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POWER FAILURE

DOES **MITOCHONDRIAL DYSFUNCTION** LIE AT THE HEART OF COMPLEX, COMMON DISEASES?

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POWER FAILURE

Does mitochondrial dysfunction lie at the heart of common, complex diseases like cancer and autism?

By Megan Scudellari

Mitochondria are tiny. A single human cell can contain hundreds to thousands of these potato-shaped organelles, depending on the tissue type. They power the biochemical reactions in our cells through the production of adenosine triphosphate (ATP).

These oft-overlooked furnaces, not studied in earnest until the 1970s, are now the subject of intense scrutiny for their potentially central role in common, complex diseases. They may be, scientists say, pivotal to the etiology of diseases such as cancer and Alzheimer's, epidemics against which researchers and companies have spent billions of dollars but made arguably little progress.

But not everyone agrees with the mitochondrial hypothesis. Complex diseases are simply that, some researchers argue—complex. While mitochondria are essential to human physiology, there has not been sufficient evidence to prove that mitochondrial dysfunction plays a causative role in complex diseases. When it is implicated, debate ensues over whether errors in energy production contribute to disease pathology or are simply a consequence of it. "The question remains, as it should, how often [are mitochondria] a major player?" asks Marvin Natowicz, a clinician specializing in autism and mitochondrial disease at the Cleveland Clinic in Ohio.

It doesn't help that studies of human mitochondrial function are invasive, costly, and lengthy. But over the last five years, a growing number of papers by researchers around the world have implicated dysfunctional mitochondria in many elusive diseases, including Parkinson's, autism, and aging. And leading the charge is an unlikely champion, a respected and renowned member of the National Academy of Sciences who is simultaneously a self-proclaimed radical and zealot: a man about whom colleagues hesitate to comment, a maverick known for mounting a soapbox to hold forth on the "vital force," Eastern medicine, and $E=mc^2$.

On a brisk February morning, Douglas Wallace walks through the halls of the Center for Mitochondrial and Epigenomic Medicine, a new research center at the Children's Hospital of Philadelphia, spouting philosophy. "Every one of the diseases we can't solve is absolutely logical if we put energy at the center," he says. "I believed that in 1970 and I believe it now."

A short, cheery man with gold-rimmed glasses, a yellow and green paisley tie and oversize pants held up by blue suspenders, Wallace is a founder of the field of human mitochondrial genetics. As a researcher he has published over 230 papers and is consulted by clinicians about some of the world's trickiest diseases. But he is also a man on a mission to convince the scientific establishment that they've got it all wrong.

Medicine fails to solve many of today's common, complex diseases, Wallace asserts, because the fundamental paradigm is wrong: the medical establishment has spent far too long focusing on anatomy and ignoring energy—specifically, mitochondria.

It has been his tune for more than 30 years, though it's often fallen on deaf ears in the scientific community. But today, the idea that energy deficiency plays a major role in human disease appears to be gaining momentum, as more and more papers link

KEVIN HAND

Douglas Wallace analyzes frozen patient cell lines to identify molecular defects affecting mitochondrial function that lead to disease.

mitochondrial dysfunction to disease. The shift has prompted Children's Hospital to put their money behind his research, and has caused many in the community to wonder: Is Doug Wallace crazy? Or is he right?

A Pandora's box of mutations

Mitochondria generate energy in the form of ATP by combining nutrients and oxygen in a chemical reaction called oxidative phosphorylation (OXPHOS). The mitochondrion is hypothesized to have originated as a bacterium engulfed by another cell some two billion years ago. Mitochondrial DNA (mtDNA) is circular, with 37 genes, 13 of which encode subunits of enzymes involved in OXPHOS and so are analogous to the wiring diagram for a power plant. (See "Mitochondria at Work" on opposite page.) More than a thousand additional genes in the nucleus of the cell (nDNA) are involved in the maintenance, growth, and replication of mitochondria, and around 80 of those nuclear genes code for proteins involved directly in OXPHOS. While nDNA is inherited from both the mother and the father, in 1980 Wallace demonstrated that human mtDNA is inherited only from the mother.

