

## **A Narrative Review on Gene Therapy in Pediatric Neurological Disorders-A Promising New Avenue in Twenty First Century**

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**Received:** April 08, 2019; **Published:** April 30, 2019

### **Abstract**

Central nervous system is a rather complex site for gene therapy, as it contains neurons, astrocytes and oligodendrocytes and they have discrete and intricate interconnections between them establishing a delicate balance. However, with significant advances in scientific technology and development of new viral vectors, now gene therapy has a greater promise for pediatric neurological disorders, especially for certain neurodegenerative diseases, which still remains to be invincible by other pharmacological modalities. Adeno associated vector is the predominant vector used for gene therapy currently. In X linked ALD, late infantile MLD, late infantile NCL, Canavan disease results of human trials have been published and the results are somewhat promising except for NCL. Ongoing clinical trials in several LSDs like MPS type III, Fabry disease, Pompeii disease are currently active. In mice models, several other neurodevelopmental disorders have also been tested successfully for gene therapy

**Keywords:** *Gene Therapy; Pediatrics; Neurological Disorders*

### **Introduction**

Central nervous system is a rather complex site for gene therapy, as it contains neurons, astrocytes and oligodendrocytes and they have discrete and intricate interconnections between them establishing a delicate balance. However, with significant advances in scientific technology and development of new viral vectors, now gene therapy has a greater promise for pediatric neurological disorders, especially for certain neurodegenerative diseases, which still remains to be invincible by other pharmacological modalities [1].

### **History**

History of gene therapy in pediatric population dates back to 1980s. The first gene therapy was attempted in beta thalassemia, which was not successful. First successful gene therapy was done in a girl suffering from SCID with ADA deficiency on 14<sup>th</sup> September 1990. However, the child continued to receive recombinant enzyme even after gene therapy, raising doubts on the success of gene therapy. Later on however a French group demonstrated long term success of gene therapy in children with X linked immunodeficiency in 1990s. Two of these patients, however, developed leukemia like state thereby confirming the risk of malignancy due to gene therapy. To further add on, Jesse Gelsinger, a patient who participated in clinical trial for ornithine transcarbamylase deficiency, died on fourth day of gene therapy, probably due to severe immune reaction to the adenovirus used as a vector for carrying the gene. The child had acute lung injury, hepatic failure and coagulopathy following gene therapy and expired subsequently [2].

### **Type of gene therapy**

Gene therapy can be a somatic cell gene therapy and germ line gene therapy. In somatic cell gene therapy, the gene is transferred into bone marrow cells, peripheral blood or even directly intracerebrally. On the other hand, in germ line gene therapy, the gene is transferred

into sperms or ovum and thereby making them heritable to next generation. Due to safety and ethical concerns, all the current researches are limited to somatic cell gene therapy. Gene therapy is divided into *in vivo* and *ex vivo* types depending on whether genetic correction occurs inside or outside the body [3].

### **In vivo gene therapy**

In this the vector first enters into the target cell, then the therapeutic gene enters into the target cell's nucleus and then the functional protein is expressed thereby returning the cell to normal state. The therapeutic gene carried by the vector can remain free in the nucleus as extrachromosomal material or it can be integrated into the genome [4].

### **Ex vivo gene therapy**

The viral vector is altered so that it cannot reproduce; the targeted genetic material is inserted into its genome. Then the viral vector is mixed with the cells collected from the patient and reinfused after transduction. Then these cells divide *in vivo* and produce the functional enzyme/protein.

### **Mechanism of action of gene therapy**

Gene therapy is basically to correct defective genes responsible for genetic disorder by one of the following approaches [5]:

1. A normal gene could be inserted into a nonspecific location within the genome to replace the nonfunctional gene (most common).
2. An abnormal gene could be swapped for a normal gene homologous recombination.
3. An abnormal gene could be repaired through selective reverse mutation.
4. Regulation (degree to which a gene is turned on or off) of a particular gene could be altered.

### **Viral vectors**

#### **Characteristics of ideal viral vector for gene therapy [5]:**

1. Highly specific for the targeted cell
2. Capable of efficiently delivering into target cell
3. Can be produced as purified in large quantities at high concentration
4. Minimal allergic reaction or inflammation
5. Safe for the patient and the environment
6. Long term expression, preferably for life long.

