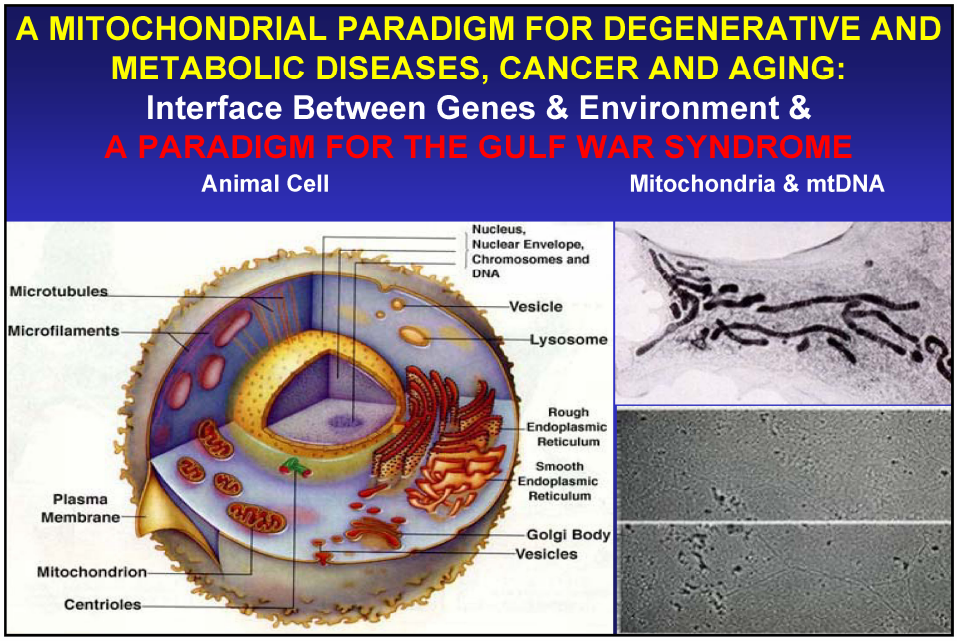
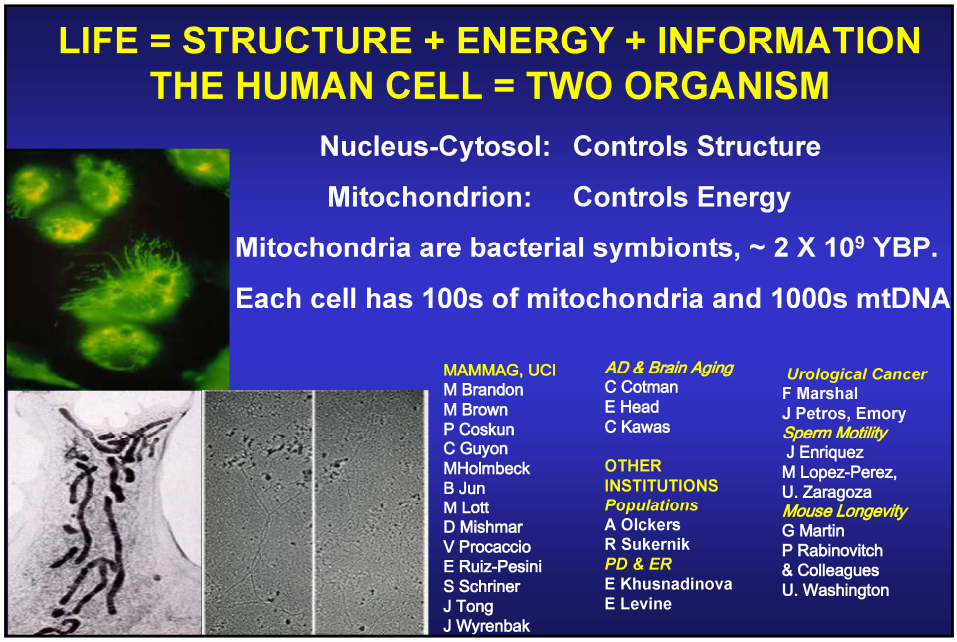
Presentation 2 – Douglas Wallace

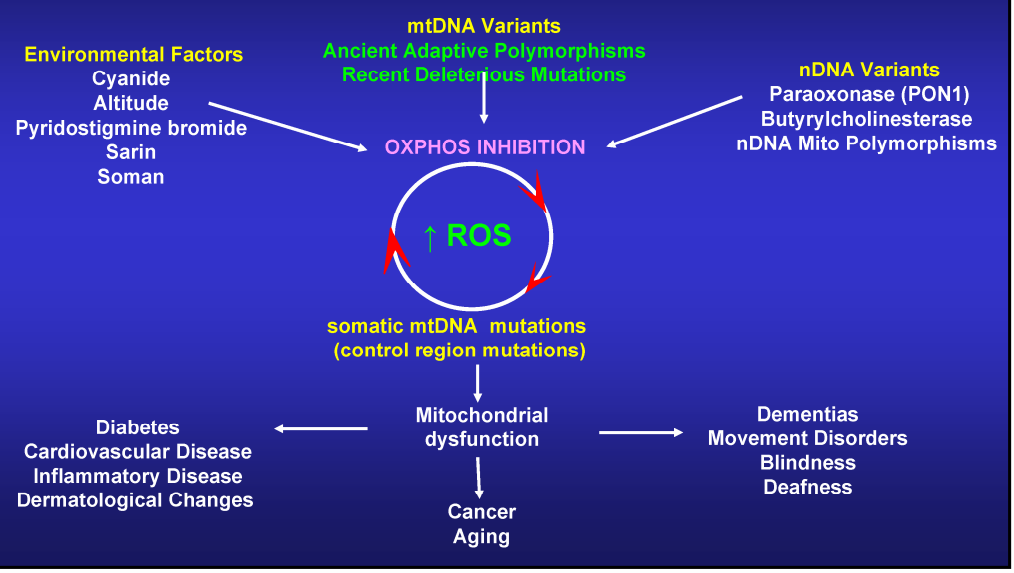


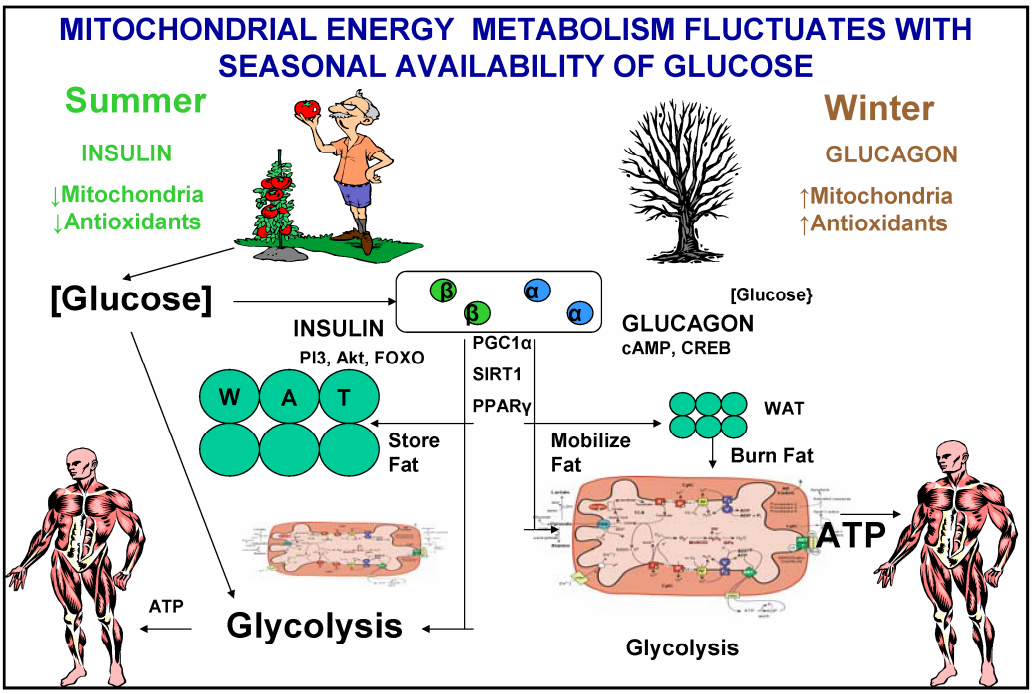


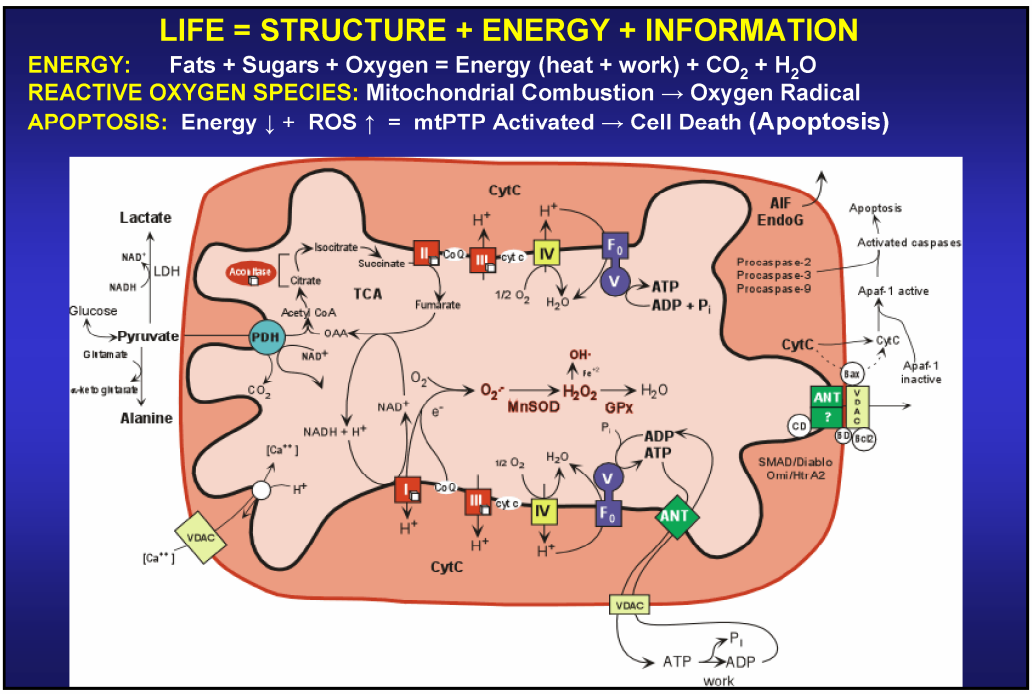
GULF WAR SYNDROME & MITOCHONDRIAL DISEASE

Symptom	Long Term Vietnam (AO) WWII (radiation)	Short Term GWS	Short & Long Term Mito Dis
Migraines		+	mtDNA-tRNA, eg 3243
Depression	+	+	mtDNA Δ + tRNA
Memory	+	+	mtDNA-protein synthesis
Basal Ganglia-Leigh/PD		+	mtDNA + nDNA
Motor-Neuron (ALS)		+	hypothesized mitochondrial
Dysarthria		+	mtDNA-protein synthesis
Vestibular-Auditory		+	mtDNA-protein synthesis
Neuro-Ophthalmological		+	mtDNA-various
Muscle Fatigue		+	mtDNA-protein synthesis
Muscle Pain		+	mtDNA-protein synthesis
Gastro-intestinal		+	mtDNA-prot syn + MINIG
Skin Rash		+	mtDNA-various
Asthma		+	hypothesized mitochondrial
Hypertension		+	mtDNA-protein snythesis
Diabetes	+		mtDNA-prot syn, eg tRNA 3243
Cardiovascular	+		mtDNA-prot syn, eg tRNA 3243
Fetal Loss		+	mtDNA-various
Peri-natal Problems	+	+	mtDNA + nDNA

