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REVIEW ARTICLE

Gene therapy for lysosomal storage diseases and peroxisomal diseases

Toya Ohashi[1]

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Abstract

Gene therapies for lysosomal storage diseases (LSD) and peroxisomal diseases (PD) are rapidly advancing. Most LSDs and PDs are characterized by brain involvement, prompting the development of therapies targeting the brain. There are two types of gene therapy for brain involvement in LSD and PD, i.e., the direct transfer of a therapeutic gene into brain cells and hematopoietic stem cell-targeted gene therapy. The rationale for the latter approach is that brain microglia are derived from hematopoietic cells. Thus, gene-corrected hematopoietic cells migrate into the brain and differentiate into microglial cells. These gene-corrected microglial cells correct the metabolic defects associated with LSD and reduce inflammation in PD and LSD, leading to a clinical benefit. Gene editing technology has recently been applied in this area and a trial focused on LSD is currently ongoing. Although these approaches are still under investigation, very encouraging results have been obtained. This review provides an overview of recently developed gene therapies for various LSDs and PDs, including the results of clinical trials, with an emphasis on the benefits of this approach for these diseases.

Introduction

Inborn errors of metabolism (IEM) are characterized by genetic mutations resulting in enzyme deficiencies, thereby disrupting metabolic pathways. This causes various clinical symptoms. The primary cause of IEM is a dysfunction of a single gene; thus, they are strong candidates for gene therapy. However, initial gene therapy studies were not highly successful owing to the use of immature technology. In addition, a patient with an ornithine transcarbamylase deficiency died due to an adverse event in an ethically and medically inappropriate trial. This halted the development of gene therapies for IEM. In addition, a patient developed hematological malignancy in a gene therapy clinical trial for primary immune deficiencies as a result of insertional mutagenesis [1]. However, the recent development of vector technology and improvements in our understanding of stem cell biology have opened up new avenues for gene therapy for various diseases, including IEM. In fact, three gene therapy products have been approved in western countries (Table 1).

Lysosomal storage diseases (LSD) and peroxisomal diseases (PD) are types of IEM and excellent targets for gene therapy. Many patients of LSD and PD exhibit brain involvement. Current therapeutic strategies for LSD and PD are limited, especially for cases with brain involvement.

The first gene therapy trial for an LSD focused on Gaucher disease in the late 1990s [2]. In this trial, the wild-type cDNA of a deficient enzyme, glucocerebrosidase, was introduced into patient-derived CD34-positive hematopoietic stem cells (HSCs) using an oncoretrovirus and transplanted into the same patient without any preconditioning. The expression of the missing enzyme was transient and the trial was not successful.

Table 1 Approved gene therapy products

Name of drug	Vector	Gene	Indication
Glyvera	AAV	*LPL*	LPL deficiency
Strimvelis	Retrovirus	*ADA*	ADA deficiency
Luxturna	AAV	*RPE65*	Leber's congenital amaurosis

LPL lipoprotein lipase, *ADA* adenosine deaminase

✉ Toya Ohashi
tohashi@jikei.ac.jp

[1] Division of Gene Therapy, Research Center for Medical Sciences/ Department of Pediatrics, The Jikei University School of Medicine, Tokyo, Japan

In this review, we describe the development of gene therapies for LSD and PD.

Adrenoleukodystrophy (ALD)

ALD is the one of the most prevalent PDs. Peroxisomes are cellular organelles that mainly function in the beta-oxidation of very long chain fatty acids (VLCFA). The primary cause of ALD is a deficiency in ALDP (adrenoleukodystrophy protein), which is encoded by the *ABCD1* gene. ALDP is expressed in the peroxisome membrane and may be responsible for the trafficking of VLCFA into peroxisomes. Thus, in ALD, VLCFA cannot enter peroxisomes, resulting in elevated serum levels of VLCFA.

ALD is inherited in an X-linked recessive manner; thus, most patients are male. However, females occasionally suffer from ALD and usually exhibit milder phenotypes. ALD is classified into seven clinical types depending on the clinical presentation and age of onset (Table 2). Childhood cerebral ALD (CCALD) is the most prevalent form of ALD, with an onset of ~2.5–10 years. The first clinical signs of CCALD include a visual disturbance, hearing difficulty, gait disturbance, and intellectual deterioration. Within a few years, almost all patients become bed-ridden.