In 1988, Wallace took our understanding of mtDNA a step further: He discovered, for the first time, that mutations in mtDNA cause disease. He identified a point mutation in a protein subunit that results in Leber's hereditary optic neuropathy, a form of midlife blindness.[1] Shortly after, Wallace identified an mtDNA mutation associated with a form of progressive epilepsy accompanied by muscle weakness.[2] It was his first glimpse into a Pandora's box of diseases caused by mutations in that small, circular DNA. Today more than 400 point mutations, as well as innumerable mtDNA rearrangements, are linked to heart and muscle disease, epilepsy, deafness, blindness, anemia, and more.[3] In addition, mutations in nDNA genes can cause mitochondrial disease, as can combinations of nDNA and mtDNA mutations.[4]

"At the moment, there are about 120 different [mitochondrial] genetic disorders, and there are probably as many again to be discovered," says David Thorburn, head of mitochondrial research at the Murdoch Childrens Research Institute in Victoria, Australia. A primary mitochondrial disease—one caused by a mutation in mtDNA—is not easy to diagnose and often involves many organ systems, including heart, brain, muscle, and gastrointestinal tract. "We used to say, if three or more systems are involved, think mitochondria," says Marni Falk, a pediatrician at Children's Hospital of Philadelphia and a leading mitochondrial researcher. In addition to the production of ATP, mitochondria regulate calcium control in the cell and guide cell death. "They're like the conductor of the orchestra," says Falk. "When they're not working, all is disrupted."

Sadly, there is a dearth of therapies for well over 95 percent of primary mitochondrial disease cases. "The treatments we hoped would prove effective have been really disappointing," says Marc Yudkoff, chief of child development and rehabilitation medicine at Children's Hospital. "The area of mitochondrial disease has become our most pressing concern." Children's Hospital is a hub of research into primary mitochondrial diseases and treatments for their victims, with hundreds of cases referred each year—and "the pace is increasing," says Falk. In 2007, she established a mitochondrial research group at the hospital, bringing together over 175 specialists in numerous fields—from endocrinology to anesthesiology to hematology to surgery—to spark collaborations to identify new biomarkers and treatments for such diseases.

But beyond the need for therapies and research into primary mitochondrial diseases, Wallace believes there is an even larger, unrecognized chasm in the medical community. Over the last fifty years, despite billions of dollars in funding, the medical community has failed to discover causes or treatments for many common, complex diseases: heart disease, Alzheimer's, autism, and more. Wallace attributes that continuing failure to the fact that clinicians and researchers base medical training and treatments on anatomy. If someone has a headache, for example, doctors look to the head. If the patient has chest pain, a clinician examines the heart or lungs. Doctors are taught organ-specific medicine in school, and the NIH still organizes its research centers based largely on organ systems: the National Eye Institute, the National Heart, Blood, and Lung Institute, and the National Institute of Diabetes and Digestive and Kidney Diseases, for example. But life is structure plus energy, argues Wallace, and we've been missing the second half of that equation.

It is "self-evident" in some ways, says Yudkoff. Disease is caused by a loss of organization—by entropy—which is essentially a loss of energy. "On a basic physical and chemical level, it's not arguable," he says. Thorburn adds, "The whole area is fascinating and plausible. [Wallace] has done some pioneering work. He sells it very hard, but people are very much interested in the ideas and following up on them."

To Wallace, looking at complex diseases through the lens of mitochondria makes everything clearer. "I'm not saying anything

ROBERT NERONI

done before isn't good. It's just not complete," says Wallace. Especially, he believes, when trying to understand the elusive link between disease, genetics, and the environment.

Genome-wide association studies (GWAS), a popular tool to find genetic variations associated with a particular disease, have for the most part had limited success. Their failures are often blamed on confounding environmental factors. Mitochondria, notes Wallace, are that missing factor: they act as a direct link between our genes and the environment, taking in calories and oxygen (products of the environment), and producing ATP and acetyl coenzyme A, two molecules involved in the regulation of most biochemical reactions, including gene expression.[5] In addition, during OXPHOS, mitochondria generate reactive oxygen species (ROS), the smoke from the furnace. At low levels, the ROS provide a critical signaling system from the mitochondrion to the cytosol and nucleus. However, at high levels, these free radicals cause significant damage to the cell and organelles, especially to the mitochondria themselves. Consequently, mtDNA has a much higher mutation rate than nuclear DNA. Thus, the most common genetic changes caused by the environment are mutations in mtDNA, says Wallace. These inheritable changes, plus mitochondrial regulation of nuclear DNA gene expression by ATP and acetyl coenzyme A, are the major factors contributing to predisposition to the common diseases, argues Wallace.[6] Yet GWAS only analyze nDNA, not mtDNA. "GWAS are wonderful," says Wallace. "The problem is they don't include energetics."

