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Precision Therapies in Neurodevelopmental Disorders

Update on Gene Therapies

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KEYWORDS

- Precision medicine
- Neurodevelopmental disorders
- Gene therapy
- Antisense oligonucleotide
- Adeno-associated virus

KEY POINTS

- Neurodevelopmental disorders (NDD) refer to a collection of rare disorders that manifest during infancy, characterized by developmental delays across multiple domains, and often manifested with neuropsychiatric and neurologic disorders, including autism spectrum disorder, epilepsy, intellectual disability, movement disorder, and attention-deficit/hyperactivity disorder.
- Genetics play an important role in the cause of NDD, and new genomic technologies have identified more than 100 significant gene associations.
- There are multiple pathways involving neurocognitive functions and other developmental domains that are usually found to be disrupted by pathogenic mutations.
- Although genome-editing technologies (ie, CRISPR/Cas9) are evolving to achieve better accuracy and efficiency, gene therapy is more mature and applicable.
- Gene therapy can stop the impact of a gene mutation at the level of DNA or downstream RNA to preserve the underlying complex functions associated with that gene.

INTRODUCTION

Neurodevelopmental disorders (NDD) refer to a collection of rare disorders that manifest during infancy [1,2], characterized by developmental delays across multiple domains and often manifested with neuropsychiatric and neurologic disorders, including autism spectrum disorder (ASD), epilepsy, intellectual disability (ID), movement disorder, and attention-

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deficit/hyperactivity disorder (ADHD). The collective prevalence of NDD is high with a global prevalence of between 3% and 5% [3–5].

NDD encompass a spectrum of disorders, and under the *Diagnostic and Statistical Manual of Mental Disorders* (Fifth edition), the diagnostic criteria require assessment of cognitive capacity using the IQ as well as adaptive functioning [6]. The diagnostic complexities of NDD often arise from wide spectrum of comorbidities in NDD; epilepsy is one such comorbidity, with 20% of people with ASD also receiving this diagnosis. Similarly, the prevalence of ASD among Fragile X cases is approximately 50% [7,8]. Apart from IQ, the gold standard for the diagnosis of ASD includes the Autism Diagnostic Observation Schedule, a standardized instrument that uses the observational assessment of social interaction, repetitive behaviors, communication, and play skills [9]. For epilepsy cases, electroencephalography, measuring electrophysiological rhythms of the brain, and high-resolution brain imaging, such as MRI, are typically used to identify brain functional and/or structural abnormalities.

Genetics play an important role in the cause of NDD [1,2,10], and new genomic technologies have identified more than 100 significant gene associations. For numerous NDD comorbidity cohorts (eg, ASD, epilepsy, ID), the genetic contribution to these individual diseases has been independently confirmed through cohort studies [11–13]. Clinical genetic testing is considered a first-tier test for diagnosing rare/de novo genetic variants that may contribute to a patient's NDD [14]. In the last 2 decades, genetic tests have been adopted as highly impactful diagnostic tests in the clinic. A recent report suggests that more than 250 genes reported by multiple independent studies have strong association with NDD [15]. Rare/de novo single nucleotide variants (SNVs) and copy number variations (CNVs) were found to be associated with NDD. Although there is no single high-penetrant mutation found in all NDD cases, a small number of genes (*SCN2A*, *CHD8*, *POGZ*, *STXBP1*, *mTOR*, and so forth) and loci (16p11.2, 15q13.3, and so forth) have been shown to be enriched for rare/de novo mutations [10,16–18]. In most clinics, exome sequencing is now routinely conducted as a first-tier test. Exome sequencing scans all functional units (exons) in the human genome and identifies SNVs. Similarly, clinical microarray is another genetic test that scans the entire genome to identify large CNVs [2,10]. Large-scale exome or whole-genome sequencing studies suggest that the combined (SNV and CNV) diagnostic yield is greater than 40% for ASD [17,19], epilepsy [20,21], and ID [22,23].

Although the recent progress in genetic diagnosis is significant, there is a lack of therapeutic interventions. The unique phenotypic spectrum of each child and the heterogeneous spectrum of mutations in a large number of genes especially complicate the detection of underlying disease pathways. There is no single common pathway that can explain the clinical manifestation of NDD that can be targeted for drug development. Instead, there are multiple pathways involving neurocognitive functions and other developmental domains that are usually found to be disrupted by pathogenic mutations [2,16,17]. To avoid the complexities of "one-size-fits-all" therapy, the concept of precision medicine has emerged as a major force in accelerating therapeutics in NDD.

