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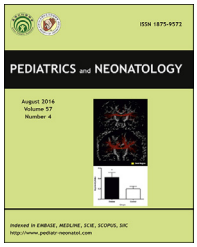

Review Article

Adeno-associated virus vector-based gene therapies for pediatric diseases

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Gene therapy using adeno-associated virus (AAV) is a rapidly developing technology with widespread treatment potential. AAV2 vectors injected directly into the brain by stereotaxic brain surgery have shown good results in treating aromatic L-amino acid decarboxylase deficiency. Moreover, gene therapy using the AAV9 vector, which crosses the blood–brain barrier, has been performed in more than 2000 patients worldwide as a disease-modifying therapy for spinal muscular atrophy. AAV vectors have been applied to the development of gene therapies for various pediatric diseases. Gene therapy trials for hemophilia and ornithine transcarbamylase deficiency are underway. Clinical trials are planned for glucose transporter I deficiency, Niemann-Pick disease type C, and spinocerebellar ataxia type 1. The genome of AAV vectors is located in the episome and is rarely integrated into chromosomes, making the vectors safe. However, serious adverse events such as hepatic failure and thrombotic microangiopathy have been reported, and ongoing studies are focusing on developing more efficient vectors to reduce required dosages.

1. Gene therapy advances in clinical applications

The concept of gene therapy in which nucleic acids are introduced into cells to restore their functions was proposed over 50 years ago.[1] Therapeutic gene can be delivered directly to the brain, liver, and muscles (*in vivo*) or to hematopoietic stem cells (HSCs) cultured outside the body and then returned to the body (*ex vivo*). In both cases, the virus is modified and used as a vector (carrier) to efficiently introduce the gene into the cells.[2] In *in vivo* gene therapy, adeno-associated virus (AAV)-derived vectors are primarily used,[2] and lentiviral vectors have been applied in *ex vivo* gene therapy.[3] Developments in *ex vivo* gene therapies to manage neurological diseases include cell products for metachromatic leukodystrophy and

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adrenoleukodystrophy in which the causative genes, *ARSA* and *ABCD1*, respectively, were transduced into HSCs by lentiviral vectors, which has been approved by the European Medicines Agency.[4] In these cases, microglia differentiated from the transduced HSCs are expected to enter the brain and exhibit enzymatic activity. However, it is not sufficient to control the long-term exacerbation of neurological symptoms, and the development of more potent gene therapy is desired. In this review, we describe the status of current research on *in vivo* gene therapies in children using AAV vectors including our work (Fig. 1).

2. AAV vector

AAVs are classified as belonging to the Parvoviridae family and have a single-stranded DNA genome of 4.7 kb in a 25 nm capsid. A large number of genotypes have been isolated, including AAV9, which crosses the blood–brain barrier.[5] The genomes of AAV vectors that have entered cells are located in the nuclear episome and are rarely integrated into chromosomes, thus ensuring the safety of AAV vectors. In non-dividing cells such as neurons, the transduced genes are expressed for long periods without being lost. In fact, in a monkey model of Parkinson's disease, the enzyme gene introduced into the putamen was expressed for more than 15 years.[6] In vector construction, inverted terminal repeats (ITRs) are left at both ends of the genome, and the target therapeutic gene is inserted between the ITRs together with a promoter and a poly(A) sequence. In 2005, brain tissues of mice injected with type 8 AAV (AAV8) vectors via the tail vein showed that marker genes were expressed in neurons of the cerebral cortex, striatum, hippocampus, and cerebellum, indicating that the AAV8 vector penetrates the blood–brain barrier.[7] Subsequently, it was reported that intravenous administration of type 9 AAV (AAV9) vector to newborn mice resulted in highly efficient gene transfer to neurons in the spinal cord, and AAV9 became a target of interest as a gene transfer vector that can penetrate the blood–brain barrier.[8] Then after, it was shown that intravenous administration of the AAV9 vector can transduce neurons in a wide range of regions in adult animals, including mice, cats, pigs, and monkeys, and the application of gene therapy to neurological diseases has rapidly progressed. Currently, research is underway to develop more efficient AAV vectors by artificially modifying capsid proteins.[9]

