Trends in Molecular Medicine

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Review

Gene therapies for neurogenetic disorders

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Pathogenic variants in over 1700 genes can cause neurogenetic disorders. Monogenetic diseases are ideal targets for genetic therapies; however, the blood–brain barrier (BBB), post-mitotic neurons, and inefficient delivery platforms make gene therapies for neurogenetic diseases challenging. Following nusinersen's 2016 approval, the development of gene therapies for neurogenetic disorders has advanced rapidly, with new delivery vehicles [e.g., BBB-crossing capsids, engineered viral-like proteins, lipid nanoparticles (LNPs)] and novel therapeutic strategies (e.g., regulatory elements, novel RNA therapeutics, tRNA therapies, epigenetic and gene editing). Patient-led disease foundations have accelerated treatment development by addressing trial readiness and supporting translational research. We review the current landscape and future directions in developing gene therapies for neurogenetic disorders.

Highlights

The discovery of monogenetic neurological disorders far outpaces the development of genetic treatments due to challenges in delivery, biodistribution, safety, efficacy, and costs.

The need to cross the blood–brain barrier, the post-mitotic nature of neurons, and inefficient delivery platforms limit gene therapies for neurological diseases.

Genetic approaches such as RNA therapeutics, gene replacement, and gene editing are being investigated to treat neurogenetic diseases.

Neurogenetic therapies require a tailored approach for different diseases and potentially for different patient subgroups.

Innovation in regulatory and commercial pathways is needed to advance the translation and reduce the time and cost of developing gene therapies for neurogenetic disorders.

Current state of gene therapies for neurogenetic diseases

Neurogenetic disorders were recognized clinically decades before the first genetic cause was identified in 1986: pathogenic variants in the dystrophin gene cause Duchenne's muscular dystrophy. Since then, clinical and genetic studies have identified >1700 monogenic causes of neurological disorders [1]. However, the central nervous system (CNS) makes the development of genetic treatments particularly challenging.

The first neurogenetic therapy, the **antisense oligonucleotide (ASO)** (see Glossary) nusinersen (Spinraza, Ionis Pharmaceuticals), was approved in 2016 to treat spinal muscular atrophy. Since then, many more cell and gene therapies are in development or have been approved to treat neurogenetic disorders. This review synthesizes the current state of gene therapy development for neurogenetic disorders and highlights key issues and directions. It summarizes several modalities including ASOs, **gene replacement** delivered in viral capsids, **RNAi**, **siRNA**, **regulatory elements** to up- or downregulate gene expression, and tRNAs. We also underscore the role of patient-led foundations in advancing trial readiness and translational research. The promise of gene therapies remains largely untapped and will require advances in science and medicine as well as in regulatory and commercial pathways (Figure 1, Key figure).

Genotypes and phenotypes of neurogenetic disorders

The pathophysiology and clinical features of neurogenetic disorders are extremely heterogeneous. Some disorders are Mendelian monogenic conditions, such as Rett syndrome arising from *MECP2* variants [2]. Others, such as Alzheimer's disease, are polygenic, with multiple lifestyle and risk alleles, such as the *APOE4* allele [3], contributing to disease pathogenesis [4]. Symptoms of neurogenetic disorders can start as early as *in utero*, as exemplified by decreased fetal movement in patients with type 0 spinal muscular atrophy [5]. By contrast, genetics strongly influence adult-onset dementia and movement disorder risks. For example, glucocerebrosidase (*GBA*) variants increase the risk of Parkinson's disease, [odds ratio (OR) = 5.4 in cases vs. controls] [6,7]. Pathogenic variants in key nervous system genes can cause diverse phenotypes,

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Key figure

Elements to consider for neurogenetic treatments

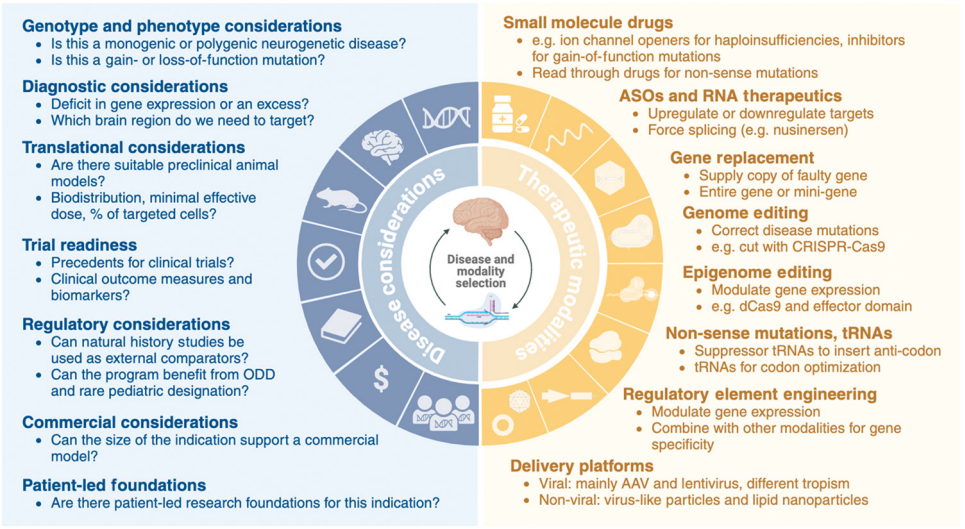

Figure 1. The development of gene therapies for neurogenetic conditions requires a tailored approach and there is no one-size-fits-all formula. Challenges to consider include genotype and phenotype, diagnostics, translational, trial readiness, and regulatory and commercial considerations, as well as the existence and strength of patient-led foundations. Modalities of choice include small molecule precision medicines, a variety of RNA therapeutics, gene replacement, genome and epigenome editing, and regulatory element engineering. Delivery platforms for these modalities can be viral and nonviral. Figure created using BioRender (www.biorender.com). Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotide; CRISPR, clustered regularly interspaced short palindromic repeats; dCas9, dead CRISPR associated protein 9; ODD, orphan drug designation.

including epileptic encephalopathies, intellectual disabilities (IDs), autism, leukodystrophies, movement disorders, psychiatric disorders, and dementias. The types of genetic variants driving these phenotypes vary from single-nucleotide variants (SNVs) or small insertion or deletion alleles to larger copy number variants (CNVs) or whole-chromosome aneuploidy (e.g., Down syndrome) [8]. While many cause impairment or functional loss of an encoded protein, others are pathogenic due to gain of function (e.g., an ion channel opening too long). Additional pathogenetic mechanisms include toxic repeat expansions [9] and epigenetic alterations [10]. Gene therapies must target the specific genetic mechanism causing the individual's disorder, which could be similar across many patients or unique.

