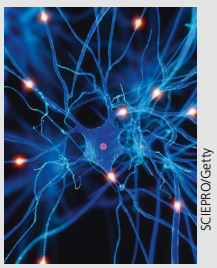
Genetics in Medicine | NEWS

NEWS

Transcriptional subunit variants result in demyelinating neuropathy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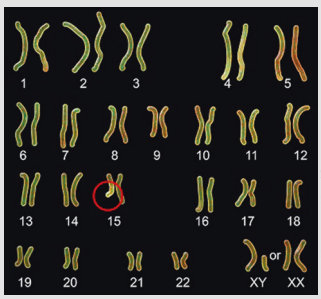
Biallelic variants in *POLR3B*, a gene that encodes the second-largest catalytic subunit of RNA polymerase III, are known to cause hypomyelinating leukodystrophy type 8. Individuals with hypomyelinating leukodystrophy type 8 present with endocrine dysfunction, ocular abnormalities, and abnormal dentition. As recently reported in the *American Journal of Human Genetics* (https://doi.org/10.1016/j.ajhg.2020.12.002), Djordjevic and colleagues found that de novo heterozygous missense variants in *POLR3B* result in demyelinating neuropathy, a disorder distinct from hypomyelinating leukodystrophy. The researchers discovered the variants from sequencing results in six unrelated individuals, who presented with clinical features that are distinct from those associated with previously reported variants. The six participants all presented with gait dysfunction ranging from mild instability to severe ataxia. Intellectual disability was mild to moderately severe in all but one individual, who showed normal development excepting early mild motor delay. This individual also displayed normal language development, while the other participants showed language delays. Two-thirds of subjects required assistance with activities of daily living and half had seizures. Notably, endocrinopathies or dentition, vision, cardiovascular, or skeletal abnormalities were not consistent features across the individuals. Electromyogram and nerve conduction studies uncovered mainly demyelinating sensory and motor neuropathies in five of the six individuals. Brain magnetic resonance imaging revealed nonspecific white matter signaling abnormalities in two individuals, but no evidence for hypomyelination or leukodystrophy was found in any individual. To determine how the de novo variants might impact POLR3B function, the researchers generated a sequence alignment and presented the variants on the yeast POL3R structure in the transcription initiation state. The modeling revealed that four of the variants cluster at the transcription bubble in an area where transcribed DNA is melted, while the remaining two are located in the exiting DNA tunnel and have direct contact with the DNA duplex. In a follow-up analysis, the team conducted affinity purification coupled with mass spectrometry in human embryonic kidney cells to assess the assembly of specific RNA pol III subunits. The analysis revealed that five of the variants impaired the association of at least one other subunit. Taken together, the data show that the *POLR3B* variants Djordjevic and colleagues identified lead to clinical features that are distinct from those of hypomyelinating leukodystrophy. The authors conclude that the findings expand the repertoire of *POLR3B*-associated disorders. —*V. L. Dengler, News Editor*

Gene therapy "unsilences" paternal *Ube3a* in Angelman syndrome model



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Variants or deletion of the maternally inherited *UBE3A* allele lead to Angelman syndrome (AS), a complex neurodevelopmental disorder. In neurons, a long noncoding RNA called *UBE3A-ATS* silences paternally inherited *UBE3A*. Potential therapeutics such as antisense nucleotides that target *UBE3A-ATS* have short half-lives and require repeated injections. Wolter and colleagues recently reported in *Nature* (https://doi.org/10.1038/s41586-020-2835-2) that they used CRISPR–Cas9 to reduce *Ube3a-ATS* expression and "unsilence" *Ube3a* in a mouse model of AS. The strategy showed that neonatal reactivation of paternal *Ube3a* can rescue many AS symptoms in mice. After screening 260 guide RNAs (gRNAs) targeting regions in or near *UBE3A-ATS*, the team found that transduction with lentivirus carrying *Staphylococcus pyogenes* Cas9 (SpCas9) and the gRNA Spjw33 in *Ube3a*$^{m+/pYFP}$ cortical neurons increased paternal *Ube3a-YFP* expression. A follow-up test in primary human neural progenitor-derived neurons with lentivirus carrying SpCas9:mCherry and adeno-associated virus type 2 (AAV2) carrying human, neuron-specific *SYN1*-driven eGFP with SpCas9 gRNAs targeting the same region as Spjw33 increased paternal *UBE3A* expression to near maternal levels. Due to the packaging limit of AAV, the researchers next utilized the smaller *Staphylococcus aureus* Cas9 with an equally effective gRNA, Sajw33. They then packaged hSYN1 promoter-SaCas9 and U6 promoter-Sajw33 into AAV9 and bilaterally injected the vector intracerebroventricularly (i.c.v.) into embryonic day 15.5 (E15.5) or postnatal day 1 (P1) *Ube3a*$^{m+/pYFP}$ mice. Animals injected at E15.5 and P1 showed biased unsilencing of paternal UBE3A-YFP in lower-layer and upper-layer cortical neurons, respectively. Injection of AS mice (*Ube3a*$^{m-/p+}$) at E15.5 and P1 unsilenced paternal UBE3A throughout the P30 and P90 brain and spinal cord. Subsequent experiments revealed that paternal UBE3A remained unsilenced in AS mice 17 months after a single E15.5 injection and that AAV viral DNA did not transfer to dams during pregnancy. Finally, the researchers assessed whether E15.5 + P1 i.c.v. injection of the SaCas9 AAV vector could rescue AS symptoms in mice modeling the disease. Treatment with the SaCas9 + Sajw33 vector increased brain weight in AS mice. Treated AS mice also showed improvements in hindlimb clasping, center time in the open field, and the rotarod test, but not in distance traveled in the open field or marble burying, compared with untreated AS mice. The authors conclude that AAV integration can unsilence *Ube3a* with enough frequency to remedy many AS symptoms in a long-term and likely permanent manner. —*V. L. Dengler, News Editor*