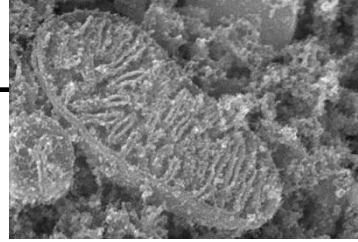
raras na criança

…

Pista diagnóstica

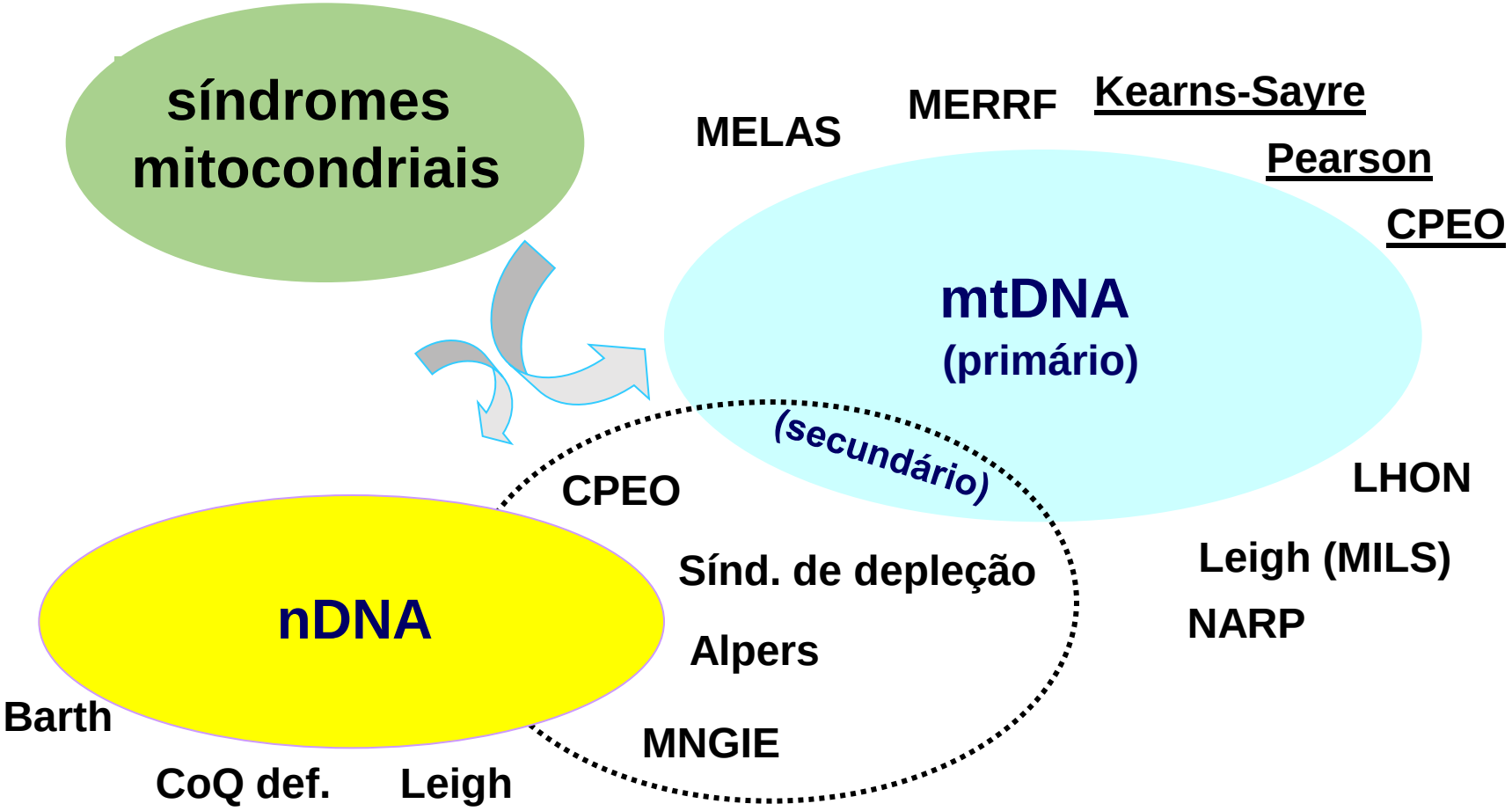
Genótipo ←/→ Fenótipo

Sobreposição clínica & genética (+++)