Therapeutic modalities

This section discusses the different approaches for neurogenetic treatments, summarizing the different ways of delivery and the different modalities in development as of January 2025. Figure 2 and Table 1 highlight the advantages and disadvantages for each modality.

Delivery platforms

The delivery of gene therapies for neurogenetic disorders is limited by the BBB. To reach the brain, gene therapies can be administered via intravenous (IV) delivery, intrathecal (IT) delivery [most commonly lumbar puncture (LP)], intracerebroventricular (ICV) delivery, or intra-cisterna

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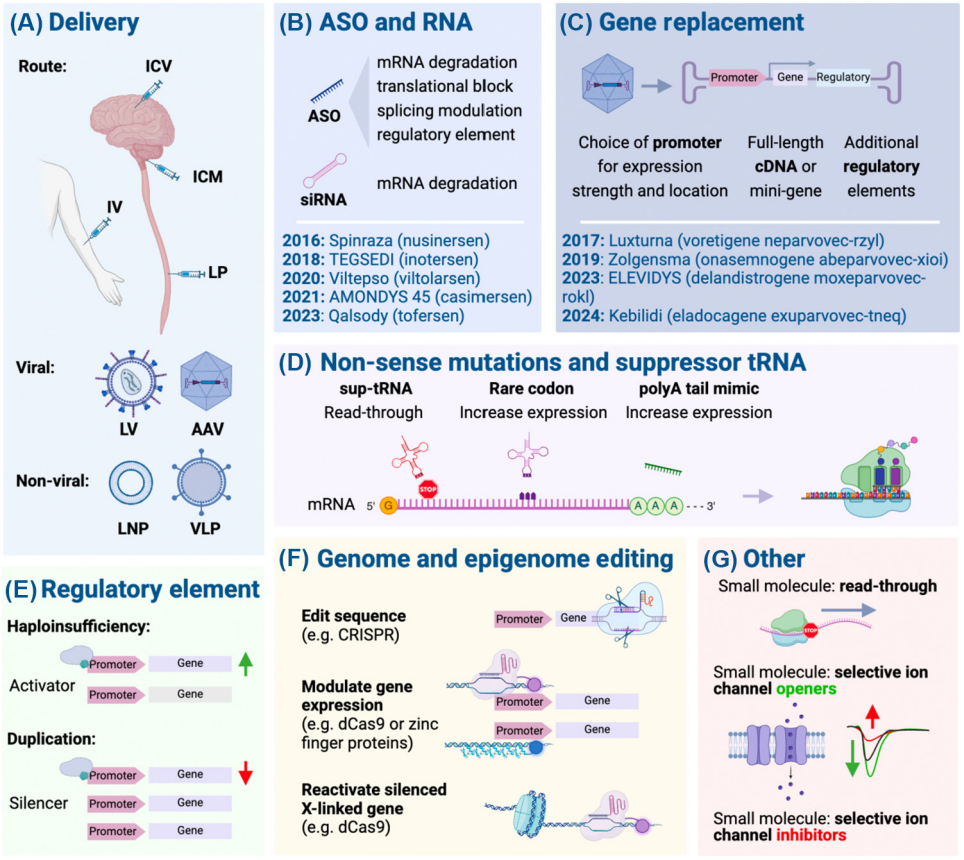

Figure 2. Approaches to neurogenetic treatments. (A) Genetic therapies for neurogenetic conditions can use different routes of administration and use viral and nonviral deliveries. (B) Antisense oligonucleotides (ASOs) and other RNA therapeutics are used to increase or decrease protein expression. (C) Gene replacement can deliver gene expression to various brain locations using viral vectors. (D) tRNAs can be used to increase protein expression by facilitating read-through of nonsense mutations, facilitating translation of rare codons, or increasing expression by binding to the polyA tail. (E) A variety of regulatory elements enable the activation or silencing of gene expression. (F) Genome and epigenome editing can edit DNA or RNA sequences or modulate gene expression. (G) Small molecule approaches can also promote nonsense mutation read-through or specifically open or close mutated channels. Approved treatments include only those approved prior to January 2025. Abbreviations: AAV, adeno-associated virus; CNS, central nervous system; CRISPR, clustered regularly interspaced short palindromic repeats; dCas9, dead CRISPR associated protein 9; ICM, intra-cisterna magna; ICV, intracerebroventricular; IV, intravenous; LNP, lipid nanoparticle; LP, lumbar puncture; LV, lentivirus; sup-tRNA, suppressor tRNA; VLP, viral-like particle. Figure created using BioRender (www.biorender.com).

magna (ICM) delivery (Figure 2A). This subsection reviews the different delivery platforms or tools used for gene therapies to treat neurogenetic conditions.

