# Prenatal Treatment Path for Angelman Syndrome and Other Neurodevelopmental Disorders

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Angelman syndrome (AS) is a rare neurodevelopmental disorder caused by mutation or deletion of the maternally inherited *UBE3A* allele. These pathogenic mutations lead to loss of maternal *UBE3A* expression in neurons. Antisense oligonucleotides and gene therapies are in development, which activate the intact but epigenetically silenced paternal *UBE3A* allele. Preclinical studies indicate that treating during the prenatal period could greatly reduce the severity of symptoms or prevent AS from developing. Genetic tests can detect the chromosome 15q11-q13 deletion that is the most common cause of AS. New, highly sensitive noninvasive prenatal tests that take advantage of single-cell genome sequencing technologies are expected to enter the clinic in the coming years and make early genetic diagnosis of AS more common. Efforts are needed to identify fetuses and newborns with maternal 15q11-q13 deletions and to phenotype these babies relative to neurotypical controls. Clinical and parent observations suggest AS symptoms are detectable in infants, including reports of problems with feeding and motor function. Quantitative phenotypes in the 0- to 1-year age range will permit a more rapid assessment of efficacy when future treatments are administered prenatally or shortly after birth. Although prenatal therapies are currently not available for AS, prenatal testing combined with prenatal treatment has the potential to revolutionize how clinicians detect and treat babies before they are symptomatic. This pioneering prenatal treatment path for AS will lay the foundation for treating other syndromic neurodevelopmental disorders. *Autism Res 2020, 13: 11–17.* © 2019 International Society for Autism Research, Wiley Periodicals, Inc.

**Lay summary:** Prenatal treatment could benefit expectant parents whose babies test positive for the chromosome microdeletion that causes Angelman syndrome (AS). Prenatal treatment is predicted to have better outcomes than treating after symptoms develop and may even prevent AS altogether. This approach could generally be applied to the treatment of other syndromic neurodevelopmental disorders.

## Overview

Angelman syndrome (AS) is characterized by developmental delays, severe intellectual disabilities, lack of speech, seizures, problems with movement and balance, and microcephaly [Pelc, Cheron, & Dan, 2008; Williams et al., 2006]. Many individuals with AS also meet the diagnostic criteria for autism [Peters, Beaudet, Madduri, & Bacino, 2004]. With a prevalence of 1:12,000 to 1:24,000 and a need for constant care across a full life span, the family burden and health care costs are high and likely exceed the now dated $2.4 M lifetime estimate for an individual with autism and intellectual disability [Buescher, Cidav, Knapp, & Mandell, 2014].

*UBE3A* is expressed biallelically in nearly all cells of the body, with the exception of neurons, where *UBE3A* is expressed only from the maternally inherited allele (Fig. 1). In most cases, AS is caused by deletion or mutation of the maternally inherited *UBE3A* allele [LaSalle, Reiter, & Chamberlain, 2015; Mabb, Judson, Zylka, & Philpot, 2011; Matsuura et al., 1997]. This biology explains why loss of the maternal allele causes AS and impairs brain function. The paternal allele is silenced by *UBE3A-ATS*, an extremely long antisense transcript that represses the paternal *UBE3A* transcript in *cis* (Fig. 1; Chamberlain & Brannan, 2001; Landers et al., 2004; Meng, Person, & Beaudet, 2012).

## Genetic Tests for AS

All of the mutation types that cause AS can be detected with routine genetic tests and provide a definitive diagnosis [Committee on, 2018; Dagli, Mueller, & Williams, 1993]. Deletion of the maternally inherited chromosome 15q11-q13 region is the most common cause of AS, is present in ~70% of cases, and is amenable to treatments that are in development (see below). This deletion typically
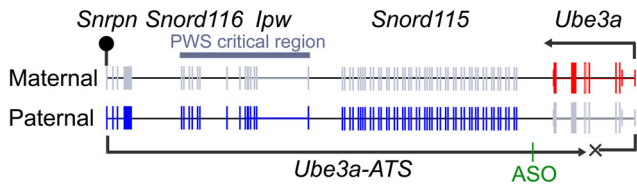
**Figure 1.** In neurons, *Ube3a-ATS* transcriptionally blocks paternal *Ube3a* in *cis*. Gray color denotes genes that are repressed. Location of Meng et al. ASO shown. Lollipop = methylated/imprinted region.

occurs *de novo* and is identified using array comparative genome hybridization and a methylation test to determine if the deletion is on the maternal or paternal chromosome. Genetic tests can also be performed prenatally in conjunction with amniocentesis or chorionic villus sampling (CVS).