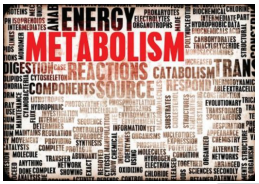


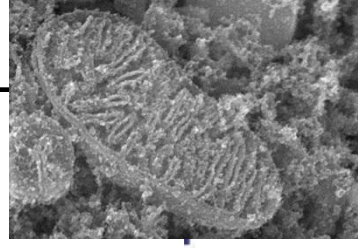
DCRM – Doenças *vs* Síndromes Mitocondriais

Neurologistas & oftalmologistas de adultos ➔ síndromes mitocondriais

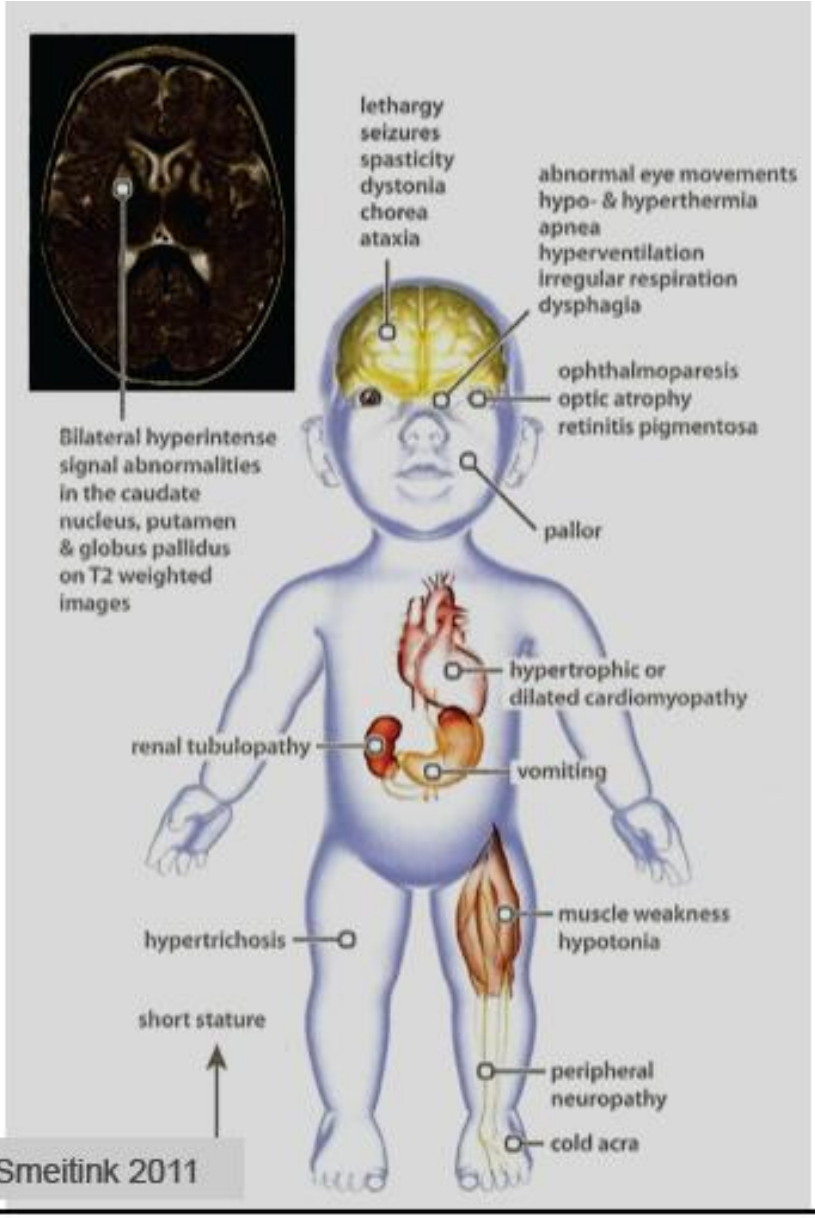


[deleção mtDNA]