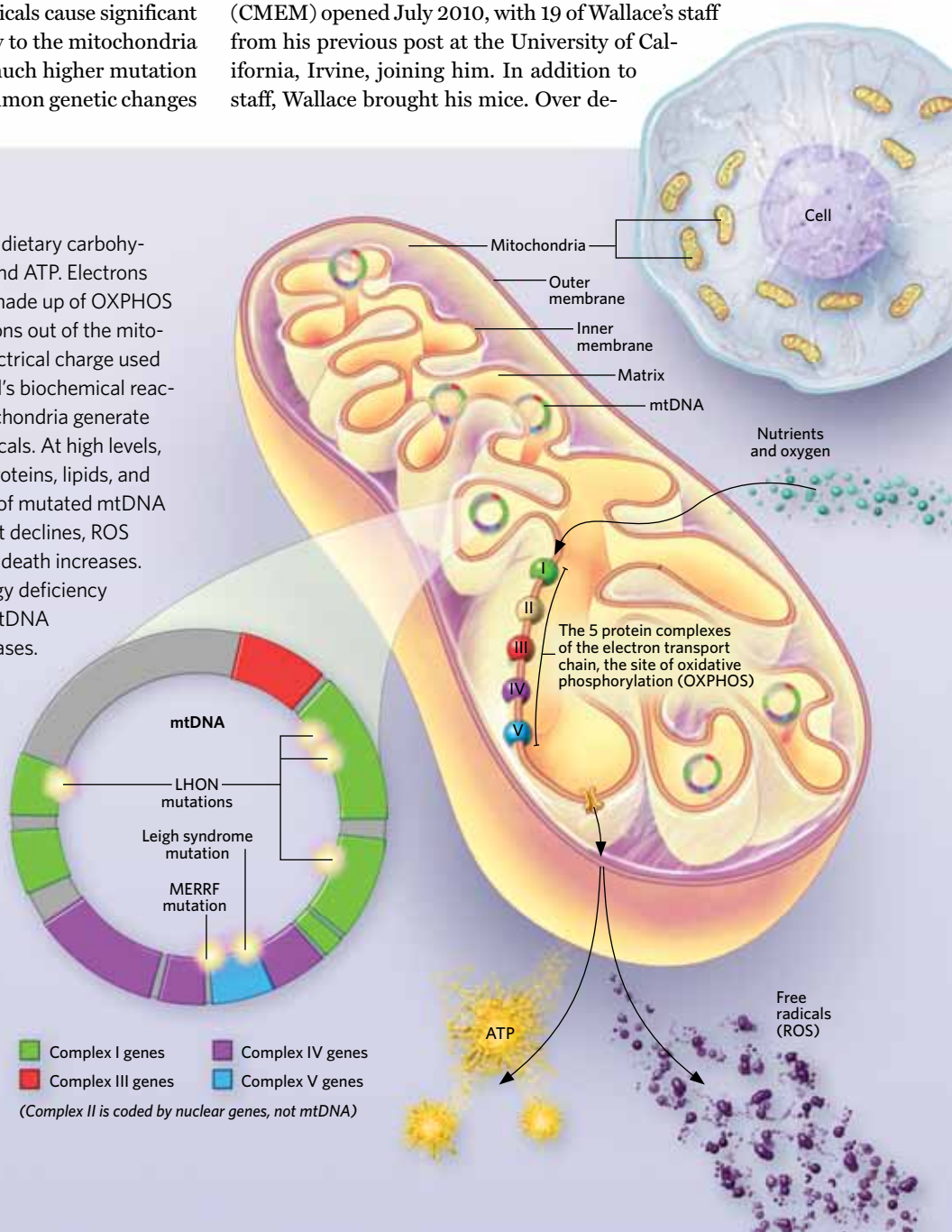
Today, Wallace finally has the backing of a major research hospital to explore these ideas and more. In 2009, while visiting Children's Hospital, Wallace spoke with Yudkoff about his desire to start a center focused on the role of mitochondria in common, complex diseases. "It was a bit like Einstein asking if he's welcome in a physics department," says Yudkoff. "There's arguably no one alive with more impeccable credentials in the field."

