DEEP DIVE

Meet the ‘mitomaniacs’ who say mitochondria matter in autism

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Illustration by Khyati Trehan

Douglas Wallace watched with anticipation as a mouse navigated chambers in a habitat in his lab in Philadelphia, Pennsylvania. For the most part, it was a typical lab mouse: a few months old, with plain black fur. But deep within its cells, it carried a single change in the DNA that runs its mitochondria, the organelles that generate the body’s energy.

Wallace, a mitochondrial geneticist at Children’s Hospital of Philadelphia, had previously discovered that a similar change could cause an eye disease in people. But no one knew whether the mutation could affect behavior and contribute to autism. To find out, Wallace had spent decades developing the mouse now on the table in front of him. He was about to have his answer. “It’s a very simple question,” Wallace says. “And it only took 30 years to get there.”

Wallace — whose son has autism — is perhaps the most passionate member of a growing group of researchers committed to the idea that mitochondria are an overlooked factor in autism and related conditions such as **fragile X syndrome**. These researchers, some of whom have adopted monikers such as ‘mitomaniac’ and — Wallace’s term — ‘mitochondriac,’ subscribe to a simple, if fundamental, idea: Mitochondria generate energy, and the brain uses a lot of it — about 20 percent, by most estimates. So it makes sense that changes in mitochondria could lead to changes in the way the brain functions or develops and, in at least some cases, to autism.

Mitomaniacs still represent a small minority of autism researchers. Studies of mitochondria’s role in autism or autism-related conditions represent just 0.4 percent of all papers in that field, according to a Web of Science search of titles and abstracts.

The field also faces challenges. Among them: showing that mitochondrial differences are a cause rather than a consequence of autism and determining how the organelles might contribute to the condition. Yet the accumulating evidence makes a potential role for mitochondria in autism hard to ignore. “I think we finally have broken through that glass window, and enough labs are now

recognizing that there is something about mitochondria that may not be a cause [of autism] in and of itself, but certainly a contributor," says **Richard Levy**, professor of anesthesiology and pediatrics at Columbia University, who studies fragile X syndrome. "The focus is now certainly on mitochondria, and I think rightly so."

Radical step:

Researchers discovered the first mitochondrial disease in 1959, and mitochondria's **ring-shaped DNA** in 1963. By 1981, they'd mapped the entire human mitochondrial genome. The first suggestion that mitochondria are linked to autism appeared in 1985, when a pair of researchers described **four autistic people with lactic acidosis**, a sign of 'mitochondrial disease' (a term for any inborn disruption to mitochondria). The researchers proposed that they had landed upon a subtype of autism arising from metabolic problems.

In 1998, neurologist **Jay Lombard** took that idea a radical step further in a three-page paper titled "**Autism: A mitochondrial disorder?**" Lombard proposed that autism is primarily a condition of altered energy metabolism in the brain, caused by problems with mitochondria. He based this theory on the presence of lactic acidosis in autistic people and signs that their mitochondria are producing low levels of the cell's energy currency: adenosine triphosphate (ATP).

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A few years later, a pediatrician named **John Jay Gargus** noticed that several children who had been referred to him for metabolic issues — recurring episodes where children became "completely washed out, listless" — also had autism, says Gargus, professor of physiology and biophysics at the University of California, Irvine. Gargus started doing mitochondrial workups to zero in on the cause and identify mitochondrial disease.

Two of the autistic children, a girl and a boy, had duplications in a stretch of DNA called 15q11-q13, a region tied to several autism-linked conditions. Gargus and his colleagues noted that the children had **high numbers of mitochondria**, but reduced energy output from these organelles, an indication that the cells are churning out mitochondria to meet energy needs.

It was the first time such 'mitochondrial hyperproliferation' had been linked to these conditions.

The results led him to start screening people with 15q variants, and eventually those with autism, for metabolites indicative of problems with mitochondria. Many showed hyperproliferation, in addition to defects in two of the five protein complexes mitochondria use to produce ATP; a small number had mutations in mitochondrial DNA (mtDNA). “I absolutely think that mitochondrial dysfunction is critical,” Gargus says. “They’re important, and I think there’s a lot of information to say they’re important, in autism.”

New energy:

Gargus’ team’s findings did not immediately resonate with **Cecilia Giulivi**, a biochemist who studies autism and related neurological conditions. But in 2004, Giulivi read Lombard’s paper in her office at the University of California, Davis MIND Institute. She’d noted that some traits associated with mitochondrial disease, such as intellectual disability, commonly co-occurred with autism. She was puzzled, then, about the lack of research probing connections between the two.