Síndrome de Leigh



S.Koene&J.Smeitink 2011

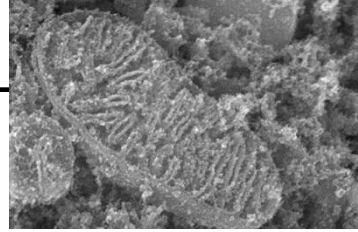
INÍCIO:
3-12 M (NN ←→ adulto)

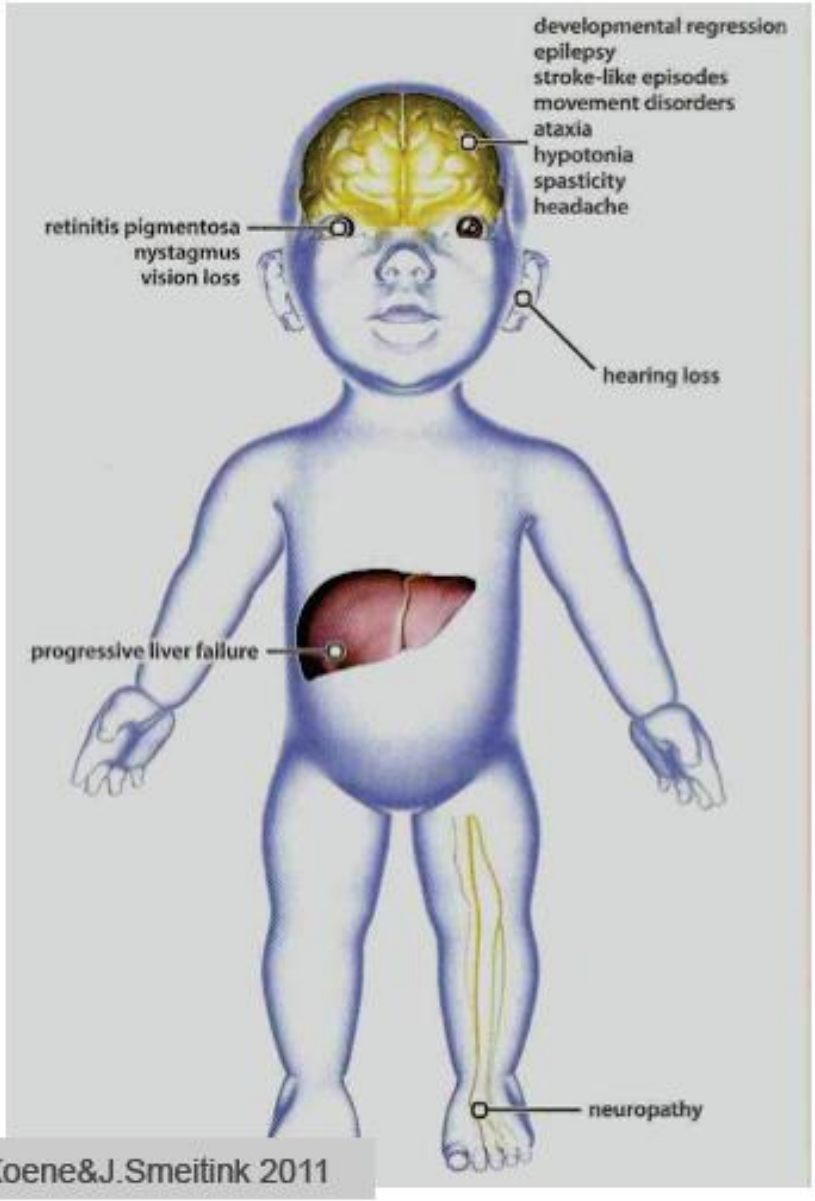
- ADPM
- *floppiness*
- Disfunção do tronco cerebral e extrapiramidal
- ± Acidose láctica
- ± doença noutros orgãos

Progressiva …

Morte-1ª decada
Insuf. Respiratória

mtDNA /nDNA / ????