The Center for Mitochondrial and Epigenomic Medicine (CMEM) opened July 2010, with 19 of Wallace's staff from his previous post at the University of California, Irvine, joining him. In addition to staff, Wallace brought his mice. Over de-

ANDREW SWIFT

MITOCHONDRIA AT WORK

Mitochondria combine hydrogen derived from dietary carbohydrates and fats with oxygen to generate heat and ATP. Electrons flowing through the electron transport chain, made up of OXPHOS complexes I through V, are used to pump protons out of the mitochondrial inner membrane. This creates an electrical charge used to generate ATP, which powers most of the cell's biochemical reactions. As a toxic by-product of OXPHOS, mitochondria generate reactive oxygen species (ROS), called free radicals. At high levels, free radicals damage mtDNA, nuclear DNA, proteins, lipids, and other molecules in the cell. As the percentage of mutated mtDNA in a cell increases, mitochondrial energy output declines, ROS production increases, and the likelihood of cell death increases. Through the work of Wallace and others, energy deficiency caused by these factors, as well as inherited mtDNA mutations, have been linked to numerous diseases.



mtDNA MUTATIONS

Mutations in the mtDNA genes can result in a wide range of symptoms. Several single-base changes in the complex I genes predispose to a person to Leber hereditary optic neuropathy (LHON), a form of inherited vision loss. Mutations in the complex V ATP synthase 6 gene can cause retinal problems when few mtDNA in a cell harbor the mutation, but can cause the lethal Leigh syndrome when many mtDNA in a cell have the mutation. Mutations in the ribosomal and transfer RNA genes in the mtDNA can predispose to deafness, muscle and heart disease, strokes, diabetes, Alzheimer's and Parkinson's. For example, a mutation in the tRNA(Lys) gene can cause a form of epilepsy together with muscle symptoms known as myoclonic epilepsy and ragged-red fiber disease (MERRF).

cades, he has created numerous mouse models in an effort to prove a direct cause-and-effect relationship between mitochondrial defects and common diseases. In 1997, for example, he created a mouse deficient in Ant1, a nuclear-encoded protein involved in ATP synthesis, loss of which produces debilitating heart and muscle disease.[7] He has also created mice harboring mtDNAs with a single base change in the mtDNA *COI* gene. These animals develop heart and muscle disease as well as other symptoms, demonstrating that a single mtDNA mutation is sufficient to cause degenerative disease. Today, Wallace has over 3,000 mice with different mitochondrial defects serving as models for diseases including diabetes, hypertension, blindness, and neurological problems.

The Center, now stocked with mice and staff, could not have opened at a more opportune time. Today there is a "renaissance" of researchers considering the role of energy and mitochondria in common disease, says Falk. "In the last decade, there's been an explosion of research," she says.

Still, while studies of nuclear DNA implicate new genes in complex diseases every day, mtDNA studies are far fewer and more difficult to perform, typically requiring an invasive muscle biopsy and an analysis of the percentage of mutated mitochondria within a cell or population of cells. To catch the attention of the medical community, every last scrap of research will be needed. Extraordinary claims, as they say, require extraordinary evidence.

Altered metabolism in complex diseases

In 1982, an illicit chemist in Northern California synthesized and sold a bad batch of a narcotic called "China White." Four drug addicts injected the contaminated dope and subsequently developed tremors, rigidity, and loss of balance—it appeared as if they had Parkinson's disease. The drug, researchers later discovered, was actually MPTP, a neurotoxin that inhibits the first step of OXPHOS. "Ever since then, the role of mitochondria in Parkinson's has been hotly debated," says Doug Turnbull, a member of the mitochondrial research group at Newcastle University in the United Kingdom.

Today, more than 800 papers have analyzed the role of mitochondria in Parkinson's disease, with intriguing conclusions. The most frequently known genetic cause of Parkinson's to date is a mutation in *LRRK2*, a nuclear gene encoding an enzyme that associates with the mitochondrial outer membrane. (See "The Genes of Parkinson's Disease," *The Scientist,* February 2011.) Studies have shown the mutation is connected to impaired mitochondrial function.[8] In 2006, Turnbull and colleagues at Newcastle found significant mitochondrial DNA deletions in the substantia nigra, a brain region damaged in patients with Parkinson's disease.[9]

"Neurobiologists are too shy to accept that it is a mitochondrial disease, but it is a mitochondrial disease," says Prasanth Potluri, a self-proclaimed "mitochondriac" and research scientist at CMEM. Yet, for others, the idea is not so cut-and-dried. It's "quite controversial," said Thomas Gasser, director of the department

