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All our knowledge begins with the antisenses

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Commentary

Epilepsy is the neurological disorder defined by spontaneous recurrent seizures, which are abnormal patterns of electrical discharge in the brain. A major advance in neurology over the last 20 years is the identification of genetic variation as an important cause of epilepsy, and in particular as a cause of the epileptic encephalopathies, defined by childhood-onset, treatment-resistant epilepsy accompanied by developmental delay leading to intellectual disability. Unfortunately, this progress in genetic diagnosis has yet to translate to effective precision or targeted therapeutics. However, in this issue of the *JCI*, Li and Jancovski et al. use antisense oligonucleotides (ASO) to treat or prevent epilepsy and epilepsy-associated cognitive and behavioral comorbidities in a mouse model of *SCN2A* encephalopathy, paralogous to the recurrent human variant *SCN2A* c.5645G>A (p.R1882Q) associated with epileptic encephalopathy. These findings may inform the development of targeted or personalized therapies for what is currently an incurable and largely untreatable disorder.

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Epilepsy is the neurological disorder defined by spontaneous recurrent seizures, which are abnormal patterns of electrical discharge in the brain. A major advance in neurology over the last 20 years is the identification of genetic variation as an important cause of epilepsy, and in particular as a cause of the epileptic encephalopathies, defined by childhood-onset, treatment-resistant epilepsy accompanied by developmental delay leading to intellectual disability. Unfortunately, this progress in genetic diagnosis has yet to translate to effective precision or targeted therapeutics. However, in this issue of the *JCI*, Li and Jancovski et al. use antisense oligonucleotides (ASO) to treat or prevent epilepsy and epilepsy-associated cognitive and behavioral comorbidities in a mouse model of *SCN2A* encephalopathy, paralogous to the recurrent human variant *SCN2A* c.5645G>A (p.R1882Q) associated with epileptic encephalopathy. These findings may inform the development of targeted or personalized therapies for what is currently an incurable and largely untreatable disorder.

SCN2A-related neurodevelopmental disorders

Over 100 monogenic causes of epilepsy have so far been identified, with an enrichment for genes encoding ion channels, neurotransmitter receptors, and synaptic molecules. Among these, each of the four brain-expressed Na^+ ion channels, *SCN1A*, *2A*, *3A*, and *8A*, are established epilepsy genes. De novo pathogenic variants in *SCN2A* associate with a spectrum of neurodevelopmental disorders, including benign neonatal or infantile-onset epilepsy, autism spectrum disorder (ASD) and intellectual disability (ID), and early-onset epileptic encephalopathy (*SCN2A* encephalopathy) (1). Nav1.2-containing Na^+ channels are important for generating action potential in brain neurons and for regulating synaptic plasticity via back-propagation into neuronal dendrites (2, 3). Studies of Na^+ channels containing disease-associated Nav1.2 variants have led to the schema under which gain of function (GoF) due to missense variants in *SCN2A* (e.g., with increased persistent current) associates with epileptic encephalopathy (4–6). In contrast, loss of function (LoF) due to missense variant or *SCN2A* deletion (with less Na^+ current), causes ASD/ID that is sometimes accompanied by typically mild epilepsy. Hence, one could envision a framework in which blocking Nav1.2-containing Na^+ channels or decreasing Nav1.2 expression could treat *SCN2A* encephalopathy due to GoF variants. That seizures, in some cases, respond to Na^+ channel–blocking antiseizure medications (7, 8) provides support for this idea.

Antisense oligonucleotides for epilepsy

Enter antisense oligonucleotides (ASOs). ASOs are synthetic oligonucleotides (short strands of DNA or RNA) engineered to specifically bind to a unique target sequence (Figure 1). ASO drug candidates are designed to manipulate levels of an mRNA transcript that encodes a target protein of interest. For example, gapmer ASOs target mRNA for degradation by ribonuclease H (RNaseH). An alternative approach involves ASO binding of pre-mRNA to modulate splicing via steric hindrance (9).