S.Koene&J.Smeitink 2011

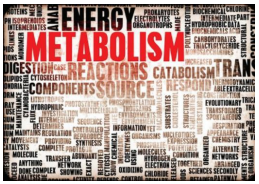
Sínd. Alpers

Início:

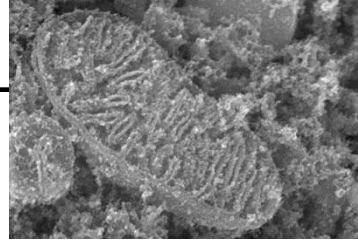
2- 4 anos (1 M ↔ > 30 anos)

- ADPM
- Regressão periódica (infecções)
- Epilepsia intratável
- Neuropatia
- Insuf Hepática (Valproato de sódio)
- ±
- Cefaleias
- Doenças do movimento
- Défice visual/ auditivo

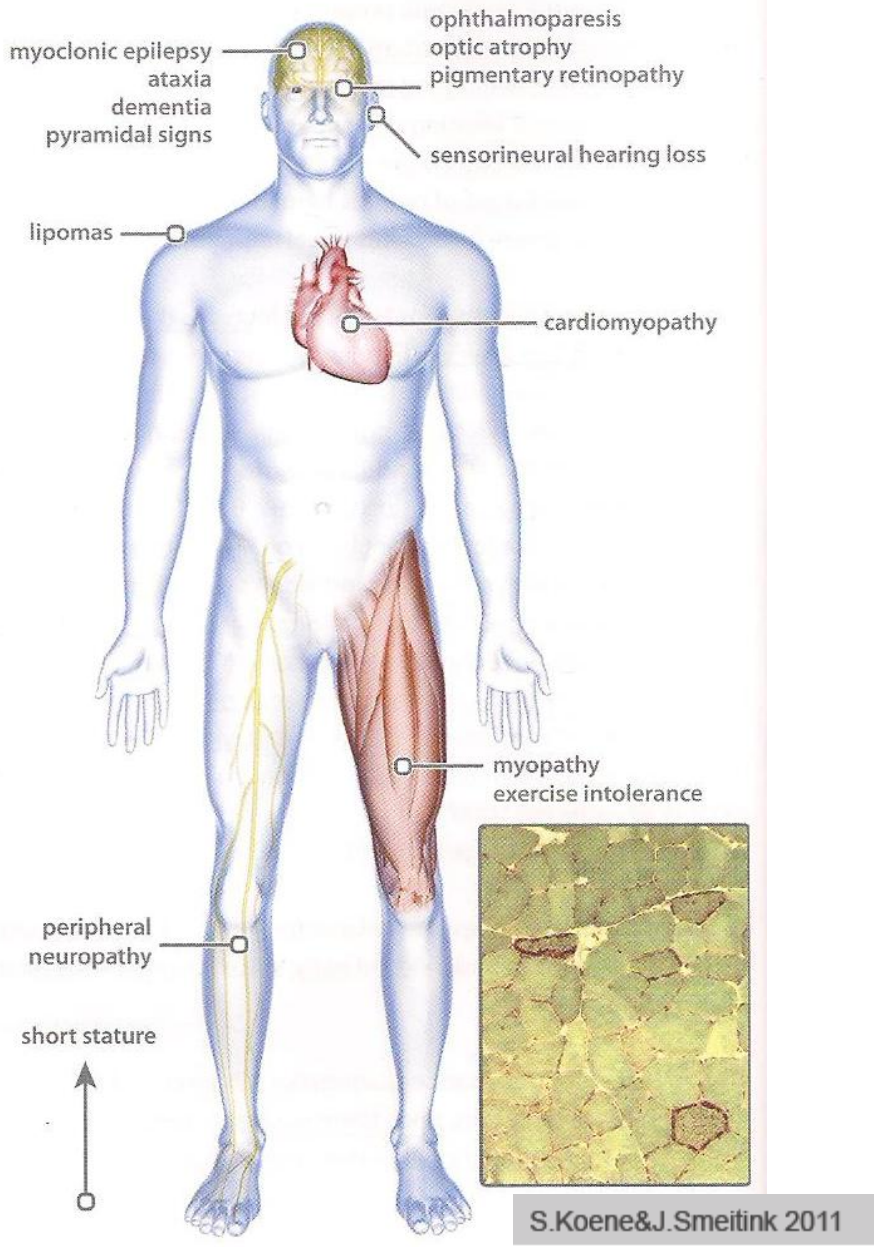
Fenótipo mais grave das Doenças relacionadas com a *POLG*



DOENÇAS MITOCONDRIAIS



MERRF – Myoclonic Epilepsy and Ragged Red Fibers



Início:

5 - 15 anos

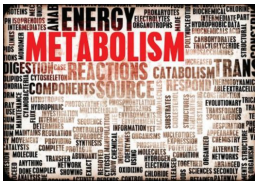
4 sintomas chave:

- Mioclonias
- Epilepsia generalizada
- Ataxia
- Lipomatose

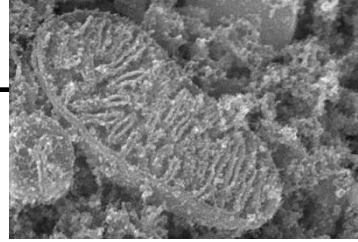
Outros sintomas:

- cardiomiopatia (WPW)
- Baixa estatura
- Surdez
- …

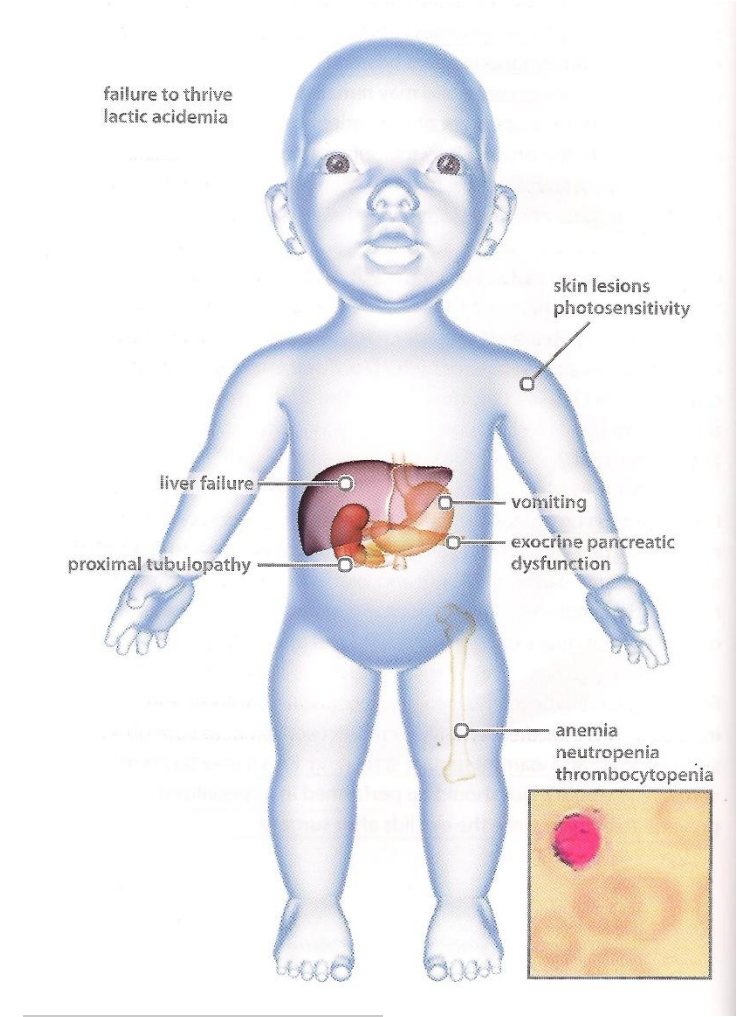
Mutações no mtDNA
(freq - m.8344 A>G)



DOENÇAS MITOCONDRIAIS



Síndrome de Pearson



S.Koene&J.Smeitink 2011

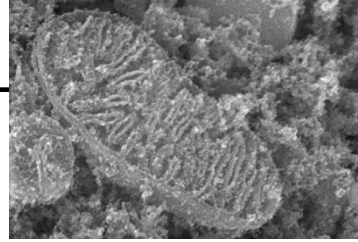
Síndrome Mitocondrial raro!