ORIGIN AND PROGRESSION OF GULF WAR SYNDROME &MITOCHONDRIAL DISEASE




MITOCHONDRIAL ENERGY METABOLISM FLUCTUATES WITH SEASONAL AVAILABILITY OF GLUCOSE
Summer
INSULIN
↓Mitochondria
↓Antioxidants
Winter
GLUCAGON
↑Mitochondria
↑Antioxidants
[Glucose]
INSULIN
PI3, Akt, FOXO
PGC1α
SIRT1
PPARγ
[Glucose}
GLUCAGON
cAMP, CREB
W A T
Store Fat
Mobilize Fat
WAT
Burn Fat
ATP
ATP
Glycolysis
Glycolysis


LIFE = STRUCTURE + ENERGY + INFORMATION
ENERGY: Fats + Sugars + Oxygen = Energy (heat + work) + CO_2 + H_2O
REACTIVE OXYGEN SPECIES: Mitochondrial Combustion → Oxygen Radical
APOPTOSIS: Energy ↓ + ROS ↑ = mtPTP Activated → Cell Death (Apoptosis)
Lactate
LDH
Glucose
Pyruvate
Alanine
PDH
TCA
Citrate
Isocitrate
Succinate
Fumarate
Acetyl CoA
OAA
CytC
AIF
EndoG
Apoptosis
Activated caspases
Procaspase-2
Procaspase-3
Procaspase-9
Apaf-1 active
Apaf-1 inactive
ATP
ADP + Pi
MnSOD
GPx
ANT
VDAC
SMAD/Diablo
Omi/Htr A2
work

ENERGY ANATOMY ACCOUNTS FOR TISSUE – SPECIFICITY OF SYSTEMIC mtDNA MUTATIONS

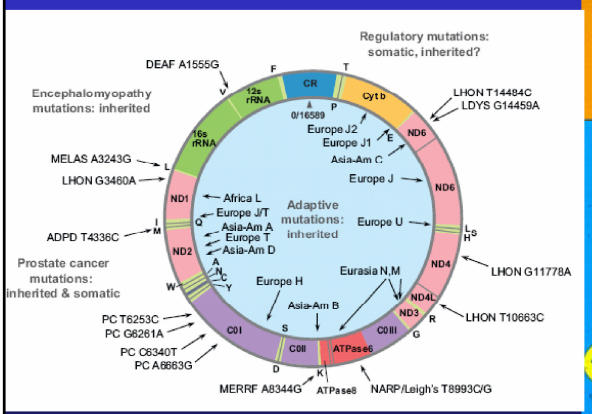
- **Energy Utilizing Tissues:**
  - Brain: Glucose or ketone bodies
  - Heart, Muscle, Renal, etc.: Glucose or fatty acids
- **Energy Storage Tissues:**
  - WAT: Energy storage for activities
  - BAT: Energy storage for thermal regulation
- **Energy Homeostasis Tissues:**
  - Liver: Glucose homeostasis
- **Energy Sensing Tissues:**
  - Purpose: Monitor & adapt to seasonal plant carbohydrates
  - Pancreatic β Cells: Glucose abundance-Insulin Signaling
  - Pancreatic α Cells: Glucose limitation-Glucagon Signaling

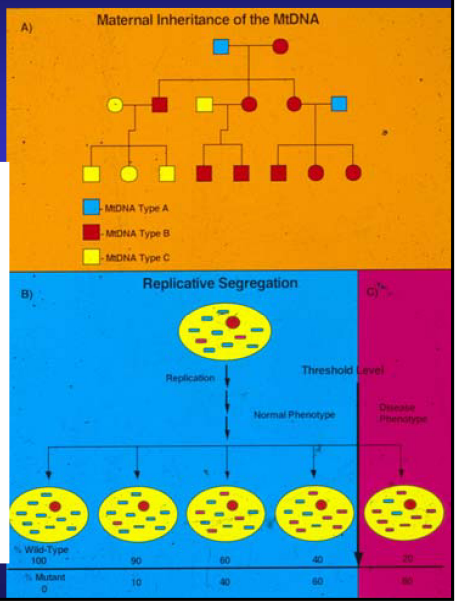
THE MITOCHONDRIA HAVE AN INDEPENDENT INFORMATION SYSTEM AND AN UNORTHODOX GENETICS

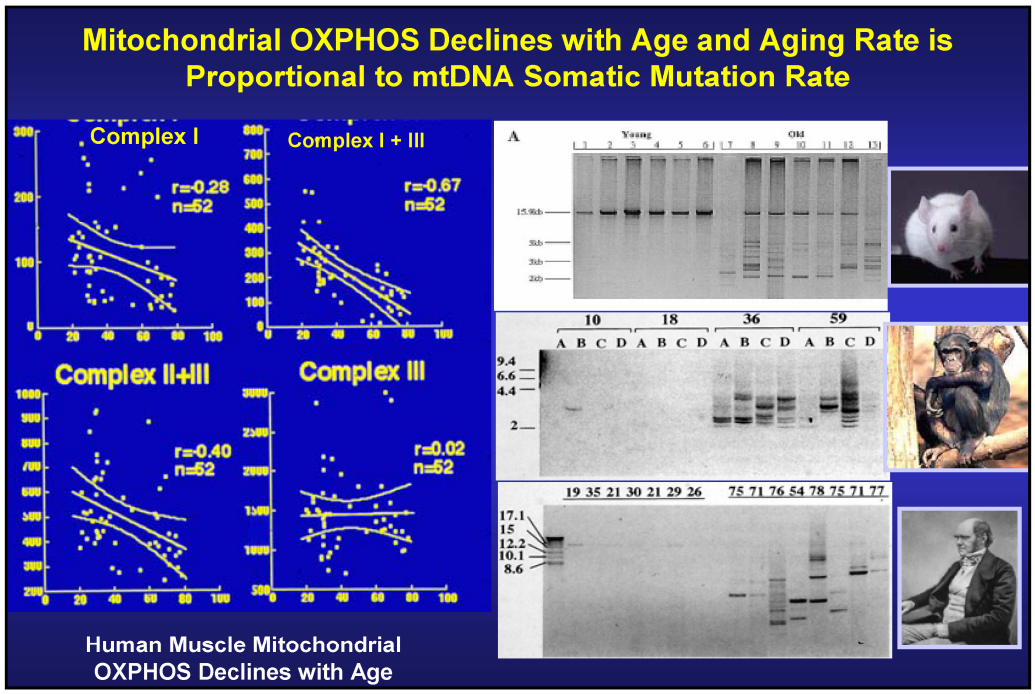
THE MITOCHONDRIAL GENOME:

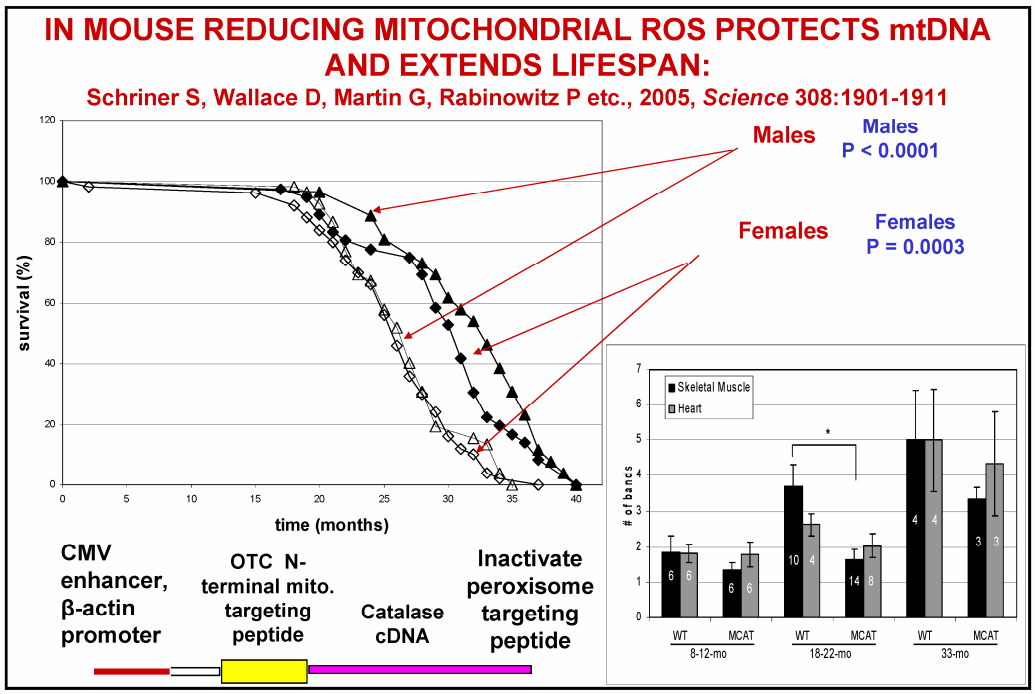
~ 1500 Chromosomal Genes

37 mtDNA Genes

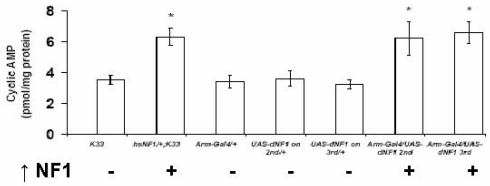


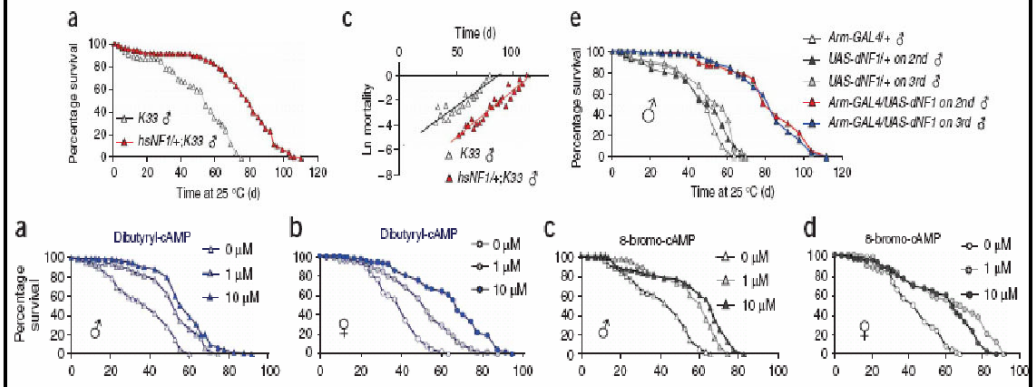



Mitochondrial OXPHOS Declines with Age and Aging Rate is Proportional to mtDNA Somatic Mutation Rate
Complex I
r=-0.28
n=52
Complex I + III
r=-0.67
n=52
Complex II+III
r=-0.40
n=52
Complex III
r=0.02
n=52
Human Muscle Mitochondrial OXPHOS Declines with Age


IN MOUSE REDUCING MITOCHONDRIAL ROS PROTECTS mtDNA AND EXTENDS LIFESPAN:
Schriner S, Wallace D, Martin G, Rabinowitz P etc., 2005, Science 308:1901-1911
survival (%)
time (months)
Males
Males
P < 0.0001
Females
Females
P = 0.0003
CMV enhancer, β-actin promoter
OTC N-terminal mito. targeting peptide
Catalase cDNA
Inactivate peroxisome targeting peptide
Skeletal Muscle
Heart
# of bancs
WT
MCAT
8-12-mo
18-22-mo
33-mo