Precision medicine is a treatment pathway that uses numerous technologies to guide individually tailored diagnosis and treatment of patients. Precision medicine is a radical shift from the idea of "one-size-fits-all," and gene therapy is one of the most effective technologies that can be used to implement precision medicine. There are currently 2 major precision genetic therapeutic approaches: genome editing (ie, CRISPR/Cas9) and gene therapy. Although genome-editing technologies are evolving to achieve better accuracy and efficiency, gene therapy is more mature and applicable. Gene therapy can stop the impact of a gene mutation at the level of DNA or downstream RNA to preserve the underlying complex functions associated with that gene. Various types of gene replacement and blocking technologies have been tried in past decades to implement precision therapy for NDD.

In this short review, the authors discuss the recent updates and successes of gene therapy technologies in various NDD.

TARGETED GENE THERAPY DRUGS IN NEURODEVELOPMENTAL DISORDERS

Although gene therapy technologies were introduced 4 decades ago, progress has been slow [24,25]. Recently, there have been success stories of the implementation of adeno-associated virus (AAV)-based gene replacement therapy and antisense oligonucleotide therapy (ASO) as potentially targeted treatments. These therapies are discussed later and summarized in Table 1.

Adeno-Associated Virus

In recent years, AAV has been shown to be one of the most effective gene replacement therapies. Despite AAV having a limited cargo capacity (approximately 4.5 kb) for the delivery of genetic material, it has

TABLE 1
List of Gene Therapy Drugs Related to Neurodevelopmental and Neurologic Genetic Disorders

Gene	Coordinate (hg38)	Condition	Drug Type	Drug Name	Targeting Pathway	PMID
SMN1	chr5: 70,925,030-70,953,012	Spinal muscular atrophy (OMIM 253300)	Adeno-associated virus 9 (AAV9)	Zolgensma	Gene replacement	31371124
			Antisense therapy	Nusinersen	mRNA interference	32180828
MFSD8	chr4: 127,917,827-127,965,173	Batten disease (OMIM 610951)	Antisense therapy	Malisen	mRNA interference	31597037
DMD	chrX: 31,119,228-33,211,519 2,092,292	Duchene muscular dystrophy (OMIM 310200)	Antisense therapy	Eteplirsen	mRNA interference	30856119

beneficial advantages (ie, transduction efficiency, long-term DNA persistence) compared with other gene therapy technologies (eg, adenoviral [~36 kb] or lentiviral vectors [~8 kb]). Introducing a wild copy of the faulty gene into the cellular environment in vitro and in vivo through AAV has been shown to be effective to restore gene functions [26,27]. AAV1 serotype was used as a backbone to develop historically the first gene therapy drug "Glybera" for lipoprotein lipase (LPL) deficiency, a multiorgan disorder with triglyceride deposition. The AAV1-based drug successfully delivered a wild-type copy of the LPL gene into human muscle cells [28]. Similarly, AAV9 technology also successfully delivered genetic material crossing the blood-brain barrier targeting neurons and astrocytes [29]. Emerging evidence suggests AAV9 uses laminin receptor for cellular transduction [30]. The AAV9-based drug "Zolgensma" is the first Food and Drug Administration (FDA) -approved AAV-delivered gene therapy used to treat spinal muscular atrophy (SMA), a genetic disorder characterized by loss of motor neurons and progressive muscle wasting owing to mutation in the *SMN1* gene. The targeted AAV9 vector replaces the nonfunctioning *SMN1* gene with a new, working copy of a human *SMN* gene. In a clinical trial comprising 12 SMA patients treated with Zolgensma, 11 patients were able to sit independently and 2 patients were able to walk [31].

Antisense Oligonucleotide Therapy

ASOs gene blocking therapy is gaining traction in developing targeted treatments. ASOs are short (21–23 bp), synthetic, single-stranded oligodeoxynucleotides that can impact RNA to reduce, restore, or modify protein expression using several molecular mechanisms. The design of an ASO is the most critical step owing to complexities in genomic context (ie, repeats, mutation) and binding affinity (ie, temperature, ASO structure). This technology allows blocking disease pathogenesis at the source by knocking out the dysfunctional gene as opposed to therapies that target downstream elements of the pathway [32]. The ASO binds with the targeted (complementary) RNA to modulate protein production. The formed DNA-RNA or RNA-RNA duplexes are usually recognized by cellular enzyme RNase H that helps degrade the targeted gene messenger RNAs (mRNAs) [33]. ASO drug development is problematic regarding the in vivo delivery into the biological system owing to rapid ASO degradation by nucleases. To alleviate this problem, scientists are now using chemically modified phosphorothioate and 2′-O-methoxyethyl ASO backbones that increase the stability against digestion from nucleases [33].