Início:
1M – 12M

Sintomas chave:

- Anemia sideroblástica/pancitopenia (com vacuolização dos precursores na MO)
- Disfunção pancreática (exócrina)
- Má progressão ponderal
- Vómitos

Deleções do mtDNA
(mais abundantes no sangue)



DCRM – Critérios de Diagnóstico

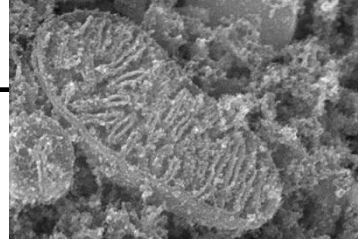
Sistemas de classificação →

- **Walker (1996)**
- **Bernier (2002)**
- **Wolff (2002)**
- **Smeitink (2006)**

DCRM

- *improvável*
- *possível*
- ***provável***
- ***definida***

Clínica compatível + Alt.patog.mtDNA/nDNA → **DCRM *definitiva***



DCRM – Critérios de Diagnóstico

- Sinais / Sintomas
- História familiar
- Rastreio laboratorial
- Rastreio envolvimento multiorgânico

Quadro clinico-laboratorial orientador

não

sim

Biópsia muscular outra…

Estudo genético

Estudo histológico

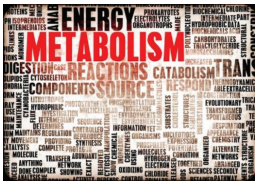
Histoquímica
Mic electrónica

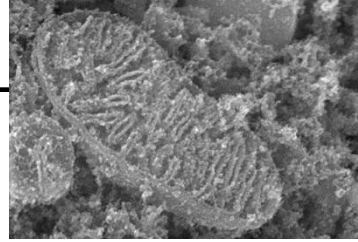
Estudo funcional

Oxidação ^{14}C metabolitos
Polarografia (O_2), ATP,
Atividade enzimática

Δ Definitivo

Δ Definido
Δ Provável
Δ Possível
Δ Improvável





Tratamento

Cuidados gerais

- Dieta adequada
- Exercício aeróbico moderado
- Evitar fármacos "perigosos"
- Tratar infecções e febre precocemente

Rastrear complicações tratáveis

- Insuf. renal
- Cardiomiopatia
- Bloqueios de condução cardíaca
- Acidose láctica

Cuidado paliativo adequado

- Abordagem multidisciplinar
- Alimentação naso-gástrica
- Suporte resp. nocturno
- Medicação anti-refluxo
- Laxantes
- Suporte auditivo
- Cirurgia para oftalmoparésia e ptose
- Apoio psicossocial

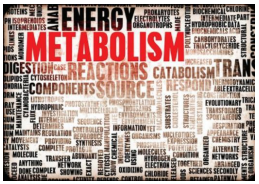
Aconselhamento genético

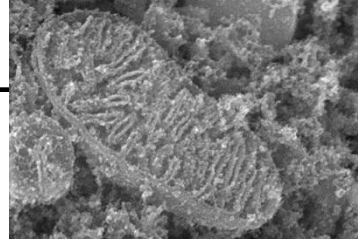
- Diagnóstico pré-natal
- Diagnóstico pré-implantação

Ensaios terapêuticos

- 6 meses com CoQ10

Adaptado de: Mitochondrial medicine - a clinical guideline - 2011-
S.Koene & J.Smeitink





Tratamento

- **Equipa multidisciplinar**
- **Sintomático (epilepsia, espasticidade, infeções....)**
- **Cofatores/ transportadores e^- / prevenção lesão *ROS* – *L-carnitina?***
- ***"stroke-like episodes" (MELAS)* – *Arginina iv (precursor NO)***
- **Evicção de fármacos/ procedimentos que interfiram com função da mitocòndria**

  *** valproato de sódio, metformina, aminoglicosideos ...**

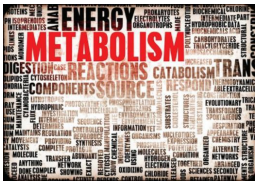
  *** transplante de orgãos (?)**
- **Recomendações dietéticas**

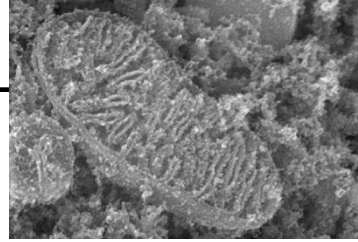
  Normal, sem excesso de calorias

  Dieta cetogénica

Não altera significativamente o curso da doença ...