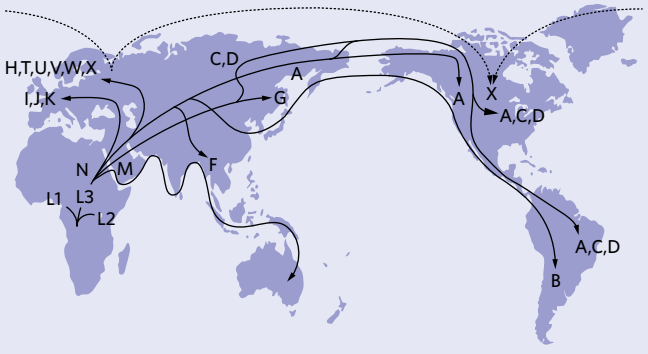
METHODS: I.D.'ING DISEASE-RELATED MUTATIONS IN MITOCHONDRIAL DNA

In the effort to identify mitochondrial DNA (mtDNA) mutations associated with human disease, a major hurdle has been the fact that there is no "normal" mtDNA sequence. As Wallace and colleagues discovered beginning in the 1980s, human populations around the world have high levels of variation in mtDNA, which can be sorted into distinct haplogroups, or branches, reflecting their geographic origins.

mtDNA in modern humans dates back to Africa some 150,000 to 200,000 years ago. Based on samples of mtDNA collected around the world over decades, Wallace's team has mapped this remarkable correlation between mtDNA variation and place of origin: as humans spread around the globe out of Africa, populations acquired adaptive mutations allowing them to thrive in different climates. (See map at right: letters denote mtDNA lineages.) In cold regions, for example, lineages acquired mtDNA mutations that resulted in a less-efficient oxidative phosphorylation system, with decreased ATP production, but increased heat production.

This high degree of mtDNA variation puts clinicians in a quandary. How can one identify which variations cause disease and which are simply the result of a person's geographic origins? For example, sequencing the mtDNA of more than 500 patients known to suffer from mitochondrial cardiomyopathy resulted in over 200 different sequence variants—far too many to identify a culprit.

To resolve the issue, Wallace and his team recently designed an automated analysis system they call MITOMASTER to compare the mtDNA sequences of patients with a database of thousands of mtDNA haplogroups. Analyzing the mtDNA sequences of 29 Italian patients with mitochondrial heart disease, the researchers identified 593 mtDNA variants, but found that 98 percent of them were haplogroup-associated. Six mutations, however, were novel and not associated with a haplogroup, suggesting they were possible disease contributors (*Eur J Hum Genet.* 19:200-07, 2011). The approach demonstrates that clinicians shouldn't be analyzing individual mtDNA sequences in isolation, and that automated systems can help researchers ferret out links between mtDNA mutations and disease pathology.



LUCY READING-IKKANDA (MAP); SOURCE: COPYRIGHT 2002 © MITOMAP.ORG

Genome-wide association studies are wonderful. The problem is they don't include energetics. —Douglas Wallace

of neurodegenerative diseases at the Hertie-Institute for Clinical Brain Research at the University of Tübingen, Germany. There are rare, recessively inherited forms of Parkinson's in which defective mitochondria certainly play a role, says Gasser, but for the more common, sporadic cases of Parkinson's, "the primary defect lies somewhere else."

There is also budding evidence for the role of mitochondrial dysfunction in other common neurodegenerative diseases. Recent studies suggest that amyloid-beta, the chief component of the characteristic plaque of Alzheimer's disease, progressively accumulates within mitochondria, acting as a direct toxin. In addition, defects in OXPHOS, including mutations in mtDNA, have been frequently associated with the disease.[10,11] And a recent study from Newcastle University found that mtDNA deletions may also be an important contributor to multiple sclerosis.[12]

In 2005, a population-based study at a school in Portugal demonstrated that seven percent of autistic children studied had disturbances in mitochondrial energy metabolism.[13] "It raised the question that disturbances of mitochondrial function might be a reasonably common finding in persons with autism," says Natowicz of the Cleveland Clinic. Yet researchers are divided over the degree to which mitochondrial dysfunction actually contributes to the autistic phenotype, and over whether people with OXPHOS disorders are a clinically distinct population of autistic individuals or no different from most who suffer from autism. More large population-based studies might answer that question, says Natowicz, but they have yet to be done. "This is a central question [in autism] that needs much more attention," he concludes.