Currently, there are more than 10 FDA-approved ASOs. One example includes nusinersen (marketed as Spinraza) for spinal muscular atrophy (10, 11), a pediatric neuromuscular disease due to reduced *SMN* protein secondary to biallelic LoF variants in the *SMN1* gene. Binding of nusinersen to an intronic splicing silencer between exons 7 and 8 of the *SMN2* pre-mRNA facilitates exon 7 inclusion into the *SMN2* mRNA and thereby increases levels of full-length, functional *SMN* protein in the central nervous system (Figure 1). There are more than 50 ASOs in various stages of clinical trials, with many others under preclinical development (12).

Stoke Therapeutics recently launched the phase 1/2a MONARCH study of STK-001 for the treatment of Dravet syndrome (ClinicalTrials.gov, NCT04442295), an epileptic encephalopathy due to heterozygous LoF variants in *SCN1A* encoding the voltage-gated Na^+ channel subunit Nav1.1. STK-001 is an ASO designed to exploit the presence of an *SCN1A* poison exon (13, 14). The small, noncoding exon contains a within-frame STOP codon such that incorporation of this exon into the full-length transcript leads to premature truncation and nonproductive splicing that triggers nonsense-mediated decay. STK-001 binds to pre-mRNA to alter splicing and block inclusion of the poison exon into the *SCN1A* mRNA, the effect of which is to increase protein expression (15, 16). The authors refer to this method as targeted augmentation of nuclear gene output (TANGO). Intracerebroventricular (i.c.v.) injection of TANGO into developing P2 or P14 *Scn1a*$^{+/-}$ mouse pups led to reduced seizures and increased survival (15).

Previously, Lenk et al. used an ASO approach to target another voltage-gated Na^+ channel gene, *Scn8a*, in a mouse model

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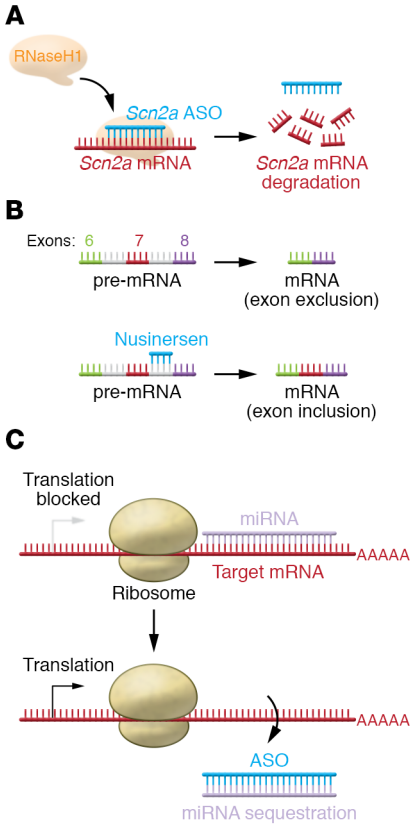

Figure 1. Antisense oligonucleotide strategies for therapeutic targeting of mRNA transcripts. (**A**) Schematic showing the basic gapmer ASO approach taken by Li and Jancovski et al. (20) to target *Scn2a* in an experimental mouse model of *SCN2A* encephalopathy. (**B**) A splice-modifying ASO as in nusinersen (Spinraza), which alters the pre-mRNA splicing of *SMN2* to facilitate integration of exon 7 into the mRNA to produce functional full-length protein. (**C**) miRNA-targeting ASO prevents miRNAs of interest from binding to a given RNA.