Diagnóstico correcto >> Aconselhamento Genético





Prognóstico

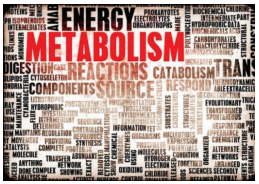
Variável …

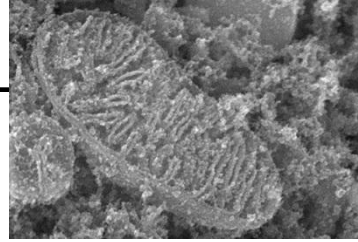
Menor sobrevivência:

- **início precoce**
- **hepático ou cardíaco**
- **acidose láctica**

… habitualmente desfavorável
Criança +++

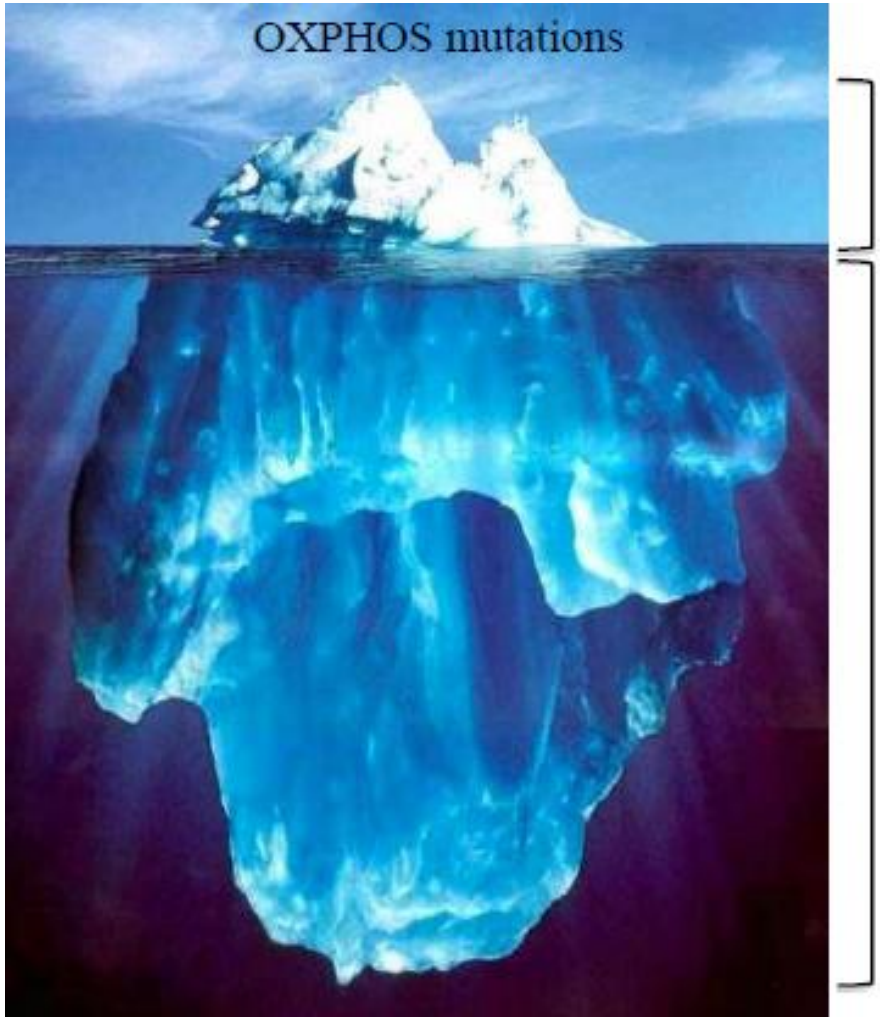
HPC- 78 doentes
Mortalidade: ~30%
(idade mediana: 8M)