3. Aromatic L-amino acid decarboxylase deficiency

Gene therapy for aromatic L-amino acid decarboxylase (AADC) deficiency is a representative example of successful gene therapy.[10,11] In this therapy, AAV vectors expressing the AADC gene were injected into the putamen by stereotaxic brain surgery. To date, this therapy has been performed in 30 patients in Taiwan, 10 patients in Japan, and 3 patients in Europe. A clinical trial in which AAV vectors are injected into the midbrain instead of the putamen has been started in the United States and Europe.[12] In the midbrain approach, AAV vectors transduce neurons in the ventral tegmental area as well as the pars compacta of the substantia nigra. The AAV vectors, however, also transduce non-dopaminergic neurons that project to various brain areas. In addition, there is a risk of lethal hemorrhage following midbrain infusion. The method of introducing the AADC gene into the putamen was originally developed as gene therapy for Parkinson's disease.[13] For Parkinson's disease, a method to simultaneously express genes for two enzymes necessary for L-dopa synthesis in addition to AADC has also been developed.[14]

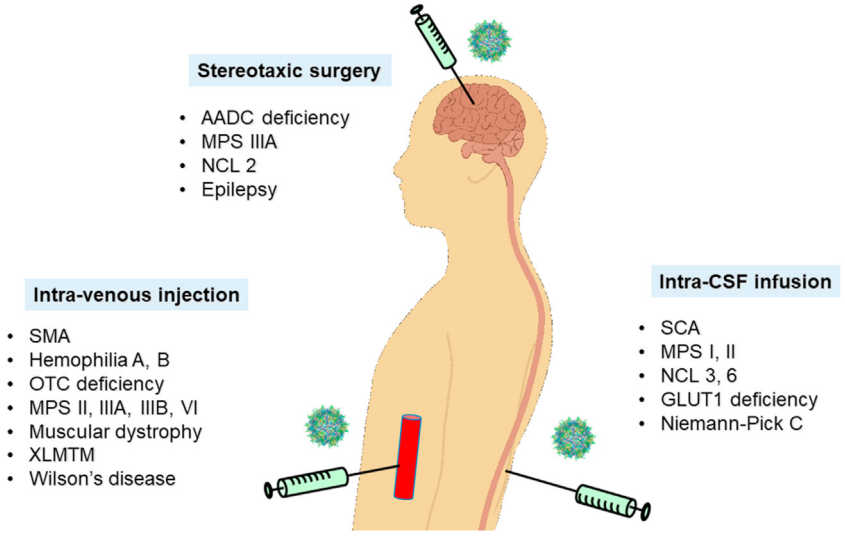

Figure 1 Various routes of administration in AAV vector-based gene therapy.
AADC, aromatic L-amino acid decarboxylase; CSF, cerebrospinal fluid; GLUT1, glucose transporter type 1; MPS, mucopolysaccharidosis; NCL, neuronal ceroid lipofuscinosis; OTC, ornithine transcarbamylase; SCA, spinocerebellar ataxia; SMA, spinal muscular atrophy; XLMTM, X-linked myotubular myopathy.

4. Spinal muscular atrophy

Spinal muscular atrophy (SMA) is caused by loss-of-function variants in the *survival motor neuron 1* (*SMN1*) gene, resulting in degeneration and loss of spinal motor neurons (spinal cord anterior horn cells). In neonatal and infantile type I SMA, 90% of patients die before the age of 2 years unless ventilatory management is provided. Intravenous administration of an AAV9 vector expressing the *SMN1* gene has demonstrated remarkable efficacy, including promoting independent walking.[15] Antisense oligonucleotides that increase the expression of functional SMN protein by targeting the mRNA of the *SMN2* gene, which differs by five nucleotides from SMN1, were previously approved as the first disease-modifying drug for this disease. However, because this drug intervention requires repeated intrathecal administration, gene therapy that can be effective with a single administration is becoming more popular. Despite the high cost of AAV9 (Onasemnogene abeparvovec) at approximately 2 million US dollars per patient, more than 2000 patients have been treated worldwide by August 2022. The efficacy of Onasemnogene abeparvovec has been reported in patients who have not yet developed the disease.[16,17] Therefore, extended newborn screening is important to initiate treatment at an early stage.