For the treatment of CCALD, hematopoietic stem cell transplantation (HSCT) is effective for central nervous system involvement. However, HSCT is only effective at the very early stage of CCALD. Additionally, this approach has some limitations, such as the insufficient availability of donors and serious side effects, including graft-versus-host disease and the rejection of donor cells.

To overcome these limitations, HSC-targeted gene therapy using lentiviral vectors has been performed in France and very promising results have been published in 2009 [3]. Two patients with CCALD (7 and 7.5 years old) with abnormal MRI findings and very mild or no clinical signs were enrolled in this proof-of-concept (POC) study. CD34-positive HSCs were mobilized by G-CSF and transduced with wild-type *ABCD1* cDNA using a lentiviral vector. The conditioning regimen was myeloablative, consisting of busulfan and cyclophosphamide. The transduced cells were used for transplantation into patients with CCALD after preconditioning. As a result, 9–14% of all cell lineages carried the therapeutic gene and the serum level of VLCFA decreased. Additionally, enhancement of gadolinium, an indicator of active demyelination, disappeared. After 14–16 months of treatment, the demyelination area was expanded, but the area stabilized thereafter. In one patient, non-verbal IQ deteriorated just after treatment but stabilized thereafter. In the other patient, a mild visual defect was observed, followed by stabilization. Other neurological signs and symptoms also stabilized. ALD is a progressive disease; thus, the stabilization of disease features strongly suggested a therapeutic effect. Additionally, these effects were very similar to those of HSCT.

Table 2 Classification of ALD

1.	Childhood cerebral form (CCALD)
2.	Adrenomyeloneuropathy (AMN),
3.	Adult cerebral form
4.	Adolescent form
5.	Adrenal insufficiency without neurologic disease
6.	Spinocerebellar form
7.	Heterozygotes

Based on the encouraging results of the POC study, a phase 2/3 clinical trial sponsored by Bluebird Bio (Cambridge, MA, USA) was performed, referred to as the Starbeam Study. This trial was a non-randomized, single-arm, multicenter study. Inclusion criteria were males, less than 17 years old, with CCALD and positive gadolinium enhancement, Loes Score 1.5–9, neurological function score <1, and no HLA-matched sibling donor. The primary endpoint was the ratio of patients free of major functional disabilities, including a loss-of-communication capacity, cortical blindness, tube-feeding, dependency on a wheel chair, loss-of-voluntary movement, and total incontinence. Secondary measurements were the neurological function score, presence of gadolinium enhancement, Loes score, and safety. The gene therapy protocol was the same as that used in the French study, the observation period was 2 years, and the follow-up period was 15 years. In total, 17 patients were enrolled in the trial and the results of an interim analysis were recently published [4]. At the time of the interim analysis, the median follow-up time was 29.4 months. Two patients died as a result of disease progression or complications from HSCT, which was performed after gene therapy due to the rapid deterioration of neurological function. The other 15 patients carried genetically modified cells with ALDP expression. There was no evidence for genotoxicity. Importantly, all 15 patients were free of major functional disabilities.

These results were very promising and the FDA has granted the Breakthrough Therapy designation to the gene therapy product (Lenti-D™) for the treatment of patients with cerebral-type ALD, a rare, serious, and life-threatening disease, in May, 2018.

Metachromatic leukodystrophy (MLD)

MLD is caused by a deficiency in the activity of the lysosomal enzyme Arylsulfatase A (ARSA), resulting in the accumulation of sulfatide in myelin-forming cells,