Prognóstico

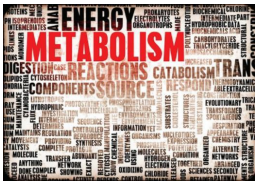
Idade vs Prognóstico



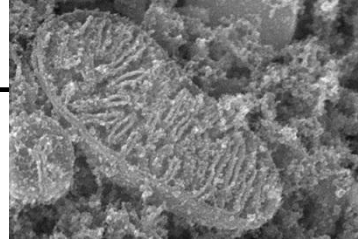
Extra-uterine survival:
disease

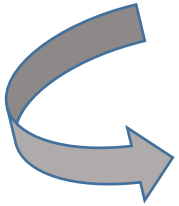
Embryonic lethal:
fetal loss

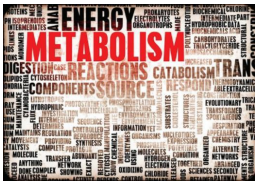
M. Zeviani 2011

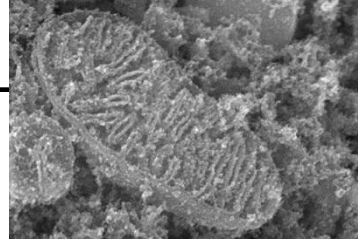


DOENÇAS MITOCONDRIAIS



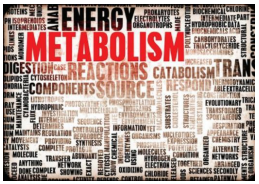


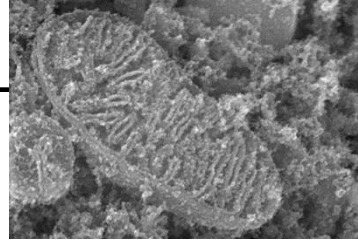




Sumário

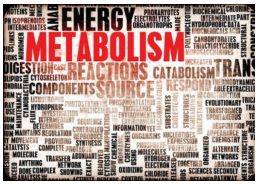
- **Podem apresentar-se:**
  - **em qualquer idade**
  - **com qualquer sinal e sintoma (qualquer órgão/ sistema)**
  - **com qualquer forma de hereditariedade**
  - **… a qualquer professional de saúde.**
- **São as doenças hereditárias do metabolismo mais frequentes.**
- **A História familiar é raramente informativa.**
- **Caracterizam-se pela sua heterogeneidade clínica de início e na evolução … podem ter um vasto diagnóstico diferencial.**
- **Envolvimento multissistémico é a sua marca, mas pode demorar vários anos a tornar-se clínico.**
- **Síndromes mitocondriais são raros, mas há sobreposição clínica.**



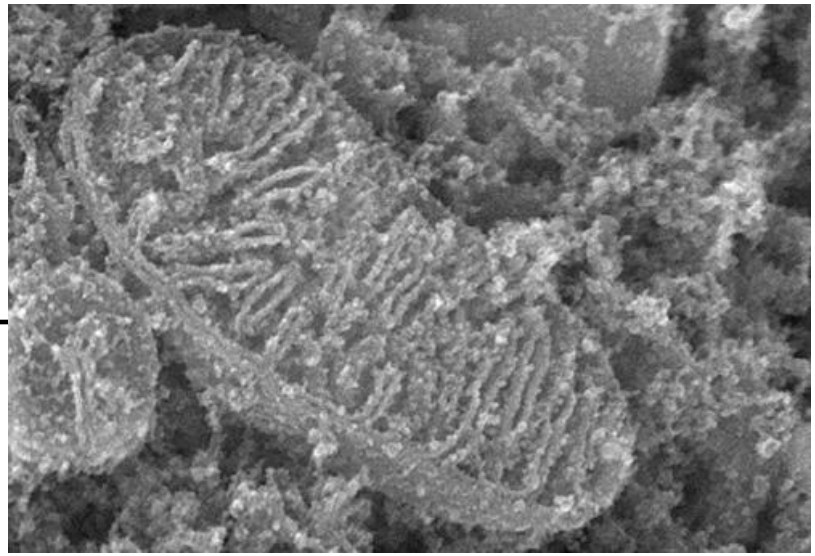


Sumário

- **Estudo etiológico é complex e multidisciplinar!**
- **Ao contrário dos adultos, nas crianças, a maioria é causada por mutações no nDNA (mtDNA ≤25%).**
- **Diagnóstico frequentemente baseado nas anomalias da actividade dos complexos enzimáticos da CRM e da morfologia da mitocôndria**
- **Progressos nos métodos de avaliação da função mitochondrial**
- **Critérios de diagnóstico para DCRM primárias - Sem *consenso*!**
- **Técnicas de análise genética recentes: *mitochondrial exom sequencing* – nem sempre com resultados claros!**
- **Não há tratamento para a maioria dos doentes.**
- **O prognóstico é geralmente mau.**
- **O Diagnóstico e o aconselhamento genético correcto são desejáveis.**



DOENÇAS MITOCONDRIAIS



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