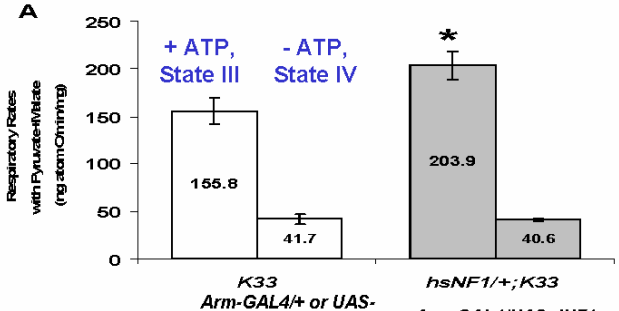
In *Drosophila* Over-Expression of NF1 INCREASES cAMP Levels & INCREASES Life Span: Exogenous cAMP has same Effect



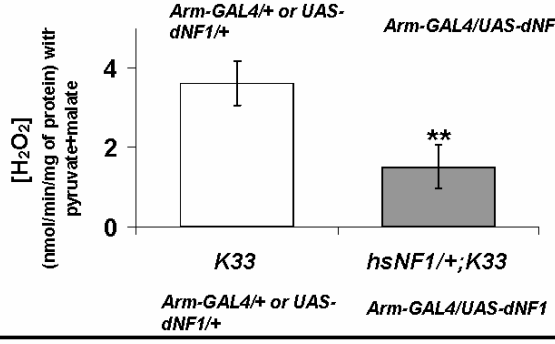


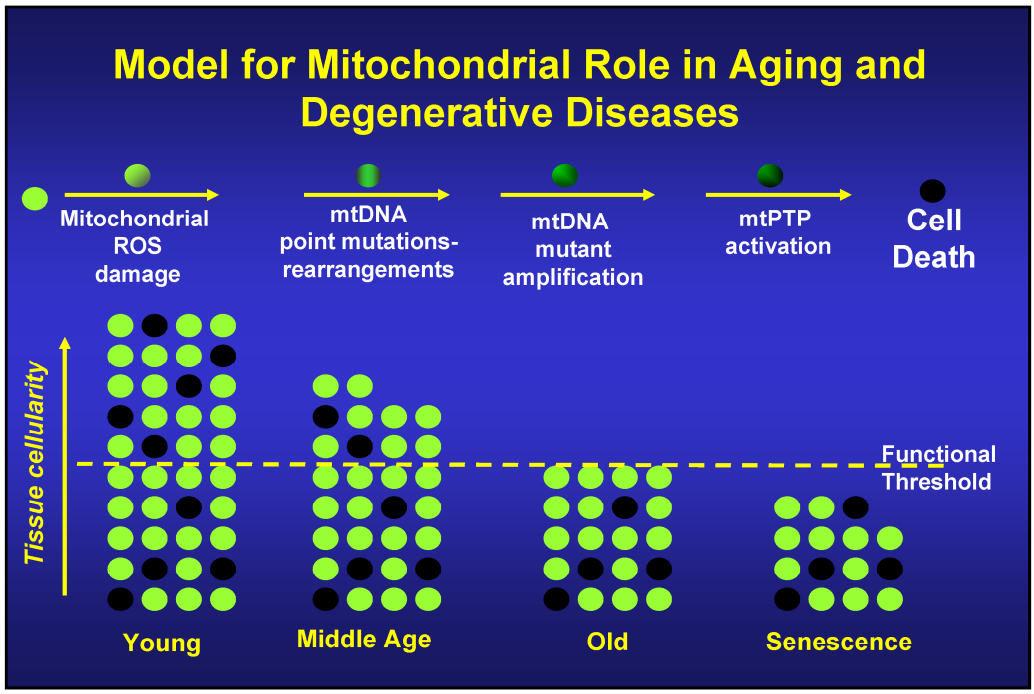
UP-REGULATION OF NF1 & cAMP INCREASES MITOCHONDRIAL COMPLEX I AND DECREASES MITOCHONDRIAL ROS

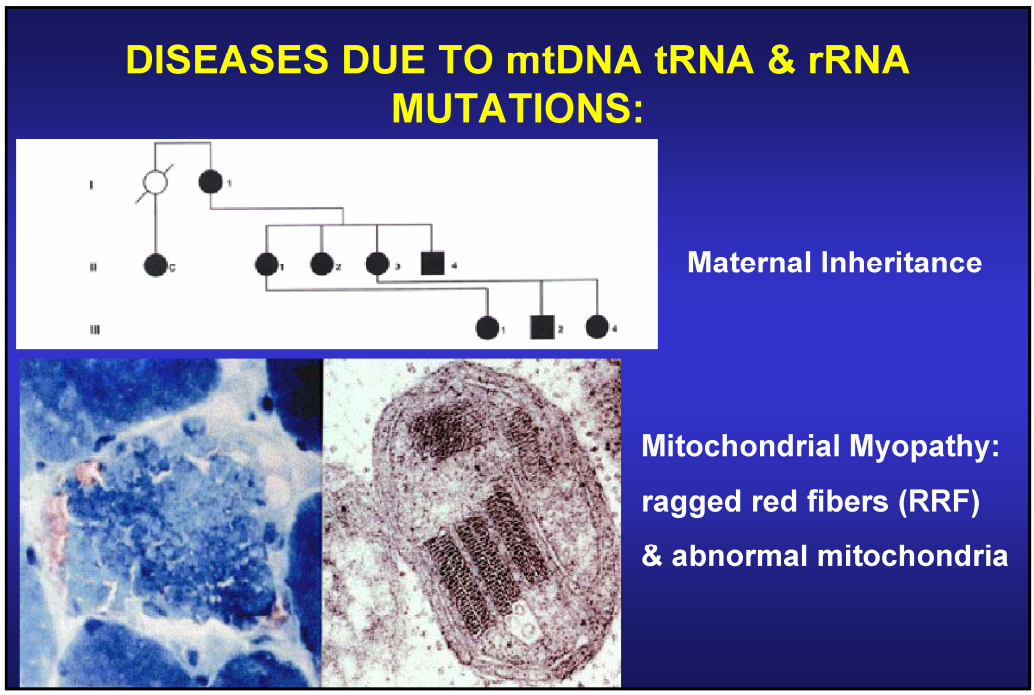
COMPLEX I STATE III RESPIRATION IS INCREASED 25-38%



MITOCHONDRIAL ROS PRODUCTION IS DECREASED 58-59%

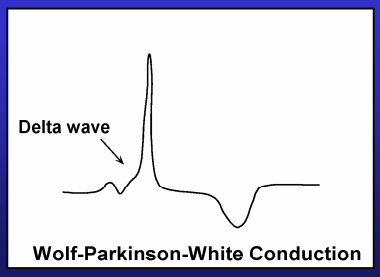



Model for Mitochondrial Role in Aging and Degenerative Diseases
Mitochondrial ROS damage
mtDNA point mutations-rearrangements
mtDNA mutant amplification
mtPTP activation
Cell Death
Tissue cellularity
Functional Threshold
Young
Middle Age
Old
Senescence


DISEASES DUE TO mtDNA tRNA & rRNA MUTATIONS:
Maternal Inheritance
Mitochondrial Myopathy:
ragged red fibers (RRF)
& abnormal mitochondria

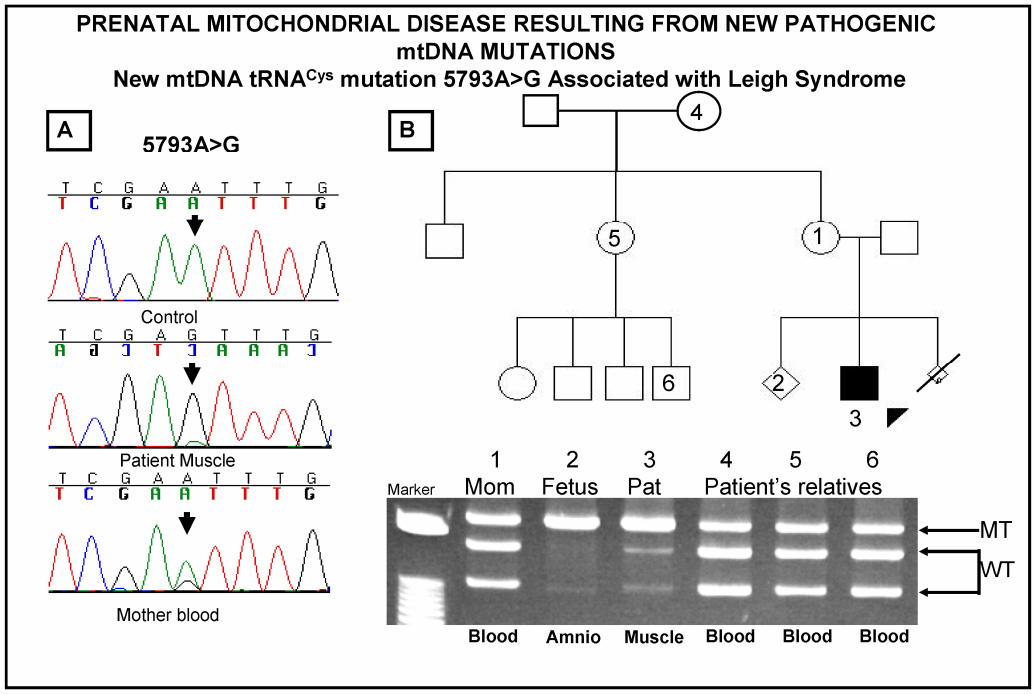
Late Teenage Onset MELAS + Hypertrophic Cardiomyopathy Associated with tRNA$^{Leu(UUR)}$ np 3243 mutation

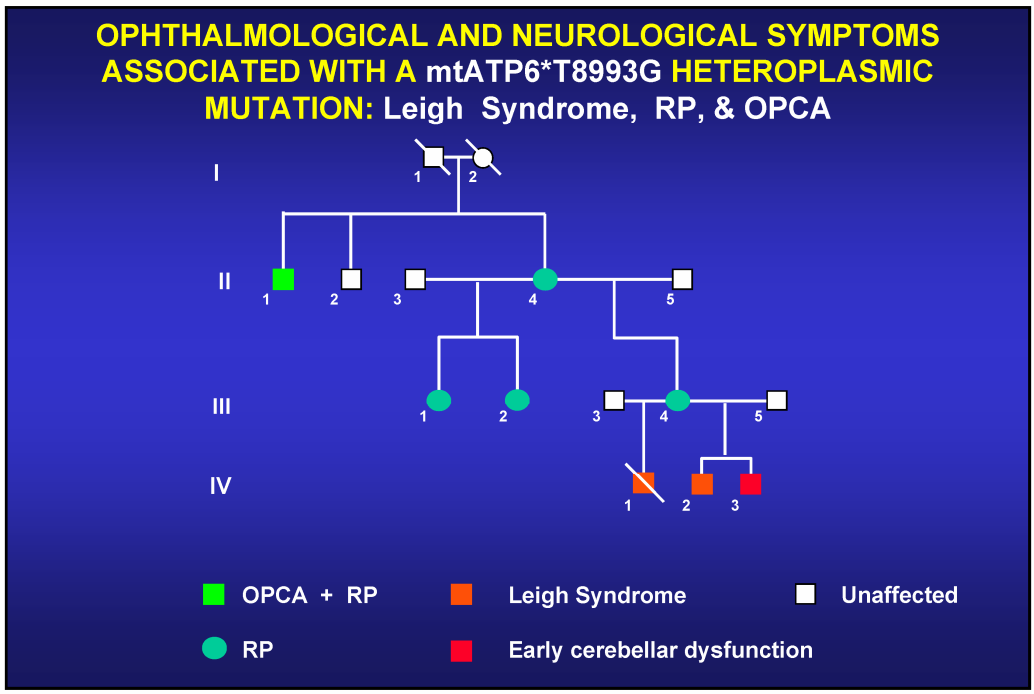
Lactic Acidosis	+	+	+	+	+	+	+	+	+	?	?	+	-
Growth Retardation	+	+	+	+	+	+	+	+	+	?	?	+	-
Dementia	+	+	+	+	+	+							
Stroke-Like Episodes	+	+	+	+	-								
Hypertrophic Cardiomyopathy	+	+	+	+	+	+							
Conduction Abnormalities	+	+		+	+	+							