She started searching for a way to test mitochondrial function in autistic children and discovered a newly launched MIND Institute study on environmental contributors to autism: the Childhood Autism Risks from Genetics and the Environment (**CHARGE**) study. Giulivi persuaded the study’s lead investigator, **Irva Hertz-Picciotto**, to let her test some of the children’s blood samples for mitochondrial function.

It was a small study, involving just 10 autistic preschoolers and 10 controls. But the autistic children’s mitochondria were **significantly less active**, producing about two-thirds less energy than those of controls, Giulivi and her colleagues found. The autistic children also had higher levels of pyruvate — a byproduct of metabolizing sugar — and more mtDNA mutations — evidence of malfunctioning mitochondria.

The findings appeared in December 2010, two months before the online publication of a meta-analysis of 68 studies showed that 5 percent of 536 autistic children from three studies have **mitochondrial disease**. (By comparison, about 0.02 percent of people in the general population have a **mitochondrial disorder**.) What’s more, many of the autistic children had other markers of mitochondrial dysfunction, including 14 percent who had high pyruvate levels across two studies in which those levels were measured. Together, the papers injected new energy into the study of mitochondria and autism, says Giulivi, a self-identified mitomaniac.

Virtually all bodily functions require a baseline of mitochondrial energy, and some require more than others. Many parts of the body can tolerate changes in mitochondria without consequence. But some organs or processes may be more sensitive — and brain development could be one of those processes. “Development to me is the marathon the brain has to run in a highly coordinated

way," Levy says. "If you've got some sort of impairment at the mitochondrial level, it can become a major deal."

Researchers have since gathered **additional metabolic clues** suggesting mitochondria function differently in some autistic people. A small 2013 study showed **low levels of energy production** by one measure in certain blood cells of 87 autistic people compared with 78 controls, a difference not seen in non-autistic people with intellectual disability or schizophrenia. In 2015, researchers using cheek swabs detected low levels of two **markers of mitochondrial activity** in 39 of 92 autistic children and overactivity in 9 of the autistic children, problems not present in any of the controls.

Some data also hint that people with mitochondrial disease have an elevated chance of having autism or autism traits. In a small 2016 study of eight people with propionic acidemia, a rare metabolic disorder, **all eight showed autism traits**, five of whom had previously been diagnosed with autism. This finding prompted the investigators to recommend screening all people with the condition for autism.

Signs of an autism connection have shown up in people's mitochondrial genomes as well. In a 2016 study, researchers analyzed mtDNA sequences from 903 trios consisting of an autistic child and the child's sibling and mother (whose mtDNA the children carry). The autistic children as a group had **twice as many 'harmful' mutations** — those that change protein function or are linked to a health condition — as their non-autistic siblings. These data suggest that mitochondrial mutations "cause autism" in a subset of autistic people, says lead investigator **Zhenglong Gu**, director of the Center for Mitochondrial Genetics and Health at the Greater Bay Area Institute of Precision Medicine in Guangzhou, China.

Other human studies have produced similar results. In a small 2018 study, researchers found **mtDNA deletions** in 10 out of 60 autistic people, compared with 2 of 60 controls. The year before, Wallace and his colleagues reported that people from any of eight mtDNA subgroups — maternal lineages of mtDNA called haplogroups — were **twice as likely** to have autism as those from the most common European haplogroup.

Slow progress:

Despite such findings, even some mitochondria researchers are hesitant to causally link mitochondria and autism. For one thing, variations in mtDNA long after birth may not be meaningful, given that mitochondria are constantly fusing and dividing, creating new organelles that may differ from the previous ones, experts say. So the mitochondria an adult or child has are not

necessarily the same as those she had in the womb, when autism is thought to develop.

In that vein, impaired mitochondrial function could be a consequence of having autism, not a cause, says **Xinyu Zhao**, professor of neuroscience at the University of Wisconsin-Madison. Even a person's diet can influence mtDNA copy number, and people with autism often have a restricted diet. "This is just a beginning," says Zhao, who has studied mitochondria in fragile X syndrome. "You really have to be careful about what exactly you are claiming."

At least one large study failed to turn up any relationship between mtDNA variation and autism. In 2012, researchers sequenced or genotyped mtDNA in nearly 1,300 autistic people and 2,600 controls, one of the biggest studies of mtDNA and autism to date, and concluded that mtDNA variations **aren't a significant contributor** to the condition. "Despite a thorough interrogation of mtDNA variation, we found no evidence to suggest a major role for mtDNA variation in [autism] susceptibility," the investigators wrote.