Noninvasive prenatal tests (NIPTs) can detect the sex of the fetus and chromosomal abnormalities as early as 10 weeks after conception [Vora & Wapner, 2018]. Currently available "cell-free" NIPTs entail isolation and sequencing of fetal-derived DNA that is circulating in the mother's blood. This simplicity, low cost, and accuracy at providing useful genetic information about the fetus has promoted increasing adoption of this test by pregnant women irrespective of maternal age [Grace, Hardisty, Dotters-Katz, Vora, & Kuller, 2016; Larion et al., 2014]. Cell-free NIPTs can also detect the 15q11-q13 deletion that causes AS (when the deletion is maternally inherited) or Prader–Willi syndrome (PWS; when the deletion is paternally inherited) [Liang et al., 2019; Wapner et al., 2015]. However, the positive predictive value is not high enough for experts to recommend cell-free NIPT for 15q11-q13 deletion testing [Petersen et al., 2017; Vora & Wapner, 2018]. Expectant mothers can "opt-in" to get 15q11-q13 deletion NIPT results, with an understanding that an invasive diagnostic procedure like CVS will be needed to confirm the diagnosis.

The increasing use of cell-free NIPTs will invariably drive rapid adoption of next-generation "cell-based" NIPTs that show specificity and accuracy values that are similar to invasive procedures [Kolvraa et al., 2016; Vossaert et al., 2018]. It has been known for over a decade that nucleated fetal-derived cells circulate in the mother's blood, but only recently has it been possible to reliably identify and isolate these cells [Christensen et al., 2005]. Cell-based NIPTs utilize cutting-edge single-cell sequencing technologies to identify mutations in these rare circulating fetal-derived cells. It is now possible to detect deletions, duplications, DNA methylation, and single nucleotide variants using single-cell DNA sequencing [Hui et al., 2018; McConnell et al., 2017]. Thus, it is technically possible to examine portions of the fetal genome and epigenome at single nucleotide resolution using a noninvasive procedure. Widespread adoption of cell-based NIPTs will undoubtedly revolutionize prenatal screening and increase the demand for early treatments for AS and for other syndromic disorders.

Newborn screening for AS is also possible, but criteria largely limit newborn screening to diseases for which early diagnosis benefits the baby, such as when a treatment or an intervention exists [Pitt, 2010]. There is reason for optimism with regard to newborn screening for AS and its "sister" imprinted disorders—PWS and Dup15q syndrome. A recent study found that PWS can be treated shortly after birth with growth hormone [Scheermeyer et al., 2017]. It might thus be possible to justify newborn screening for paternal 15q11-q13 deletion, to identify PWS newborns who would benefit from growth hormone. This test would also identify newborns with maternal 15q11-q13 deletion or duplication. Screening of this single genetic locus could thus permit early detection of three distinct neurodevelopmental disorders.

## Critical Period for Treatment

Existing genetic tests can identify individuals with a maternally inherited deletion of 15q11-q13 at any age, so a major question for the field is when to treat, particularly given that AS therapeutics are moving toward the clinic. To address this question, Elgersma and colleagues developed a way to genetically "reinstate" *Ube3a* at different ages in an AS model mouse [Silva-Santos et al., 2015]. AS model mice have reproducible phenotypes that recapitulate AS symptoms, including motor deficits, sleep disruption, seizures, and cognitive deficits [Ehlen et al., 2015; Huang et al., 2013; Jiang et al., 1998; Sonzogni et al., 2018]. Elgersma and colleagues found that early embryonic reinstatement of *Ube3a* prevented AS phenotypes across multiple domains from developing, while reinstatement at birth (postnatal day 0; P0) or 21 days after birth (P21) rescued some phenotypes (Table 1). Extrapolating to humans, P0 in a mouse corresponds to early second trimester in humans, whereas P21 corresponds to ~210 days old in humans [Workman, Charvet, Clancy, Darlington, & Finlay, 2013].

In contrast, adult *Ube3a* reinstatement only rescued hippocampal long-term potentiation, a learning and memory phenotype (Table 1; Silva-Santos et al., 2015). Likewise, antisense oligonucleotides (ASOs, described below) that unsilence paternal *Ube3a* partially treated a fear conditioning deficit in adult AS model mice [Meng et al., 2015], a phenotype that is also related to learning and memory.

These findings were reinforced by a subsequent study from Elgersma and colleagues, in which they deleted maternal *Ube3a* at different ages [Sonzogni et al., 2019]. They found that deletion of maternal *Ube3a* embryonically recapitulated all measured AS phenotypes. In contrast, deletion of maternal *Ube3a* postnatally or in adults had very few deleterious effects. Collectively, these data indicate that there

**Table 1. Phenotypes that Are Expected to Show Full (FR), Partial, or No Rescue (NR) When Paternal *Ube3a* Is Unsilenced at Different Developmental Time Periods in AS Model Mice**

| Phenotype | Developmental time period | | | |
|---|---|---|---|---|
| | Embryonic | P0 | P21 | Adult |
| Learning/memory | FR | FR | FR | FR |
| Rotarod | FR | FR | Partial | NR |
| Open field | FR | FR | NR | NR |
| Marble burying | FR | NR | NR | NR |
| Nest building | FR | NR | NR | NR |
| Forced swim | FR | NR | NR | NR |

Expectations based on data in [Meng et al., 2015; Silva-Santos et al., 2015].

is a critical period, early in development, when AS can most effectively be treated through restoration of *Ube3a*.

