VIEWPOINT

New tools strengthen old link between autism, mitochondria

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Most research into the genetic basis of autism focuses on the DNA nestled inside a cell's nucleus. Scientists unravel the DNA and study the genes that code for proteins, with the goal of identifying the molecular pathways involved. They then aim to develop treatments that target these pathways.

So far, studies have implicated hundreds of nuclear genes in autism, with about 100 genes emerging as strong candidates[1,2]. But the nucleus may not be the only source of genes involved in autism.

DNA also exists inside cellular compartments, or 'organelles,' called mitochondria. These organelles are the powerhouses of cells. They generate a molecule called adenosine triphosphate (ATP), which serves as the main source of cellular energy.

Each mitochondrion contains thousands of copies of a circular genome that codes for 13 of the 96 proteins needed to build the mitochondria's core ATP machinery. Unlike nuclear DNA, mitochondrial DNA (mtDNA) is inherited only from the mother.

This year, my colleagues and I published results suggesting that variants of mitochondrial genes contribute to autism — in some cases, by teaming up with genes in the nucleus.

Genetic puzzle:

Over the past decade, several lines of evidence have led investigators to suspect that mitochondrial genetics contributes to autism. Epidemiological analyses revealed that the incidence of mitochondrial disorders is higher in people with autism than in the general population.

Clinicians have also observed that both mitochondrial disorders and autism are marked by functional disturbances in tissues and organs that need to produce a lot of energy, such as the

brain, liver, heart and kidneys[3,4]. What's more, case reports and small studies reveal rare genetic variants — including DNA deletions or substitutions and extra copies of genes — in the mitochondrial genome of people with autism[5].

Last year, my colleagues and I applied new bioinformatics approaches to investigate a possible contribution for mtDNA. We used these new methods to analyze the mitochondrial genomes of people with autism.

Sequencing methods that decipher the '**exome**' — the protein-coding parts of the genome — often also yield information about mtDNA in cells. Under most circumstances, researchers ignore these 'off-target' data, but scientists have developed bioinformatics tools for analyzing the mtDNA generated as a byproduct of exome sequencing.

Energy generator:

Last year, researchers at Cornell University and Columbia University used these tools to evaluate the mtDNA in individuals whose exomes had been sequenced[6]. They extracted mtDNA sequences from 903 families with autism.

The researchers found that children with autism do not have more rare mutations in mtDNA than their unaffected mothers or siblings do. However, they appear to have more mutations that are harmful — ones that are likely to disrupt protein function.

In our study, we applied the same bioinformatics tools to exome mtDNA sequences from 10 families that each include more than one individual with autism[7]. Our goal was to identify mtDNA variants of interest — rare variants that occur in all affected members of a family and disrupt energy production in cells. We also looked for variants in the 83 nuclear genes that give rise to key mitochondrial proteins.

We identified several variants that merit further research. In one family, each person with autism has two rare mtDNA variants of a gene called MT-ND5, which encodes a piece of the respiratory chain complex (RCC) — the machinery that generates ATP.

We suspect that these changes contribute to autism in this family because the same two variants have been associated with mitochondrial disease. In addition, cell-culture studies show that mitochondria harboring these two variants have impaired energy production[8,9].

Studying synergy:

In another family, we found a pair of variants in genes that code for protein components of the RCC. Four people with autism from this family carry a rare variant of the mitochondrial gene MT-

ATP6 and a rare variant of the nuclear gene NDUFS4. Bioinformatics tools predict that these variants affect protein function, although their effects have not yet been studied in cells.

Our findings suggest that the flaw in the mitochondrial gene may team up with the flaw in the nuclear gene to disrupt energy production. If so, this is an example of a postulated phenomenon known as 'synergistic heterozygosity.' In this phenomenon, a combination of two or more individually innocuous defects in a biological process disrupts the process enough to cause a disorder[10].

We plan to investigate other possible contributions of mitochondrial genes to autism, as well as possible synergies between mitochondrial and nuclear genes. Research into synergistic heterozygosity requires data on the functions of a large number of variants and their combinations. We don't yet have the technology to test gene function at the speed necessary for such experiments[11].

Scientists should also closely examine people with autism who harbor variants for proteins in the RCC for signs of mitochondrial disorders. The assessments should include testing of multiple organ systems, measures of various biochemical markers and brain imaging[12].

Some therapies for mitochondrial diseases exist, and others are in development[13]. A better understanding of how mitochondria contribute to autism could rapidly lead to new treatments and improved outcomes for some individuals on the spectrum.

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REFERENCES:

1. de la Torre-Ubieta L. *et al*. *Nature Med.* **22**, 345-361 (2016) **PubMed**
2. Sanders S.J. *et al. Neuron* **87**, 1215-1233 (2015) **PubMed**
3. Chinnery P.F. *Gene Reviews* (2000) **Full text**
4. Frye R.E. and D.A. Rossignol *Pediatr. Res*. **69**, 41R-47R (2011) **PubMed**
5. Rossignol D.A. and R.E. Frye. *Mol. Psychiatry* **17**, 290-314 (2012) **PubMed**
6. Wang Y. *et al*. *PLoS Genet*. **12**, e1006391 (2016) **PubMed**
7. Patowary A. *et al*. *Autism Res.* Epub ahead of print (2017) **PubMed**
8. Petruzzella V. *et al. Hum. Mol. Genet.* **21**, 3753-3764 (2012) **PubMed**
9. McKenzie M. *et al. J. Biol. Chem.* **282**, 36845-36852 (2007) **PubMed**
10. Vockley J. *et al*. *Mol. Genet. Metab*. **71**, 10-88 (2000) **PubMed**
11. Gasperini M. *et al. Nat. Protoc.* **11**, 1782-1787 (2016) **PubMed**
12. Parikh S. *et al*. *Genet. Med.* **17**, 689-701 (2015) **PubMed**

13. Distelmaier F. *et al*. *Brain* **140**, e11 (2017) **PubMed**