Gene therapies involve various strategies to modulate gene expression, including reducing gene expression via RNAi [11], editing [12], or epigenetic silencing [13,14]. They can also enhance gene expression through epigenetic activation, correction of gene mutations via base editing [15] or prime editing [16], or the delivery and expression, *in trans*, of a functional transcript that underlies the disease [17,18]. There are multiple tools to deliver these gene therapies, which include viral and nonviral approaches (Figure 2A), the former most often accomplished with recombinant **adeno-associated virus (AAV)**- or **lentivirus (LV)**-based vectors. Differences between AAVs and LVs include their genomes (AAV – single-stranded or double-stranded DNA; LV – single-stranded

Glossary

Adeno-associated viruses (AAVs): small viral carriers devoid of viral genes; can infect dividing and quiescent cells, and DNA persists extrachromosomally (ecDNA) without genomic integration.
Antisense oligonucleotides (ASOs): short, single-stranded, chemically modified oligonucleotide molecules that bind to RNA and alter RNA structure or function.
***Cis*-regulation therapy (CRT):** alters enhancer–promoter interactions or methylation status to restore transcription by increasing or decreasing gene expression.
DNA methyltransferase (DNMT): enzyme that adds methyl groups to promote cytosines to deactivate genes.
Epigenome editing: modifies the methylation status of promoters to activate (demethylate cytosines) or deactivate (methylate cytosines) without modifying the DNA sequence.
Gene replacement: cDNA encoding the protein deficient in a genetic disease, transmitted via a viral vector or in normal cells that secrete that missing protein into the affected tissue.
Haploinsufficiency: one copy of a gene is inactivated or deleted and the remaining copy is insufficient for normal functions.
Lentivirus (LV): retrovirus that is used in research to introduce a new gene into or block the expression of a gene using RNAi in human or animal cells.
Nonsense mutations: DNA mutations that create a premature stop codon in mRNA.
Premature termination codons (PTCs): mutations that transform amino acid codons into stop codons, causing truncated mRNA and protein truncation.
Regulatory elements: noncoding DNA that binds transcription factors (enhancers and repressors) that regulate gene expression at transcription or post-transcription; used to treat haploinsufficiency disorders or reduce toxic mRNAs or proteins.
RNAi: RNA interference; process to silence genes by binding mRNA and preventing translation to proteins (see siRNA).
siRNA: small interfering RNA; noncoding double-stranded RNAs that regulate gene expression. Inhibit translation in ribosomes and destroy target mRNAs by ribonucleases

RNA), the nature of the virion (AAV – protein capsid; LV – enveloped), the fate of the viral genome after transduction (delivery of the recombinant viral material to the nucleus), and different limits on deliverable genetic payload sizes (AAV – 4.8 kb; LV – 7.5 kb). AAVs do not integrate into the genome unless free DNA ends are present due to breakage or via targeted nuclease activity [19]. LVs naturally integrate into the genome; LVs naturally integrate into the genome and are usually used in dividing cells where long-term expression is desired in daughter cells [20]. Both LVs and AAVs can be engineered to alter their tropism for desired cell or tissue targeting [21–23], an emerging methodology to improve their safety and efficacy in treating human disorders. Components of AAVs, LVs, and adenoviruses can be used to manufacture virus-like particles (VLPs) to deliver gene therapy [16,24–26]. Other nonviral approaches including LNPs [27,28] are exceptionally robust at delivering DNA or RNA payloads to liver and, like viruses, there are advances in engineering to target other tissues (Figure 2A) [17,29–31]. The most extensively used delivery approaches are IT delivery of ASOs, and IV or ICV delivery of AAV-based gene therapies.

Suppressor tRNAs (sup-tRNAs): engineered tRNAs to deliver correct the amino acid at nonsense mutations.
Ten-eleven translocation (TET) dioxygenases: enzymes that activate genes via demethylating promoter cytosines.

ASO and RNA therapeutics

ASOs are an emerging treatment modality for neurogenetic diseases, with several approved drugs and many more in development. ASOs are short, single-stranded, chemically modified oligonucleotide molecules that bind to RNA through Watson–Crick base pairing and subsequently alter RNA structure or function. ASOs can be designed to result in decreases or increases in target RNA and protein levels through mechanisms such as endogenous nuclease-mediated RNA degradation, splicing alterations, translational blocking, and regulatory element alterations, making them a versatile modality for neurogenetic disorders (Figure 2B). ASOs do not cross the BBB and are delivered by IT injection into the cerebrospinal fluid (CSF) where, through diffusion, broad delivery to most areas of the brain and spinal cord and to neurons and glial cells is achieved [32]. This delivery has proved feasible and tolerable due to the long half-life of ASOs in the CNS, with over 15 000 patients treated and some for >10 years (https://www.spinraza.com/) (Table 1). The first approved ASO for a neurological disorder was nusinersen (Spinraza) for spinal muscular atrophy [33,34]. Nusinersen acts by altering the splicing of SMN2 pre-mRNA to increase the amount of full-length, active SMN protein deficient in patients with genetic mutations in the SMN1 gene. Tofersen (Qalsody) was recently approved for the treatment of SOD-1 related amyotrophic lateral sclerosis (ALS), acting by degrading SOD1 mRNA and decreasing toxic SOD1 protein [35]. Additional ASOs in clinical development include for Alexander disease, ALS, Alzheimer's disease, Angelman syndrome, Dravet syndrome, Huntington's disease and spinobulbar ataxias, Parkinson's disease, and prion disease, with many more in preclinical development and research. Until recently, ASOs have been the only proven RNA modality for treating neurogenetic disorders, but emerging data with conjugated siRNA have overcome the delivery challenges that were previous barriers for this RNA degradation modality for the CNS and show promise (Figure 2B).

Gene replacement

Gene replacement usually involves a synthetic DNA transcribed by reverse transcriptase from mRNA (i.e., cDNA) encoding the protein that is deficient in a genetic disease, transmitted via a viral vector or in normal cells that can secrete that missing protein into the affected tissue (Figure 2C and Table 1). Gene replacement strategies may be full-length cDNA if all coding regions fit into the delivery vehicle. Examples in clinical trials include gene replacement for inherited hemoglobinopathies [36,37], deficiencies in lysosomal proteins where loss of function can impact peripheral tissues [38] or the brain [39], deficiencies in proteins in vision [40], hearing [41], or other sensory disorders, or deficiencies principally impacting the brain [42,43]. For those disorders wherein the gene product is very large (e.g., muscular dystrophy), gene replacement involves miniaturized protein versions with functional sequences that fit within a viral vector [44]. When considering gene replacement therapies, important factors include selecting the appropriate