## Therapeutic Development

Therapeutic approaches are being pursued that restore *Ube3a* expression in neurons and hence target the root cause of AS. One approach is based on unsilencing the functional but epigenetically silenced paternal *Ube3a* allele in neurons. Unsilencing approaches have a key advantage over other approaches. Namely, unsilencing approaches drive expression of paternal *Ube3a* from the endogenous promoter, which is predicted to permit proper isoform distribution and proper UBE3A protein levels at each stage of life. Work in our laboratory, jointly with others, provided the first evidence that the paternal *Ube3a* allele can be unsilenced with a class of drugs that are used clinically to treat cancer, namely topoisomerase inhibitors [Huang et al., 2012]. These drugs repress the extremely long *Ube3a-ATS* transcript, permitting expression of paternal *Ube3a* in mouse and human neurons [King et al., 2013]. However, topoisomerase inhibitors are not suitable for life-long use because of known side-effects in humans, and these drugs downregulate many other long gene transcripts, some of which are important for brain development and synaptic function [King et al., 2013; Mabb et al., 2014].

Subsequently, Beaudet and colleagues unsilenced paternal *Ube3a* by targeting *Ube3a-ATS* with ASOs (Fig. 1) [Meng et al., 2015]. ASOs rescued one behavioral phenotype in AS model mice but required repeated invasive spinal injections every few months, which is not ideal for a pediatric onset disorder that lasts a lifetime. An artificial transcriptional repressor was also developed that binds to *Ube3a-ATS* and partially unsilenced paternal *Ube3a* in the mouse brain [Bailus et al., 2016]. No behavioral studies were performed in this latter study. Like ASOs, this protein repressor had to be injected repeatedly—three times per week for 4 weeks, and this protein downregulated

genes in the PWS critical region, which is not a desirable side-effect.

It may be possible to overcome shortcomings of topoisomerase inhibitors, ASOs, and protein-based repressors using new genome editing technologies like CRISPR/Cas9. When packaged into adeno-associated virus (AAV) gene therapy vectors, Cas9 can be delivered to animals and treat diseases for significantly longer periods of time [Nelson et al., 2016].

Another approach is based on using AAV to deliver *UBE3A* to individuals with AS, a so-called gene replacement therapy. AAV-based gene replacement therapies have an acceptable safety profile in humans [Hocquemiller, Giersch, Audrain, Parker, & Cartier, 2016; Hudry & Vandenberghe, 2019] and drive gene expression in the human and primate brain for a decade or more [Sehara et al., 2017; Tuszynski et al., 2015]. Gene replacement therapies are used clinically to treat other single gene disorders, including spinal muscular atrophy, a pediatric-onset neurological disorder [Mendell et al., 2017]. However, challenges remain, particularly with regard to selecting the appropriate *UBE3A* isoform [Avagliano Trezza et al., 2019] and with selecting a promoter that drives *UBE3A* expression at the correct levels across the life span, without overshooting normal expression levels. It is well established that *UBE3A* levels must be tightly maintained within a narrow range to permit normal brain development, as evidenced by the fact that the loss of maternal *UBE3A* causes AS while duplication of *UBE3A* increases autism and neuropsychiatric disorder risk [LaSalle et al., 2015; Noor et al., 2015]. Traditional gene replacement therapies typically drive expression of a single isoform at abnormally high levels, with variability between cells influenced by vector copy number.

## Prenatal Treatment Path

The benefits and risks associated with treating AS at different ages will ultimately need to be evaluated by institutional review boards and government regulators. However, the studies described above indicate that the optimal age to treat AS is prenatally. Pathogenic mutations that cause or increase risk for AS can be detected prenatally, further enabling proactive prenatal treatment. Given the necessity and sufficiency of *Ube3a* over a narrow prenatal window, prenatal administration of a gene therapy vector has the potential to greatly diminish the severity of AS, and if transduction efficiency is high, prevent AS from developing.

Indeed, studies with rare "AS mosaics" suggest some behavioral recovery can be achieved even if *UBE3A* is restored in a small percentage of all neurons. AS individuals that are mosaic show variable loss of methylation at the maternal imprinting center, which leads to inactivation of maternal *UBE3A* in a variable number of cells. AS mosaics show symptoms that are classified as

"exceptionally mild" when as few as 10% of all blood cells contain normal levels of methylation [Brockmann, Bohm, & Burger, 2002; Carson, Bird, Childers, Wheeler, & Duis, 2019; Le Fevre et al., 2017; Nazlican et al., 2004]. Mild phenotypes include near normal speech, near normal motor performance, and lack of seizures. Assuming mosaicism in blood cells is reflective of mosaicism in the brain, restoring functional *UBE3A* in as few as 10% of all brain neurons could largely prevent severe AS symptoms from developing. Changes of this magnitude would greatly improve the quality of life for individuals with AS and their caregivers.

