A MITOCHONDRIAL ETIOLOGY OF COMPLEX DISEASES

Why can’t we understand and cure the common diseases?

Neuropsychiatric Disorders: Autism, ADHD, Schizophrenia, Bipolar Disease, Stress Response, Alzheimer Disease, Parkinson Disease, ALS, Multiple Sclerosis, Blindness, Deafness…

Heart-Muscle: Cardiomyopathy, Myopathy, Chronic Fatigue…

Visceral: Renal, Hepatic, Immunological…

Metabolic: Diabetes, Obesity, Cardiovascular Disease…

Cancer & Aging

Western medicine has approached the common diseases primarily from an anatomical and Mendelian perspective, but

Life = Anatomy + Energetics + Information

Consequently, the role of bioenergetics and non-Mendelian bioenergetic inheritance has been largely ignored.

Our hypothesis is that bioenergetic dysfunction lies at the nexus of the genetic and environmental “causes” of the “common-complex” diseases.
Information Flow in The Cell

DNA

Stored Genetic Information

Replication

Transcription

RNA

Working Copy of Gene (message)

Translation

mRNA + Ribosomes (interpreting structures)

PROTEINS

Yield Structure (skin, hair)
DNA Genetic Code Dictates Amino Acid Identity and Order

DNA Sequence Is the Genetic Code.

GCA AGA GAT AAT TGT... Growing Protein Chain
THE EUKARYOTIC CELL IS COMPOSED OF TWO ORGANISMS

THE MITOCHONDRIA SPECIALIZED IN ENERGY
1000s of mtDNAs + ~1500 nDNA genes
ENERGY: Fats + Sugars + Oxygen = Energy (heat + work) + CO₂ + H₂O

REDOX BALANCE: Thiol-Disulfide Regulation of Pathways and Transcription Factors.

REACTIVE OXYGEN SPECIES (ROS): Oxygen Radicals + Signal Transduction.

Ca²⁺ REGULATION: Regulates Cytosol Ca²⁺, Metabolism, & mtPTP.

APOPTOSIS: Energy ↓ + ROS ↑ = mtPTP Activated → Cell Death (Apoptosis).

EPIGENOMIC REGULATION: Mito. (ATP, acetyl CoA, SAM, α-ketoglutarate) modify epigenome.

MITOCHONDRIAL FUNCTION IS CENTRAL TO HEALTH

ANT1 = heart, muscle, brain.
ANT2 = systemic.
THE MITOCHONDRIAL GENOME IS DISTRIBUTED ACROSS THE nDNA & mtDNA

THE MITOCHONDRIAL GENOME:
~ 1500 nDNA Genes
Dispersed Across the Chromosomes
+ 37 mtDNA Genes

High Mutation Rate: ROS
THE BODY’S ANATLOMY IS DEFINED BY ENERGY
Origin of Tissue-Specific Disease

• **Energy Utilizing Tissues:**
  – **Brain:** High demand-Low reserve.
    ~2% body weight but uses ~20% of the O₂.
  – **Heart, Muscle, Renal, etc.:** Constant demand-High reserve.

• **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 abundant-Insulin signaling.
  – **Pancreatic α Cells:** Glucose limitation-Glucagon signaling.
A MITOCHONDRIAL ETIOLOGY OF COMPLEX DISEASES

Environmental Factors
- Energy Sources
  - Carbohydrates, Fats, Amino Acids
- Energy Uses
  - Growth, Maintenance, Reproduction
  - Toxins

Metabolic
- Type II Diabetes, Obesity
- Hypertension, CVD
- Stress
  - Thermal, Trauma

Trauma & Sepsis, Inflammation & Immunity
- MS, Type I Diabetes
  - (Tregs, DAMPs, NLRP3, TLRs, HMGB1)
- Infection Predisposition
  - MAVS (mt αViral Sigans)

mtDNA Variants
- Ancient Adaptive Polymorphisms
- Recent Deleterious Mutations

nDNA Variation
- Mutations
  - Deleterious Mutations, Mito Gene Polymorphisms
- Epigenomics
  - Histone Modifications, Signal Transduction, Redox Controls