> "I think it would be quite shocking to many people to say metabolic changes are key to brain function." Elizabeth Ann Jonas

Connecting mtDNA mutations to specific outcomes is challenging, experts say. Mitochondria affect, and are affected by, a long list of biological processes, making it exceedingly difficult to tie a particular aspect of them to autism, says **Gaia Novarino**, professor of neuroscience at the Institute of Science and Technology in Klosterneuburg, Austria. "You are putting something very complex — that is, autism — with something that is extremely complex, mitochondrial function, with something else that is brain development, which is again very complex," she says. "You make a really intriguing but also difficult and complex system to understand."

What's more, the expansive nature of both mitochondrial disease and autism makes connecting the two fraught at best, says **Bruce Barshop**, professor of pediatrics at the University of California, San Diego. "It's a difficult question, because autism is so hard to define, and mitochondrial disease is so broadly manifesting," Barshop says. The vast majority of people with mitochondrial disease do not fall on the autism spectrum, he adds.

Conversely, mitochondrial problems are not likely to be singularly responsible for autism in anyone, says **Elissar Andari**, assistant professor of psychiatry at the University of Toledo College of Medicine in Ohio. "It's a behavioral and brain or a genetic and environmental disorder. There's no way there's one factor that's causing it."

And when mitochondria contribute to the condition, it would be good to know what that looks like, Andari says. Perhaps mitochondrial problems lead to an elevated chance of seizures or other specific traits, she says. “We really need to understand and define the clinical characteristics of individuals with autism who have these mitochondrial deficits.”

Partly as a result of such knowledge gaps, the mitomaniacs all say they’ve had trouble convincing the wider autism field to take mitochondria seriously. “I think it would be quite shocking to many people to say metabolic changes are key to brain function,” says **Elizabeth Ann Jonas**, professor of internal medicine and neuroscience at Yale University, who works on fragile X mice. “But I think it’s really, really important, and I think that it’s not well accepted because it’s so difficult.”

Central hub:

Wallace — who earlier this year was denied a grant to continue his mouse studies of mitochondrial function in autism — is not dissuaded by difficulty. He thinks most forms of autism are tied to mitochondria, and that many autism-linked genes play as-yet undiscovered roles in mitochondrial function. Some mouse models of autism support the latter idea. Mice missing a stretch of DNA called 22q11.2, a mutation associated with autism, have both **misshapen mitochondria** and neurons with structural deficits, a 2019 study showed. Giulivi and her colleagues reported in 2012 that mice that under-express the autism-linked gene **PTEN** also have **low levels of a protein** mitochondria use to produce energy.

Similarly, mice missing **FMR1**, the fragile X syndrome gene, show **signs of metabolic stress** and reduced expression of two genes that help mitochondria fuse together, something they often must do to meet energy demands, Zhao and her colleagues showed in 2019. Findings such as this suggest that many underlying causes of autism produce mitochondrial dysfunction, making mitochondria a “central hub” of the condition, Jonas says.

Some mitomaniacs also contend that mitochondria could help account for the many autism-linked traits and challenges that arise outside of the brain, such as gastrointestinal issues and motor problems. After all, virtually all parts of the body rely on mitochondria, to varying degrees. For example, mouse models of two different autism-linked conditions have **different body weights**, burn calories differently and have different metabolites in their blood than both control mice and each other, according to a study published in March.

The influence of mitochondria may even extend beyond autism, Novarino says. “Certainly it looks like mitochondria and mitochondrial DNA is an emerging pathway or emerging cause of autism, but I don’t think it’s specific to autism,” she says. “It’s an emerging part of brain development.”

Mitochondrial problems are likely to have the most impact during mid-fetal development, when autism is thought to arise. In one theory, inborn mitochondrial disease makes the developing brain more susceptible to other environmental hits, causing changes that lead to autism, Gu says. “Somehow their mitochondrial homeostasis is not as stable or as robust as other kids,’” he says. “So any stress — it could be a virus, it could be environmental stress — can trigger this whole different development.”

Gu and his team are analyzing cord blood from 1,000 children with autism, as well as mtDNA samples from their mothers, to see if the mutations appear prenatally. If so, it’s more likely the mutations are influencing the condition. Preliminary results seem to support his 2016 findings, Gu says.