oligodendrocytes. As a result, oligodendrocytes are degraded, leading to the progression of demyelination. The primary cause of MLD is a mutation in the *ARSA* gene; MLD is inherited in an autosomal recessive manner. The main clinical feature is progressive central and peripheral nervous system deterioration. MLD is divided into three clinical phenotypes, the late infantile form (LI), early juvenile form (EJ), and adult form, according to the age of onset and severity. The LI form is the most representative phenotype of MLD, and it usually develops before 2 years old. The disease progresses rapidly and patients become bed-ridden within a few years. Like ALD, HSCT at the early stage is currently the only effective treatment. However, there are limitations of HSCT, as described for ALD. As an alternative, targeted gene therapy using a lentivirus vector was performed in Italy, and the results of this POC study were published in 2013 [5]. Three presymptomatic patients whose siblings were diagnosed with LI MLD were enrolled in the study. The methods for gene therapy were very similar to those used for ALD gene therapy. The normal *ARSA* cDNA was introduced into CD34-positive HSCs by a lentiviral vector and re-introduced to patients who received myeloablative preconditioning using busulfan. The observation period was 18–24 months. A total of 45–80% of hematopoietic progenitor cell colonies contained the vector sequence, and ARSA activity levels in CD15-positive cells and CD14-positive cells were greater than wild-type levels. Moreover, ARSA activity was also observed in the cerebrospinal fluid. Motor function and cognitive function were maintained and were much better than those of siblings at the same age.

These observations were very encouraging; thus, a phase I/II study was started using six additional patients; the study was sponsored by GSK (https://clinicaltrials.gov/ct2/show/study/NCT03392987?term=gene+therapy&cond=MLD&rank=3), and the results of an ad hoc analysis were published in 2016 [6]. The inclusion criteria were patients with presymptomatic or early-stage MLD who have siblings with MLD. The nine patients included six with the LI form without any clinical symptoms, two with the EJ form at a very early stage, and one unclassified case without any clinical symptoms. No graft failure was observed in all patients, and no serious treatment-related adverse events were observed. On average, 60.4% (range 14.0–95.6%) of hematopoietic colonies contained the vector sequence. No clonal expiation of hematopoietic cells was observed and hematopoietic reconstitution was polyclonal. ARSA activity in CD15-positive cells was elevated in all patients. Moreover, ARSA activity in the cerebrospinal fluid was also increased. Skin biopsy was performed after 2 years, revealing reductions in sulfatide accumulation in Schwann cells, indicating that the gene therapy approach was effective for demyelination in the peripheral nerve. This observation was consistent with the improvement or stabilization of the nerve conduction velocity in most patients. In particular, the nerve conduction velocity improved in 3 out of 9 patients, stabilized in 4 out of 9 patients, and deteriorated in 2 out of 9 patients. The nerve conduction velocity was higher in patients than in untreated siblings with MLD. The MRI scores (where higher values indicate a worse condition) were lower in the treatment group than in the untreated control MLD cases, except in one case who had a high MRI score at baseline. The effect on the GMFM score was very encouraging. As expected, an effect on the GMFM score was not observed in patients who had a high MRI score at baseline. Surprisingly, the developmental GMFM score curve was highly similar to that of healthy children. The IQ scores were within the normal range, except in one case. This study is on-going and the final results will be available by 2025.

Mucopolysaccharidosis (MPS) type III A and B

MPS type III (MPS III) is characterized by the accumulation of glycosaminoglycan (heparan sulfate) in neural cells. Thus, the main affected organ in MPS III is the brain, and somatic involvement is less than that of other MPS types. This clinical feature prompted the development of brain-targeted gene therapy for MPS III. Deficiencies in *N*-sulfoglucosamine sulfohydrolase (SGSH) and alpha-*N*-acetylglucosaminidase are found in MPS IIIA and MPS IIIB, respectively. Gene therapy trials for MPS III were conducted for MPS IIIA and MPS IIIB by different sponsors using similar approaches, including the use of an AAV vector. The MPS IIIA trial was sponsored by a French company, Lysogene (Neuilly-sur-Seine, France)." (https://clinicaltrials.gov/ct2/show/NCT02053064?term=AAV&cond=MPS+III+A&rank=2). The primary outcome was adverse events and the secondary outcomes were: (1) neurological and cognitive changes based on clinical status and standardized neurocognitive and behavioral assessments, (2) changes in disease biomarkers, and (3) indicators of the immune response.

The results of the phase I/II trial were published in 2014 [7]. The AAV vector (serotype 10) carrying cDNA for *SGSH* and sulfatase-modifying factor (SUMF1) was generated. This vector was administered intracerebrally to four children who were able to walk, but exhibited cognitive disabilities. The vector was injected into six brain areas, white matter anterior, medial, and posterior to the basal ganglia on both sides of the brain. No treatment-related adverse events were observed. With respect to efficacy, some data suggested improved or stabilized clinical parameters in some patients.