5. Lysosomal disease

Diseases caused by single enzyme deficiency have long been considered good candidates for gene therapy. Clinical trials using AAV vectors are underway mainly in the United States for mucopolysaccharidosis types I, II, IIIA, and IIIB, as well as for ceroid lipofuscinosis types 2, 3, and 6.[18-21] In the case of mucopolysaccharidosis type I and II, genome editing has also been attempted and clinical trials have been initiated.[22-24] Because hematopoietic stem cell transplantation is a primary therapeutic option for mucopolysaccharidosis, *ex vivo* gene therapy using lentivirus is also under development.[25,26] In Niemann-Pick type C (NPC1), which is caused by a genetic variant of lysosomal lipid transport protein, the therapeutic effects of various AAV vectors and promoters using animal models have been investigated. We showed the therapeutic effects on systemic organs such as the heart and liver as well as the central nervous system when the AAV vector expressing *NPC1* was injected into the lateral ventricle and cisterna magna.[27] Based on the beneficial results, we are planning a clinical trial for NPC1.

6. Duchenne muscular dystrophy

Progressive muscular dystrophy caused by the deficiency of dystrophin protein has been difficult to treat with gene therapy using AAV vectors because of the large size of the gene. To address this limitation, micro-dystrophin and mini-dystrophin have been developed and are now used in clinical applications. A clinical trial using AAVrh74 (SRP-9001), in which micro-dystrophin is expressed by the skeletal and cardiac muscle-specific MHCK7 promoter, is underway in the United States.[28] Following a single dose of 2.0×10^{14} vector genome (vg)/kg AAV vector was administered via a vein in the lower limb, creatine kinase levels were reduced. Extensive expression and proper localization of micro-dystrophin were also confirmed after a 3-year follow-up. However, one death due to thrombotic microangiopathy was reported in a clinical trial in which a micro-dystrophin-expressing AAV9 vector (SGT-001) was administered at 2.0×10^{14} vg/kg.[29] In a trial of the AAV9 vector (PF-06939926) carrying mini-dystrophin, one death was reported at a dose of 2.0×10^{14} vg/kg.[30]

7. X-linked myotubular myopathy

X-linked myotubular myopathy (XLMTM) is an X-linked muscle disease characterized by hypotonia of the whole body including the facial muscles, respiratory distress, feeding difficulties, and intellectual disability from the neonatal period. In a clinical trial using the AAV8 vector (AT132) carrying human MTM1, good results were initially obtained at 1.0×10^{14} vg/kg, including recovery of muscle strength, but three patients died at a higher dose of 3.0×10^{14} vg/kg. Subsequently, one death occurred even at the lower dose of 1.0×10^{14} vg/kg, and the study was discontinued.[31] XLMTM is associated with severe hepatic injury such as hepatic peliosis, and death is often caused by bleeding from the liver. It is possible that the high dose of AAV may have caused injury to the liver, which was originally fragile, but detailed analysis results are not yet available.

8. Ornithine transcarbamylase deficiency

Many congenital metabolic disorders are caused by the dysfunction of liver enzymes. One of the most common disorders that lead to central nervous system disorders is ornithine transcarbamylase (OTC) deficiency, a loss of enzyme activity in the urea cycle. OTC deficiency is an X-linked disorder that primarily occurs in males and occasionally occurs in female carriers of the disease. The onset of feeding in the neonatal period causes fulminant hyperammonemia leading to central nervous system damage and requires continuous hemodialysis. Currently, the only curative treatment is living donor liver transplantation, but gene therapy is expected to be a potential alternative to this highly invasive treatment. Clinical trials using the AAV8 vector have been initiated mainly for adults (in some cases, patients over 12 years of age) (Table 1).[32] We have developed an AAV vector that transduces human hepatocytes efficiently to facilitate treatment at an earlier stage.[33]

9. Hemophilia

Hemophilia is an inherited bleeding disorder caused by a genetic variant of blood coagulation factor VIII (hemophilia A) or factor IX (hemophilia B). Protein preparations of coagulation factors are used to treat hemorrhage, but their short half-life and frequent administration are problematic. Therefore, gene therapy using AAV vectors has been developed (Table 1). In the beginning, AAV2 vectors were injected into the muscle, but they did not show sufficient efficacy. However, a gene therapy targeting the liver was

Table 1 Clinical trials of liver-targeted gene therapy.