### **Retroviruses**

By using reverse transcriptase, it produces double stranded viral genome which integrates into human genome by *integrase*. Integrase inserts the gene anywhere because it has no specific site and may cause insertional mutagenesis. Zinc finger nuclease can be used for targeting the insertion to specific site. Vectors derived from HIV and other lentiviruses are being evaluated for safety concerns. Packaging capacity is around 8 kb [6].

### **Adenoviruses**

They have double stranded DNA genome and can cause respiratory, intestinal, and eye infections in humans. The inserted DNA is not incorporate into genome and is left free in the nucleus. It can infect slowly dividing cells. It can also spread into the surrounding cells. Immunogenic response is more severe with adenovirus as compared to AAV. Packaging capacity is around 7.5 kb [7].

### **Adeno associated virus**

It is from parvovirus family with small, single stranded DNA that inserts genetic material at a specific point on chromosome 19 with near 100% certainty. It causes no known human disease and doesn't trigger patient immune response. The gene is always "on" so the protein is constitutionally expressed, possibly even in instances when it isn't needed. Packaging capacity is around 4.5 kb (small) [7].

### **Herpes simplex virus**

It is a double stranded DNA virus that infects the neurons. It has a large genome compared to other viruses, which enables scientists to insert more than one therapeutic gene into a single virus, paving the way for treatment of disorders caused by more than one gene defect. HSV makes an ideal vector as it can infect a wide range of tissues including muscle, liver, pancreas, and nerve and lung cells. The wild type of HSV-1 virus is able to infect neurons which are not rejected by immune system. Antibodies to HSV-1 are common in humans; however, complications due to herpes infections are somewhat rare. Recent advances in AAV has produced recombinant DNAs, which gets inserted at the end of chromosomes producing episomes and thereby reducing probability of mutation along with long standing gene expression [7].

### Physical method of gene delivery into target cell

1. Gene guns (shoots DNA coated gold particles into cells by using high pressure) [8],
2. Electroporation (creation of electric field induced pores in plasma membrane),
3. Sonoporation (ultrasonic frequencies to disrupt cell membrane),
4. Magnetofection (use of magnetic particle complexed with DNA).
5. Hydrodynamic therapy.

### Chemical method of gene delivery into target cell

1. Receptor mediated gene transfer: DNA conjugated with specific protein either- viral structural protein or with liposome or both [9].
2. Oligonucleotides (to inactivate defective genes by using antisense specific to target gene).
3. Lipoplexes (made up of anionic and neutral lipids).
4. polyplexes (complex of polymers with DNA).
5. Hybrid techniques: Vibrosomes that combine liposomes with an inactivated HIV or influenza virus [9].

### Recent advances in gene therapy

RNA interference or gene silencing is a recently recognized mechanism, which is being explored now for a large number of diseases. siRNAs are small RNA molecules with homology to specific mRNA molecules and after binding to the mRNA they will suppress the translation of the defective protein and degrade RNA of particular sequence. As a result of which abnormal protein won't be produced and cell will return to normal function. A single ASO can cause significant reduction in level of mutant huntingtin, ataxin 1 and 3 and atrophin-1, thereby being effective in several trinucleotide repeat mutation with polyQ (polyglutamine disorders). Based on these principals several molecules have been developed and many of them are about to complete preclinical stage [10].

### Detailed methodology

First of all, intracellular dsRNA is recognized by an RNase III (designated as "Dicer" in Drosophila) and cleaved into siRNAs of 21 - 23 nucleotides. These siRNAs are then integrated in a complex (designated as "RISC", RNA-induced silencing complex). Each siRNA in RISC is specific and targeting to certain sequences of mRNAs, which is homologous to the integral siRNA followed by completely degradation of targeted mRNA. Actually, the target mRNA is cleaved in the center of the sequence complementary to the siRNA. As a result, when AAV carrying the siRNA gains entry into neuronal cell, rapid degradation of the target mRNA and decreased protein expression was observed. However, only a few leukodystrophies follow autosomal dominant inheritance. siRNA associated gene therapy has been done with some success in SCA type 1, 3 and 6 which can also be seen in pediatric population [11].