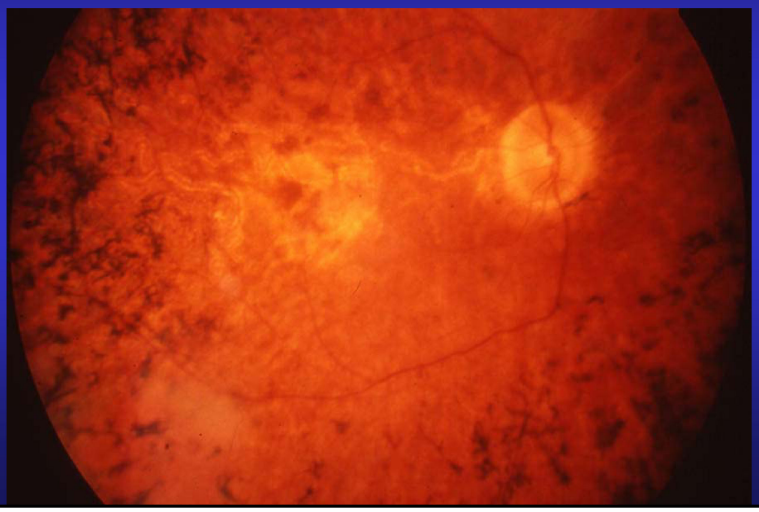
Variation in mtDNA tRNA$^{Leu(UUR)}$ np 3243 Mutation Levels has been Associated with Type I and Type II Diabetes & Autism

% Mutant	Phenotype
70-95	MELAS, Cardiomyopathy, CPEO
10-30	Diabetes, I and II (multiple studies)
1-10	Autism

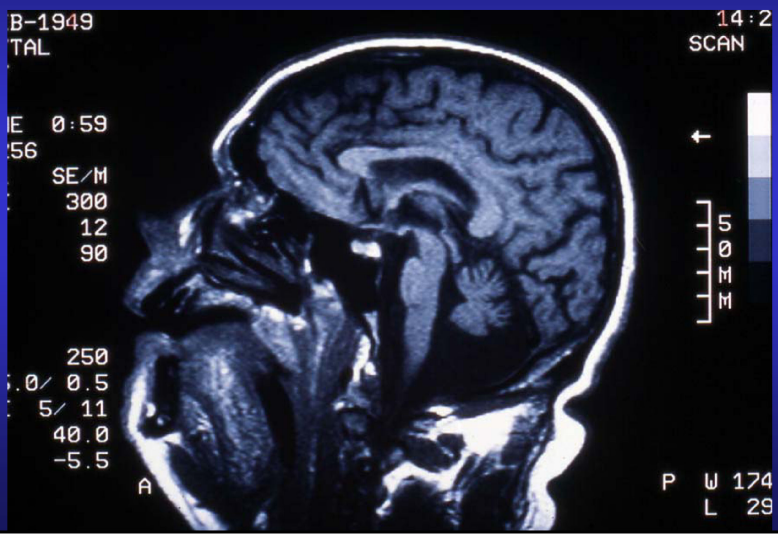

PRENATAL MITOCHONDRIAL DISEASE RESULTING FROM NEW PATHOGENIC mtDNA MUTATIONS
New mtDNA tRNA^Cys mutation 5793A>G Associated with Leigh Syndrome
A
5793A>G
Control
Patient Muscle
Mother blood
B
1 2 3 4 5 6
Marker
Mom
Fetus
Pat
Patient's relatives
MT
WT
Blood
Amnio
Muscle
Blood
Blood
Blood

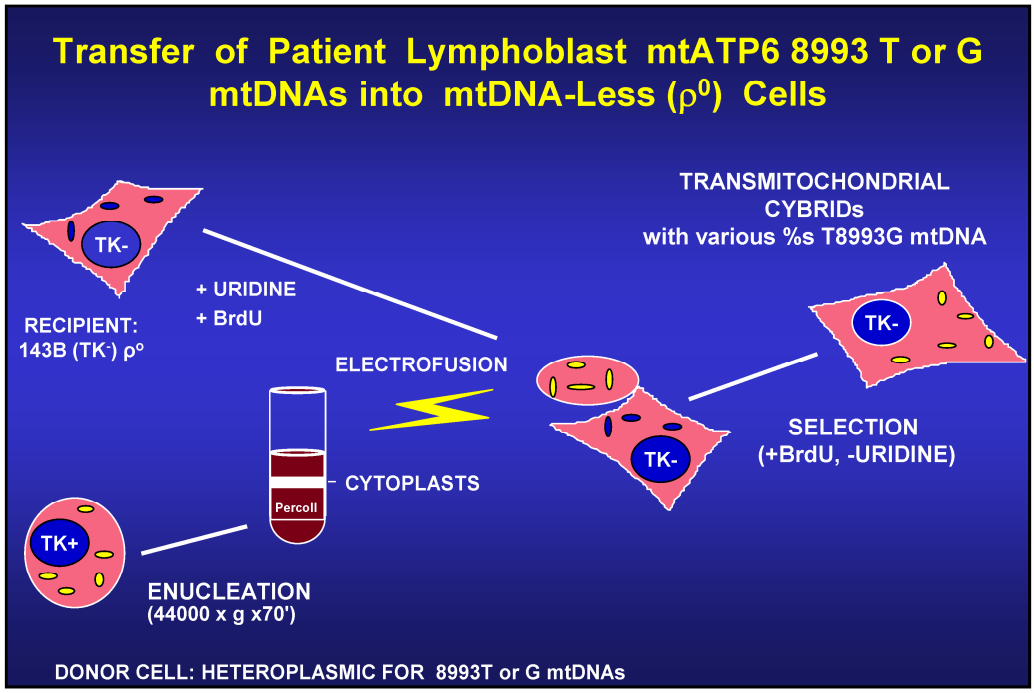

OPHTHALMOLOGICAL AND NEUROLOGICAL SYMPTOMS ASSOCIATED WITH A mtATP6*T8993G HETEROPLASMIC MUTATION: Leigh Syndrome, RP, & OPCA
I
II
III
IV
OPCA + RP
RP
Leigh Syndrome
Early cerebellar dysfunction
Unaffected

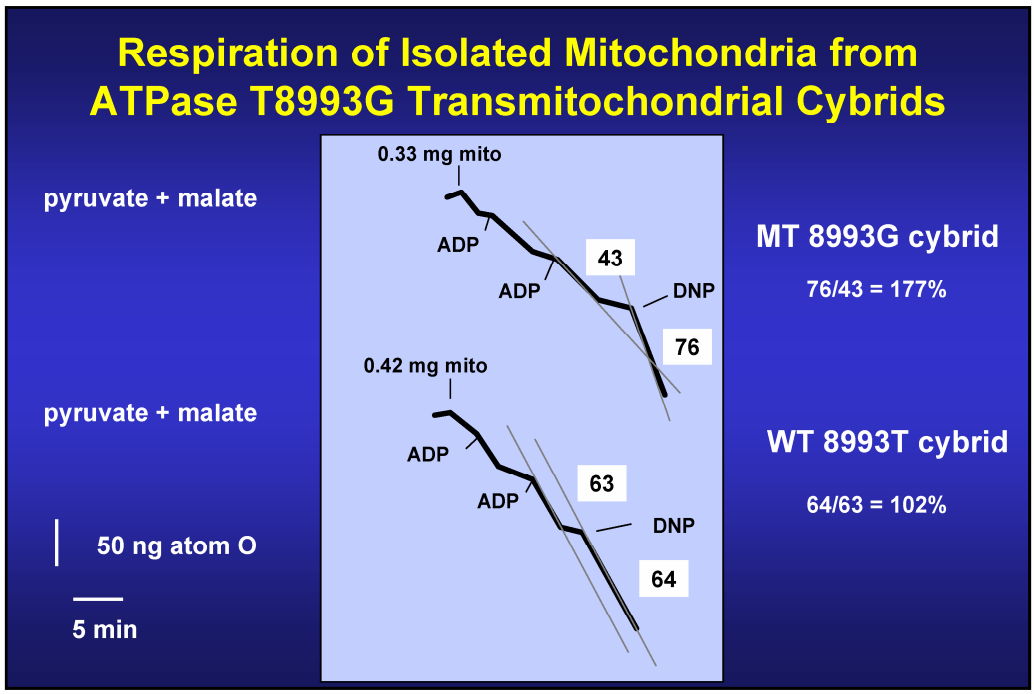
Spicular RP & Macular Degeneration Assocuaed with the mtATP6 T8993G Mutation



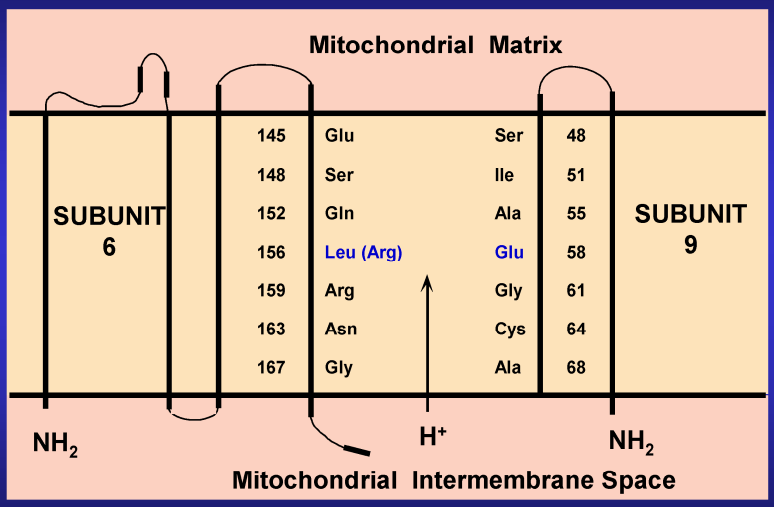
Olivopontocerebellar Atrophy Associated with the mtATP6 T8993G Mutation




Transfer of Patient Lymphoblast mtATP6 8993 T or G mtDNAs into mtDNA-Less (ρ0) Cells
TRANSMITOCHONDRIAL CYBRIDs with various %s T8993G mtDNA
TK-
+ URIDINE
+ BrdU
RECIPIENT: 143B (TK-) ρo
ELECTROFUSION
TK-
TK-
SELECTION (+BrdU, -URIDINE)
Percoll
CYTOPLASTS
TK+
ENUCLEATION (44000 x g x70')
DONOR CELL: HETEROPLASMIC FOR 8993T or G mtDNAs


