



Adult Gene Therapy for Epilepsy in a Model of Angelman Syndrome: Hope or Hype?

Antisense Oligonucleotide Therapy Rescues Disturbed Brain Rhythms and Sleep in Juvenile and Adult Mouse Models of Angelman Syndrome

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UBE3A encodes ubiquitin protein ligase E3A, and in neurons its expression from the paternal allele is repressed by the UBE3A antisense transcript (UBE3A-ATS). This leaves neurons susceptible to loss-of-function of maternal UBE3A. Indeed, Angelman syndrome, a severe neurodevelopmental disorder, is caused by maternal UBE3A deficiency. A promising therapeutic approach to treating Angelman syndrome is to reactivate the intact paternal UBE3A by suppressing UBE3A-ATS. Prior studies show that many neurological phenotypes of maternal Ube3a knockout mice can only be rescued by reinstating Ube3a expression in early development, indicating a restricted therapeutic window for Angelman syndrome. Here, we report that reducing Ube3a-ATS by antisense oligonucleotides in juvenile or adult maternal Ube3a knockout mice rescues the abnormal electroencephalogram (EEG) rhythms and sleep disturbance, two prominent clinical features of Angelman syndrome. Importantly, the degree of phenotypic improvement correlates with the increase of Ube3a protein levels. These results indicate that the therapeutic window of genetic therapies for Angelman syndrome is broader than previously thought, and EEG power spectrum and sleep architecture should be used to evaluate the clinical efficacy of therapies.

Commentary

Angelman syndrome (AS) is a neuropsychiatric disorder with developmental delay, speech impairment, motor and balance deficiencies, sleep disturbances, and unique personality features.^{1,2} A very common feature is epilepsy, with the most frequently observed seizure types being atypical absences, generalized tonic-clonic, atonic or myoclonic seizures.³ A majority (70%) of patients have a maternal chromosomal deletion of 15q11.2-13, and these deletion patients have more severe and intractable epilepsy than nondeletion cases.^{2,4,5} The maternal deletion region is paternally inactivated, leading to severe loss of function of genes in this region: E6AP-E3 ubiquitin protein ligase (UBE3A), ATP10C, HERC2, and multiple GABA_A receptor subunits, including GABRB3, GABRA5, and GABRG3.^{6,7} In 1997 two studies found mutations in UBE3A in a small fraction of Angelman syndrome cases, even in the absence of 15q11.2-13 deletions,^{2,7} supporting the conclusion that UBE3A is an Angelman syndrome gene. Despite the fact that other 15q11-q13 genes play important roles in Angelman syndrome,^{6,8} there has been a major focus on the role of UBE3A since these genetic discoveries. Gene therapies focusing on restoration of UBE3A function have progressed to clinical trials.

Early preclinical UBE3A gene therapy trials in mice showed efficacy, but only with very early treatment, before the second day of life (reviewed in supplemental files of study by Lee et al⁹). Here Lee and colleagues⁹ used a novel gene therapy approach and show partial restoration of function with antisense oligonucleotide gene knockdown approaches in *juvenile*, and to a lesser extent in *adult* mice engineered with maternal UBE3A deficiency. As the paternal copy of UBE3A is repressed by a specific endogenous antisense sequence (UBE3A-ATS), genetic restoration of the paternal copy through exogenous antisense oligonucleotides (ASOs) targeting UBE3A-ATS is possible; this approach was adopted by Lee and colleagues.

First, the authors showed that each of 2 different ASOs increased UBE3A expression, especially in 3-week-old mice. With one ASO (UBE3A-as) UBE3A protein levels were restored to near control levels in multiple brain regions, with effects lasting at least 10 weeks. This suggests that the gene therapy approach can be both powerful and durable. A second ASO also restored protein expression, but to a lesser extent. The authors further showed that levels of UBE3A-ATS in the brain were inversely correlated with UBE3A. This is the first demonstration that Angelman syndrome gene therapy could be



effective even outside of the neonatal period. This is important because Angelman syndrome is often not diagnosed until significantly later in development—efficacy in juvenile mice suggests potential benefits when treating young patients. The authors also tested the ASOs in young adult mice (9 weeks old) and found some restoration of brain UBE3A protein, with the restoration being weaker and briefer than in juvenile mice.