Prenatal testing can be performed around the end of the first trimester, and results are returned early in the second trimester. Thus, not inclusive of preimplantation genetic testing, the earliest practical age to treat is during the second trimester. This is the period when cortical neurogenesis ends [Meyer, Schaaps, Moreau, & Goffinet, 2000]. A minimally invasive ultrasound-guided injection procedure was recently developed to deliver an AAV gene therapy vector to the brain of second trimester rhesus macaques—a nonhuman primate whose gestation period is similar to that of humans [Massaro et al., 2018].

Prenatal treatment with a gene therapy vector has other advantages. Mammals, including humans, develop immune responses to AAV and its cargo when injected into adults [Vandamme, Adjali, & Mingozzi, 2017]. This immune response is known to reduce treatment efficacy over time. However, prenatal or early postnatal injection of AAV results in immune tolerance—meaning the virus and cargo are seen as self, allowing for persistent expression with no immune response [Hinderer et al., 2015; Hordeaux et al., 2019; Tai et al., 2015]. Treatment during this immune tolerant period is ideal, especially if non-mammalian proteins like CAS9 are used as part of the gene therapy [Nelson et al., 2019]. As additional advantages, the brain is smaller in a fetus, so significantly less gene therapy vector will need to be manufactured and administered, potentially reducing costs. And, AAV is effective at transducing >50% of all neurons when injected intracerebroventricularly into newborn mice [Chakrabarty et al., 2013]. As noted above, this age in mice is equivalent to the second trimester in humans. Whether AAV transduces neurons as efficiently when injected prenatally into nonhuman primates is currently unclear.

There are parallels between prenatal treatment of AS and prenatal treatment of spina bifida—a neurodevelopmental disorder that, like AS, results in severe, life-long neurological deficits if not treated [Fletcher & Brei, 2010]. Prenatal neurosurgeries to treat spina bifida have been performed for over two decades [Tulipan & Bruner, 1998], the risks are tolerated, and patient outcomes are significantly better than when surgeries are performed after birth [Adzick et al., 2011]. This procedure involves creating an incision in the mother's abdomen and uterus to access the fetus. The bioethics associated with performing invasive maternal–fetal surgeries to treat nonlethal fetal conditions has been extensively reviewed [Chervenak & McCullough, 2007; Lyerly, Gates, Cefalo, & Sugarman, 2001]. The bioethics associated with treating AS prenatally, using a less invasive intra-cerebroventricular injection procedure [Massaro et al., 2018], is expected to be comparable.

## Limitations to Early Treatment

One of the main limitations to prenatal treatment of AS is that the regulatory path and safety profile for the fetus and mother has not been established. But as biomedical history has taught us, the prenatal period is not off-limits for new treatments that benefit the baby, including fetal blood transfusions and fetal neurosurgeries. Regulators base decisions on safety and efficacy data. Encouragingly, recent draft guidance from the U.S. Food and Drug Administration seeks to broaden the definition of the neonatal period to include preterm newborns born during the second trimester [Administration, 2019]. As a logical next step, clinicians may be able to evaluate the safety and efficacy of drug and biological therapeutics in second- or third-trimester fetuses, provided risks to the mother are considered and assessed. The reproducible and disease-modeling phenotypes in AS model mice, coupled with the ability to deliver gene therapies to fetal nonhuman primates [Conlon et al., 2016; Massaro et al., 2018], should allow for the necessary safety data to be collected.

Additionally, we currently lack a quantitative understanding of symptoms that differ between maternal 15q11-q13 deletion positive and neurotypical babies in the 0- to 1-year range, which limits rapid assessment of efficacy following prenatal treatment. Feeding problems and muscle hypotonia are evident in AS infants [Dagli et al., 1993; Pelc et al., 2008], but these symptoms were identified retrospectively and hence need to be quantitatively evaluated in a prospective manner. In the absence of neonatal natural history data, commonly studied AS symptoms that are apparent by 1–2 years of age, such as motor deficits, language impairment, and cognitive delays, could serve as primary end points in clinical trials. Extending the follow-up window would permit the use of standard developmental assessment tools. A small number of maternal 15q11-q13 deletion positive fetuses are being detected through traditional invasive prenatal genetic testing and could be enrolled in natural history studies focused on the 0- to 1-year old range. The number of fetuses that test positive for this deletion is expected to increase as next-generation cell-based NIPTs come online, which will increase the demand for prenatal treatment options.

## Applicability to Other Syndromic Neurodevelopmental Disorders

Significant progress has been made at identifying new syndromic forms of autism. As examples, *de novo* loss-of-function mutations in *CHD8* and *DYRK1* cause syndromic forms of autism [Bernier et al., 2014; van Bon et al., 2016]. A variant of CRISPR/Cas9 that does not cut the genome was recently packaged into an AAV gene therapy vector and used to rescue phenotypes caused by *Sim1* haploinsufficiency in mice [Matharu et al., 2019]. Assuming the penetrance of syndromic autism gene mutations is high and the critical period for treatment is early, it may be possible to develop gene therapies to treat these and other syndromic forms of autism caused by gene haploinsufficiency. Ultimately, efforts to treat AS prenatally could pioneer a future where sensitive noninvasive prenatal genetic tests are coupled with gene therapies to offer disease-modifying treatments for a diversity of syndromic neurodevelopmental disorders.

