Study finds children with autism have mitochondrial dysfunction

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Children with autism are far more likely to have deficits in their ability to produce cellular energy than are typically developing children, a new study by researchers at UC Davis has found. The study, published today in the *Journal of the American Medical Association* (JAMA), found that cumulative damage and oxidative stress in mitochondria, the cell's energy producer, could influence both the onset and severity of autism, suggesting a strong link between autism and mitochondrial defects.

After the heart, the brain is the most voracious consumer of energy in the body. The authors propose that deficiencies in the ability to fuel brain neurons might lead to some of the cognitive impairments associated with autism. Mitochondria are the primary source of energy production in cells and carry their own set of genetic instructions, mitochondrial DNA (mtDNA), to carry out aerobic respiration. Dysfunction in mitochondria already is associated with a number of other neurological conditions, including Parkinson's disease, Alzheimer's disease, schizophrenia and bipolar disorder.

"Children with mitochondrial diseases may present exercise intolerance, seizures and cognitive decline, among other conditions. Some will manifest disease symptoms and some will appear as sporadic cases," said Cecilia Giulivi, the study's lead author and professor in the Department of Molecular Biosciences in the School of Veterinary Medicine at UC Davis. "Many of these characteristics are shared by children with autism."

The researchers stress that these new findings, which may help physicians provide early diagnoses, do not identify the cause or the effects of autism, which affects as many as 1 in every 110 children in the United States, according to the U.S. Centers for Disease Control and Prevention.

While previous studies have revealed hints of a connection between autism and mitochondrial dysfunction, these reports have been either anecdotal or involved tissues that might not be representative of neural metabolism.

"It is remarkable that evidence of mitochondrial dysfunction and changes in mitochondrial DNA were detected in the blood of these young children with autism," said Geraldine Dawson, chief science officer of Autism Speaks, which provided funding for the study. "One of the challenges has been that it has been difficult to diagnose mitochondrial dysfunction because it usually requires a muscle biopsy. If we could screen for these metabolic problems with a blood test, it would be a big step forward."

For the study, Giulivi and her colleagues recruited 10 autistic children aged 2 to 5, and 10 age-matched typically developing children from similar backgrounds. The children were randomly selected from Northern California subjects who previously had participated in the 1,600-participant Childhood Autism Risk from Genetics and the Environment (CHARGE) Study and who also consented to return for a subsequent study known as CHARGE-BACK, conducted by the UC Davis Center for Children's Environmental Health and Disease Prevention.

The children with autism met stringent diagnostic criteria for autism as defined by the two most widely used and rigorous assessment tools. Though the total number of children studied was small, it is generally

representative of the much larger CHARGE cohort, and that increases the significance of the study results, the authors said.

The researchers obtained blood samples from each child and analyzed the metabolic pathways of mitochondria in immune cells called lymphocytes. Previous studies sampled mitochondria obtained from muscle, but the mitochondrial dysfunction sometimes is not expressed in muscle. Muscle cells can generate much of their energy through anaerobic glycolysis, which does not involve mitochondria. By contrast, lymphocytes, and to a greater extent brain neurons, rely more heavily on the aerobic respiration conducted by mitochondria.

The researchers found that mitochondria from children with autism consumed far less oxygen than mitochondria from the group of control children, a sign of lowered mitochondrial activity. For example, the oxygen consumption of one critical mitochondrial enzyme complex, NADH oxidase, in autistic children was only a third of that found in control children.

"A 66 percent decrease is significant," Giulivi said. "When these levels are lower, you have less capability to produce ATP (adenosine triphosphate) to pay for cellular work. Even if this decrease is considered moderate, deficits in mitochondrial energy output do not have to be dismissed, for they could be exacerbated or evidenced during the perinatal period but appear subclinical in the adult years."

Reduced mitochondrial enzyme function proved widespread among the autistic children. Eighty percent had lowered activity in NADH oxidase than did controls, while 60 percent, 40 percent and 30 percent had low activity in succinate oxidase, ATPase and cytochrome c oxidase, respectively. The researchers went on to isolate the origins of these defects by assessing the activity of each of the five enzyme complexes involved in mitochondrial respiration. Complex I was the site of the most

common deficiency, found in 60 percent of autistic subjects, and occurred five out of six times in combination with Complex V. Other children had problems in Complexes III and IV.

Levels of pyruvate, the raw material mitochondria transform into cellular energy, also were elevated in the blood plasma of autistic children. This suggests the mitochondria of children with autism are unable to process pyruvate fast enough to keep up with the demand for energy, pointing to a novel deficiency at the level of an enzyme named pyruvate dehydrogenase.

Mitochondria also are the main intracellular source of oxygen free radicals. Free radicals are very reactive species that can harm cellular structures, including DNA. Cells are able to repair typical levels of such oxidative damage. Giulivi and her colleagues found that hydrogen peroxide levels in autistic children were twice as high as in normal children. As a result, the cells of children with autism were exposed to higher oxidative stress.

Mitochondria often respond to oxidative stress by making extra copies of their own DNA. The strategy helps ensure that some normal genes are present even if others have been damaged by oxidation. The researchers found higher mtDNA copy numbers in the lymphocytes of half of the children with autism. These children carried equally high numbers of mtDNA sets in their granulocytes, another type of immune cell, demonstrating that these effects were not limited to a specific cell type. Two of the five children also had deletions in their mtDNA genes, whereas none of the control children showed deletions.

Taken together, the various abnormalities, defects and levels of malfunction measured in the mitochondria of autistic children imply that oxidative stress in these organelles could be influencing autism's onset.

"The various dysfunctions we measured are probably even more extreme in brain cells, which rely exclusively on mitochondria for energy," said Isaac Pessah, director of the Center for Children's Environmental Health and Disease Prevention, a UC Davis MIND Institute researcher and professor of molecular biosciences at the UC Davis School of Veterinary Medicine.

Giulivi cautions that these findings do not amount to establishing a cause for autism.

"We took a snapshot of the mitochondrial dysfunction when the children were 2-to-5 years old. Whether this happened before they were born or after, this study can't tell us," she said. "However, the research furthers the understanding of autism on several fronts and may, if replicated, be used to help physicians diagnose the problem earlier."

"Pediatricians need to be aware of this issue so that they can ask the right questions to determine whether children with autism have vision or hearing problems or myopathies," Giulivi said. Exercise intolerance in the form of muscle cramps during intensive physical activity is one of the characteristics of mitochondrial myopathies.

The chemical fingerprints of mitochondrial dysfunction also may hold potential as a diagnostic tool. Giulivi and colleagues are now examining the mitochondrial DNA of their subjects more closely to pinpoint more precise differences between autistic and non-autistic children.

"If we find some kind of blood marker that is consistent with and unique to children with autism, maybe we can change the way we diagnose this difficult-to-assess condition," she said.

The study also helps refine the search for autism's origins.

"The real challenge now is to try and understand the role of mitochondrial dysfunction in children with autism," Pessah said. "For instance, many environmental stressors can cause mitochondrial damage. Depending on when a child was exposed, maternally or neonatally, and how severe that exposure was, it might explain the range of the symptoms of autism."

"This important exploratory research addresses in a rigorous way an emerging hypothesis about potential mitochondrial dysfunction and autism," said Cindy Lawler, program director at the National Institute of Environmental Health Sciences (NIEHS), which provided funding for the study. "Additional research in this area could ultimately lead to prevention or intervention efforts for this serious developmental disorder."

More information: *JAMA*. 2010;304[21]:2389-2396.

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