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Gene Therapy and Battens Disease

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Abstract

Late Infantile Neuronal Ceroid Lipofuscinoses is an inherited neurodegenerative condition caused by a mutation in the *CLN2* gene that codes for an enzyme, tripeptidyl peptidase I (TPP-1). Deficiencies in TPP-1 lead to protein accumulation within lysosomes and subsequent neuronal death, which produce the clinical features of the disease.

Gene therapy is considered a potential treatment option to allow functional administration of *CLN2* to restore TPP-1 activity and distribution in the CNS. Adeno- associated viruses are being trialed as a vector for gene therapy delivery. They are relatively safe and efficacious in their ability to mediate long-term gene expression at high levels of activity. This parallels improvements in both functional and clinical outcomes in human and animal models.

This article outlines the potential clinical benefits of using gene therapy, and discusses some of the limitations of the trials to date.

Keywords: Gene therapy; Vector; Protein; Databases; Gene

Introduction

The Neuronal Ceroid Lipofuscinoses (NCL's), also known as Battens disease, are a collection of congenital neurodegenerative conditions that span from prenatal life to late adulthood with an incidence of 1:12,500 [1]. They comprise of at least 8 autosomal recessive disorders defined by having a mutation in a *CLN* gene, either coding for an enzyme (CLN1 and CLN2) or a trans membrane protein (CLN3, CLN5, CLN6 and CLN8) [2] with all disorders having common clinical features, including progressive visual loss to blindness, seizures, speech disturbances, motor degeneration and intellectual decline, leading to early death [3].

This essay considers the efficacy of gene therapy as a future treatment of Late Infantile Neuronal Ceroid Lipofuscinoses (LINCL) through the evaluation of data derived from both human and animal studies.

LINCL has an incidence of 0.36-0.46 per 100,000 with an age of onset of between 2-4 years, and death commonly anticipated in the early teenage years. LINCL is caused by a mutation of the *CLN2* gene on chromosome 11p15 [4], of which 98 mutations are known [5], three of which account for the majority of cases (Figure 1) [4].

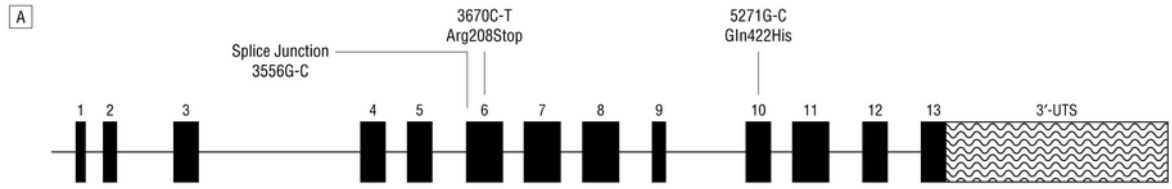

Figure 1: The positions of the three most common mutations of the *CLN2* gene in LINCL. They include a splice site, nonsense and missense mutation (left to right).

The normal product of *CLN2* is tripeptidyl peptidase I (TPP-1), which functions within the lysosome to degrade N-terminal tripeptides from their substrates. Therefore deficiencies lead to an accumulation, in particular of subunit C of mitochondrial ATP synthase causing subsequent neuronal and retinal cell death. This accumulation is seen on UV imaging as auto fluorescent storage and on electron microscopy as curvilinear bodies [6]. Gene therapy is an attractive prospect for long-term therapy in LINCL because it is a

monogenetic disorder. The process involves introducing *CLN2* human complementary DNA (cDNA) into the central nervous system under the control of a promoter and in a suitable vector [4].