For fragile X syndrome, researchers have a more specific hypothesis. Early in development, mitochondria have channels in their inner membranes through which protons leak, much like the hole near the top of a bathroom sink prevents the sink from overflowing. Later in development, the holes close, prompting a switch in metabolic processes. If that doesn’t happen, or happens too late, **synapses may not form correctly**, Jonas has shown in human cells. And in fragile X mice, mitochondria remain leaky — and must work extra hard to keep energy levels stable, Jonas and Levy have shown. “We think that’s a normal developmental stage that just has never closed,” Jonas says.

If so, it may be possible to treat fragile X by closing that leak, something Jonas and her colleagues did by bathing mouse neurons in dexpramipexole, a drug tested (unsuccessfully) in people with amyotrophic lateral sclerosis. The drug binds to the enzyme that synthesizes ATP, which may close the leak and thereby **improve mitochondrial function**, although the process is still unclear. Injecting fragile X mice with dexpramipexole reduced excessive grooming and nest shredding, a proxy for **repetitive behaviors** in people.

Such a treatment might also ease difficulties associated with other forms of autism, Jonas says. The leak doesn’t appear to be directly related to the FMRP mutation, she says, and it’s likely that mitochondria are crucial for the early development of **synapses**, a process that lays the groundwork for the brain’s circuitry.

Another potential treatment for issues associated with autism is a compound called M1 that boosts mitochondrial fusion. When Zhao treated her fragile X mice with M1, they became more social and had better memory. And in unpublished work presented at the 2021 Society for Neuroscience meeting, researchers found that injecting Rett syndrome mice with the oral anesthetic dyclonine made the mice live longer and **improved their mitochondrial function**, as well as their breathing, motor skills and limb strength. These effects were comparable to experimentally boosting expression of the antioxidant enzyme catalase in the animals.

Yet few drugs that target mitochondria are in human trials for Rett, fragile X or other forms of

autism. As of now, the options are limited. “We don’t have a lot of good fixes for mitochondria,” Gargus says. “That’s the real problem.”

Another problem is drawing a direct line from mitochondrial defects to autism. Jonas is looking into whether such defects, for instance the persistent leak she described, alter nuclear gene expression in neurons. She and her colleagues are also examining, in cells and mice, whether closing the leak with drugs could nudge the brain into reforming altered circuits. Zhao is studying human neurons to better understand how FMR1 might regulate mitochondrial fusion; she aims to trace pathways that connect mitochondria to autism and thereby identify treatment targets.

Genetic source:

Wallace’s strategy is to edit a mitochondrial gene in mice and observe the effects on behavior. To do so, he first had to find a way to edit mtDNA. The abundance of mitochondria, even within a single cell, makes it difficult to introduce an edit into all or even a substantial portion of them. And the main gene-editing tool, CRISPR, cannot even get inside mitochondria. “All this kind of makes the field progress slower than it should,” Gu says.

Wallace’s trick: Isolate an mtDNA mutation of interest in a mouse cell line. Strip the mutant cell of its nucleus. Then fuse the mtDNA-containing cytoplasm to a mouse stem cell treated to destroy its resident mtDNA. The result is a stem cell with the mutant mtDNA. In 2008, he injected stem cells bearing a mutation in the mitchondrial gene ND6 into a mouse embryo and implanted the embryo into a surrogate mother. He then screened the pups for the mutation; the female pups later passed it along to their offspring. The process ensures the mito-mice have no biological differences from controls other than a mutation in this single mitochondrial gene.

> “We’ve proven that energy in and of itself is sufficient to cause autism.” Douglas Wallace.

The new mouse model is likely to “open the door” for other researchers to study how mtDNA mutations affect traits and behaviors, says **Frank Castora**, professor of physiological sciences at Eastern Virginia Medical School in Norfolk, who studies mitochondria’s role in autism, among other conditions. “I would be surprised if we don’t see some other mouse colonies being developed that are going to have other mitochondrial DNA mutations,” he says.

ND6 codes for a protein necessary to produce ATP, so any resulting changes in a mouse's health or behavior would have to originate from its production or use of energy. In 2015, the researchers began evaluating the mice for behaviors that echo autism traits. Four years passed before they, and journal editors, were confident of the results: The ND6 mice did not show a preference for a new mouse over a rock, indicating low social motivation. The animals also spent an unusual amount of time burying marbles in their cage, a type of rodent repetitive behavior, and 'froze' in an open area unusually often, suggesting they are anxious. In the brain, the ND6 mice showed low neural activity and signs of lethargic energy production from mitochondria.

It's hard to know what these results mean for people. "Is that autism?" Gargus says of the mouse behavior. But Wallace believes these data are powerful support for the idea that mitochondria matter. "We've proven that energy in and of itself is sufficient to cause autism," he says.

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