The first FDA-approved ASO drug was introduced in 1998, for the treatment of cytomegalovirus retinitis [34]. Currently, there are 3 ASO therapies that have received FDA approval for the treatment of SMA, Batten disease, and Duchenne muscular dystrophy (DMD). DMD is an X-linked disease of progressive muscle wasting resulting in premature death. Approximately 14% of affected patients carry a stop mutation at exon 51 of the *DMD* gene. The ASO drug "Exondys 51" or eteplirsen targets this mutation in RNAs and allows for skipping of the mutated exon and production of a shortened protein rather than none. Another ASO therapy "Milasen" was designed for an extremely rare condition, late infantile-onset neuronal ceroid lipofuscinosis, or Batten disease, where a pathogenic mutation in the *MFSD8* gene leads to a severe neurodegenerative disorder in the first 5 years of life [35]. This drug was successfully administered to a young girl, and it was designed for an "N of 1" sample, a great example of future precision medicine of NDD. ASO therapy has also recently been approved for SMA; Nusinersen is an ASO-derived drug that redirects the splicing of *SMN2* mRNA, the nonfunctional version of *SMN1*, and promotes inclusion of exon 7 that usually is excluded in patient *SMN2* mRNA. The designed ASO binds to the pre-mRNA by Watson-Crick base pairing and through block recognition of splicing factors, thereby controlling the formation of mature mRNA. This mechanism has increased the inclusion of exon 7 to restore production of SMN protein and therefore rescue the phenotype [36]. Animal models and multiphase clinical trials of Nusinersen showed significant increases of SMN protein and the reversal of phenotypes in SMA cases [37].

RISKS AND CHALLENGES OF GENE THERAPY

Gene therapy technologies for the treatment of genetic disorders have been hindered by numerous challenges, including unwanted side effects owing to the immune system invoking innate or adaptive immune responses to the foreign gene product [38]. This issue remains pertinent until today, and technological advancement is necessary to control or avoid immune response against major gene therapy technologies (ie, AAV, ASO) for stable and efficient delivery of gene products. Another major challenge is tissue specificity that hinders transfection rate of gene transport vectors into the cells of different organs. Variability has also been observed within different serotypes of AAV; for

example, AAV6 has been shown to have increased transduction rate in skeletal muscle [39], and AAV4 has displayed preference for the central nervous system [40].

There are lingering challenges involving ASO technology also. The most prominent challenge is the short lifespan of ASO in the cellular environment; ASO decays rapidly because of the presence of endogenous nucleases that degrade it, thus reducing its titer within the cellular environment. Despite attempts at improvement of lifespan using modified chemistry, the sensitivity and specificity of ASO are still unknown within most tissue types. Another challenge with ASO technology is the intracellular off target mRNA binding that can lead to specific inhibition of gene expression [41].

Perhaps the main limitation to the utilization of gene therapy in NDD is the large number of chromosomal abnormalities involved in these disorders. For instance, a major risk factor for NDD is CNVs, and each CNV (deletion/duplication) comprises multiple genes, making it impractical to use gene therapy to compensate or mitigate molecular instability causes by multiple gene disruptions. To date, there is no large retrospective study on the long-term effects of using the FDA-approved gene therapy drugs, and there is a lack of gold-standard guidelines for outcome measures. Finally, gene therapy drugs are currently the most expensive drugs globally, and the cost-effectiveness is unlikely to improve because of their limited use in a relatively small number of patients.

FUTURE OF NEURODEVELOPMENTAL DISORDERS GENETIC THERAPIES

Late-stage development of clinical trials is underway for gene therapies for the treatment of several NDD, including Dravet syndrome, Angelman syndrome, and Rett syndrome, targeting *SCN1A*, *UBE3A*, and *MECP2* genes, respectively [42]. Genome editing tools, CRISPR/Cas9 and others, have the potential to design or aid future targeted drugs by manipulation of DNA or RNA. Mutation reversal (alteration) during fetal development may become a possibility when higher accuracy and efficiency are achieved. The combination of gene therapy with CRISPR/Cas9 technology can be a very effective tool for in vivo delivery and precision targeting [43]. These emerging technologies complement each other's strengths and have the potential to accelerate drug development in NDD.

The future therapeutic development should integrate the use of artificial intelligence (AI) to power precision medicine in NDD [4]. AI algorithms have the capacity to handle massive large data sets (ie, genomics, transcriptomics, proteomics, and clinical records) to decipher information related to a given hypothesis. Machine learning algorithms applying neural networks are becoming a major tool to identify drug molecules for numerous diseases [4,44,45]. Most recently, a new algorithm has been proposed that combines neural networks with a genetic algorithm to conduct massive optimization in search of biological molecules [46]. AI also can be used to best predict repurposed drug candidates for the treatment of NDD.

A combination of CRISPR/Cas9, AI, and gene therapy over the next decade has the potential to significantly accelerate drug development for NDD.

DISCLOSURE

The authors have nothing to disclose.

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