Respiration of Isolated Mitochondria from ATPase T8993G Transmitochondrial Cybrids
pyruvate + malate
0.33 mg mito
ADP
ADP
43
DNP
76
MT 8993G cybrid
76/43 = 177%
pyruvate + malate
0.42 mg mito
ADP
ADP
63
DNP
64
WT 8993T cybrid
64/63 = 102%
50 ng atom O
5 min

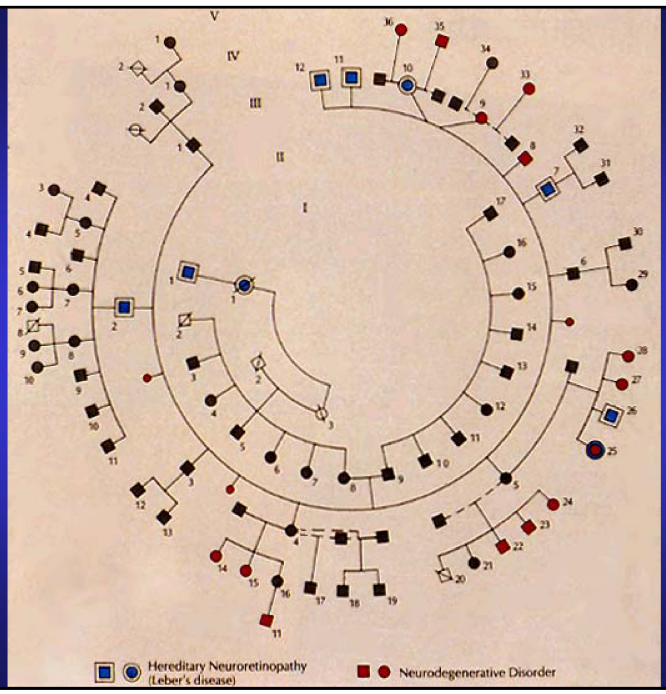
Molecular Consequence of the mtATP6 T8993G Mutation



Tatuch, Y. & Robinson, B.H. (1992) Biochem. Biophys. Res. Comm. 192:124-128

Maternal LHON & Dystonia Pedigree

Due to mtDNA ND6 np 14459 mutation



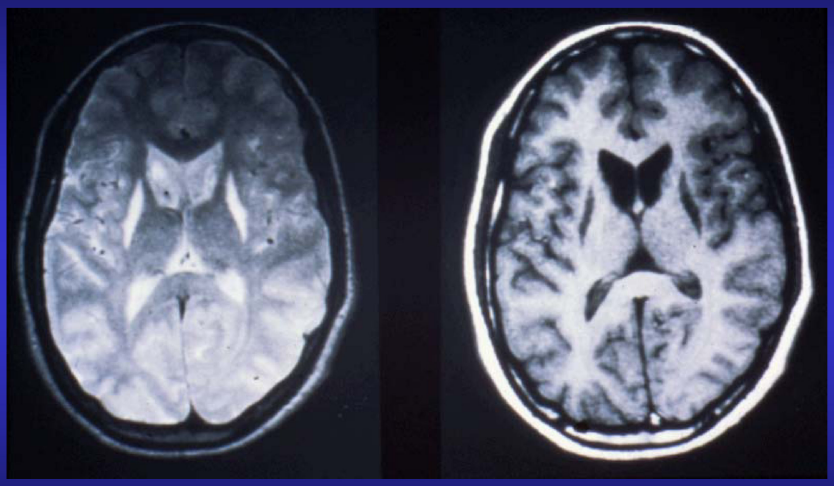
LHON = Mid-Life, Sudden-Onset, Central Vision Loss

Generalized Dystonia & Leber's Hereditary Optic Neuropathy (LHON)

mtDNA mutation at np 14459 in ND6

Generalized Dystonia in Proband

The ND6 np 14459 Mutation Causes Bilateral Sriatal Necrosis

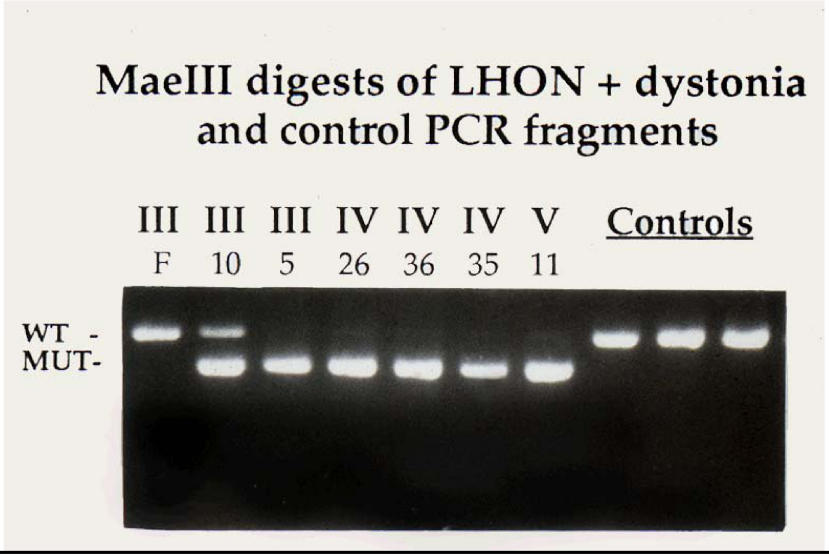


Phylogenetic Comparisons of ND6 np 14459 & np 14484 Amino Acid Mutations

					64								72		
LHON + Dystonia	L	G	G	M	M	V	V	F	G	Y	T	T	V	M	A
LHON	L	G	G	M	V	V	V	F	G	Y	T	T	A	M	A
Human	L	G	G	M	M	V	V	F	G	Y	T	T	A	M	A
Cow	L	G	G	M	M	V	V	F	G	Y	T	T	A	M	A
Fin whale	L	G	G	M	L	V	V	F	G	Y	T	T	A	M	A
Mouse	L	G	G	M	L	V	V	F	G	Y	T	T	A	M	A
Rat	L	G	G	M	L	V	V	F	G	Y	T	T	A	M	A
Xenopus	L	G	G	M	L	V	V	F	A	Y	S	A	A	R	A
Sea Urchin	I	G	G	M	L	V	V	F	P	Y	S	S	A	I	S
Chicken	L	G	G	M	L	V	V	F	V	Y	S	V	S	L	A
Quail	L	G	G	M	L	V	V	F	V	Y	S	V	S	L	A
Fruit fly	L	G	G	M	L	V	L	F	I	Y	V	T	S	L	A
C. elegans	L	S	G	I	F	V	I	L	V	Y	F	S	S	L	S
A. suum	L	S	G	I	F	V	I	L	V	Y	F	S	S	L	S
					14484								14459		

LHON & Dystonia, mtDNA ND6 np 14459

Heteroplasmy versus Symptoms



Leber's Hereditary Optic Neuropathy (LHON) Mutations have Varying Biochemical Severities

Mutation	Gene	Heteroplasmy	~ % Patients	Complex I Defect	AA Conserv.
14459A	ND6	Ht/Hm	5	Very Severe	High
3460A	ND1	Ht/Hm	15	Severe	Moderate
11778A	ND4	Ht/Hm	50	Moderate	Moderate
14484C	ND6	Hm	15	Mild	Low
10663C	ND4L	Hm	<1	Very Mild	Low

Association of the LHON 11778, 14484 & 10663 Mutations with Haplogroup J

11778 Patients % (J / Total)	14484 Patients % (J / Total)	10663 Patient %(J/Total
29 (17 / 58)	79 (30 / 38)	100 (3/3)

Europeans
% (J / Total)

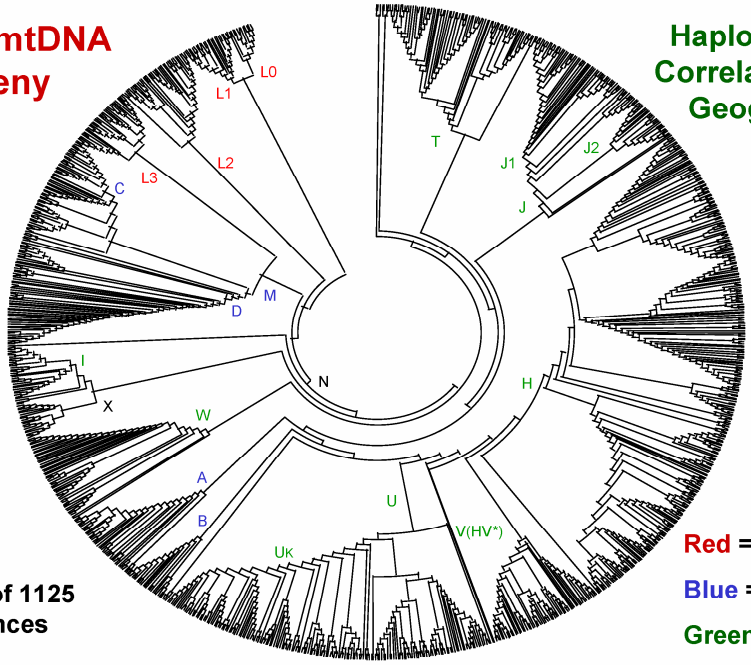
9.5 (39 / 409)

ANCIENT mtDNA VARIATION PERMITTED HUMAN ADAPTATION TO NEW ENVIRONMENTS, BUT NOW PREDISPOSES TO COMMON DISEASES

Surveying Global mtDNA Variation

Global mtDNA Phylogeny

Haplogroups Correlate with Geographic Origin



NJ Tree of 1125 Sequences

Red = Africa

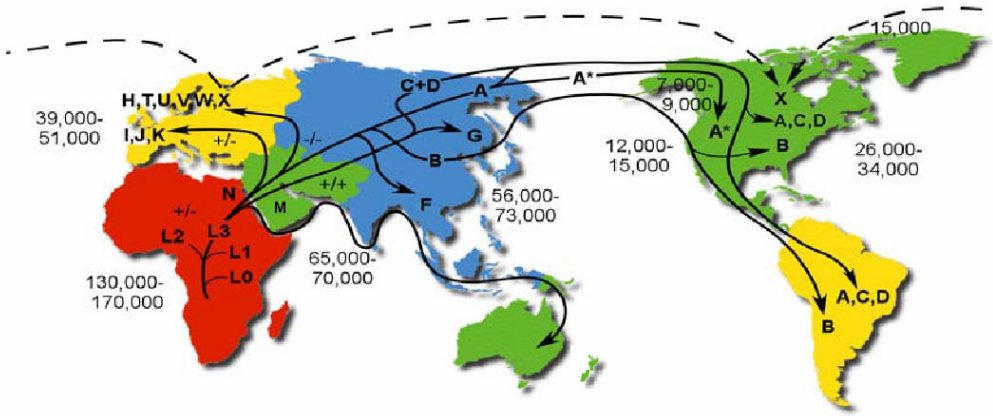
Blue = Asia

Green = Europe

DISTRIBUTION OF mtDNAs & HIMAN MIGRATIONS:

Two Striking Discontinuities:

Tropical Africa vs Temperate Eurasia & Temperate Eurasia vs Arctic



+/-, +/+, or -/- = Dde I 10394 / Alu I 10397
* = Rsa I 16239

Wallace DC, 2005, Ann Rev Genet 39:359-407

Mutation rate = 2.2 – 2.9% / MYR
Time estimates are YBP.