Table 1. Advantages and disadvantages of genetic therapy modalities

Modality	Advantage	Disadvantage	RoA[a] and biodistribution	Comment
ASO	Safety and efficacy established in CNS disorders (e.g., spinal muscular atrophy) Can discontinue if ineffective or toxic Dose can be increased or decreased	Delivery (see next column) Repeated IT dosing (often sedated) ASO sequences may cause inflammation (immunogenicity) – may cause hydrocephalus, cranial nerve injury, and lower extremity weakness due to spinal inflammation	Delivered via LP (IT) Limited distribution to deep brain (e.g., thalamus, basal ganglia) Ongoing efforts to conjugate with receptor-specific antibodies for improved biodistribution or with brain-penetrating peptides to enable BBB crossing	Usually well tolerated; requires repeated dosing indefinitely
Gene replacement	Single administration can permanently restore protein expression Episomal vectors with favorable safety profile; minimal risk for insertion and mutagenesis; less potential for innate immune reactions than integrating viral vectors	Potential for under- or overdosing Cannot re-dose with same or alternative AAV due to neutralizing antibodies Potential for AAV immunogenicity and toxicity Limited capacity of AAV (4700 bp)	Several RoAs: ICV, ICM, IT LP, or IV for BBB-crossing capsids Variable distribution in brain based on delivery vehicle, RoA, baseline levels of neutralizing antibodies, and individual CSF flow Ongoing efforts to engineer evolved AAV capsids with improved biodistribution or BBB-crossing properties, and to conjugate AAVs with brain-penetrating antibodies of peptides	Novel engineered capsids may distribute widely to brain via IV administration or parenchymal delivery
Gene editing	Can treat diverse genetic disorders Can repair SNPs and indels Can be delivered with LNPs or other nonviral modalities	Challenges for CNS delivery vehicles and distribution Limited cargo capacity of AAV Risk of genetic mutations or chromosomal translocation with active nucleases; potential off-target effects No re-dosing due to viral capsid immunogenicity; potential for CRISPR enzyme or zinc finger immunogenicity Potential for AAV immunogenicity and toxicity	Too large to package in a single AAV Potential delivery using two or three AAVs (same as for gene replacement) Ongoing efforts for viral-free delivery such as Couragene's STEP technology Alternatives: LNPs and engineered viral-like proteins allow larger payloads but none currently available for IT delivery	No clinical trials for CNS gene editing due to large cargo size, distribution, and immunogenicity
Epigenetic editing	Can potentially treat X-linked, imprinting, nucleotide repeat disorders, and cancer DNA sequence remains intact Sustained effects in animal models Fine-tuned gene regulation: key for dose-sensitive diseases (e.g., Rett syndrome) No off-target at the transcriptional level	Unknown duration of effect, especially in non-human primates and humans Potential off-target effects; potential for CRISPR enzyme or zinc finger immunogenicity Potential for AAV immunogenicity and toxicity	Too large to package in a single AAV Potential delivery using two AAVs or nonviral alternatives (same as for gene replacement) Ongoing efforts for viral-free delivery such as Couragene's STEP technology Alternatives: LNPs and engineered viral-like proteins allow larger payloads but none currently available for IT delivery	Most applicable for X-linked disorders (e.g., Rett syndrome, fragile X, CDKL5 deficiency disorder), imprinting disorders (e.g., Angelman, Prader–-Willi), nucleotide repeat disorders (e.g., Huntington's, C9orf72), and interference disorders (e.g., PCDH19)
tRNA suppressors	Potential therapy for premature stop codons in diverse genetic disorders; mutation agnostic tRNAs are small and can fit easily in AAV No risk of protein overexpression Can treat disorders due to mutations in large genes that cannot be treated with gene replacement	Challenges to CNS delivery and distribution depending on delivery vehicle Duration of expression dependent on vector Unknown off-target read-through Potential for AAV immunogenicity and toxicity	Plans for AAV delivery; several RoA: ICV, ICM, IT (LP), or IV for BBB-crossing capsids	
tRNA codon optimization and tethered	Can treat haploinsufficiency disorders; mutation agnostic Can treat disorders due to	Challenges to CNS delivery and distribution depending on delivery vehicle	Envisioned for delivery via AAV; several RoA: ICV, ICM, IT (LP), or IV for BBB-crossing capsids	

(continued on next page)

Table 1. (continued)

Modality	Advantage	Disadvantage	RoA[a] and biodistribution	Comment
polyA binding protein	mutations in large genes that cannot be treated with gene replacement	Efficacy *in vivo* not yet defined Expression duration depends on vector Unknown off-target read-through Potential for AAV immunogenicity and toxicity		
CRT	Can treat diverse haploinsufficiency disorders or gain-of-function and toxic mRNA or protein disorders (e.g., nucleotide repeat disorders) by up- or downregulating gene expression, respectively	Potential off-target effects; potential for dCas[b] or zing finger immunogenicity Cannot be re-dosed; potential for under- or over-treatment Potential for AAV immunogenicity and toxicity	Envisioned for delivery via AAV; several RoA: ICV, ICM, IT (LP), or IV for BBB-crossing capsids	'Hit and run' (treatment and degradation of therapeutic) potential for down- and upregulation of target genes

[a]Abbreviation: RoA, route of administration.
[b]dCas: enzymatically inactive, used to target DNA sequence with single guide RNA (sgRNA).

promoter to regulate gene expression and restricting the missing gene product to specific subpopulations, such as inhibitory interneurons as proposed in Dravet syndrome [45]. Additionally, it is essential to choose the most effective protein isoform for the affected tissues, whether that involves neurons or neuronal and glial cells (Figure 2C).

Nonsense mutations and suppressor tRNAs

Nonsense mutations are common causes of human disease, introducing **premature termination codons (PTCs)**, which lack a corresponding tRNA for decoding, like natural stop codons. This truncates mRNAs and proteins, leading to disease. Engineered tRNA can correct PTCs (Figure 2D). tRNAs allow ribosomes to translate mRNA into proteins. The four nucleotides in mRNA encode 64 unique triplets ($4^3 = 64$) or codons; 61 sense codons specify 20 amino acids through base-pairing with complementary tRNA anticodons. The UGA, UAA, and UAG stop codons signal the end of protein synthesis, recognized by the eRF1 release factor protein.