OXPHOS DYSFUNCTION
- ↓ ENERGY, ↑ ROS, Δ REBOX, Δ Ca++

mtDNA Damage & Somatic Mutations

PROGRESSIVE BIOENERGETIC DECLINE
- Apoptosis

Neuropsychiatric Disorders
- ASD, Psychiatric Disorders
- AD, PD, Blindness, Deafness
- Cardiomyopathy
  - Hypertrophic & Dilated
  - Muscle
  - Myalgia, Fatigability
  - Renal Failure

Cancer
- Energy Production, ROS & Redox

Infection Predisposition
- MAVS (mt αViral Sigans)

Penetration & Expressivity
- Delayed-Onset & Progression,
  - Aging

Aging
- Penetration & Expressivity
  - Delayed-Onset & Progression,
mtDNA GENE MUTATIONS GIVE VARIABLE PHENOTYPES
THE SAME mtDNA tRNA\textsuperscript{Leu(UUR)} np A3243G MUTATION CAUSES DIFFERENT DISEASES
10-30% Autism & Type I & II diabetes;
> 70% mutation myopathy, cardiomyopathy & MELAS;
~100% Leigh Syndrome & perinatal lethality

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<th>Condition</th>
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MULTIPHASIC NUCLEAR RESPONSES TO CHANGING mtDNA 3243A>G HETEROPLASMY EXPLAINS PHENOTYPIC VARIATION

LEIGH SYNDROME & PERINATAL LETHALITY

NEUODEGENERATION 50-90%

DIABETES & AUTISM 20-30%

NORMAL 0%

ρ°
LEBER HEREDITARY OPTIC NEUROPATHY MATERNAL INHERITANCE, MALE BIAS, & BACKGROUND REGULATION OF EXPRESSIVITY

Penetrance of Milder Mutations exacerbated by Haplogroup J or ND1 T3394C (Y30H)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gene</th>
<th>~ % Patients</th>
<th>Complex I Defect</th>
<th>AA Cons</th>
<th>% Hplgr J</th>
<th>ND1 3394C</th>
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<tr>
<td>3460A</td>
<td>ND1</td>
<td>15</td>
<td>Severe</td>
<td>Moderate</td>
<td>~10</td>
<td>-</td>
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<tr>
<td>11778A</td>
<td>ND4</td>
<td>50</td>
<td>Moderate</td>
<td>Moderate</td>
<td>29</td>
<td>+</td>
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<tr>
<td>14484C</td>
<td>ND6</td>
<td>15</td>
<td>Mild</td>
<td>Low</td>
<td>79</td>
<td>+</td>
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mtDNA ND4 np 11778 G>A arginine 340 to histidine

MATERNALLY INHERITED OPTIC NEUROPATHY, VARIABLE PENETRANCE, & 4:1 MALE BIAS
ANCIENT mtDNA VARIANTS PREDISPOSE TO COMMON DISEASES
mtDNA VARIATION CORRELATES WITH THE GEOGRAPHIC LOCATIONS OF INDIGENOUS PEOPLES.

Groups of Related mtDNA Haplotypes (Haplogroups) were Founded by Adaptive Variants that Permitted Migration into New Environments.

Mutation rate = 2.2 – 2.9% / MYR
Time estimates are YBP.
TRANSITIONAL HAPLOGROUPS
FOUNDED BY FUNCTIONAL MUTATIONS

Nodal Founder mtDNA Mutations for Macro-haplogroup N

ND3 G10398A A114T
ATP6 G8701A A59T

Membrane Potential, Altered Ca++
Kazuno A et al., 2006, PloS Genet. 2:1167
NODAL SUBSTITUTIONS ALTERING CONSERVED AMINO ACIDS INITIATE EACH mtDNA HAPLOGROUP
EUROPEAN HAPLOGROUPS T & J

<table>
<thead>
<tr>
<th>Haplogroup (Hplgr)</th>
<th>Gene</th>
<th>npΔ</th>
<th>CI %</th>
<th>Function (Funct)</th>
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<td>T</td>
<td>ND2</td>
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<tr>
<td>J1</td>
<td>Cytb</td>
<td>14798C</td>
<td>79</td>
<td>Qi</td>
</tr>
<tr>
<td>J2</td>
<td>Cytb</td>
<td>15257A</td>
<td>95</td>
<td>Qo</td>
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The image contains a diagram of genetic sequences and a text summarizing the findings of the European Association of tRNA^{Gln} A4336G & ND1 (M31V) with Alzheimer & Parkinson Disease.