TRANSITIONAL HAPLOGROUPS FOUNDED BY FUNCTIONAL MUTATIONS

Ruiz-Pesini E, Mishmar D, & Wallace DC

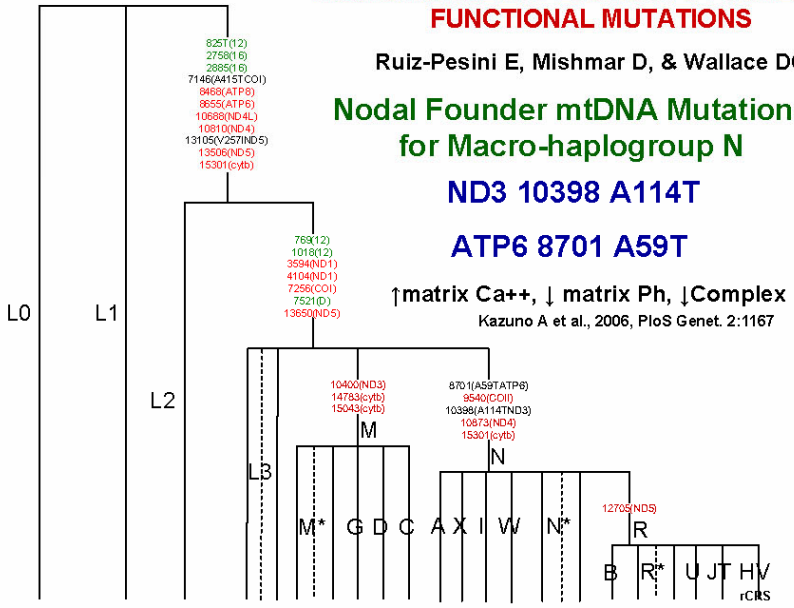
Nodal Founder mtDNA Mutations for Macro-haplogroup N

ND3 10398 A114T

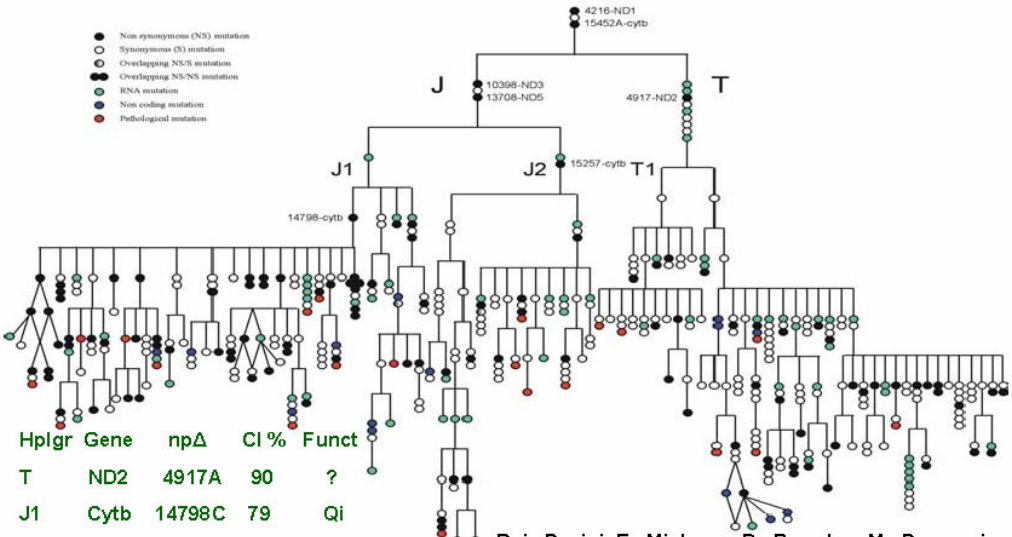
ATP6 8701 A59T

↑matrix Ca++, ↓ matrix Ph, ↓Complex I SpAc

Kazuno A et al., 2006, PloS Genet. 2:1167



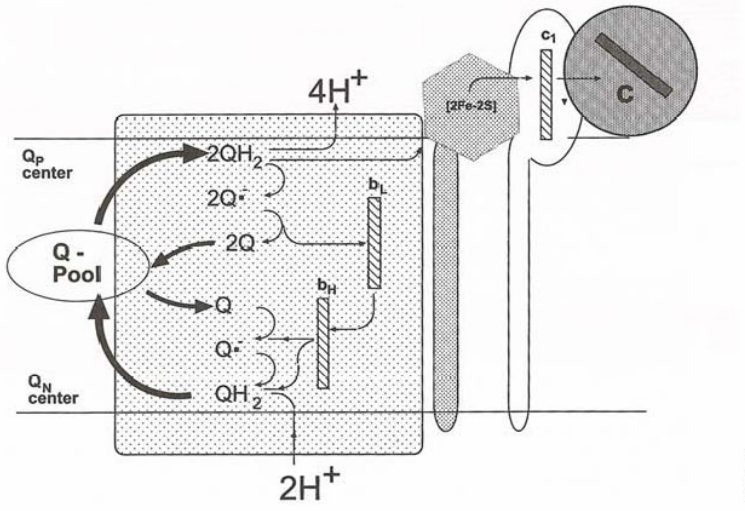
EUROPEAN HAPLOGROUPS T & J NODAL AMINO ACID SUBSTITUTION



Hplgr	Gene	npΔ	Cl %	Funct
T	ND2	4917A	90	?
J1	Cytb	14798C	79	Qi
J2	Cytb	15257A	95	Qo

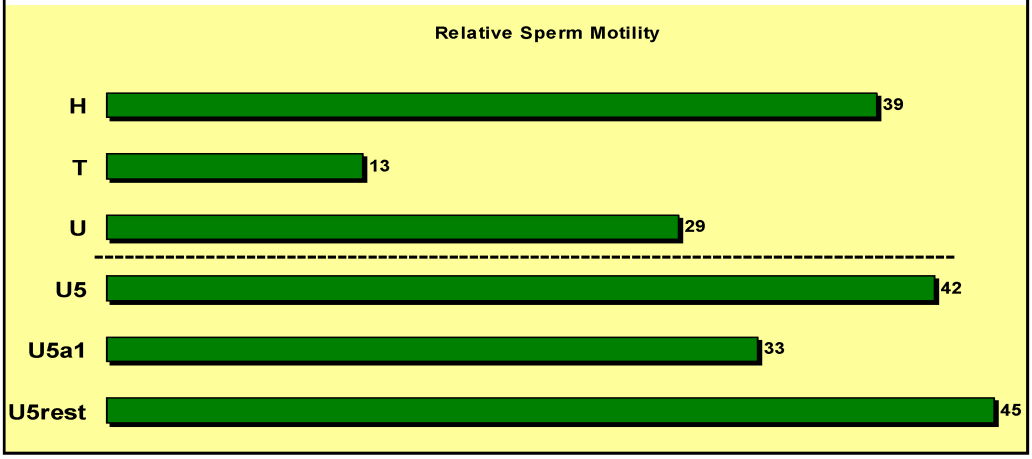
Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V., and Wallace, D.C. *Science* 303:223-226 (2004).

Coupling of Electron Transport to Proton Pumping: Coenzyme Q Cycle of Complex III [bc1 complex]



Scheffler, 1999, Mitochondria, Wiley.

RELATIONSHIP BETWEEN EUROPEAN HAPLOGROUP AND SPERM MOTILITY (% NAST)



mtDNA Genotypes Correlate with Finnish Elite Endurance versus Sprinter Athletes

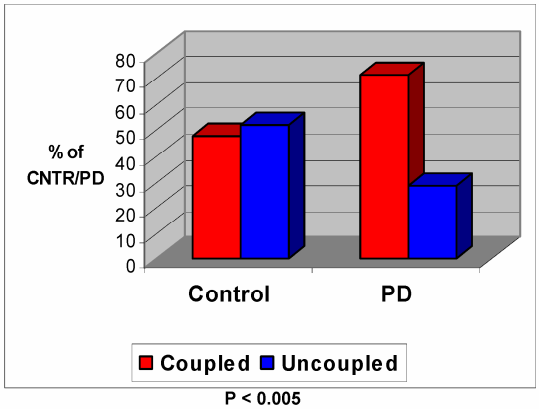
A Nieme A & K Majamaa, 2005, Euro. J. Hum. Genet. 13:965-969.

Table 2 Mitochondrial DNA haplogroup and subhaplogroup frequencies among the endurance and sprint athletes

Haplogroup	*Endurance (n = 52)*		*Sprint (n = 89)*		*Controls (n = 1060)*	
	n	*%*	*n*	*%*	*n*	*%*
H	27	52	42	47	508	48
H1	13	25	22	25	ND	ND
H2	3	5.8	2	2.2	ND	ND
H3	1	1.9	3	3.4	ND	ND
H*	10	19	15	17	ND	ND
V[a]	3	5.8	7	7.9	51	4.8
V1	0	0	2	2.2	ND	ND
V*	2	3.8	5	5.6	ND	ND
Pre-V	1	1.9	0	0	ND	ND
U	11	21	13	15	253	24
U5	9	17	13	15	ND	ND
U8	1	1.9	0	0	ND	ND
U*	1	1.9	0	0	ND	ND
K	0	0	8	9.0	48	4.5
K1[b]	0	0	5	5.6	ND	ND
K*	0	0	3	3.4	ND	ND
T	3	5.8	4	4.5	38	3.6
T1	2	3.8	2	2.2	ND	ND
T2	1	1.9	2	2.2	ND	ND
J	1	1.9	6	6.7	51	4.8
J1	1	1.9	4	4.5	ND	ND
J2	0	0	2	2.2	ND	ND
W	3	5.8	6	6.7	47	4.4
W1	1	1.9	3	3.4	ND	ND
W2	2	3.8	1	1.1	ND	ND
W*	0	0	2	2.2	ND	ND
I	4	7.7	0	0	30	2.8
I1	2	3.8	0	0	ND	ND
I2	2	3.8	0	0	ND	ND
X	0	0	2	2.3	12	1.1
Others	0	0	1	1.1	22	2.1

COLD ADAPTED mtDNAS ARE PROTECTIVE OF PARKINSON DISEASE: Uncoupled Mitochondria Make More Heat and Less ROS

Kushnadinov E & Wallace DC



P < 0.005

HG	PD (TATARS) [FREQ (%)]	CNTRL (TATARS) [FREQ (%)]
H	62 (40%)	37 (20%)
U	24 (16%)	69 (37%)
Uk	2 (1%)	15 (8%)
JT	16 (11%)	23 (14%)
TOTAL	153	183