Disease	NCT number	Study description	AAV	Vector dose (vg/kg)	Phase
OTC Deficiency	05345171	DTX301	scAAV8OTC	n/a	III
	05092685	HORACE	AAVLK03hOTC	6×10^{11} (low) 2×10^{12} (intermediate) 6×10^{12} (high)	I/II
	04909346	OTC Deficiency, GSDIa, Wilson Disease	n/a	n/a	n/a
	02991144	DTX301	scAAV8OTC	2×10^{11} (low) 6×10^{12} (intermediate) 1×10^{13} (high)	I/II
Hemophilia A	02576795	BMN-270-201	AAV5-FVIII-BDD	6×10^{12}, 2×10^{13}, 6×10^{13}	I/II
	03392974	BMN-270-302		4×10^{13}	III
	03370913	BMN-270-301		6×10^{13}	III
	03520712	BMN-270-203		6×10^{13}	I/II
	03003533	SPK-8011-101	AAV-SPARK200-FVIII-BDD	5×10^{11}, 1×10^{12}, 2×10^{12}	I/II
	03432520	SPK-8011-LTFU(SPK-8011 extension study)			I/II
	03734588	SPK-8016-101 (Dose finding pre FVIII inhibitor study)		n/a	I/II
	03001830	GO-8	AAV2/8-HLP-FVIIIV3	6×10^{11}, 2×10^{12}, 6×10^{12}	I
	03061201	SB-525-1603	AAV2/6-FVIII-BDD	9×10^{11}, 2×10^{12}, 1×10^{13}, 3×10^{13}	I/II
	03370172	BAX-888	AAV8-FVIII-BDD	n/a	I/II
	03588299	BAY2599023 (DTX201)	AAVhu37	n/a	I/II
Hemophilia B	00979238	n/a	scAAV2/8-LP1-hFIXco	2×10^{11}, 6×10^{11}, 2×10^{12}	I
	01687608	AskBio009	AAV8.sc-TTR-FIXco-Padua	2×10^{11}, 1×10^{12}, 3×10^{12}	I/II
	02396342	AMT-060-01	AAV5-FIXco-wt	5×10^{12}, 2×10^{13}	I/II
	03489291	AMT-061-01	AAV5-FIXco-Padua	2×10^{13}	II
	03569891	AMT-061-02 (HOPE-B)			III
	02484092	SPK-9001-101	AAV5-FIXco-Padua	5×10^{11}	II
	03307980	SPK-9001-LTFU-101(SPK-9001 extension study)			II
	03861273	BENEGENE-2	AAV Spark100 hFIX Padua	n/a	III
	03369444	FLT-180a	AAVS3-FIXco-adua	6×10^{11}, 2×10^{12}	I
	03641703	FLT180a LTFU(FLT180a extension study)			I/II

co, codon-optimized; FVIII-BDD, B-domain deleted FVIII; n/a, not applicable; sc, self-complementary; vg, vector genomes.

developed, and a single administration of the AAV2 vector resulted in the long-term maintenance of coagulation factors in the blood and eliminated the need for administering coagulation factor preparations.[34,35] Furthermore, a method of inserting therapeutic genes into the target genome sequence via genome editing has been developed to maintain the therapeutic effect even when hepatocytes divide and proliferate.[36]

10. Spinocerebellar ataxia

In Spinocerebellar ataxia type 1, levels of high molecular group box 1 (HMGB1), a DNA structural regulator protein, are decreased in neurons, leading to cerebellar ataxia and neuronal cell death via impaired DNA repair and transcription. We are planning a clinical trial in which an AAV vector expressing HMGB1 is injected into the cerebellum.[37]

In addition, in Spinocerebellar ataxia type 6, the CAG repeats in the coding region of the transcription factor alpha1ACT in the calcium voltage-gated channel subunit alpha1 A gene (*CACNA1A*) is abnormally elongated and disrupts neuronal cell function. We demonstrated that AAV vectors expressing miR-3191-5p selectively inhibited alpha1ACT mRNA translation and rescued from the degeneration of Purkinje cells in a mouse model.[38]

11. Epilepsy

In the absence of a clear link to a single gene variant, several methods have been investigated for effectively managing epilepsy, including induction of inhibitory synapses, suppression of neuronal hyperexcitability by gene transfer of inhibitory neurotransmitters, and repair of tissues by gene transfer of neurotrophic factors.[39] Although AAV vectors can be delivered to neurons in broad areas of the brain by intrathecal or intravascular administration,[40] stereotaxic neurosurgery to inject the vectors around the epileptic focus would be a shortcut to clinical application.