Adeno-associated viruses are considered the ideal vector as they can transduce post mitotic cells, mediate long-term gene expression and have an excellent safety record [7]. Therefore, these vectors are considered in this essay as potential delivery vectors for the treatment of LINCL. The challenge is whether using a suitable AAV vector allows sufficient activity, expression and distribution of TPP-1 to destroy existing lysosomal storage protein, prevent its on-going formation and consequently halt the progression of the disease. One way of maximizing enzyme distribution is through cross correction using mannose-6-phosphate pathway (Figure 2) [4,8], this means that CLN2 cDNA does not need not be introduced to the whole of the CNS [8]. The target for gene expression is 1-5% normal expression of the gene product which is sufficient to reverse pathology in other conditions [9].

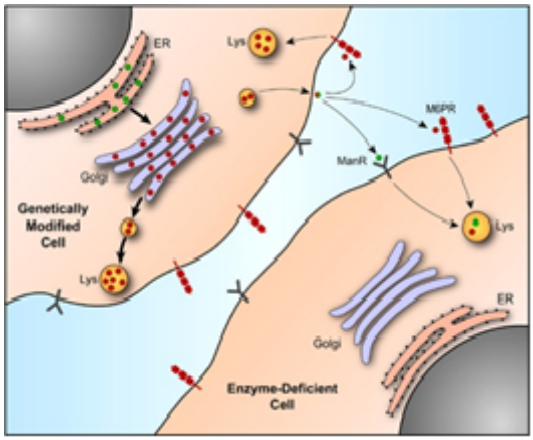

Figure 2: Cross-correction whereby genetically modified cells can produce enzyme that can be taken up by neighboring unmodified cells through surface mannose 6-phosphate receptors.

Methods

The identification of clinical and experimental articles was achieved through PubMed, Google Scholar, Science Direct and Elsevier online databases. Inclusion criteria for this study was that they should explore AAV mediated gene therapy in LINCL with at least 6 month follow up and should try to relate TPP-1 parameters with either histological or clinical signs of the disease.

Results

This study of literature review revealed three animal studies and one clinical study.

The study of therapy in animal trials:

The earliest demonstration of gene therapy in LINCL found in this study literature search considered the use of AAV2 (AAV serotype 2) mediated *CLN2* gene transfer to rodent and non-human primate brains [10]. The primary measures were TPP-1 protein expression, distribution and activity. The use of non-human primate is useful for assessing gene therapy use in humans due to our common ancestry linking our conserved proteins, and also for evaluating clinical benefit and safety.

The vectors used in the study included AAV2Hcln2 (primates), AAV2-Rcln2 (R-rat CLN2 DNA) and AAV2 Null (control). Administration of AAV2-Rcln2 and NULL into the rat CNS was done through unilateral injection into the left striatum, frontal cortex, parietal cortex or cerebellum. The dosage was $10^9/10^{10}$ particles of AAV2Rcln2 or AVV2 Null with assessments of primary measures performed at 1,2,4,8,12 and 18 months during which brains were harvested. In the non -human primates (African green monkeys) vector administration was done through six burr holes (3 per hemisphere) into 12 cortical locations. Injection sites included the head of the caudate nucleus, the body of the caudate and hippocampal formation. Dosages of 3.6×10^{11} particles of either AAV2Hcln2 or AAV2 Null were used with assessments performed at weeks 5 and 13, during which brains were harvested.

Results from the article suggest successful long term gene expression following delivery to rat and non-human primate brain compared to controls. TPP-1 expression in rats was present at 18 months (Figure 3), and for at least 3 months in the brains of non-human primates-the longest time-period examined to date. TPP-1 distribution within the non-human primate however, was confined to the site of injection, suggesting axonal retrograde transport was not present and so unlikely to provide generalized benefit.

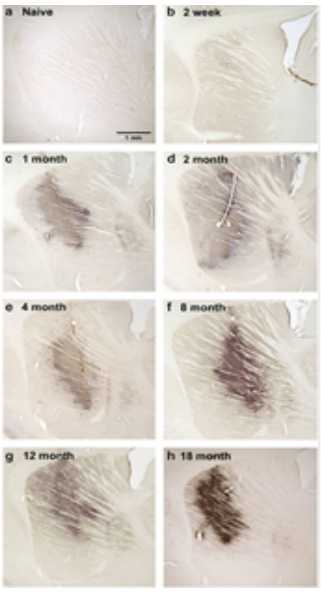

Figure 3: TPP-1 detection within the striatum at time intervals 1,2,4,8,12 and 18 month following *AAV2-CLN2* gene transfer.