## References

Adzick, N. S., Thom, E. A., Spong, C. Y., Brock, J. W., 3rd, Burrows, P. K., Johnson, M. P., … Investigators, M. (2011). A randomized trial of prenatal versus postnatal repair of myelomeningocele. The New England Journal of Medicine, 364(11), 993–1004. https://doi.org/10.1056/NEJMoa1014379

Avagliano Trezza, R., Sonzogni, M., Bossuyt, S. N. V., Zampeta, F. I., Punt, A. M., van den Berg, M., … Elgersma, Y. (2019). Loss of nuclear UBE3A causes electrophysiological and behavioral deficits in mice and is associated with Angelman syndrome. Nature Neuroscience, 22, 1235–1247. https://doi.org/10.1038/s41593-019-0425-0

Bailus, B. J., Pyles, B., McAlister, M. M., O'Geen, H., Lockwood, S. H., Adams, A. N., … Segal, D. J. (2016). Protein delivery of an artificial transcription factor restores widespread Ube3a expression in an Angelman syndrome mouse brain. Molecular Therapy, 24(3), 548–555. https://doi.org/10.1038/mt.2015.236

Bernier, R., Golzio, C., Xiong, B., Stessman, H. A., Coe, B. P., Penn, O., … Eichler, E. E. (2014). Disruptive CHD8 mutations define a subtype of autism early in development. Cell, 158(2), 263–276. https://doi.org/10.1016/j.cell.2014.06.017

Brockmann, K., Bohm, R., & Burger, J. (2002). Exceptionally mild Angelman syndrome phenotype associated with an incomplete imprinting defect. Journal of Medical Genetics, 39(9), e51–e551.

Buescher, A. V., Cidav, Z., Knapp, M., & Mandell, D. S. (2014). Costs of autism spectrum disorders in the United Kingdom and the United States. JAMA Pediatrics, 168(8), 721–728. https://doi.org/10.1001/jamapediatrics.2014.210

Carson, R. P., Bird, L., Childers, A. K., Wheeler, F., & Duis, J. (2019). Preserved expressive language as a phenotypic determinant of mosaic Angelman syndrome. Molecular Genetics & Genomic Medicine, e837. https://doi.org/10.1002/mgg3.837

Chakrabarty, P., Rosario, A., Cruz, P., Siemienski, Z., Ceballos-Diaz, C., Crosby, K., … Levites, Y. (2013). Capsid serotype and timing of injection determines AAV transduction in the neonatal mice brain. PLoS One, 8(6), e67680. https://doi.org/10.1371/journal.pone.0067680

Chamberlain, S. J., & Brannan, C. I. (2001). The Prader-Willi syndrome imprinting center activates the paternally expressed murine Ube3a antisense transcript but represses paternal Ube3a. Genomics, 73(3), 316–322. https://doi.org/10.1006/geno.2001.6543

Chervenak, F. A., & McCullough, L. B. (2007). Ethics of maternal-fetal surgery. Seminars in Fetal & Neonatal Medicine, 12(6), 426–431. https://doi.org/10.1016/j.siny.2007.06.001

Christensen, B., Philip, J., Kolvraa, S., Lykke-Hansen, L., Hromadnikova, I., Gohel, D., … Djursing, H. (2005). Fetal cells in maternal blood: a comparison of methods for cell isolation and identification. Fetal Diagnosis and Therapy, 20(2), 106–112. https://doi.org/10.1159/000082432

Committee on, G. (2018). ACOG technology assessment in obstetrics and gynecology no. 14: Modern genetics in obstetrics and gynecology. Obstetrics and Gynecology, 132(3), e143–e168. https://doi.org/10.1097/AOG.0000000000002831

Conlon, T. J., Mah, C. S., Pacak, C. A., Rucker Henninger, M. B., Erger, K. E., Jorgensen, M. L., … Byrne, B. J. (2016). Transfer of therapeutic genes into fetal rhesus monkeys using recombinant adeno-associated type I viral vectors. Human Gene Therapy. Clinical Development, 27(4), 152–159. https://doi.org/10.1089/humc.2016.119

Dagli, A. I., Mueller, J., & Williams, C. A. (1993). Angelman Syndrome. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), GeneReviews((R)). Seattle, WA: University of Washington.

Ehlen, J. C., Jones, K. A., Pinckney, L., Gray, C. L., Burette, S., Weinberg, R. J., … DeBruyne, J. P. (2015). Maternal Ube3a loss disrupts sleep homeostasis but leaves circadian rhythmicity largely intact. The Journal of Neuroscience, 35(40), 13587–13598. https://doi.org/10.1523/JNEUROSCI.2194-15.2015

Fletcher, J. M., & Brei, T. J. (2010). Introduction: Spina bifida—A multidisciplinary perspective. Developmental Disabilities Research Reviews, 16(1), 1–5. https://doi.org/10.1002/ddrr.101

Food and Drug Administration. (2019). General clinical pharmacology considerations for neonatal studies for drugs and biological products guidance for industry. Rockville, MD: Center for Drug Evaluation and Research.