of *SCN8A* encephalopathy (17). Mice expressing the *Scn8a*-p.Arg1872Trp missense variant (18) are healthy prior to seizure onset, which occurs at P14–P16, then die within 24 hours. Na^+ channels containing Nav1.6-Arg1872Trp exhibit GoF. Hence, the authors generated an *Scn8a* ASO that decreased both WT and variant transcripts, but not other Na^+ channel transcripts. It is important to note that individuals who are heterozygous for LoF variants or lack *SCN8A* due to deletion show mild intellectual disability with or without ataxia. Moreover, an *Scn8a* null mutation (lacking any functional Nav1.6) in mice is lethal (19); hence, marked reduction in *Scn8a* may prove detrimental (similar to the situation in Li and Jancovski et al. regarding *Scn2a*; ref. 20). Lenk et al. (17) attempted to titrate the ASO dose to partially reduce *Scn8a* mRNA and Nav1.6 protein while still maintaining sufficient levels to observe a therapeutic effect. *Scn8a* ASO delivered via i.c.v. injection at P2 prolonged survival and delayed epilepsy onset in *Scn8a*-p.Arg1872Trp mice. Mice eventually exhibited epilepsy and death; however, repeated dosing prolonged survival (17).

ASO for *SCN2A* encephalopathy

In this issue of the *JCI*, Li and Jancovski et al. (20) used a gapmer ASO targeting *Scn2a* mRNA to treat or prevent epilepsy and epilepsy-associated cognitive and behavioral comorbidities in a mouse model of *SCN2A* encephalopathy. *Scn2a*-p.R1883Q mice (hereafter, Q/+ mice) are homologous to the recurrent human variant *SCN2A* c.5645G>A (p.R1882Q) associated with epileptic encephalopathy (1). This variant produced Na^+ currents in heterologous cell systems with increased persistent Na^+ current and, hence, likely GoF. Q/+ mice exhibited a severe, early-onset seizure phenotype, with spontaneous tonic seizures seen as early as P1, death as early as P13, and median survival time of 18 days. All mice were deceased by P30. Since the authors could not propagate mice with this phenotype, they cleverly generated an experimental animal model in which a targeting vector was delivered to mouse embryonic stem cells to yield 100% ES cell–derived Q/+ founder mice after implantation into and delivery by WT surrogate host dams.

The authors confirmed that i.c.v. injection of the *Scn2a* ASO successfully downregulated *Scn2a* mRNA and Nav1.2 protein (both variant and WT) with no off-target effect on other voltage-gated Na^+ channels. An effective dose was determined for 50% reduction (ED_{50}) of *Scn2a* mRNA levels at different ages. A single i.c.v. injection of ED_{50} *Scn2a* ASO into P1 Q/+ mice prolonged median survival to P47 with approximately 10% of mice surviving to the experimental endpoint at P80, compared with control ASO. *Scn2a* ASO almost completely eliminated seizures as determined via continuous video EEG at various time points. Repeated administration of *Scn2a* ASO further extended survival. *Scn2a* ASO ED_{50} i.c.v. injection at P14–P16 also extended lifespan, suggesting that treatment could be effective if initiated after symptom onset. Treated Q/+ mice underwent a battery of behavioral and cognitive testing and minimal abnormalities were identified. However, *Scn2a* ASO ED_{80} did result in impaired motor function as well as behavioral changes relative to untreated WT mice (comparison to untreated Q/+ mice was impossible as all mice were deceased at the relevant time points).

ED_{50} i.c.v. injection into WT mice at P1 did not affect body weight or survival, but ED_{80} injection led to decreased body weight and early mortality, consistent with early lethality observed in *Scn2a*$^{-/-}$ mice. WT mice injected with ED_{50} did spend less time in the closed arm of an elevated plus maze and demonstrated impairments on a grid walk assay.

These results show that *Scn2a* ASO can treat or prevent epilepsy and epilepsy-associated comorbidities, and prolong survival in an experimental mouse model of *SCN2A* encephalopathy with minimal apparent side effects if closely titrated, which could be achieved in the preclinical system but may be more difficult in humans.

Such results are impressive. However, one question that was not fully addressed by Li and Jancovski et al. is the exact mechanism whereby *Scn2a* ASO exerts this effect at the neuronal and network levels, and if the observed effects were actually due to downregulation of variant *Scn2a*. The authors provided some data demonstrating relatively subtle increases in the excitability of pyramidal neurons in layer 2/3 primary somatosensory neocortex in acute brain slices prepared from P10–P12 Q/+ mice. Neuronal excitability was accompanied by or due to increased input resistance and a lower rheobase (the minimal current injection required to generate an action potential). It seems unlikely that the profound phenotype in the Q/+ mice was due to this particular electrophysiological abnormality. Demonstration of cellular and circuit abnormalities would provide further support for the biological premise.