In contrast, rat TPP-1 distribution was found to be stable within the striatum at each checkpoint and throughout striatal circuitry>8 months (e.g. *Substantia niagra*) of the injected hemisphere with some spread to the contralateral hemisphere. This suggests cross correction was taking place as confirmed in *in vitro* studies. However, in both populations TPP-1 protein activity was found to exceed the accepted target of 5% [9] with rat TPP-1 activity varying at 0.24 and primate at 0.55 fold increases to controls respectively.

This study supports the idea that AAV2 mediated *CLN2* gene delivery is a good candidate for treatment of LINCL. This evidence shows that it can fulfill the requirements of potential therapeutics such as long term expression, high TPP-1 activity and major distribution. The discrepancy however, between rat and primate TPP-1 distribution could be a result of the different time points of assessment used in both. It should be considered that widespread distribution in rats was found only after 8 months, suggesting longer term experimentation in primates should be allowed. Furthermore these results were achieved in healthy animals, and as such no comparisons between TPP-1 activity and hallmarks of the condition could be made however, Passini et al. found that 0.5 fold TPP-1 activity in control LINCL mice was able to produce marked reduction in auto fluorescent storage and curvilinear bodies [11], making these results important.

Whilst the previous article shows AAV2 mediated-*CLN2* delivery in LINCL has potential [10], the effect of different AAV serotypes have on CLN2 cDNA transfer and subsequent TPP-1 distribution and expression has been considered. This article considered AAV-Rh10-Rhesus non-human primate serotype and AAV2, 5 and 8 human serotypes in an animal model [12].

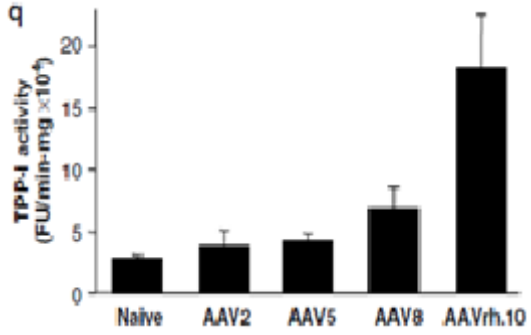

Figure 4: Comparison of the expression of TPP-1 according to choice of AAV-CLN2 vector administered into the striatum of rats.

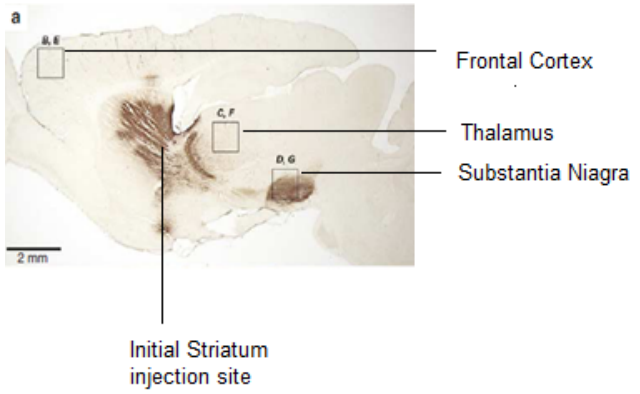

Figure 5: Distribution of TPP-1 from initial striatal injection site at 4 weeks following AAVrh.10-CLN2 administration.