Grace, M. R., Hardisty, E., Dotters-Katz, S. K., Vora, N. L., & Kuller, J. A. (2016). Cell-free DNA screening: Complexities

and challenges of clinical implementation. Obstetrical & Gynecological Survey, 71(8), 477–487. https://doi.org/10.1097/OGX.0000000000000342

Hinderer, C., Bell, P., Louboutin, J. P., Zhu, Y., Yu, H., Lin, G., … Wilson, J. M. (2015). Neonatal systemic AAV induces tolerance to CNS gene therapy in MPS I dogs and nonhuman primates. Molecular Therapy, 23(8), 1298–1307. https://doi.org/10.1038/mt.2015.99

Hocquemiller, M., Giersch, L., Audrain, M., Parker, S., & Cartier, N. (2016). Adeno-associated virus-based gene therapy for CNS diseases. Human Gene Therapy, 27(7), 478–496. https://doi.org/10.1089/hum.2016.087

Hordeaux, J., Hinderer, C., Buza, E. L., Louboutin, J. P., Jahan, T., Bell, P., … Wilson, J. M. (2019). Safe and sustained expression of human iduronidase after intrathecal administration of adeno-associated virus serotype 9 in infant rhesus monkeys. Human Gene Therapy, 30, 957–966. https://doi.org/10.1089/hum.2019.012

Huang, H. S., Allen, J. A., Mabb, A. M., King, I. F., Miriyala, J., Taylor-Blake, B., … Philpot, B. D. (2012). Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. Nature, 481(7380), 185–189. https://doi.org/10.1038/nature10726

Huang, H. S., Burns, A. J., Nonneman, R. J., Baker, L. K., Riddick, N. V., Nikolova, V. D., … Moy, S. S. (2013). Behavioral deficits in an Angelman syndrome model: effects of genetic background and age. Behavioural Brain Research, 243, 79–90. https://doi.org/10.1016/j.bbr.2012.12.052

Hudry, E., & Vandenberghe, L. H. (2019). Therapeutic AAV gene transfer to the nervous system: A clinical reality. Neuron, 102 (1), 263. https://doi.org/10.1016/j.neuron.2019.03.020

Hui, T., Cao, Q., Wegrzyn-Woltosz, J., O'Neill, K., Hammond, C. A., Knapp, D., … Hirst, M. (2018). High-resolution single-cell DNA methylation measurements reveal epigenetically distinct hematopoietic stem cell subpopulations. Stem Cell Reports, 11(2), 578–592. https://doi.org/10.1016/j.stemcr.2018.07.003

Jiang, Y. H., Armstrong, D., Albrecht, U., Atkins, C. M., Noebels, J. L., Eichele, G., … Beaudet, A. L. (1998). Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron, 21(4), 799–811.

King, I. F., Yandava, C. N., Mabb, A. M., Hsiao, J. S., Huang, H. S., Pearson, B. L., … Zylka, M. J. (2013). Topoisomerases facilitate transcription of long genes linked to autism. Nature, 501(7465), 58–62. https://doi.org/10.1038/nature12504 nature12504 [pii].

Kolvraa, S., Singh, R., Normand, E. A., Qdaisat, S., van den Veyver, I. B., Jackson, L., … Beaudet, A. L. (2016). Genome-wide copy number analysis on DNA from fetal cells isolated from the blood of pregnant women. Prenatal Diagnosis, 36 (12), 1127–1134. https://doi.org/10.1002/pd.4948

Landers, M., Bancescu, D. L., Le Meur, E., Rougeulle, C., Glatt-Deeley, H., Brannan, C., … Lalande, M. (2004). Regulation of the large (approximately 1000 kb) imprinted murine Ube3a antisense transcript by alternative exons upstream of Snurf/Snrpn. Nucleic Acids Research, 32(11), 3480–3492. https://doi.org/10.1093/nar/gkh670

Larion, S., Warsof, S. L., Romary, L., Mlynarczyk, M., Peleg, D., & Abuhamad, A. Z. (2014). Uptake of noninvasive prenatal testing at a large academic referral center. Am J Obstet Gynecol, 211(6), 651–657. https://doi.org/10.1016/j.ajog.2014.06.038

LaSalle, J. M., Reiter, L. T., & Chamberlain, S. J. (2015). Epigenetic regulation of UBE3A and roles in human neurodevelopmental disorders. Epigenomics, 7(7), 1213–1228. https://doi.org/10.2217/epi.15.70

Le Fevre, A., Beygo, J., Silveira, C., Kamien, B., Clayton-Smith, J., Colley, A., … Dudding-Byth, T. (2017). Atypical Angelman syndrome due to a mosaic imprinting defect: Case reports and review of the literature. American Journal of Medical Genetics. Part A, 173(3), 753–757. https://doi.org/10.1002/ajmg.a.38072

Liang, D., Cram, D. S., Tan, H., Linpeng, S., Liu, Y., Sun, H., … Wu, L. (2019). Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. Genetics in Medicine. https://doi.org/10.1038/s41436-019-0467-4

Lyerly, A. D., Gates, E. A., Cefalo, R. C., & Sugarman, J. (2001). Toward the ethical evaluation and use of maternal-fetal surgery. Obstetrics and Gynecology, 98(4), 689–697.

