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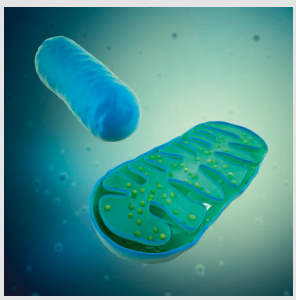
NEWS

Duplication allele may underlie fatal mitochondrial disorder



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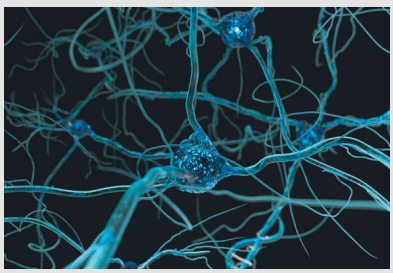
As recently reported in *American Journal of Human Genetics* (https://doi.org/10.1016/j.ajhg.2020.01.007), Gunning and colleagues found a monoallelic reciprocal duplication at the *ATAD3* locus in five unrelated neonates with a lethal metabolic disorder. The infants presented with cardiomyopathy, corneal clouding, encephalopathy, hypotonia, and seizures. None survived past 6 weeks of age. Exome sequencing revealed two de novo intergenic duplications at the *ATAD3* locus, a gene cluster composed of three paralogs, *ATAD3A, ATAD3B*, and *ATAD3C*. Variants in *ATAD3* are associated with pontocerebellar hypoplasia, hereditary spastic paraplegia, and a neurological disorder known as Harel–Yoon syndrome, as well as mitochondrial disease. *ATAD3A* and *ATAD3B* are nearly identical protein-coding genes, but whether *ATAD3C* is expressed is unknown. *ATAD3A* produces a transmembrane ATPase that localizes to contact sites between the inner and outer mitochondrial membranes. ATAD3 dysfunction and deficiency have been shown to affect mitochondrial morphology and fission dynamics, loss of cristae, and impaired mitochondrial DNA and cholesterol metabolism. The researchers determined that the duplications likely occurred through nonallelic homologous recombination between highly similar regions in *ATAD3A* and *ATAD3C*. Polymerase chain reaction (PCR) and Sanger sequencing confirmed the duplications via a 1.2-kb proband-specific amplicon with a 5′ end from exon 10 of *ATAD3A* and a 3′ end from intron 7 of *ATAD3C*. Sanger sequencing of a reverse transcription PCR product derived from one subject's fibroblast RNA produced a sequence identical to the predicted fusion transcript. Western blot analysis suggested that the fusion protein was expressed and stable, but in silico analyses revealed it lacks key functional residues. The team then assessed mitochondrial morphology and cholesterol levels from the subject's fibroblasts. Filipin staining revealed that free-unesterified cholesterol was significantly higher than that in controls and similar to cells deficient in ATAD3. The subject's fibroblasts also possessed features associated with *ATAD3* cluster deletions, including mitochondrial aggregations, swollen organelles, and mitochondrial DNA accumulations. The authors deduce that the ATAD3A-C fusion protein is dysfunctional and perturbs cholesterol metabolism. The findings extend the spectrum of *ATAD3*-related disorders. Because the subjects did not show obvious mitochondrial distress, Gunning and colleagues emphasize the consideration of mitochondrial genes in atypical cases and advise evaluation of the *ATAD3* locus for severe neonatal disorders of unknown origin that are negative for mitochondrial variants and mitochondrial nuclear genome panels. —*V. L. Dengler, News Editor*

Intellectual disability–associated gene *TBC1D24* maintains excitatory synapses



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Numerous signaling proteins decorate dendritic spines, protrusions located at the ends of branch-like structures on neurons where synapses transmit information. Dysfunctional dendritic spine proteins may lead to cognitive deficits and intellectual disability (ID). Human genetic studies have found that diverse variants in the *TBC1D24* gene are associated with epilepsy and ID. Despite TBC1D24's high expression in the human brain, the protein's physiological function is not well understood. Recently in *PLOS Genetics* (https://doi.org/10.1371/journal.pgen.1008587), Lin and colleagues reported that TBC1D24 is required for the maintenance of dendritic spines with roles in learning and memory. Using an exogenously expressed FLAG-tagged construct, the researchers first determined that TBC1D24 localizes to dendritic spines in dissociated rodent hippocampal neurons and confirmed the findings via immunofluorescence staining for endogenous TBC1D24. They also found that TBC1D24 colocalizes with PSD-95, a postsynaptic protein, indicating that TBC1D24 is expressed at postsynaptic sites of excitatory synapses. Knockdown of TBC1D24 with short hairpin RNAs (shRNAs) in cultured hippocampal neurons reduced the number of excitatory synapses and dendritic spines. An RNAi-resistant TBC1D24 rescued the loss. Together the results suggest that TBC1D24 is essential for maintaining dendritic spine numbers and excitatory synapses in vitro. In a subsequent experiment, Lin and team discovered that TBC1D24 maintains dendritic spines partially by suppressing the activity of the small GTPase ARF6. The team then confirmed that knockdown of TBC1D24 reduces dendritic spine density of hippocampal neurons in adult mice, using adeno-associated viral delivery of TBC1D24 shRNA via stereotaxic injection. Open-field tests revealed that mice with depleted TBC1D24 levels traveled farther than control mice and hugged the periphery of the arena as they moved, displaying increased anxiety. In a test of contextual fear conditioning, mice injected with TBC1D24 shRNA froze less after conditioning, suggesting impaired learning. Finally, the researchers investigated the effects of an ID-associated missense variant (c.751T>C) in *TBC1D24* that results in an amino acid substitution, F251L. The team generated knock-in mice harboring the variant. Homozygous mice died within 21 days, but heterozygous mice survived to adulthood. Reflecting previous experiments, the F251L heterozygous knock-in mice possessed fewer dendritic spines in hippocampal CA1 neurons and displayed reduced fear memory following contextual fear conditioning. The authors conclude that dendritic spine maintenance is a plausible pathogenic mechanism contributing to cognitive deficits from *TBC1D24* variants. —*V. L. Dengler, News Editor*

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