Coupled

↑ATP, ↓ Heat, ↑ ROS

Uncoupled

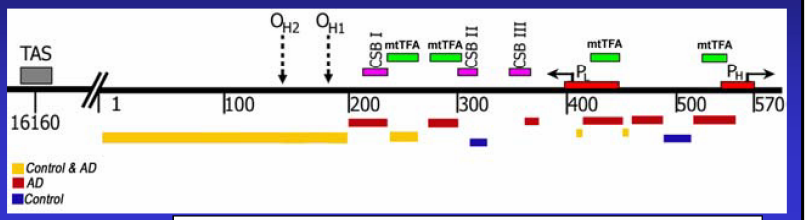
↓ ATP, ↑ Heat, ↓ ROS

P(H) = 0.0007
P(Uk) = 0.0005
P(U) = 0.003

Somatic mtDNA CR Mutations are Increased in Alzheimer Disease Brains

mtDNA control region
Regulatory elements
on HVR2

Regional distribution
Of mutation
accumulation
in AD and control



Frequency of T414G CR Mutation

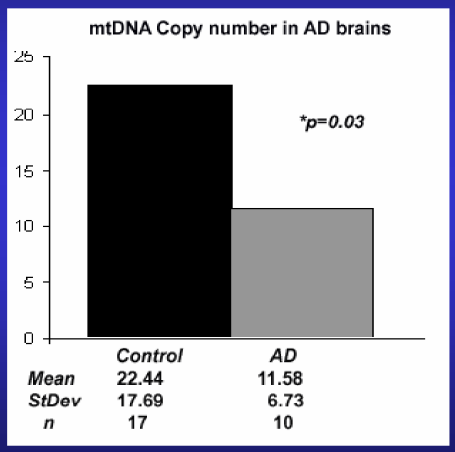
AD Brains 65%

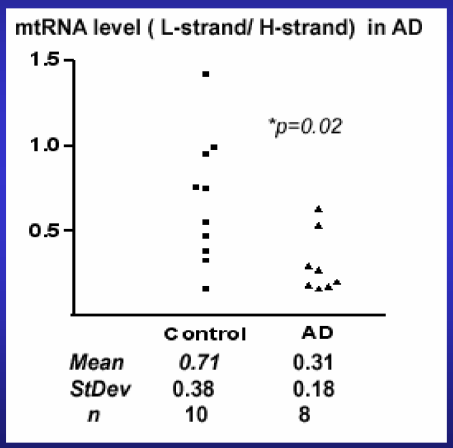
CNTR Brains 0%

Michikawa et al. (1999) *Science* 286:774-779, Murdock et al. (2000) *NAR* 28:4350-4355,

LOCATION	Regulatory elements	LENGTH	AD no. of mutation	CTR no. of mutation
213-235	CSB I	22	7	0
233-260	mtTFA	27	5	4
276-303	mtTFA	27	5	1
299-315	CSB II	16	2	3
346-363	CSB III	17	1	1
392-445	LSP	53	8	1
418-445	mtTFA	27	4	0
523-551	mtTFA	27	3	0
545-567	HSP	22	1	0

Increased mtDNA CR Mutations in AD Brains Correlate with Reduced mtDNA and ND6 mRNA Levels





Cancer in an Age-Related Disease Involving mtDNA Mutations: COI Mutations in Prostate Cancer

Germline COI Mutations

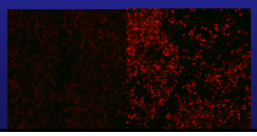
Patients	Controls	Population
10.6 %	0 %	5.5 %
(n=260)	(n=54)	(n=1327)

Recurrent Germline Mutants

Mutation	AA Change	CI	# Patients	Haplogroup:
T6253C	M117T	69	3	H
G6261A	A120T	97	6	J, T, L1, N
C6340T	T146I	79	2	H, N
A6663G	I125V	95	5	O, L2

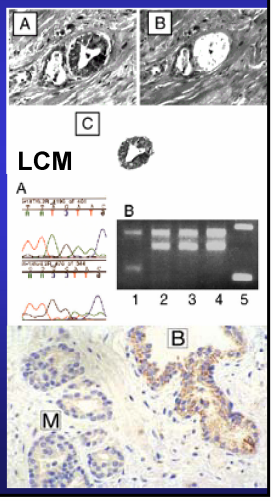
Increased ROS in Tumors with mtDNA Mutants

PC3(mtT8993T)



PC3(mtT8993G)

Somatic COI Mutations



MANY SOMATIC TUMOR mtDNA MUTATIONS ARE THE SAME AS ANCIENT ADAPTIVE POPULATION VARIANTS

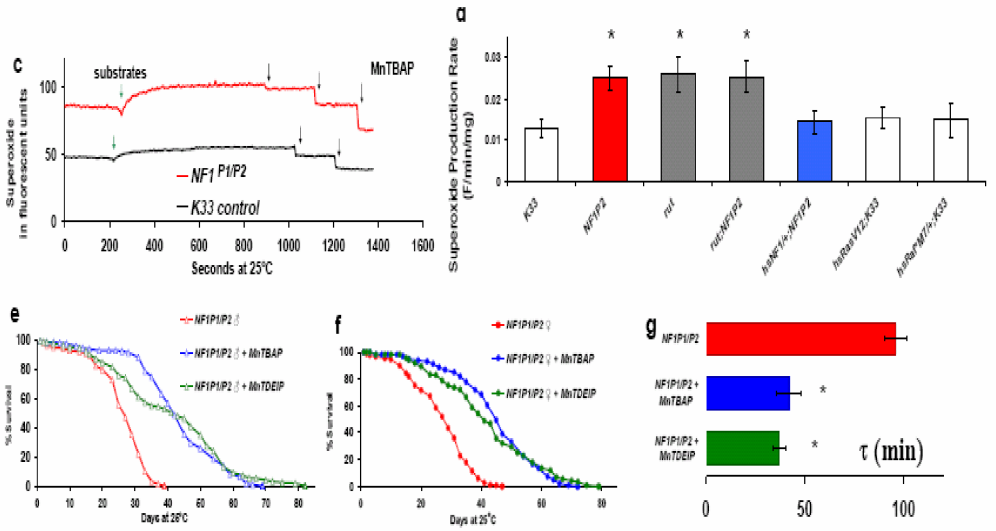
mtDNA Genes	#Pop/#Som	%
Polypeptide	22/42	52
rRNA & tRNA	10/19	52
CR	71/84	85

		Tumor			Population	
Nucleotide Position	Gene	Type	Normal	Tumor	# / 2533	Tumor Base Haplogroup
12308	$tRNA^{Leu(CUN)}$	Prostate	A	G	165	U
1811	16S rRNA	Head/Neck	A	G	98	U1811
7521	$tRNA^{Asp}$	Thyroid	G	A	121	L1 – L2
10398	ND3	Thyroid	A	G	936	L0-L3, UK, B, M, C, D, G
8701	ATP6	Thyroid	A	G	733	L0-L3,M, C, D, G, Z
13708	ND5	Breast	G	A	155	A, D, J, X

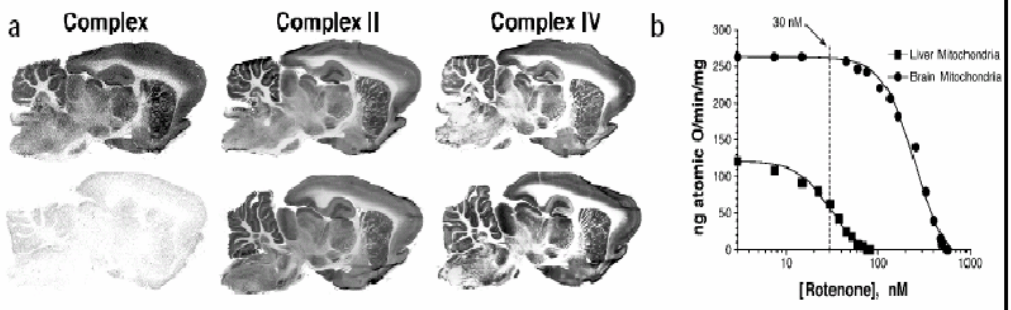
12308 = Jeronimo C et al, 2001, Oncogene 20:5195-5198; 1811 =Fliss et al, 2000, Science 287:2017-2019; 7521 + 10398 = Yeh JJ, et al, 2000, Oncogene 19:2060-2066; 8701 = Maximo V, et al, 2002, A J Path 160:1857-1865; 13708 = Parrella P, et al, 2001, Cancer Res 61:7623-7626.

In *Drosophila NF1* Mutants Increase Mitochondrial ROS and Reduced Life Span: Catalytic Antioxidants can Rescue

Tong J, Schriner S, McCleary D, Day B, & Wallace DC *Nature Genetics* (in Press)

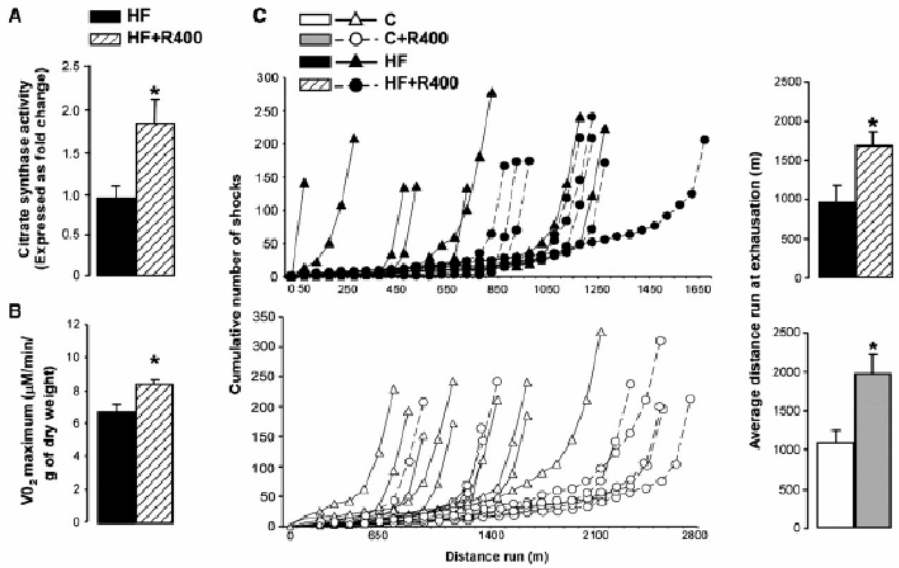


PESTICIDE (ROTENONE) INHIBITION OF OXPHOS COMPLEX I INDUCES DOPAMINERGIC NEURONAL DEATH IN THE SUBSTANTIA NIGRA EQIVALENT TO PARKINSON DISEASE



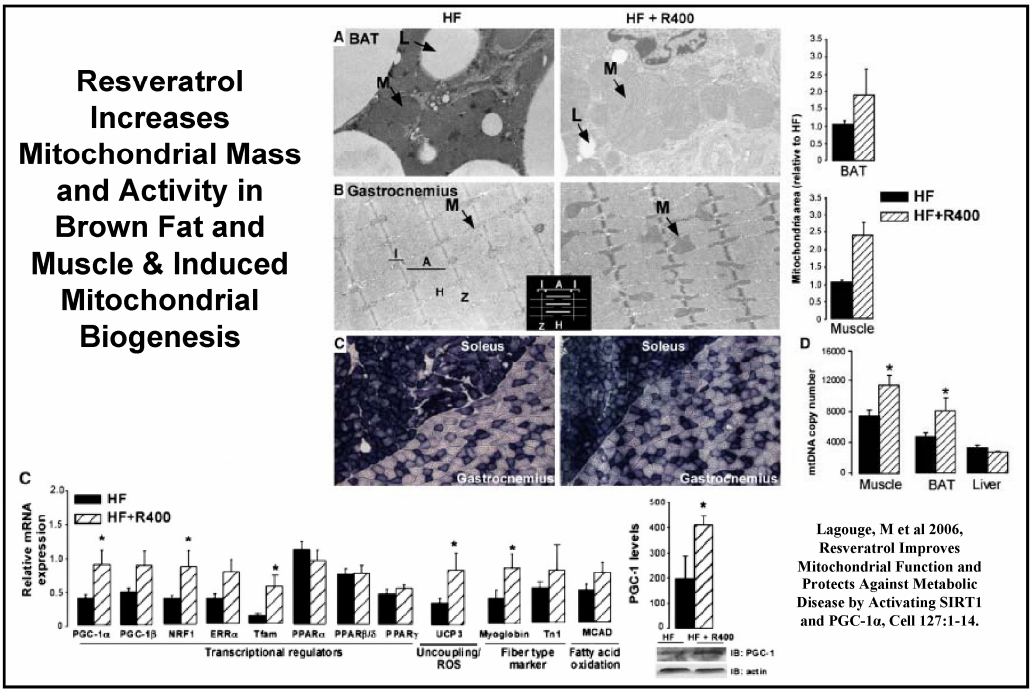
Betarbet, R et al, 2000, Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neurosci.* 3:1301-1306.