Suppressor tRNAs (sup-tRNAs) can be engineered to insert the correct amino acid at nonsense mutations, leading to read-through of nonsense mutations and completion of the polypeptide chain (Figure 2D and Table 1) [46]. Nineteen sup-tRNAs are required to correct all possible PTCs resulting from nonsense mutations, but most mutations can be addressed with four or five sup-tRNAs. An anticodon of a PTC can replace the mutational tRNA PTC with the 'missing' amino acid. sup-tRNAs selectively read PTCs rather than normal termination codons. Differentiating between premature from native stop codons is a challenge that could help to address other questions.

tRNAs offer versatility, as a single therapy could treat multiple diseases. sup-tRNAs are ideal for 'Goldilocks genes', such as MECP2 in Rett syndrome, where sup-tRNAs target only naturally transcribed mRNAs and prevent overexpression to maintain physiological levels of gene expression. sup-tRNAs are only 78 nucleotides and are ideal to treat disorders resulting from mutations in large genes such as dystrophin, cystic fibrosis transmembrane conductance regulator (CFTR), or Titan.

tRNA therapies can also optimize codons, as optimal codons are translated more rapidly, increasing mRNA stability and the number of protein copies from an mRNA (Figure 2D and Table 1) [46,47]. Thus, by providing more of the rare codons, mRNA translation speed and mRNA stability translate into more functional protein. Finally, other RNA therapies can use proteins that bind to the mRNA polyA tail to prolong mRNA half-life and increase the amount of protein translated from a single mRNA thread (Figure 2D) [46]. Because the development of tRNA therapeutics is still in preclinical

stages, there is no proof yet that these modalities lead to durable expression or of their off-target rate. As with other modalities, tRNA therapeutics are likely to require AAV vectors for delivery into the CNS, also adding challenges to CNS delivery and biodistribution.

Regulatory element engineering

Neurogenetic disorders caused by gene dosage, such as **haploinsufficiency** (only one functional gene copy instead of two) or gene duplications, can be rescued by targeting their regulatory elements with nuclease-deficient gene editing systems to upregulate or downregulate gene expression (Figure 2E and Table 1). This ***cis*-regulation therapy (CRT)** [48] can rescue haploinsufficient obesity [49,50], epilepsy [51], or autism/neurodevelopmental diseases [52] in cellular and mouse models. Changing gene expression by altering enhancer–promoter interactions or changing a regulatory element's methylation status can also yield therapeutic transcriptional changes (Figure 2E) [48]. Advantages of CRT include: (i) it uses the gene's regulatory machinery to rescue and avoid ectopic expression; (ii) it can fit into a single AAV to target large genes whose cDNA would exceed the AAV's 4700-bp packaging capacity [53]; (iii) it provides tissue/cell type specificity (i.e., targeting of a hypothalamus enhancer upregulated expression only in the hypothalamus [49] or parvalbumin-positive inhibitory interneurons [54]); and (iv) it is a 'one-and-done' approach, potentially requiring a single lifetime therapy. To get CRT into the clinic, advances in safety (e.g., nonimmunogenic, nontoxic) and dosage and delivery optimization are needed.

Genome and epigenome editing

Applications of clustered regularly interspersed palindromic repeats (CRISPR) systems have transformed the exploration and editing of the genome. Most commonly employing an RNA-guided Cas9 nuclease to induce targeted double-strand breaks, CRISPR systems can disrupt gene function and precisely correct disease mutations (Figure 2F and Table 1) [55]. Building on the technology's programmability, researchers have created fusion proteins using a catalytically inactive dead Cas9 (dCas9) nuclease as a DNA targeting module and various effector domains, such as deaminase enzymes to precisely correct specific variants without introducing double-stranded breaks [56,57]. Zinc finger DNA binding domains can also be engineered as nucleases or targeting modules to precisely deliver regulatory elements (Figure 2F). Despite promising results in mouse models [58], scaling delivery to the human brain remains a major challenge and toxicity in non-human primate brains remain largely unexplored. Other technologies like RNA editing have not yet been applied to the brain.

dCas9 can be linked to enzymatic regulators of the epigenome to make precise manipulations, altering gene expression without changing the underlying DNA sequence or relying on DNA repair [59–62]. The methylation status of the DNA cytosine-5 residue is a key epigenetic regulator of mammalian gene expression [63]. Dysregulation of DNA methylation contributes to, or causes, human diseases including cancer, neurological disorders, and cardiovascular diseases [64,65]. Changes in DNA methylation, particularly in regions rich in CpG dinucleotide sequences (i.e., promoters) can silence genes when a **DNA methyltransferase (DNMT)** family enzyme adds methyl groups to cytosines, and activate genes via demethylating cytosine-5 via oxidative reactions catalyzed by **ten-eleven translocation (TET) dioxygenases** [64,66,67]. dCas9 fused to the catalytic domains of Dnmt3a or Tet1 can edit DNA methylation at precise genomic loci [63,68–73]. These tools can silence gene expression, reactivate gene via heterochromatin modifications, manipulate chromatin architecture, and reverse disease phenotypes *in vitro* by the targeted manipulation of DNA methylation [74–77].

Some types of human diseases are particularly suitable for **epigenome editing**. The first are X-linked diseases caused by heterozygous mutations of genes on the X chromosome, such as

MECP2 in Rett syndrome. Reactivation of the healthy and wild-type allele of these X genes by epigenome editing is a promising therapeutic approach (Figure 2F). The second are nucleotide repeat disorders in which a few nucleotide sequences are expanded to silence genes or produce abnormal transcripts and proteins that aggregate and cause cell dysfunction and death. Epigenome editing-based reactivation or suppression of repeats could reverse transcription defects and restore normal functions. Finally, imprinting disorders such as Angelman's and Prader–Willi syndromes [78] are also amenable to epigenome editing to reactivate the allele-specific wild-type imprinted genes for phenotypical rescue. One of the first neurogenetic therapies of this class to progress into a Phase 1/2 clinical trial combines epigenetic targeting using zinc fingers with an engineered transcription factor regulatory element to treat a neurodevelopmental disorder caused by a genetic haploinsufficiency (ETX101 gene therapy; NCT05419492[i]). Most other modalities of genetic and epigenetic editing for the CNS are still in preclinical stages.