### Problems associated with gene therapy in neurological disorders

1. Blood brain barrier: It is difficult for vectors to cross BBB and infect neurons. However, HSC targeted stem cells enter into CNS, converted into microglia cells. They can provide the enzymes to the nearby cells [8].
2. Short term expression of the gene [12].
3. Immunity development against viral vector making the vector inefficient with repeated use.
4. Some of the genes are too large to be packaged into the suitable vector.

5. Some of the vectors are associated with serious leukemia like side effects, hence, retroviral vectors are now not used since 2013.
6. Some of the genes if overexpressed then can cause neuronal damage as with MECP2 gene for Rett syndrome [12].
7. Intracerebral insertion although has better efficacy, can have serious side effects. Also, the number of sites, dose of vector and the method of insertion are not standardized. Intrathecal or intraventricular administration does not necessarily produce the same effect as intracerebral administration.
8. Another problem with gene therapy is that one does not have control over where the gene will be inserted into the genome. The location of a gene in the genome is of importance for the degree of expression of the gene and for the regulation of the gene (the so-called «position effect»), and thus the gene regulatory aspects are always uncertain after gene therapy [12].

### Human studies on gene therapy in pediatric and adult neurological diseases

Mice models with gene therapy have been successfully tried in preclinical studies in the following disease. For few diseases human trials have been successfully completed and results have been described in the table below. For some of other diseases, phase I/II clinical trials have been started and the result is yet to be published [13].

#### Leucodystrophies

Gene therapy has been somewhat successful in following leucodystrophies

- X linked ALD
- Late infantile MLD
- Late infantile and infantile NCL
- Krabbe's disease
- Pelizaeus Merzbacher disease (no significant success in preclinical study).

#### Lysosomal storage disease

- Gaucher disease
- Sanfilippo disease type A and B
- MPS type I
- Alpha mannosidosis
- Pompei disease
- Fabry disease [14].

#### Other grey matter DBD

- Late infantile and infantile NCL.

#### Other neurodevelopmental diseases

- Rett syndrome
- Angelman syndrome
- Fragile X syndrome [13].

However, gene therapy for these diseases has more logistic difficulties as compared to leucodystrophies and other monogenic diseases [12].

#### Adult human clinical studies were successful to some extent with gene therapy in following diseases [15]:

- Alzheimer disease

- Parkinson disease
- Familial amyotrophic lateral sclerosis
- Chronic pain
- Malignant brain tumor like gliomatosis cerebri.

**Important non-neurological diseases**

- Hemoglobinopathies
- X linked and other primary immunodeficiencies
- Leber congenital Amaurosis
- Age related macular degeneration
- Familial hypercholesterolemia [15].

**Brief description of important ongoing clinical studies on gene therapy in various neurological disorders**

Various successful clinical studies on gene therapy in several neurological disorders in children have been described in table 1. Few other studies worth mentioning have been described below.

Author, year journal	Study population, disease	Intervention	Result
Bainbridge., et al. 2015, <i>NEJM</i>	Phase I/II open-label trial, 12 participants, 6-23 year, Leber’s Congenital amaurosis	Safety and efficacy of gene therapy with recombinant AAV 2/2 (rAAV2/2) vector carrying the RPE65 cDNA  4 received a lower dose 8 received a higher dose	6 cases had improvement in vision, peaking at 6 to 12 months after treatment and then declining.  No improvement in ERG.  3 participants had intraocular inflammation, and two had clinically significant deterioration of visual acuity.  No durable robust effect
Worgfall., et al. may 2008, <i>Journal of Human Genetics</i>	LINCL, 10 children, 18 months follow up	AA2 vector expressing the human CLN2 cDNA (AAV2CUH-CLN2) administered to 12 locations in the CNS	Neurological rating scale showed no significant difference as compared to untreated patients  4 of 10 patients had mild humoral immune response  One patient had status epilepticus and died on day 49 of trial  No AAV associated serious adverse effect  Measured rates of decline of all MRI parameters in quantitative MRI brain were slower
Leone., et al. <i>Science Translational medicine.</i> 2012	Canavan disease, 13 children	AAV vector carrying ASPA gene (AAV2-ASPA). Intraparenchymal delivery at six brain infusion sites.	No long-term adverse events related to the AAV2 vector.  Statistically significant changes in predefined surrogate markers of disease progression and clinical assessment subscores.  Decrease in elevated NAA in the brain and slowed progression of brain atrophy, with improvement in seizure frequency and with stabilization of overall clinical status.
Biffi., et al. <i>Science</i> , 2013	3 presymptomatic children with genetic, biochemical, and neurophysiological evidence of late infantile MLD.	Lentiviral vector to transfer a functional ARSA gene into autologous hematopoietic stem cells (HSCs).	High enzyme expression throughout hematopoietic lineages and in cerebrospinal fluid.  Evidence of aberrant clonal behavior.  The disease did not manifest or progress in the three patients 7 to 21 months beyond the predicted age of symptom onset.
Cartier., et al. <i>Science</i> , 2009	2 ALD children without donor for HSCT	Hematopoietic stem cell targeted gene therapy  <i>Ex vivo</i> genetically corrected autologous CD34+ cells with a lentiviral vector encoding wild-type ABCD1, re-infused into circulation after myeloablative treatment.	At 30 months follow up hematological stem cell transduction was confirmed  Clinical follow up at 16 months, progression of cerebral demyelination was halted similar to allogenic HSCT proving clinical efficacy