Resveratrol from Red Wine Induces Endurance and Oxidative Capacity 100%



Lagouge, M et al 2006, Resveratrol Improves Mitochondrial Function and Protects Against Metabolic Disease by Activating SIRT1 and PGC-1α, Cell 127:1-14.

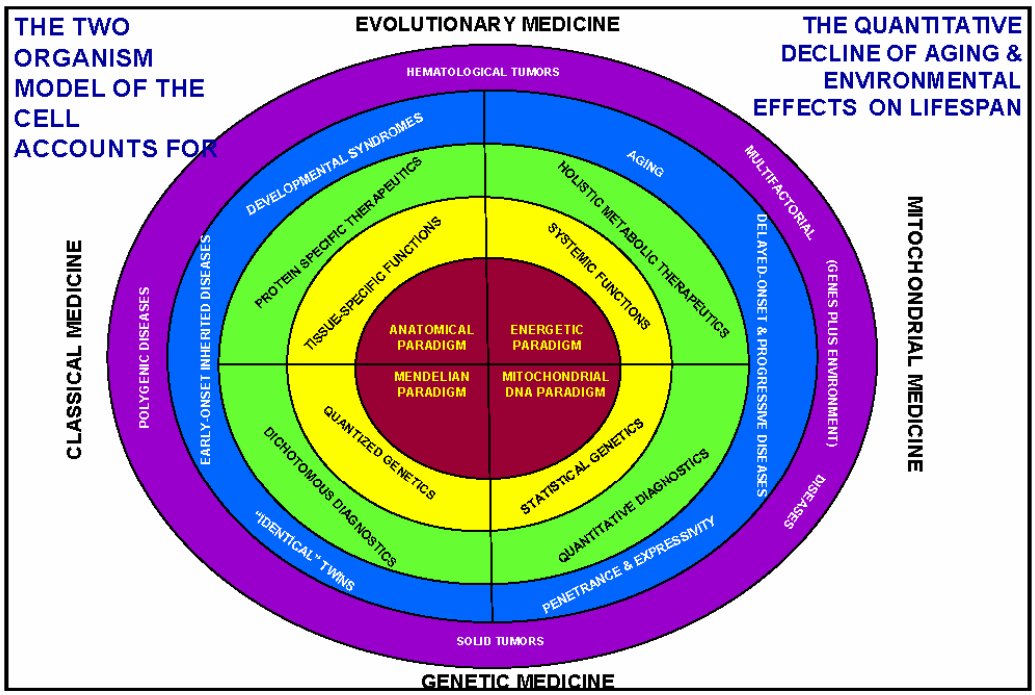
Resveratrol Increases Mitochondrial Mass and Activity in Brown Fat and Muscle & Induced Mitochondrial Biogenesis

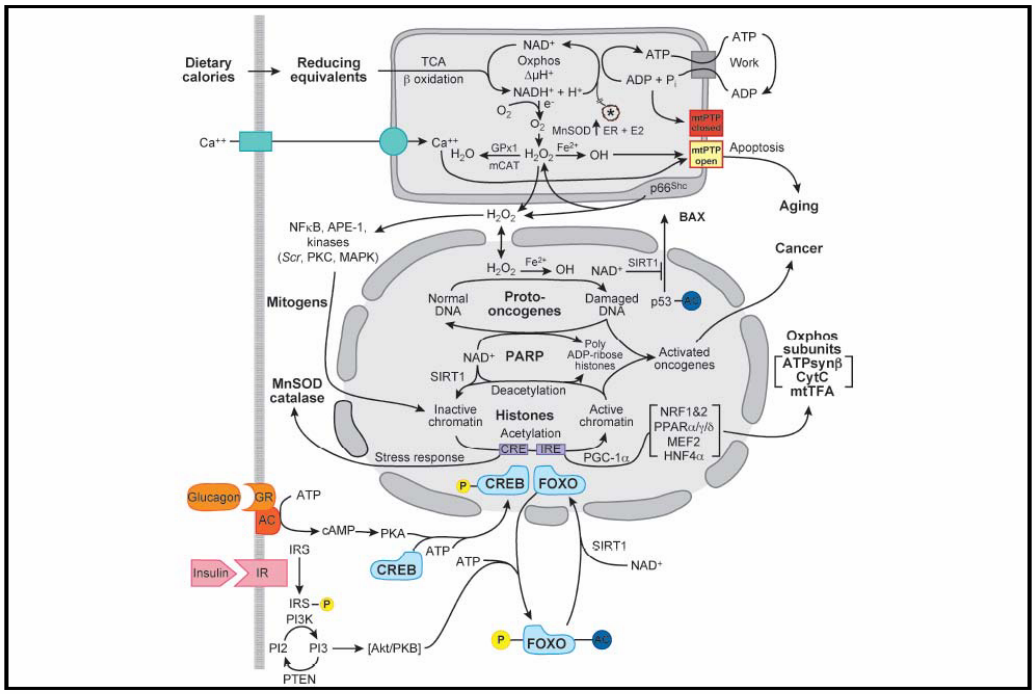


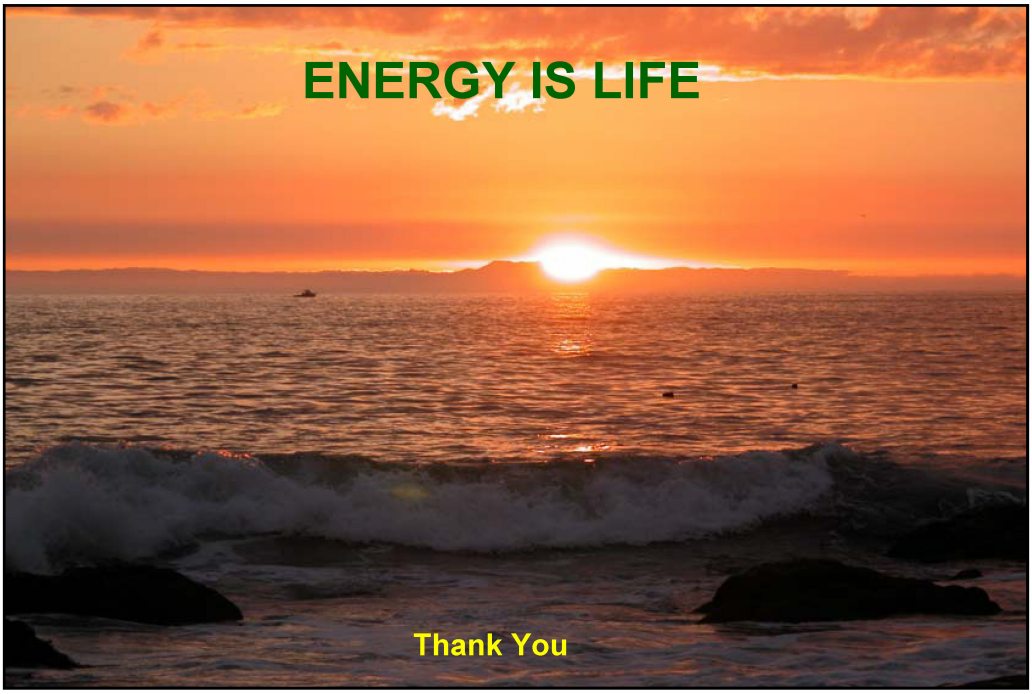
Lagouge, M et al 2006, Resveratrol Improves Mitochondrial Function and Protects Against Metabolic Disease by Activating SIRT1 and PGC-1α, Cell 127:1-14.

MITOCHONDRIAL INTOXICANTS AND THERAPEUTICS

Target	Intoxicants	Therapeutics
mtDNA	Ethidium Bromide mtDNA Mutations Radiation	Resveratrol mDNA Gene Transfer Estrogen
Physiology	ROS Generators & OXPHOS Inhibitors Pesticides & Poisons Rotenone MPTP Pyridostigmine Bromide ? Sarin Organophosphates & carbamides OXPHOS Poisins Cyanide CO Toxins & OXPHOs Inhibitors Antimycin A Oligomycin Atracyloside	OXPHOS Enhancers PPARγ Rosagilazone SIRT1/PGC-1α Agonists Resveratrol OXPHOS Substrates Succinate, CoQ_{10}, Carnitine Antioxidants Lipoic Acid, CoQ_{10}, Vit. E, Vit. C Anti-apoptotics Benzodiazapines


THE TWO ORGANISM MODEL OF THE CELL ACCOUNTS FOR
EVOLUTIONARY MEDICINE
THE QUANTITATIVE DECLINE OF AGING & ENVIRONMENTAL EFFECTS ON LIFESPAN
CLASSICAL MEDICINE
MITOCHONDRIAL MEDICINE
GENETIC MEDICINE
HEMATOLOGICAL TUMORS
SOLID TUMORS
POLYGENIC DISEASES
EARLY-ONSET INHERITED DISEASES
DEVELOPMENTAL SYNDROMES
AGING
MULTIFACTORIAL
(GENES PLUS ENVIRONMENT)
DISEASES
DELAYED-ONSET & PROGRESSIVE DISEASES
PROTEIN SPECIFIC THERAPEUTICS
HOLISTIC METABOLIC THERAPEUTICS
TISSUE-SPECIFIC FUNCTIONS
SYSTEMIC FUNCTIONS
ANATOMICAL PARADIGM
ENERGETIC PARADIGM
MENDELIAN PARADIGM
MITOCHONDRIAL DNA PARADIGM
QUANTIZED GENETICS
STATISTICAL GENETICS
DICHOTOMOUS DIAGNOSTICS
QUANTITATIVE DIAGNOSTICS
"IDENTICAL" TWINS
PENETRANCE & EXPRESSIVITY


Dietary calories
Reducing equivalents
TCA
β oxidation
NAD+
Oxphos
ΔμH+
NADH+ + H+
ATP
ADP + Pi
Work
ADP
O2
e-
O2
MnSOD
ER + E2
mtPTP closed
mtPTP open
Apoptosis
Ca++
Ca++
H2O
GPx1
mCAT
H2O2
Fe2+
OH
p66Shc
Aging
NFκB, APE-1, kinases (Scr, PKC, MAPK)
H2O2
BAX
Cancer
H2O2
Fe2+
OH
NAD+
SIRT1
Mitogens
Normal DNA
Proto-oncogenes
Damaged DNA
p53
NAD+
PARP
Poly ADP-ribose histones
Activated oncogenes
Oxphos subunits
ATPsynβ
CytC
mtTFA
SIRT1
Deacetylation
MnSOD catalase
Inactive chromatin
Histones
Acetylation
Active chromatin
NRF1&2
PPARα/γ/δ
MEF2
HNF4α
CRE
IRE
PGC-1α
Stress response
CREB
FOXO
Glucagon
GR
AC
ATP
cAMP
PKA
IRS
Insulin
IR
CREB
ATP
ATP
SIRT1
NAD+
IRS
PI3K
PI2
PI3
[Akt/PKB]
PTEN
FOXO


ENERGY IS LIFE
Thank You