The MPS IIIB trial was sponsored by a Dutch company, uniQure Biopharma B.V. (Amsterdam, The Netherlands)."

(https://clinicaltrials.gov/ct2/show/NCT03300453?term=AAV&cond=MPS&III+A&rank=1). The primary outcome was safety, as assessed by (1) the number of participants with treatment-related (serious) adverse events determined by continuous evaluations of changes from baseline and (2) an aggregation of multiple measurements to derive the number of participants with abnormal laboratory values and/or adverse events related to the treatment. The secondary outcome was the number of participants with brain atrophy, white matter lesions, and other lesions, as assessed by cerebral MRI. The results of the phase 1/2 trial for MPS IIIB using the AAV vector were published in 2017 [8]. The AAV 2/5 vector was injected into multiple areas of the brain after immunosuppressive therapy. Four patients were included in this study; 117 adverse events were observed and 6 were severe. Neurocognitive progression improved in all patients compared with the natural history of the disease. In addition, enzyme activity increased in the cerebrospinal fluid. The youngest patients obtained the most benefits. These results are encouraging, but further assessments of safety and efficacy are necessary.

Pompe disease

Pompe disease is characterized by a deficiency in alfa-glucosidase (GAA) activity, resulting in the accumulation of glycogen in muscle cells and the heart. This causes cardiac failure, muscle weakness, and respiratory failure. To improve respiratory dysfunction in Pompe disease, AAV carrying *GAA* cDNA was directly injected into the diaphragms of nine patients with Pompe disease. This trial was sponsored by the University of Florida (https://clinicaltrials.gov/ct2/show/NCT00976352?term=gene+therapy&cond=Pompe+Disease&rank=2). The results of the phase I/II study were published [9]. The primary outcome measure was safety and the secondary outcome measures included maximal inspiratory pressure. The results indicated that this gene therapy is safe and has a modest effect.

Gene editing

Gene editing technologies are currently being developed and are very promising. In current gene therapies, it is not possible to control the location of therapeutic gene insertion in the host genome. This leads to a risk of hematopoietic cell malignancy, which was observed in a trial of primary immune deficiencies. However, if gene editing technology is employed, the insertion site of a therapeutic gene can be precisely controlled, thereby avoiding the risk of insertional mutagenesis. To take advantage of this property, the biotech company Sangamo Fab (Richmond, CA, USA) developed a new technology in which a therapeutic gene is targeted to the albumin locus by gene editing (in this case, Zinc finger nuclease technology) and is transcribed by a very strong albumin promotor, resulting in substantial expression of the gene product in liver cells [10]. This approach is highly suitable for diseases requiring protein supplementation therapy, such as hemophilia and LSDs [11]. Using this technology, preclinical data have been translated to humans and patients with MPS II have been treated. The results are not yet published, but seem to be very encouraging.

Fabry disease

Another trial for an LSD is ongoing. Fabry disease is characterized by deficient activity of alfa-galactosidase A, resulting in accumulation of glycolipids, such as globotriaosylceramide, are accumulated in various tissues. Renal, cardiac, cerebrovasulular events will occur. Enzyme replacement therapy was currently available, but there are many limitations. To overcome these limitation, an HSC-targeted gene therapy using a lentiviral vector is underway; the trial is sponsored by Avrobio, Inc (Cambridge, MA, USA). The preliminary results were very encouraging (ESGCT annual meeting abstract, Lausanne, 2018)

Conclusion

Gene therapies for LSD and PD have rapidly been developed in the past 5 years. Many companies focused on the development of gene therapies for LSDs have been founded and have performed clinical trials. In some cases, very positive results have been obtained. Recently developments in gene therapies for LSD and PD can be attributed to successful collaborations between academia and these newly established companies. This kind of collaboration will be very important for the continued development of gene therapies for LSD, PA, and other diseases.

Compliance with ethical standards

Conflict of interest T. Ohashi have active research support from Sanofi Genzyme Corporation, Sumitomo Dainippon Pharma and Avrobio, Inc. . These activities have been fully disclosed and are managed under Memorandum of Understanding with the Conflict of Interest Resolution Board of the Jikei University School of Medicine.

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