### Key Findings

- **Haplogroup H5a**
  - One event = ancient polymorphism

- **ND1 A3397G Met 31Val**
  - Two independent events = recent deleterious mutation

- **Genetic Variations**
  - AD = 3.3%
  - PD = 5.3%
  - AD+PD = 6.8%
  - CNTL = 0.4%
mtDNA AMINO ACID SUBSTITUTIONS CAN BE POSITIVE OR NEGATIVE DEPENDING ON CONTEXT
M [ND3 10398G (114A)] IS ENRICHED IN TIBET & [ND1 3394C (30H)] IS PATHOGENIC ON N BUT ADAPTIVE ON M IN TIBETANS
**THE ND1 3394C (30H) MUTATION CAUSES A COMPLEX I DEFICIENCY ON N HAPLOGROUPS B4 & F1, BUT IS DOES NOT IMPAIR COMPLEX I ON M HAPLOTYPE M9**

**COMPLEX I SPECIFIC ACTIVITY**

![Graph showing NADH: coenzyme Q oxidoreductase activity](graph)

Blood Platelets X 143B (TK-) po + BrdU, - uridine → Cybrids
ASSOCIATION BETWEEN mtDNA HAPLOGROUPS AND AUTISM SPECTRUM DISORDER RELATIVE TO HAPLOGROUP RO

Illumina 550 GWAS data set. Generalized Linear Modeling Analysis
Results with GEE Solution for the AGRE Family mtDNA SNPs Relative to R0 (H-HV-V)

<table>
<thead>
<tr>
<th>Continent Co-Variant</th>
<th>Haplogroup</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-Value</th>
<th>Benjamini-Hochberg Correction</th>
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<td>Europeans</td>
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<td>T</td>
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<td>N+</td>
<td>2.02</td>
<td>1.19-3.42</td>
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<td>Africans</td>
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<td>1.01</td>
<td>0.63-1.61</td>
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<td>0.98</td>
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<td>Gender (Male)</td>
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<td>3.93</td>
<td>3.3-4.67</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>

(N_{ALL}=4041, N_{ASD}=1624, 933 families with 1-12 members)
(reference haplogroup = HHV+, N_{ALL}=1792, N_{ASD}=712)

Covariates: haplogroups, sex.
ASSOCIATIONS BETWEEN mtDNA HAPLOGROUPS & COMMON DISEASES

• NEURODEGENERATIVE DISEASES
  – Autism
  – Alzheimer Disease
  – Parkinson Disease
  – Macular Degeneration
  – Familial Amyloidosis with Polyneuropathy
  – Migraine
  – Psychiatric Disorders

• NEUROLOGICAL DISEASES
  – Stoke

• METABOLIC DISEASES
  – Diabetes
  – Cardiovascular Disease
  – Metabolic Syndrome

• INFLAMMATORY & INFECTIOUS DISEASES
  – Sepsis
  – IgE Levels
  – Asthma
  – AIDS progression
  – Anti-AIDS HAAT* Lipodystrophy
  – Osteoarthritis

• AGING

• CANCERS

• ATHLETIC PERFORMANCE (L0>L3>N>H>J-U-T)
  * HAAT- highly active anti-retroviral therapy
HETEROPLASMIC mtDNA MUTATIONS SEGREGATE RAPIDLY
A HUMAN HETEROPLASMIC mtDNA ND6 G14600A P25L MUTATION SEGREGATES TO GIVE VARIOUS PHENOTYPES

Leigh Syndrome

Lactic Acidosis
SN Deafness
Epileptic Seizures
Metabolic Failure
Muscle Myopathy
Cerebral atrophy
Death

Optic Atrophy
Cerebellar Atrophy

Malfatti –Zevani  
*Brain (2007), 130, 1894–1904*
MOUSE MODELS OF mtDNA DISEASE:

ND6 = NEURODEGENERATION
COI = CARDIOMYOPATHY & METABOLIC SYNDROME
129 + NZB = NEUROPSYCHIATRIC DISEASE

Mouse ND6 nt G13997A (P25L) = Human ND6 G14600A (P25L),
COI nt 6589 T>C (V421A), &
129 + NZB Heteroplasy