Naive Rats were used initially for the comparison of serotypes following unilateral AAV administration into the left striatum, with evaluations made at 4 weeks for TPP-1 activity and distribution in the brain. Results clearly showed that different AAV serotypes could produce different TPP-1 activity and expression (Figure 4). AAVrh10 was able to produce activity up to 6.4 times the naïve endogenous levels and was found to be statistically significant when compared to both AAV2 and AAV5 serotypes ($p<0.05$). Furthermore, while AAV2Hcln2 was confined to the site of injection, AAVrh.10 was found to fill most of the striatum, achieving similar TPP-1 activity in the *Subtantia niagra* as in the initial injection site, suggesting axonal transport had occurred (Figure 5).

Based on these promising results, 7 week old CLN2-/-mice received bilateral administration of AAVrh.10 in 4 locations (thalamus, upper and lower striatum and cerebellum) with untreated CLN2-/-mice serving as controls. Primary measures were quantification of auto-fluorescence and behavioral assessments including gait, balance beam test, performance on grip strength test and overall morbidity/mortality.

The success of AAVrh.10 CLN2 was shown by the therapeutic outcomes it produced. Auto fluorescence in CLN2-/-mice at 18-22 weeks was reduced in several key areas including the striatum, thalamus and cerebellum, with reductions of 44%,42% and 41% respectively. These histological observations paralleled improvements in motor function, including gait, where reduced limb dragging and improved coordination were noted. Moreover, there were improvements in the balance beam test that in untreated controls show an age dependent decrease, as well as delay in the deterioration of grip strength ($p<0.01$ compared to untreated).

These positive results translated into enhanced survival in mice, with a median survival of 162 days, compared with untreated CLN2-/-mice of 128 days ($p<0.05$) (Figure 6).

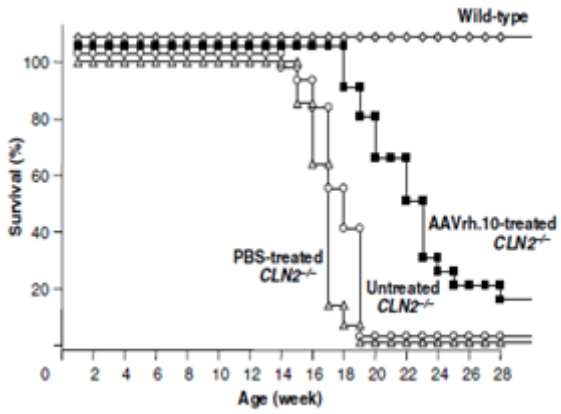

Figure 6: Kaplan Meier curve showing the effect of AAV.rh10-CLN2 on survival in CLN2-/-mice when compared to untreated controls.

This article indicates that different AAV serotypes offer different therapeutic outcomes, for example AAVrh.10 is able to provide long term expression with no effect from the immune system. This is a major consideration as AAV2 epitopes have been implicated in immune responses, and could cause theoretical elimination of transduced cells [13].

Also, the distribution of AAVRh.10 suggests axonal transport is an important mechanism for global correction of TPP1 deficiency

throughout the CNS, has taken place, with this transport of AAVRh.10 being more rapid than AAV2 of the first article. This suggests greater and earlier administration of vector would allow for improved CNS correction and survival, especially when we consider the onset of pathology is as early as 5-6 weeks in mice [14]. However, further follow up must be done to demonstrate that motor improvements are maintained, due to the rapid progression of this disease in untreated models. But as these findings parallel Griffey et al. [15] who used AAVRh.10 in Infantile NCL, it is unsurprising that this vector has been considered for human trials [16].

An important discussion point for the use of gene therapy in LINCL patients is how timing of therapy may influence functional and survival outcomes. This is important since AVVRh.10 was capable of producing functional improvement but had little effect on survival in 7 week old mice [12]. As such Salazar et al. tried to assess functional improvement using the vector AAV1-Hcln2, following its administration into pre and post symptomatic male CLN2 mutant mice at 4 (n=20) and 11 weeks (n=20) respectively compared to controls [17].