An important control or complementary experiment would be to treat another mouse epilepsy model with *Scn2a* ASO, such as a model of acquired epilepsy. Perhaps *Scn2a* ASO exerts a nonspecific antiseizure effect? As noted above, many

antiseizure medications act via Na^+ channel blockade, and Na^+ channel–blocking agents show efficacy in treating seizures in some cases of *SCN2A* encephalopathy. Perhaps the *Scn2a* ASO acts nonspecifically as a Na^+ channel–blocking antiseizure medication. An allele-specific ASO (targeting only the variant allele) could address this question and could potentially achieve the same or greater effect with fewer off-target effects, yet would have even more narrow clinical applicability.

ASO delivered intrathecally can target the peripheral nervous system in humans as demonstrated by the success of Spinraza. But in the examples described above for TANGO in *Scn1a*$^{+/-}$ mice, *Scn8a* ASO for *Scn8a* encephalopathy, and *Scn2a* ASO for *Scn2a* encephalopathy in Li and Jancovski et al., the compound was delivered via i.c.v. Li and Jancovski et al. show widespread distribution of ASO in mouse brain that is not likely achievable in humans via intrathecal injection as ASOs cross the blood-brain barrier inefficiently. It would be useful to know the extent of distribution needed in mouse brain to achieve the therapeutic effects observed by Li and Jancovski et al.

Considering safety and efficacy

An initial question for the future is whether ASO therapy is safe, and it largely appears to be. However, for some genes, including for epilepsy-associated ion channel genes, both GoF and LoF are pathogenic. In the case of *SCN2A*, LoF is an important cause of ASD/ID. Could an *SCN2A* ASO convert patients from an epileptic encephalopathy to an ASD/ID phenotype? Allele-specific ASO could potentially avoid this issue.

It is also known that plastic changes occur in brain circuits in response to pathogenic variants of disease-associated genes. Two recent papers show that the electrical excitability of Nav1.2-expressing excitatory neurons in striatum and neocortex is actually enhanced, rather than impaired, in mice lacking *Scn2a* (21, 22), due to complex interactions between and compensatory reorganization of Na^+ and potassium (K^+) currents. What changes might occur during development in *Scn2a* encephalopathy remains unknown. However, the safety and efficacy of a given ASO could depend on the timing of delivery during development. It is possible that there is a critical period within which knockdown of *SCN2A* might determine a given clinical outcome. This therapeutic window may make early and rapid genetic diagnosis in the epilepsies more critical.

Considering equity

Finally, an important question for the future is the potential scope across which ASO therapies can eventually be made available given that there are over 700 genetic causes of intellectual and developmental disability. The San Diego, California, USA–based nonprofit company n-Lorem, working with Ionis Pharmaceuticals (which contributed to the Li and Jancovski et al. paper), aims to develop ASO-based therapies for children with ultrarare diseases affecting one or a small handful of patients, and offer such therapies for free (free, at least, to the patient). It remains to be seen how the field can and should best allocate resources and address issues related to equity of access to this promising therapy.

ASOs potentially open an exciting avenue toward the treatment of rare neurological diseases that might previously have been considered unapproachable via other therapies. Questions remain related to equity of access: how should targets be prioritized for therapy development, and based on what criteria? Disease severity, lack of available alternative therapies such as candidate small molecules, existence of robust natural history data, knowledge of the ASO target and disease pathomechanisms, and total number of patients who might stand to benefit, could factor into such calculations.

Nevertheless, early studies applying ASO technology to the treatment of epileptic encephalopathy and other neurodevelopmental disorders, including the study by Li and Jancovski et al., provide hope for a better tomorrow for patients with epilepsy and their families.

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