11.1. Dravet syndrome

Dravet syndrome is caused by a dysfunction of voltage-gated sodium channel 1.1 (NaV 1.1). The SNCN1A gene encoding NaV 1.1 is approximately 6 kb in length and cannot be inserted into AAV vectors. Therefore, the gene for a short voltage-gated sodium channel accessory subunit (NaV β1) was loaded into an AAV vector and injected into the Scn1a $\pm$ mice, resulting in prolonged survival and reduced seizure frequency.41 In addition, in Scn1a $\pm$ mice, researchers have attempted to introduce dCAS9 bound to VP16, a transcription activator, together with a guide RNA using an AAV vector and initiate binding to the promoter region of NaV1.1α to enhance transcription.[42,43]

11.2. Glucose transporter 1 deficiency

In Glucose transporter 1 deficiency (GLUT1D), variants in the *SLC2A1* gene impair glucose transport to brain tissues, resulting in epileptic seizures. Because patients with GLUT1D have intractable epilepsy and are forced to follow a high-fat, low-carbohydrate, low-protein diet for the rest of their lives, it is hoped that gene therapy will provide curative treatment. We have demonstrated that intrathecal administration of AAV vectors that express the *SLC2A1* gene under the *SLC2A1* core promoter transduced vascular endothelial cells and neurons in broad areas of the pig brain.[44]

12. Wilson's disease

Wilson's disease is an inherited disorder of copper metabolism associated with variants in *ATP7B* gene. Gene therapy based on AAV vectors has been developed for Wilson's disease using mouse models.[45,46] Addressing the relatively large size of *ATP7B* cDNA, a dual AAV vector approach using split intein technology or mini-ATP7B, with four out of six metal binding domains deleted, was designed.[47,48] Two phase I/II AAV-based clinical trials are currently ongoing (NCT04537377, NCT04884815).

13. Adverse events caused by AAV vectors

In the AAV9-based vector, Onasemnogene abeparvovec, for spinal muscular atrophy, even when prednisolone is administered prophylactically at 1 mg/kg, liver function tests (ALT, AST, bilirubin) are increased in many cases.[49] In addition, severe thrombotic microangiopathy has been reported, although it is rare. The pathogenesis of these adverse events has not been precisely elucidated. An immune response to the capsid protein of AAV has been postulated. The dosage of onasemnogene abeparvovec is 1.1×10^{14} vg/kg for patients under 2 years of age, and most patients weigh less than 10 kg. In the trials conducted in older children with Duchenne muscular dystrophy and XLMTM, the vector dose may be excessive. When the viral capsid load reaches a certain threshold, proliferation of capsid-specific activated T cells may cause hepatotoxicity.[50,51] Intrathecal administration of AAV vectors can cause a neuronal loss in the dorsal root ganglia due to strong gene expression.[52] In fact, a case of temporary sensory disturbance has been reported after gene therapy for amyotrophic lateral sclerosis.[53] However, in most cases, it is presumed to be clinically unproblematic.

Most of the DNA introduced by AAV vectors is localized to episomes and is very unlikely to be incorporated into chromosomes. The increased risk of hepatocellular carcinoma reported in animals has not been demonstrated in humans.[54] However, long-term follow-up is required, especially in children.

Despite these challenges, gene therapy with AAV vectors is expected to be clinically applicable as a condition-modifying approach to treating many pediatric diseases for which no effective treatment is available.

Declaration of competing interest

S.M. owns equity in a gene therapy company (Gene Therapy Research Institution) that commercializes the use of AAV vectors for gene therapy applications. Since the work in this manuscript may increase the value of these commercial holdings, S.M. has a potential conflict of interest.