Mice in the experimental group, received injections into 6 regions of the brain including the striatum and hippocampus of the right hemisphere and the motor cortex of the left. These were then randomized to two separate cohorts:

1. Age matched cohort (n=10 per group)

2. Survival cohort (n=10 per group)

At 19 weeks the age matched cohort, and controls were killed after undergoing functional tests. These included biweekly accelerating rotarod tests and gait analysis at 18 weeks. The surviving cohort, which underwent the same analysis, was left until they died naturally or until 47 weeks upon which they were killed.

Results showed that auto fluorescent storage in 19 week old mice, regardless of being treated before or after symptom onset, showed similar storage levels to wild-type controls. TPP-1 activity varied between 10-100 times that of wild-type controls. In terms of degeneration, mice treated at 11 weeks were found to have a similar but less pronounced pattern of degeneration compared to untreated CLN2 mice, while mice treated at 4 weeks showed little neural degeneration. This implies an enhanced protective function when treatment is administered before symptoms emerge.

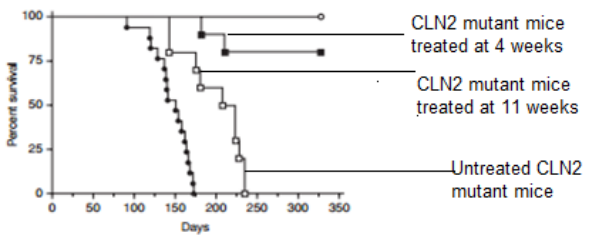

Figure 7: Median survival of mice after treatment with AAV1-Hcln2 compared to untreated controls.

This difference in degeneration accounts for the differences in motor performance seen between the two cohorts. At 16 weeks of age, the 11 week group saw a continuous decline in performance on the Rotarod test, whilst 4 week group were seen to be identical to untreated wild-type controls. Furthermore, gait analysis at 18 weeks of age showed improvements but only the 4 week group showed equivalency to wild-type controls. These observations culminating in a clear difference in lifespan depending on the time treatment was administered (Figure 7).

This study supports the idea of early therapy in LINCL patients, and raises the question of whether neonatal gene transfer should be considered, as early therapy may be able to prevent disease onset as in other lysosomal storage diseases [18]. Furthermore, these results explain the poor increased survival observed in 7 week old mice treated with AAVRh.10, as neuronal degeneration and progression beyond a point can only be slowed and not reversed further suggesting the need for early treatment. As this Article has been cited by many articles and published in molecular therapy a peer reviewed journal, these observations are valid.

The assessment of treatment in clinical trials

The key importance in assessing gene therapy is whether the results found in animal models can be reproduced within humans. Currently, very few human studies exist in this area although some are within the recruitment phase [16,19] and others are ongoing.

One human trial evaluated the use of AAV2 vector to transfer human *cCLN2* cDNA to the CNS of 10 children with LINCL aged between 3 and 10 with five different mutation types [20]. The study was an 18 month follow-up to vector administration with a primary outcome measure being neurological assessment of disease status using the modified Hamburg LINCL scale [1]. Secondary measures were quantitative CNS Magnetic Resonance Imaging assessment of the brain including grey matter and ventricular volume. Control comparisons were made with data from 4 independent untreated LINCL children who had been assessed twice at 1 year intervals.

Participants received an average dose of 2.5×10^{12} particles ($1.8\text{-}3.2 \times 10^{12}$) of the AAV2Hcln2 vector. The dose was shared equally through 6 burr holes (3 in each hemisphere) of the cranial vault, and 12 cortical locations were targeted.

Assessments were made on days 7 and 14 and at 1,6,12 and 18 months after therapy. Adverse effects were assessed at 2 and 3 months.

While surgery itself recorded no evidence of adverse effects, post therapy highlighted 60 serious and 94 non-serious complications, the majority occurring within the first 2 weeks after therapy. Serious events, including seizures and myoclonus were considered to be consequences of drug administration, whereas vomiting and thrombocytosis (non-serious examples) were not considered to be caused by the operation or by tolerance to the therapy. Out of the 10 patients, 2 died, one of unknown reasons and the other during the study period from status epileptics, a known complication of late LINCL.