Other modalities

Small molecules can be used as precision therapies to correct genetic mutations such as premature stop codons and to restore function in ion channels or receptors adversely affected by genetic variants (Figure 2G). Small molecules – or translation readthrough-inducing drugs – restore full-length protein expression by inserting an amino acid instead of prematurely terminating the protein and leading to nonsense-mediated decay of the mRNA transcript or producing truncated proteins that can be toxic or nonfunctional [79]. Small molecules can be used to target variant-specific changes in currents (e.g., persistent or resurgent sodium currents in SCN8A and SCN2A gain-of-function disorders) [80,81]. Finally, as cell therapies (e.g., allogenic inhibitory interneuron therapies into the hippocampus) emerge to treat epilepsy [82], lessons may be translated from chimeric antigen receptor T cell (CAR-T) therapies [83], where small molecules can be applied across ten points in the T cell life cycle to improve safety and efficacy and reduce costs [83].

Translational considerations

Gene therapies for neurogenetic conditions require a tailored approach and there is no one-size-fits-all formula (Figure 1). Development begins with the therapy's intended use (target product profile) and works backwards. Factors such as specific brain regions to target and the type of genetic therapy being used influence the choice of modality and delivery. These decisions, in turn, affect translational steps such as the use of preclinical animal models. While transgenic mice are commonly used, larger species like dogs (CLN2 Batten) or sheep (Tay–Sachs) are preferred for specific disorders. If human and mouse gene sequences differ, mice with a humanized gene can be used. Preclinical safety for gene therapies is more complex than for small molecules due to the interaction of multiple components (capsid, transgene, gene product), often requiring non-human primates for testing. Early regulatory feedback is important for gene therapies for neurogenetic conditions (Box 1). Key translational questions common to all modalities include determining the biodistribution, minimal effective dose, and necessary percentage of targeted cells and the identification of target engagement and response biomarkers.

Trial readiness considerations

When a gene therapy is developed for a neurogenetic disorder, choices must be made about clinical trial design, including specific populations to be studied and the trial readiness of the population. Important considerations include the patients' age, sex, genetic diagnosis, type of mutation (e.g., loss or gain of function, premature stop codon, location in gene), level of severity, comorbid disorders, and current medication.

For companies deciding which neurogenetic disorder to develop a gene therapy for, trial readiness is a key consideration (Figure 3). For example, companies often favor disorders with larger

Box 1. Regulatory considerations

Neurogenetic conditions can be ultrarare, impacting very few patients, or common, affecting millions of patients with the same variant(s) (e.g., carrying both ApoE4 alleles, 2% of the population). In other cases, a similar genetic variant in different genes might be addressable by the same therapeutics, as in the case of premature stop codons, which account for 12% of all point mutations. Ultimately, the size of the indication determines future commercial viability and regulatory standards.

Many neurogenetic diseases are commercially viable. Since regulatory standards were similar for common and rare indications, regulatory changes have 'adjusted the bar' to allow an easier and less expensive path for rare disorders. For example, platform technologies can target multiple genes or gene regions (e.g., in gene editing) with minimal design changes. In May 2024, the US FDA released a Platform Technology Designation Program draft guidance enabling sponsors to leverage prior knowledge in applications using the same platform technology to reduce the burden to approvals. Another challenge awaiting regulatory changes is the requirement for lengthy placebo or sham-control groups for trials in rare neurogenetic conditions. This poses recruitment and ethical challenges where the disease might be progressive and impact mainly children. Natural history studies as external comparators for therapy approval remain underused, although there are some successful examples such as in the approval of cerliponase alfa (Brineura, BioMarin). Regulatory innovation is needed in this space.

For commercially nonviable ultrarare disease, or for bespoke therapeutics such as patient-specific ASOs, clinical trials are used to treat those patients without any future commercialization of the therapy. One regulatory path that supports these trials is the use of open-investigational new drug applications (INDs) to enable investigator-initiated clinical trials without a commercial sponsor. An FDA guidance from December 2021 also simplified the IND standards for N-of-1 ASO programs (e.g., requiring only one species for toxicology testing). There is still a need to regulate cases where small groups can be treated with the ASO, falling outside the 'individualized investigational ASOs' guidance, and for non-US territories that lack equivalent frameworks. In other cases, sponsors have used rare pediatric designations to obtain an FDA Priority Review Voucher on approval, which can be sold and have been trading at about US$100–150M, and which can therefore offset the limited market of these ultrarare conditions.

populations, making them more economically viable. However, a smaller patient population in which the advocacy group has a list of patients willing to consider a gene therapy together with natural history data and patient records, and with an actively engaged patient population, can be more valuable than a larger population without such data. A disease concept model informs trial endpoints, such as coordination, walking, and seizure control, that must be relevant to the affected patients and families, measurable with tools that are reliable (consistent) and valid (accurate), and sensitive to change as the therapeutic goal is improving function and quality of life. A more 'trial-ready' advocacy group will de-risk factors that often delay or subvert gene therapies, as rapid and sufficient enrollment is crucial to reduce trial costs and ensure adequate power to distinguish signal from noise.

Commercial considerations

A burgeoning interest in research and therapies for rare and neurogenetic diseases reflects scientific advances in drug target and disease with reduced timelines and costs. The explosion in genetic testing, particularly in pediatrics, has refined the definition of genetic disorders and increased patient identification, creating a growing commercial space. Commercially, ASOs resemble small molecule drugs, with similar manufacturing costs and timelines, and pricing similar to other rare disease treatments that can greatly reduce morbidity and mortality for severely ill patients. For gene therapies, higher cost combined with manufacturing and regulatory hurdles, and long timelines, create reimbursement challenges for a one-time therapy (Box 1). In addition, since many neurogenetic diseases are ultrarare, a development and commercial model to support smaller patient groups will require innovative strategies.