**Table 1:** Clinical studies describing successful gene therapy in various neurological disorders of childhood.

### Neuronal ceroid lipofuscinosis

Sands, *et al.* first successfully tried in mice model of infantile NCL. Intracranial injections of AAV2 expressing human recombinant PPT1. PPT1 activity increased to 15% of normal levels. Reduction in auto-fluorescent storage was demonstrated in result. In another study, intravitreal injection of AAV2-PPT1 into INCL mice - expression of PPT1 activity and restoration of retinal function was found after gene therapy [16]. PPT1 activity was also detected in the brain of intravitreally injected INCL due to anterograde axonal transport. AAV2/9 can cross the neonatal and adult blood- brain barrier and transduce CNS cells, hence it is preferred. AAVrh.10-CLN2 has been approved for use in a Phase I clinical trial, which has recently begun recruiting patients. Also, plan is being done for prenatal transfer of the INCL gene in antenatally detected cases [16].

### Pompei disease

Phase I/II trial for Pompe disease- GAA gene transfer into muscle to establish the safety and appropriate dose [11].

### Amyotrophic lateral sclerosis

Phase I clinical trial for familial ALS patients with SOD1 mutation utilizing ASO. phase I/II trial with AAV associated with SGSH and SUMF1 cDNA for Sanfilippo type A disease. Direct injection of gene material SF301 into both sides of brain in a single neurosurgical session has been planned in this study. Primary objective of this study is to measure safety and tolerability. Secondary objective is to measure the efficacy (Desired sample size n = 4) [14].

### Alzheimer disease

A phase II clinical trial is recruiting patients with early Alzheimer disease for treatment with CERE 110(AAV containing cDNA of NGF gene). Three different approaches have been described for Parkinson's disease:

1. Insertion of AAV2-GAD into subthalamus-efficacy already proven in placebo controlled trial.
2. Neuroprotection-phase IIb study is ongoing with CERE 120(AAV with neurotrophic factor) [15].
3. Insertion of AAV with AADC gene into the striatum-phase II study is about to begin [12].

### Other miscellaneous diseases

NP2 (replication defective HSV with PENK gene for preproenkephalin)-phase II trial for subjects with chronic pain has been recruiting participants and phase I trial for HER2 positive glioblastoma multiforme and retroviral replicating vector (RRV) in recurrent malignant glioma are also recruiting patients currently. Fomivirsen for CMV infection and Pegaptanib for Age related macular degeneration. ASOs have also been found to have proven efficacy [17].

### Conclusion

Apart from Duchenne muscular dystrophy and spinal muscular atrophy, no recent FDA approved gene therapy drug available apart from research settings till date. With the results of the ongoing trials, future prospective of gene therapy is going to change, especially for leucodystrophies like ALD and MLD as well as lysosomal storage disease, in which even partial availability of protein can have significant clinical benefit.

### Conflict of Interest

Nil.

### Source of Funding

Nil.

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**Volume 2 Issue 3 May 2019**

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