Did restoration of UBE3A levels improve neurobehavioral outcomes? The authors recorded EEG to test for correlates of sleep and hyperexcitability. Quantitative sleep EEG in Angelman Syndrome patients⁶ has documented increased broad spectrum (1–32 Hz) power, with peak differences in the delta range near 3 Hz. This is consistent with findings in Angelman syndrome patients of persistent slow and sharp waves during sleep resembling continuous spike and slow waves.⁴ The maternal UBE3A deficient mice in the current study also showed subtle sleep defects. Increases in alpha and low beta power were observed in juvenile mice, yet unlike in human patients, no changes in delta were seen. Species specific sleep-related spectral power differences were restored with UBE3A-as, with effects lasting up to 10 weeks. Smaller, more variable EEG changes were seen during sleep in adult mice—these, however, were *not* improved by UBE3A-as treatment. Finally, polyspikes, 1 to 2 second long sequences of brief EEG spikes with potential relevance to epilepsy, occur frequently (~ every 10 hours) in UBE3A deficient mice, but their incidence was not affected by UBE3A-as treatment.

In summary, this paper extends gene therapeutic approaches to older mice in models of Angelman syndrome. Unsilencing paternal UBE3A genes increased UBE3A protein expression and produced partial restoration of function. The authors thus provide evidence that the therapeutic window can be extended beyond embryonic and early neonatal stages to juvenile, and to a lesser extent, young adult stages. A strength of the paper is the rigorous analysis of protein and gene expression in the mouse brain, with careful documentation of the extent and duration of gene therapeutic effects on UBE3A, which can extend for many weeks. Another strength is the finding of subtle sleep differences in juvenile mice that were restored by gene therapy. Weaknesses and opportunities for further work include limitations of the preclinical animal model (see next), the decreased efficacy in adult compared to juvenile mice indicating that there may be a limited therapeutic window, the failure to affect hyperexcitability (polyspikes), and the limited improvement in sleep EEG only observed in juvenile mice.

Is Angelman syndrome a single-gene disorder? This is a critical question when considering gene therapies. The authors of the studies originally documenting UBE3A mutations in some Angelman cases were circumspect in their findings. For example, one study's authors noted that UBE3A is *an* Angelman gene—concluding in the abstract (emphasis mine) that “Our results demonstrate that UBE3A mutations are *one* cause of AS...”¹⁰—and in the other paper “...we conclude that loss-of-function mutations in UBE3A *can lead to* AS.”¹¹ Neither of these reports ruled out the possibility that other genes in the 15q deletion region are important contributors to pathology.

Although the original authors may have not intended this outcome, these papers are often cited as evidence that UBE3A is *the* Angelman gene. In fact, research in the field has gone on to focus almost exclusively on loss of UBE3A function as the sole cause of Angelman syndrome. This premise, unfortunately, is undermined by 2 issues. First, a large number of AS patients, so called class V, have no loss of the UBE3A gene, either through deletion or mutation.^{2,7} While these patients are possibly misdiagnosed, overall these considerations indicate that a focus on genetic rescue of UBE3A function needs to be further considered, especially regarding epilepsy. The second and related issue is that other genes in the deletion region may play major roles in the disease.^{6,8}

If AS is not a single-gene disorder, and other alleles in the deletion region such as GABA_A receptor subunits contribute to pathology, then there are major implications. First, animal models that exclusively focus on loss of UBE3A function may not fully recapitulate the human disease. As discussed above, severe epilepsy is manifest mainly in patients with the most common cause—those with maternal chromosomal deletions.^{2–5} In addition, other Angelman deleted genes, for example, GABA receptors, likely contribute to sleep disturbances and epilepsy in affected patients,⁶ and perhaps in cognitive developmental delay. Severe seizures, a very common issue for human AS patients, are not observed in UBE3A mutant mice.⁹ Instead, only mild EEG abnormalities are reported (polyspikes and altered seizure susceptibility). Similarly, cognitive deficits are not a reliable feature in UBE3A mutant mice (see authors' response to reviewers,⁹ always available for *eLife* papers). A related second major concern is that gene therapies tested in such monogenetic models may have limited ability to predict the expected improvement in the vast majority of patients: those with altered gene function beyond UBE3A deficiency. Note that in discussions of current clinical trials,¹² it is appreciated that UBE3A restoration might still be of clinical interest even if it only partially rescues AS deficits. If monogenetic mouse models have important limitations, are there alternative, and potentially more informative, preclinical models available? At least one mouse model that mimics 15q11–13 deletions has been created, and affected mice do show spontaneous seizures and cognitive deficits with hidden platform and fear conditioning tests.¹³ Arguably, further clinical trials should await evidence of gene therapeutic efficacy in robust preclinical models with documented epileptic and cognitive deficits.

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Declaration of Conflicting Interests

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