References

1. Friedmann T, Roblin R. Gene therapy for human genetic disease? *Science* 1972;**175**:949—55.
2. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther* 2021;**6**:53.
3. Tucci F, Galimberti S, Naldini L, Valsecchi MG, Aiuti A. A systematic review and meta-analysis of gene therapy with hematopoietic stem and progenitor cells for monogenic disorders. *Nat Commun* 2022;**13**:1315.
4. Federico A, de Visser M. New disease-modifying therapies for two genetic childhood-onset neurometabolic disorders (metachromatic leucodystrophy and adrenoleucodystrophy). *Neurol Sci* 2021;**42**:2603—6.

5. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov* 2019;**18**:358–78.
6. Sehara Y, Fujimoto KI, Ikeguchi K, Katakai Y, Ono F, Takino N, et al. Persistent expression of dopamine-synthesizing enzymes 15 Years after gene transfer in a primate model of Parkinson's disease. *Hum Gene Ther Clin Dev* 2017;**28**:74–9.
7. Nakai H, Fuess S, Storm TA, Muramatsu S, Nara Y, Kay MA. Unrestricted hepatocyte transduction with adeno-associated virus serotype 8 vectors in mice. *J Virol* 2005;**79**:214–24.
8. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol* 2009;**27**:59–65.
9. Macdonald J, Marx J, Büning H. Capsid-engineering for central nervous system-directed gene therapy with adeno-associated virus vectors. *Hum Gene Ther* 2021;**32**:1096–119.
10. Tai CH, Lee NC, Chien YH, Byrne BJ, Muramatsu SI, Tseng SH, et al. Long-term efficacy and safety of eladocagene exuparvovec in patients with AADC deficiency. *Mol Ther* 2022;**30**:509–18.
11. Kojima K, Nakajima T, Taga N, Miyauchi A, Kato M, Matsumoto A, et al. Gene therapy improves motor and mental function of aromatic l-amino acid decarboxylase deficiency. *Brain* 2019;**142**:322–33.
12. Pearson TS, Gupta N, San Sebastian W, Imamura-Ching J, Viehoever A, Grijalvo-Perez A, et al. Gene therapy for aromatic L-amino acid decarboxylase deficiency by MR-guided direct delivery of AAV2-AADC to midbrain dopaminergic neurons. *Nat Commun* 2021;**12**:4251.
13. Hwu PW, Kiening K, Anselm I, Compton DR, Nakajima T, Opladen T, et al. Gene therapy in the putamen for curing AADC deficiency and Parkinson's disease. *EMBO Mol Med* 2021;**13**:e14712.
14. Muramatsu S, Fujimoto K, Ikeguchi K, Shizuma N, Kawasaki K, Ono F, et al. Behavioral recovery in a primate model of Parkinson's disease by triple transduction of striatal cells with adeno-associated viral vectors expressing dopamine-synthesizing enzymes. *Hum Gene Ther* 2002;**13**:345–54.
15. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. *N Engl J Med* 2017;**377**:1713–22.
16. Strauss KA, Farrar MA, Muntoni F, Saito K, Mendell JR, Servais L, et al. Onasemnogene abeparvovec for presymptomatic infants with two copies of SMN2 at risk for spinal muscular atrophy type 1: the Phase III SPR1NT trial. *Nat Med* 2022;**28**:1381–9.
17. Strauss KA, Farrar MA, Muntoni F, Saito K, Mendell JR, Servais L, et al. Onasemnogene abeparvovec for presymptomatic infants with three copies of SMN2 at risk for spinal muscular atrophy: the Phase III SPR1NT trial. *Nat Med* 2022;**28**:1390–7.
18. Zhu D, Schieferecke AJ, Lopez PA, Schaffer DV. Adeno-associated virus vector for central nervous system gene therapy. *Trends Mol Med* 2021;**27**:524–37.
19. Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther* 2008;**19**:463–74.
20. Kleine Holthaus SM, Aristorena M, Maswood R, Semenyuk O, Hoke J, Hare A, et al. Gene therapy targeting the inner retina rescues the retinal phenotype in a mouse model of CLN3 Batten disease. *Hum Gene Ther* 2020;**31**:709–18.
21. Jin X, Su J, Zhao Q, Li R, Xiao J, Zhong X, et al. Liver-directed gene therapy corrects neurologic disease in a murine model of mucopolysaccharidosis type I-Hurler. *Mol Ther Methods Clin Dev* 2022;**25**:370–81.
22. Ou L, Przybilla MJ, Ahlat O, Kim S, Overn P, Jarnes J, et al. A highly efficacious PS gene editing system corrects metabolic and neurological complications of mucopolysaccharidosis type I. *Mol Ther* 2020;**28**:1442–54.