Primary assessments demonstrated that gene therapy had an effect on the progression of disease, with an improved modified Hamburg scale score in treated subjects monitored for>6 months, and a disease progression rate which was significantly slower than in controls. This difference was shown by the mean rate of change of the modified Hamburg scale in the treated and untreated groups ($P<0.05$) (Figure 8).

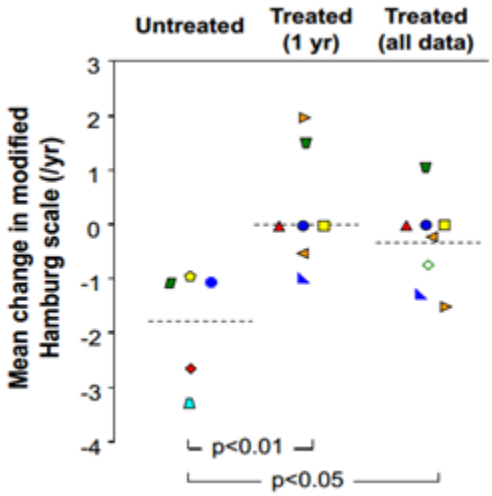

Figure 8: Scatter plot to show changes in mean Hamburg score after AAV2Hcln2 treatment.

Secondary measures showed that the MRI parameters indicated a decreased rate of decline, comparable with the primary outcomes however, there was no statistical significance between groups including in the grey matter volume as a percentage of total brain volume with-2.6%/year loss of volume compared to -2.84%/year in controls (p=0.8).

This article suggests that AAV2 mediated gene therapy can provide deceleration of disease progression regardless of severity and mutation type in humans, while suggesting minimal effects on TPP-1 activity and distribution in MRI parameters that on the basis of Sondhi et al. [12] may be found using other AAV serotypes.

Limitations however to this study include the small study population size and unmatched, non-randomized groups which question the articles validity due to potential selection bias [21]. Also, it is generally considered that the Weill Cornell LINCL scale, featuring feeding, gait, motor and language scores, gives a better representation of LINCL progression than the modified Hamburg scale used in this study [22]. The Weill Cornell scale also correlates better with imaging results, implying that the study has measurement bias [21].

Conclusion

In conclusion of this research has indicated that gene therapy offers a potential therapy in LINCL, by being able to relieve the histopathological burdens of this disease but also in mediating functional improvements in animal models and in the one human study evaluated here. However, an area of improvement remains in the distribution that AAV mediated *CLN2* gene transfer can achieve allowing full utilization of axonal transport and cross correction to take place. This may suggest the need for multiple injections and surgeries to improve the duration of expression and further research into how different AAV serotypes can mediate improved lifespan as some contradictions exist (e.g. AAV2) but also the safety of their use. As such clinical trials are being currently conducted and planned to assess this.

Other potential treatments considered include stem cell therapy, as this would have the benefit of allowing production of TPP-1 but also replacement of the lost neural tissue that is found in this disease. Early evidence from its use in infantile NCL mice have shown to be well tolerated and able to restore enzyme activity to 4.4% of normal at 160-188 post- transplant, and correctly integrate into the CNS [23]. As such, perhaps future research should allow a combination of these treatments to produce additional benefits.

As of now no current treatment exists and the safety and long term effects of gene transfer in humans is being assessed perhaps instead considerations should be made concerning prenatal screening as is it would allow us to address ethical issues in allowing children to be born, knowing the bleak prognosis and profound suffering that the afflicted will face, especially when we consider that screening takes place for less severe but more frequent genetic conditions.