Mabb, A. M., Judson, M. C., Zylka, M. J., & Philpot, B. D. (2011). Angelman syndrome: insights into genomic imprinting and neurodevelopmental phenotypes. Trends in Neurosciences, 34(6), 293–303.

Mabb, A. M., Kullmann, P. H., Twomey, M. A., Miriyala, J., Philpot, B. D., & Zylka, M. J. (2014). Topoisomerase 1 inhibition reversibly impairs synaptic function. Proceedings of the National Academy of Sciences of the United States of America, 111(48), 17290–17295. https://doi.org/10.1073/pnas.1413204111

Massaro, G., Mattar, C. N. Z., Wong, A. M. S., Sirka, E., Buckley, S. M. K., Herbert, B. R., … Rahim, A. A. (2018). Fetal gene therapy for neurodegenerative disease of infants. Nature Medicine, 24(9), 1317–1323. https://doi.org/10.1038/s41591-018-0106-7

Matharu, N., Rattanasopha, S., Tamura, S., Maliskova, L., Wang, Y., Bernard, A., … Ahituv, N. (2019). CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. Science, 363(6424), eaau0629. https://doi.org/10.1126/science.aau0629

Matsuura, T., Sutcliffe, J. S., Fang, P., Galjaard, R. J., Jiang, Y. H., Benton, C. S., … Beaudet, A. L. (1997). De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nature Genetics, 15(1), 74–77.

McConnell, M. J., Moran, J. V., Abyzov, A., Akbarian, S., Bae, T., Cortes-Ciriano, I., … Brain Somatic Mosaicism, N. (2017). Intersection of diverse neuronal genomes and neuropsychiatric disease: The brain somatic mosaicism network. Science, 356(6336), eaal1641. https://doi.org/10.1126/science.aal1641

Mendell, J. R., Al-Zaidy, S., Shell, R., Arnold, W. D., Rodino-Klapac, L. R., Prior, T. W., … Kaspar, B. K. (2017). Single-dose gene-replacement therapy for spinal muscular atrophy. The New England Journal of Medicine, 377(18), 1713–1722. https://doi.org/10.1056/NEJMoa1706198

Meng, L., Person, R. E., & Beaudet, A. L. (2012). Ube3a-ATS is an atypical RNA polymerase II transcript that represses the paternal expression of Ube3a. Human Molecular Genetics, 21(13), 3001–3012. https://doi.org/10.1093/hmg/dds130

Meng, L., Ward, A. J., Chun, S., Bennett, C. F., Beaudet, A. L., & Rigo, F. (2015). Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. Nature, 518(7539), 409–412. https://doi.org/10.1038/nature13975

Meyer, G., Schaaps, J. P., Moreau, L., & Goffinet, A. M. (2000). Embryonic and early fetal development of the human neocortex. The Journal of Neuroscience, 20(5), 1858–1868.

Nazlican, H., Zeschnigk, M., Claussen, U., Michel, S., Boehringer, S., Gillessen-Kaesbach, G., … Horsthemke, B. (2004). Somatic mosaicism in patients with Angelman syndrome and an imprinting defect. Human Molecular Genetics, 13(21), 2547–2555. https://doi.org/10.1093/hmg/ddh296

Nelson, C. E., Hakim, C. H., Ousterout, D. G., Thakore, P. I., Moreb, E. A., Castellanos Rivera, R. M., … Gersbach, C. A. (2016). In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science, 351(6271), 403–407. https://doi.org/10.1126/science.aad5143

Nelson, C. E., Wu, Y., Gemberling, M. P., Oliver, M. L., Waller, M. A., Bohning, J. D., … Gersbach, C. A. (2019). Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy. Nature Medicine, 25(3), 427–432. https://doi.org/10.1038/s41591-019-0344-3

Noor, A., Dupuis, L., Mittal, K., Lionel, A. C., Marshall, C. R., Scherer, S. W., … Stavropoulos, D. J. (2015). 15q11.2 duplication encompassing only the UBE3A gene is associated with developmental delay and neuropsychiatric phenotypes. Human Mutation, 36(7), 689–693. https://doi.org/10.1002/humu.22800

Pelc, K., Cheron, G., & Dan, B. (2008). Behavior and neuropsychiatric manifestations in Angelman syndrome. Neuropsychiatric Disease and Treatment, 4(3), 577–584.

Peters, S. U., Beaudet, A. L., Madduri, N., & Bacino, C. A. (2004). Autism in Angelman syndrome: implications for autism research. Clinical Genetics, 66(6), 530–536. https://doi.org/10.1111/j.1399-0004.2004.00362.x

Petersen, A. K., Cheung, S. W., Smith, J. L., Bi, W., Ward, P. A., Peacock, S., … Breman, A. M. (2017). Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. American Journal of Obstetrics and Gynecology, 217(6), 691 e691–691 e696. https://doi.org/10.1016/j.ajog.2017.10.005

Pitt, J. J. (2010). Newborn screening. Clinical Biochemist Reviews, 31(2), 57–68.