23. Harmatz P, Lau HA, Heldermon C, Leslie N, Foo CWP, Vaidya SA, et al. EMPOWERS: a phase 1/2 clinical trial of SB-318 ZFN-mediated *in vivo* human genome editing for treatment of MPS I (Hurler syndrome). *Mol Genet Metabol* 2019;**126**:S68.
24. Muenzer J, Prada CE, Burton BK, Lau H, Ficicioglu C, Foo CW, et al. CHAMPIONS: a phase 1/2 clinical trial with dose escalation of SB-913 ZFN-mediated *in vivo* human genome editing for treatment of MPS II (Hunter syndrome). *Mol Genet Metabol* 2019;**126**:S104.
25. Gentner B, Tucci F, Galimberti S, Fumagalli F, De Pellegrin M, Silvani P, et al. Hematopoietic stem- and progenitor-cell gene therapy for Hurler syndrome. *N Engl J Med* 2021;**385**:1929–40.
26. Shimada Y, Ishii N, Higuchi T, Goto M, Ohashi T, Kobayashi H. A novel preclinical model of mucopolysaccharidosis type II for developing human hematopoietic stem cell gene therapy. *Gene Ther* 2022. https://doi.org/10.1038/s41434-022-00357-y.
27. Kurokawa Y, Osaka H, Kouga T, Jimbo E, Muramatsu K, Nakamura S, et al. Gene therapy in a mouse model of Niemann-pick disease type C1. *Hum Gene Ther* 2021;**32**:589–98.
28. Mendell JR, Sahenk Z, Lehman K, Nease C, Lowes LP, Miller NF, et al. Assessment of systemic delivery of rAAVrh74.MHCK7.-micro-dystrophin in children with Duchenne muscular dystrophy: a Nonrandomized controlled trial. *JAMA Neurol* 2020;**77**:1122–31.
29. Gene therapy at the crossroads. *Nat Biotechnol* 2022;**40**:621.
30. Philippidis A. Food and drug administration lifts clinical Hold on pfizer Duchenne muscular dystrophy gene therapy linked to patient death. *Hum Gene Ther* 2022;**33**:573–6.
31. Philippidis A. Fourth boy dies in clinical trial of Astellas' AT132. *Hum Gene Ther* 2021;**32**:1008–10.
32. Wang L, Warzecha CC, Kistner A, Chichester JA, Bell P, Buza EL, et al. Prednisolone reduces the interferon response to AAV in cynomolgus macaques and may increase liver gene expression. *Mol Ther Methods Clin Dev* 2022;**24**:292–305.
33. Ito M, Takino N, Nomura T, Kan A, Muramatsu S. Engineered adeno-associated virus 3 vector with reduced reactivity to serum antibodies. *Sci Rep* 2021;**11**:9322.
34. Fong S, Yates B, Sihn CR, Mattis AN, Mitchell N, Liu S, et al. Interindividual variability in transgene mRNA and protein production following adeno-associated virus gene therapy for hemophilia A. *Nat Med* 2022;**28**:789–97.
35. Xue F, Li H, Wu X, Zhang F, Tang D, Chen Y, et al. Safety and activity of an engineered, liver-tropic adeno-associated virus vector expressing a hyperactive Padua factor IX administered with prophylactic glucocorticoids in patients with haemophilia B: a single-centre, single-arm, phase 1, pilot trial. *Lancet Haematol* 2022;**9**:e504–13.
36. Ohmori T, Nagao Y, Mizukami H, Sakata A, Muramatsu SI, Ozawa K, et al. CRISPR/Cas9-mediated genome editing via postnatal administration of AAV vector cures haemophilia B mice. *Sci Rep* 2017;**7**:4159.
37. Ito H, Fujita K, Tagawa K, Chen X, Homma H, Sasabe T, et al. HMGB1 facilitates repair of mitochondrial DNA damage and extends the lifespan of mutant ataxin-1 knock-in mice. *EMBO Mol Med* 2015;**7**:78–101.
38. Miyazaki Y, Du X, Muramatsu S, Gomez CM. An miRNA-mediated therapy for SCA6 blocks IRES-driven translation of the CACNA1A second cistron. *Sci Transl Med* 2016;**8**:347ra94.
39. Shaimardanova AA, Chulpanova DS, Mullagulova AI, Afawi Z, Gamirova RG, Solovyeva VV, et al. Gene and cell therapy for epilepsy: a mini review. *Front Mol Neurosci* 2022;**15**:868531.
40. Oguro K, Shimazaki K, Yokota H, Onuki Y, Murashima Y, Kawai K, et al. Global brain delivery of neuroligin 2 gene ameliorates seizures in a mouse model of epilepsy. *J Gene Med* 2022;**24**:e3402.
41. Niibori Y, Lee SJ, Minassian BA, Hampson DR. Sexually divergent mortality and partial phenotypic rescue after gene