Patient-led disease foundations

Most neurogenetic conditions are rare diseases. Over the years, rare disease patient groups have evolved from advocacy to actively driving research into therapeutics, and a new figure of 'patient-led research foundations' has emerged where the focus is specifically on research, and not on patient advocacy. Some of these organizations have established the foundational knowledge

Clinician's corner

Neurogenetics syndromes are an ever-expanding range of disorders with monogenetic or polygenic etiologies. Over 15 000 genes are translated in the human brain. More than 1000 are associated with epilepsy. Variants in most brain-translated genes influence developing and mature brain functions.

The diversity of clinical genetic tests poses challenges for neurologists. Chromosomal microarray analysis can identify DNA sequence insertions or deletions, whether both chromosomal regions came from one parent, or whether parts of different chromosomes are the same.

Targeted sequencing panels can detect changes in genes associated with epilepsy, autism, ataxia, and other disorders. Whole-exome sequencing (WES) evaluates all protein-coding genes and whole-genome sequencing (WGS) evaluates the entire genome. Since WES and WGS identify many variants in each individual, laboratories prioritize variants in genes that match the patient's disorder. Variants are categorized as pathogenic, likely pathogenic, of uncertain significance (VUS), likely benign, or benign. Interpretations can vary between laboratories. VUSs are common means and indicate that the variant may or may not contribute to the disorder.

The traditional view of clinicians that genetic tests are 'for geneticists' and 'of unclear value' for their patients is no longer tenable. Identifying a genetic cause can greatly impact patient care.

Patients with spinal muscular atrophy (SMN1 gene variants) or Duchenne's muscular dystrophy [dystrophin (DMD) gene variants] can be treated with FDA-approved gene therapies. Dravet syndrome patients with loss-of-function SCN1A mutations can be treated with FDA-approved medications (e.g., fenfluramine, cannabidiol, stiripentol) or enroll in gene therapy trials; they should also avoid sodium channel-blocking medications such as carbamazepine, phenytoin, and lacosamide.

In patients with SCN2A developmental epileptic encephalopathy, one must distinguish gain- from loss-of-function variants. Sodium channel-blocking

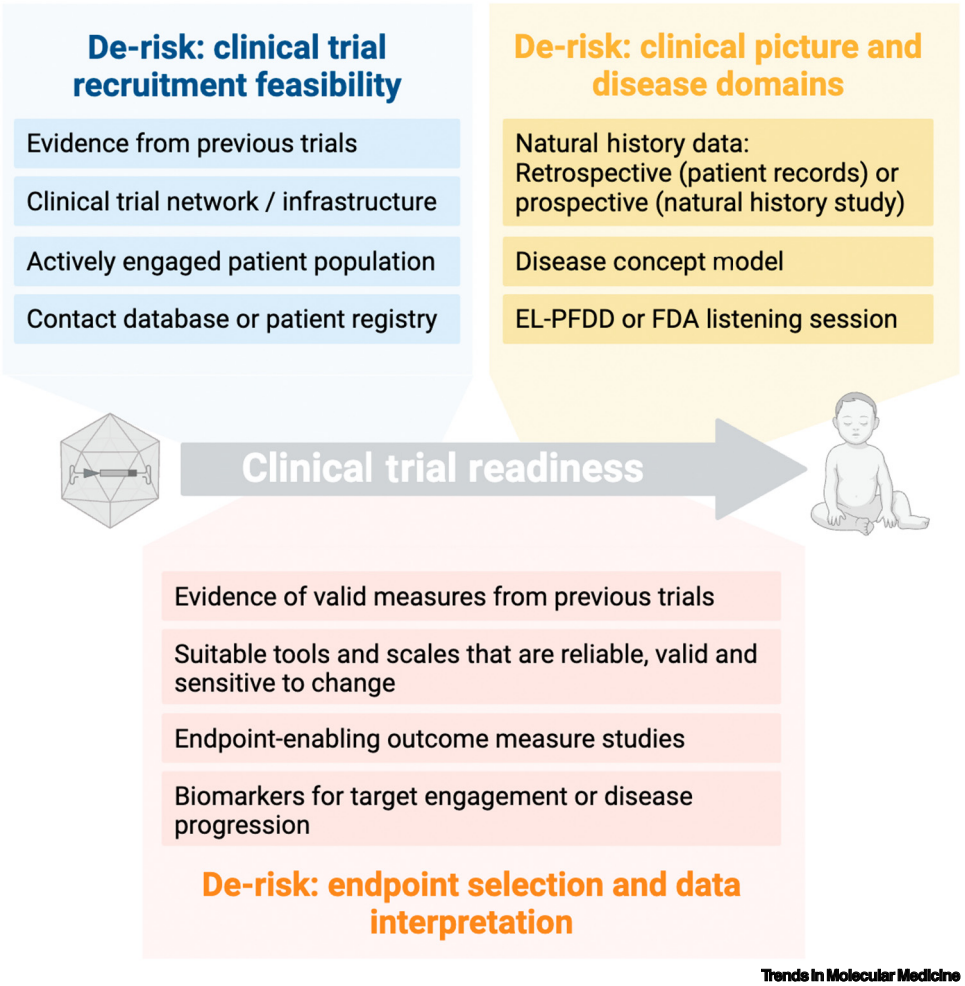

Figure 3. Clinical trial readiness considerations. Many neurogenetic conditions are rare diseases with no or few previous clinical trials. For companies deciding which neurogenetic disorder to develop a gene therapy for, trial readiness is a key consideration. Trial readiness requires de-risking three main areas: recruitment feasibility, clinical presentation documentation, and endpoint selection. Figure created using BioRender (www.biorender.com). Abbreviation: EL-PFDD, externally-led patient-focused drug development.

and research tools for their rare disease field, while others have initiated therapeutic programs taking the role of biotech companies (Box 2).