But nowhere is the study of altered metabolism more popular than in cancer research. Researchers have long observed that metabolism in tumors is different from metabolism in noncancerous cells, possibly because cancer cells must accommodate the increased metabolic demands of rapid cell proliferation. The study of metabolism in cancer cells has "exploded" over the last five years, says Eyal Gottlieb, a researcher at the Beatson Institute for Cancer Research in Glasgow, Scotland. There are hundreds of papers describing mitochondrial DNA mutations in cancer, including Wallace's own work identifying mtDNA mutations in prostate cancer.[14] But alterations in mitochondrial DNA and function could be a consequence of a cancerous phenotype, rather than the cause. There are some instances where scientists have demonstrated a direct causal role of mitochondria dysfunction in cancer,[15] but such cases are, at the moment, "the exception to the rule," says Gottlieb. Still, he adds, "There is a link there, even if we don't fully understand it."

For now, the role of mitochondria in common diseases continues to be investigated in numerous studies. Still, the majority of clinicians in all of these fields—even in Parkinson's, where the evidence seems strongest—have not embraced Wallace's paradigm-shifting theory.

A new concept of medicine

Wallace walks down a long, empty corridor. To the right, row after row of sparkling lab benches stand empty and waiting. Today, only twenty-one people fill one of the four lab bays that will make up the new center, but already the team is tackling projects in metabolic syndrome, cancer, heart disease, and aging. Wallace has plans to hire more new faculty plus support staff this year.

He reaches the end of the corridor and turns, walking into one of the center's new conference rooms, which appropriately overlooks a silent power plant, silhouetted against the cold Philadelphia sunset. His voice has grown hoarse. He settles back into a chair. In the end, Wallace, whose mother had Alzheimer's and whose son is autistic, isn't out to criticize his colleagues, but to save lives. "I don't know how long it's going to take for people to see this is relevant," he says with a sigh, looking out at the quiet plant below. "We now have a mitochondrial, energy-based concept of medicine, which beautifully explains in a simple way all the previous inexplicable problems. Things are only complex when we don't understand them." ■

References

1. D.C. Wallace et al., "Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy," *Science*, 242:1427-30, 1988.
2. J.M. Shoffner et al., "Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation," *Cell*, 61:931-37, 1990.
3. D.C. Wallace, "Mitochondrial DNA Mutations in Disease and Aging," *Environ Mol Mutagen*, 51:440-50, 2010.
4. A. Spinazzola, M. Zeviani, "Disorders from perturbations of nuclear-mitochondrial intergenomic cross-talk," *J Intern Med*, 265:174-92, 2009.
5. D.C. Wallace, Colloquium paper: "Bioenergetics, the origins of complexity, and the ascent of man," *PNAS*, 107:8947-53, 2010.
6. D.C. Wallace, "Bioenergetics and the epigenome: interface between the environment and genes in common diseases," *Dev Disabil Res Rev*, 16:114-19, 2010.
7. B.H. Graham et al., "A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator," *Nat Genetics*, 16:226-34, 1997.
8. H. Mortiboys et al., "Mitochondrial impairment in patients with Parkinson disease with the G2019S mutation in *LRRK2*," *Neurology*, 75:2017-20, 2010.
9. A. Bender et al., "High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease," *Nat Genet*, 38:515-17, 2006.
10. P.E. Coskun et al., "Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication," *PNAS*, 101:10726–31, 2004.
11. P.E. Coskun, "Systemic mitochondrial dysfunction and the etiology of Alzheimer's disease and Down syndrome dementia," *J Alzheimers Dis*, 20 Suppl 2:S293-310, 2010. **F1000 ID 3857960, http://bit.ly/MitoAlzDown**
12. G.R. Campbell et al., "Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis," *Ann Neurol*, 69:481-492, 2011. **F1000 ID 6325956, http://bit.ly/MitoMS**
13. G. Oliveria et al., "Mitochondrial dysfunction in autism spectrum disorders: a population-based study," *Dev Med Child Neurol*, 47:185-59, 2005.
14. J.A. Petros et al., "mtDNA mutations increase tumorigenicity in prostate cancer," *PNAS*, 102:719-24, 2005.
15. D.C. Wallace, "A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, and Cancer: A Dawn for Evolutionary Medicine," *Ann Rev Genet*, 39:359-407, 2005.