therapy in a mouse model of dravet syndrome. *Hum Gene Ther* 2020;**31**:339–51.
42. Colasante G, Lignani G, Brusco S, Di Berardino C, Carpenter J, Giannelli S, et al. dCas9-Based Scn1a gene activation restores inhibitory interneuron excitability and Attenuates seizures in Dravet syndrome mice. *Mol Ther* 2020;**28**:235–53.
43. Yamagata T, Raveau M, Kobayashi K, Miyamoto H, Tatsukawa T, Ogiwara I, et al. CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons ameliorates epileptic and behavioral phenotypes of Dravet syndrome model mice. *Neurobiol Dis* 2020;**141**:104954.
44. Nakamura S, Osaka H, Muramatsu SI, Takino N, Ito M, Jimbo EF, et al. Intra-cisterna magna delivery of an AAV vector with the GLUT1 promoter in a pig recapitulates the physiological expression of SLC2A1. *Gene Ther* 2021;**28**:329–38.
45. Greig JA, Nordin JML, Smith MK, Ashley SN, Draper C, Zhu Y, et al. A gene therapy approach to improve copper metabolism and prevent liver damage in a mouse model of Wilson disease. *Hum Gene Ther Clin Dev* 2019;**30**:29–39.
46. Murillo O, Luqui DM, Gazquez C, Martinez-Espartosa D, Navarro-Blasco I, Monreal JI, et al. Long-term metabolic correction of Wilson's disease in a murine model by gene therapy. *J Hepatol* 2016;**64**:419–26.
47. Padula A, Petruzzelli R, Philbert SA, Church SJ, Esposito F, Campione S, et al. Full-length ATP7B reconstituted through protein trans-splicing corrects Wilson disease in mice. *Mol Ther Methods Clin Dev* 2022;**26**:495–504.
48. Murillo O, Collantes M, Gazquez C, Moreno D, Hernandez-Alcoceba R, Barberia M, et al. High value of 64Cu as a tool to evaluate the restoration of physiological copper excretion after gene therapy in Wilson's disease. *Mol Ther Methods Clin Dev* 2022;**26**:98–106.
49. Chand D, Mohr F, McMillan H, Tukov FF, Montgomery K, Kleyn A, et al. Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy. *J Hepatol* 2021;**74**:560–6.
50. Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated in vivo gene therapy. *Mol Ther Methods Clin Dev* 2018;**8**:87–104.
51. Yang TY, Braun M, Lembke W, McBlane F, Kamerud J, DeWall S, et al. Immunogenicity assessment of AAV-based gene therapies: an IQ consortium industry white paper. *Mol Ther Methods Clin Dev* 2022;**26**:471–94.
52. Hordeaux J, Buza EL, Dyer C, Goode T, Mitchell TW, Richman L, et al. Adeno-associated virus-induced dorsal root ganglion pathology. *Hum Gene Ther* 2020;**31**:808–18.
53. Mueller C, Berry JD, McKenna-Yasek DM, Gernoux G, Owegi MA, Pothier LM, et al. SOD1 suppression with adeno-associated virus and MicroRNA in familial ALS. *N Engl J Med* 2020;**383**:151–8.
54. Sabatino DE, Bushman FD, Chandler RJ, Crystal RG, Davidson BL, Dolmetsch R, et al. Evaluating the state of the science for adeno-associated virus integration: an integrated perspective. *Mol Ther* 2022;**30**:2646–63.