Within research, patient-led research foundations play a unique role in solving the key translational questions in their fields and building the key animal models and research tools, making them available license free. This breath of scope and open-access agenda is unique to patient organizations and cannot be replaced by academic institutions or biopharmaceutical companies. Patient-led research foundations play a critical role in enabling the development of genetic approaches for neurogenetic conditions.

Concluding remarks

Advances in molecular biology have been translated into diverse gene therapy options for neurogenetic disorders. Early success with ASOs and AAV-based gene replacement therapies has expanded the range of treatable disorders and therapeutic modalities, requiring tailored

medications can improve seizure control for gain-of-function variants but can exacerbate epilepsy for loss-of-function variants. Pediatric neurologists routinely order genetic testing, but many adult neurologists do not. Thus, adults with childhood-onset disorders should be reassessed and genetic testing ordered, especially for patients with severe developmental delays and early childhood-onset epilepsy. Such results can inform best therapies and may provide opportunities to participate in clinical trials or research.

The breathtaking advances in diagnosing and treating genetic disorders mandate that insights from researchers and geneticists inform clinical practice. This knowledge can transform patient lives, especially as technologies to modify gene expression and replace or edit genes or the epigenome move to the clinic.

Box 2. Patient-led research foundations

Rare disease patient groups have evolved from serving as an inspiration for the development of therapies or facilitating research, to driving therapy development and occupying roles traditionally played by biopharma companies. Here we illustrate three examples where patient-led research foundations for a variety of neurogenetic indications have played these roles.

Case 1: gene therapy enabler. In 2014, the Rett Syndrome Research Trust (RSRT) set up a gene therapy consortium bringing together leading experts in the gene therapy and MECP2 fields. From this consortium, two gene therapies have advanced to clinical trials in Rett syndrome and became foundational programs for the companies Taysha Gene Therapies (TSHA-102) and Neurogene (NGN-401). Thus, this patient-led research foundation advanced their field from basic science into therapeutics before the biopharma industry was ready to make that transition.

Case 2: gene therapy developer. In 2017, the Foundation for Angelman Syndrome Therapeutics (FAST) created the single-asset GeneTx Biotherapeutics to develop an ASO for Angelman syndrome. In 2019, GeneTx signed a partnership with the biotech Ultragenyx to advance GTX-102 into clinical trials, ultimately leading to GeneTx's acquisition by Ultragenyx in 2022. In this example, the patient-led research foundation played the role of an early-stage biotech company, developing the program to the stage where it was licensed to a larger organization.

Case 3: gene therapy developer and marketer. The patient-led CureSPG50 Foundation developed a gene therapy for SPG50, an ultrarare neurogenetic condition affecting less than 100 patients, and later founded Elpida Therapeutics to commercialize the gene therapy. Their program is moving to Phase 3 trials, and the company is seeking Rare Pediatric Designations (which trigger a Priority Review Voucher on approval) to offset the reduced commercial potential of the ultrarare indication. In this example, the patient-led research foundation – and the socially responsible corporation they created – fully replaced the role of biopharma companies in this ultrarare field.

Outstanding questions

What regions or cell types should be targeted?

Can we control the expression of 'Goldilocks genes' where over- or under-expression cause disease?

What is the long-term safety and efficacy of gene therapies?

Can we control costs and immunosuppression effects of BBB-crossing AAV therapies?

Will VLPs and LNPs address cost, cargo limits, and biodistribution issues?

Can regulatory changes facilitate new platform technologies?

What manufacturing changes are needed to advance neurogenetic gene therapies?

How can patient-led research foundations accelerate genetic therapy development?

approaches for each condition. Novel modalities include gene and mRNA editing, read-through of premature stop codons, and the use of various approaches to regulate gene expression. In the next 5–10 years, ASOs and AAV-based gene replacement therapies administered directly into the CNS are expected to dominate the landscape, becoming more standard therapeutic approaches. Regulatory adaptations for platform technologies may streamline approvals, but pricing and reimbursement challenges are likely to increase. Other modalities such as gene and mRNA editing, along with epigenetic editing, might see the first approvals within a decade. Systemic and nonviral delivery for neurological conditions may take longer than a decade to develop.

The future of gene therapies for brain disorders depends on improved delivery, precise dosing, and safer, less invasive, and cost-effective modalities (see Outstanding questions). While we are likely to learn much about the durability and safety of these approaches in the coming years, other outstanding questions such as the key target brain regions and protein levels will need to be answered on a case-by-case basis. Diseases with strong patient-led research foundations are more likely to solve these disease-specific questions and encourage therapy development. Advances in neurogenetic therapies offer transformative potential for monogenic disorders, shifting the field from palliative care to disease-modifying treatments. Fully realizing this potential will require additional scientific, regulatory, and commercial innovation.

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Declaration of interests

O.D. has equity ownership in Regel Therapeutics, Epitor Therapeutics, Actio Bio, Script Therapeutics, Tevard Biosciences, Praxis Precision Therapeutics, and Cirsium Biosciences. He is on the board of Regel Therapeutics, Epitor Therapeutics, Scrtip Therapeutics, and Cirsium Biosciences. He is a Scientific Advisor for all of the aforementioned companies. He has

patents relevant to regulatory elements and CRISPR gene editing. J.C. has equity in Tevard Biosciences and patents on tRNA therapeutics. X.S.L. has equity in Epitor Therapeutics and patents on epigenetic editing. Y.W. has equity in and is the CEO of Mahzi Therapeutics. B.L.D. serves on the advisory board of Latus Biosciences, Patch Bio, Spirovant Biosciences, Resilience, and Carbon Biosciences and has sponsored research from Roche, Latus, and Spirovant. A.M. has no conflicts. K.M.B. is an employee of Falcon Bio and a BOD member of Armatus Bio. R.A-N. is an advisor to LatusBio and serves on a Data and Safety Monitoring Board (DSMB) for AskBio.

Resources

[i]https://clinicaltrials.gov/study/NCT05419492

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