Scheermeyer, E., Harris, M., Hughes, I., Crock, P. A., Ambler, G., Verge, C. F., … collaboration, O. (2017). Low dose growth hormone treatment in infants and toddlers with Prader-Willi syndrome is comparable to higher dosage regimens. Growth Hormone & IGF Research, 34, 1–7. https://doi.org/10.1016/j.ghir.2017.03.001

Sehara, Y., Fujimoto, K. I., Ikeguchi, K., Katakai, Y., Ono, F., Takino, N., … Muramatsu, S. I. (2017). Persistent expression of dopamine-synthesizing enzymes 15 years after gene transfer in a primate model of Parkinson's disease. Human Gene Therapy. Clinical Development, 28(2), 74–79. https://doi.org/10.1089/humc.2017.010

Silva-Santos, S., van Woerden, G. M., Bruinsma, C. F., Mientjes, E., Jolfaei, M. A., Distel, B., … Elgersma, Y. (2015). Ube3a reinstatement identifies distinct developmental windows in a murine Angelman syndrome model. The Journal of Clinical Investigation, 125(5), 2069–2076. https://doi.org/10.1172/JCI80554

Sonzogni, M., Hakonen, J., Bernabe Kleijn, M., Silva-Santos, S., Judson, M. C., Philpot, B. D., … Elgersma, Y. (2019). Delayed loss of UBE3A reduces the expression of Angelman syndrome-associated phenotypes. Molecular Autism, 10, 23. https://doi.org/10.1186/s13229-019-0277-1

Sonzogni, M., Wallaard, I., Santos, S. S., Kingma, J., du Mee, D., van Woerden, G. M., & Elgersma, Y. (2018). A behavioral test battery for mouse models of Angelman syndrome: a powerful tool for testing drugs and novel Ube3a mutants. Molecular Autism, 9, 47. https://doi.org/10.1186/s13229-018-0231-7

Tai, D. S., Hu, C., Lee, C. C., Martinez, M., Cantero, G., Kim, E. H., … Lipshutz, G. S. (2015). Development of operational immunologic tolerance with neonatal gene transfer in nonhuman primates: preliminary studies. Gene Therapy, 22(11), 923–930. https://doi.org/10.1038/gt.2015.65

Tulipan, N., & Bruner, J. P. (1998). Myelomeningocele repair in utero: a report of three cases. Pediatric Neurosurgery, 28(4), 177–180. https://doi.org/10.1159/000028645

Tuszynski, M. H., Yang, J. H., Barba, D., U, H. S, Bakay, R. A., Pay, M. M., … Nagahara, A. H. (2015). Nerve growth factor gene therapy: Activation of neuronal responses in Alzheimer disease. JAMA Neurology, 72(10), 1139–1147. https://doi.org/10.1001/jamaneurol.2015.1807

van Bon, B. W., Coe, B. P., Bernier, R., Green, C., Gerdts, J., Witherspoon, K., … Eichler, E. E. (2016). Disruptive de novo mutations of DYRK1A lead to a syndromic form of autism and ID. Molecular Psychiatry, 21(1), 126–132. https://doi.org/10.1038/mp.2015.5

Vandamme, C., Adjali, O., & Mingozzi, F. (2017). Unraveling the complex story of immune responses to AAV vectors trial after trial. Human Gene Therapy, 28(11), 1061–1074. https://doi.org/10.1089/hum.2017.150

Vora, N. L., & Wapner, R. J. (2018). Introducing new and emerging genetic tests into prenatal care. Seminars in Perinatology, 42(5), 283–286. https://doi.org/10.1053/j.semperi.2018.07.004

Vossaert, L., Wang, Q., Salman, R., Zhuo, X., Qu, C., Henke, D., … Beaudet, A. (2018). Reliable detection of subchromosomal deletions and duplications using cell-based noninvasive prenatal testing. Prenatal Diagnosis, 38(13), 1069–1078. https://doi.org/10.1002/pd.5377

Wapner, R. J., Babiarz, J. E., Levy, B., Stosic, M., Zimmermann, B., Sigurjonsson, S., … Benn, P. (2015). Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. American Journal of Obstetrics and Gynecology, 212(3), 332–339. https://doi.org/10.1016/j.ajog.2014.11.041

Williams, C. A., Beaudet, A. L., Clayton-Smith, J., Knoll, J. H., Kyllerman, M., Laan, L. A., … Wagstaff, J. (2006). Angelman syndrome 2005: updated consensus for diagnostic criteria. American Journal of Medical Genetics. Part A, 140(5), 413–418.

Workman, A. D., Charvet, C. J., Clancy, B., Darlington, R. B., & Finlay, B. L. (2013). Modeling transformations of neurodevelopmental sequences across mammalian species. The Journal of Neuroscience, 33(17), 7368–7383. https://doi.org/10.1523/JNEUROSCI.5746-12.2013