References

1. Lopez SP, Pearce DA (2006) Saccharomyces cerevisiae lacking Btn1p modulate vacuolar ATPase activity to regulate pH imblance in the vacuole. Journal of Biological Chemistry 281 : 10273-10280.
2. Cooper JD (2003) Progress towards understanding the neurobiology of batten disease or neuronal ceroid lipofuscinosis. Curr Opin Neurol 16: 121-128.
3. Cooper JD (2008) Moving towards therapies for Juvenile Batten Disease. Experimental Neurology 211: 329-331.
4. Sohndi D (2001) Feasibility of gene therapy for late neuronal ceroid lipofuscinosis. Arch Neurol 58: 1793-1798.
5. University College London. CLN2 mutation table.
6. Goebel HH, Wisniewski KE (2004) Current state of clinical and morphological features in human NCL. Brain Pathol 14: 61-69.
7. Chtarto A, Bockstael O, Tshibangu T, Dewitte O, Levivier M, et al. (2013) A next step in adeno-associated virus (AAV)-mediated gene therapy for neurological diseases: Regulation and targeting. British J Clin Pharmacol 76: 217-232.
8. Sands M, Davidson B (2006) Gene Therapy for lysosomal storage diseases. Mol Ther 13: 839-849.
9. Sarkar C, Zhang Z, Mukherjee AB (2011) Stop codon read-through with PTC124 induces palmitoyl protein thioesterase-1 activity, reduces thioester loads and suppresses apoptosis in cultured cells from INCL patients. Mol Genet Metab 104: 338-345.
10. Sondhi D (2005) AAV2 mediated CLN2 gene transfer to rodent and non-human primate brain results in long term TPP-1 expression compatible with therapy for LINCL. Gene Ther 12: 1618-1632.
11. Passini MA (2006) Intracranial delivery of CLN2 reduces brain pathology in a mouse model of classical late infantile neuronal ceroid lipofuscinosis. J Neurosci 26: 1334-1342.
12. Sondhi D, Hackett NR, Peterson DA, Stratton J, Baad M, et al. (2007) Enhanced survival of the LINCL mouse following CLN2 gene transfer using the rh.10 rhesus macaque-derived adeno-associated virus vector. Mol Ther 15: 481-491.
13. Madsen D, Cantwell ER, Brien OT, Johnson PA, Mahon BP (2009) Adeno associated virus serotype 2 induces cell mediated immune responses directed against multiple epitopes of the capsid protein VP1. J Gen Virol 90: 2622-2633.
14. Ezaki J, Wolfe LS, Kominami E (1995) Defect of proteolysis of mitochondrial ATP synthase subunit C in neuronal ceroid lipofuscinosis. Gerontology 41: 259-270.
15. Griffy MA (2006) CNS-directed AAV2 mediated gene therapy ameliorates functional deficits in a murine model of infantile neuronal ceroid lipofuscinosis. Mol Ther 13: 538-547.

16. Clinical Trials.gov. AAVRh.10 administered to children with late infantile neuronal ceroid lipofuscinosis with uncommon genotypes or moderate/severe impairment. (Accessed 25th March 2013).
17. Cabrera-Salazar MA, Roskelley M, Hodges BL, Yew N, Dodge JC, et al. (2007) Timing of therapeutic intervention determines functional and survival outcomes in a mouse model of late infantile batten disease. Mol Ther 15: 1782-1788.
18. Hawkins JA, Reddy AS, Sands MS (2011) Combination therapies for lysosomal storage disease: Is the whole greater than the sum of its parts? Hum Mol Genet 20: R54-60.
19. Clinical trials gov. Safety study of gene transfer vector (Rh.10) for children with late infantile neuronal ceroid lipofuscinosis.
20. Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, et al. (2008) Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. Hum Gene Ther 19: 463-474.
21. Research technical guide. Research-bias and confounding. (Accessed 3rd April 2013).
22. Worgall S, Kekatpure MV (2007) Neurological detoriation in late infantile neuronal ceroid lipofuscinosis. Neurology 69: 521-535.
23. Tamaki SJ, Jacobs Y, Dohse ME, Capela A, Cooper JD, et al. (2009) Neuroprotection by host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. Cell Stem Cell 5: 310-319.