Mouse Cell Line Mutants
ND6 13997G>A (P25L) mtDNA,
COI nt 6589 T>C (V421A) mtDNA,
129 + NZB mtDNA

Disaggregate Female ES Cells:
ND6 13997G>A (P25L) or
COI nt 6589 T>C (V421A) mtDNA,
129 + NZB Heteroplasmic mtDNAs

ES Cell cybrids

R6G

Female ES Cells:
ND6 13997G>A (P25L) or
COI nt 6589 T>C (V421A) mtDNA
129 + NZB Heteroplasmic mtDNAs

Female Chimera

R6G

Pseudopregnant mother

ND6 13997G>A (P25L),
COI nt 6589 T>C (V421A),
129 + NZB Heteroplasmic mtDNA Mice
THE mtDNA ND6 P25L MUTATION RESULTS IN ELEVATED REACTIVE SPECIES (ROS) PRODUCTION AND NEUROLOGICAL DISEASE

NEURONAL DEGENERATION

INCREASED ROS PRODUCTION, NORMAL ATP

ALTERED EEGs
THE COI V421A mtDNA MOUSE EXHIBITS MYOPATHY, CARDIOMYOPATHY & METABOLIC SYNDROME

COI (6589T>C, V421A) MISSENSE MUTATION

Mitochondrial Myopathy

Mitochondrial Cardiomyopathy

Glucose Intolerance & Insulin Resistance

**Graphs and images showing glucose and insulin levels over time.**
MATERNAL TRANSMISSION OF NORMAL mtDNA HETERPLASMY

CREATION OF NZB-129 HETEROPLASMIC MICE

91 “129” vs “NZB” mtDNAs differences: 15 aaΔ +5 tRNA +7 rRNA + 11 CR

Backcrossed 20 generations onto C57BL/6L nDNA.
Permitted nZB-129 mtDNAs to segregate.
Correlated mtDNA NZB-129 genotypes with behavior.
129-NZB mtDNA HETEROPLASMIC MICE ARE DEPRESSED & HAVE LONG TERM MEMORY DEFICITS

BIASED GERMLINE SEGREGATION OF mtDNA

SPONTANEOUS

HOMOPLASMIC
129 mtDNA
(stable)

NORMAL

ABNORMAL METABOLISM, ACTIVITY AND BEHAVIOR

HETEROPLASMIC
mtDNA
(unstable)

HOMOPLASMIC
NZB mtDNA
(stable)

NORMAL

Primary Latency (s)

Acquisition (day)
MITOCHONDRIAL ALTERATIONS MODULATE NEUROENDOCRINE RESPONSES TO ACUTE STRESS

Modulation of Corticosterone: the Hypothalamic-Pituitary-Adrenocortical (HPA) axis

Modulation Catecholamines: of the Sympathetic-Medullary Axis
ANT1 MITOCHONDRIAL DYFUNCTION IMPAIRS INTERNEURON MIGRATION

Partial Mitochondrial Defects Inhibit Tangential Inhibitory Interneuron Migration but not Radial Excitatory Neuron Migration

Cortical Function Requires Excitatory Glutamateric-Inhibitory GABAergic Neuronal Balance

Excitation-Inhibition Imbalance May Cause Epilepsy, ADHD, Autism, Manic-Depressive Disorder, etc.

Pyramidal Neurons

Interneurons

Excitatory Glutamateric Neurons Migrate Radially while the Inhibitory GABAergic Interneurons Migrate Tangentially from the Medial Ganglionic Eminence (MGE).
ANT1−/− IMPAIRS INTERNEURONAL MIGRATION = EXCITATION-INHIBITION IMBALANCE
ANT1-ND6-NNT DEFICIENCY YIELDS AUTISM ENDPHENOTYPES

ANT1−/− Inactivation Disorients Interneuron Migration

Social Interactions

ANT1 Inhibitor Bongkrekic Acic (BA) Disorients Interneuron Migration

![Image of brain sections showing interneuron migration with and without ANT1 inhibitor](image-url)
MITOCHONDRIA MEDIATE ENVIRONMENTAL-GENOMIC INTERACTION