Summer 8-11-2012

Gene Therapy for Parkinson's Disease

Kari Andersen
Pacific University

Gene Therapy for Parkinson's Disease

Abstract

Background: Parkinson's disease (PD) is a neurodegenerative disorder of unknown cause. The characteristic motor impairments of PD including resting tremor, rigidity, slowed movement, decreased dexterity, small handwriting, flexed posture, gait disorder, and imbalance predominantly arise from the loss of neurons in the substantia nigra region of the midbrain that produce the neurotransmitter dopamine. Dopamine replacement therapy provides temporary relief of motor symptoms, but chronic use leads to serious side effects and cannot prevent disease progression. This systematic review will focus upon gene therapy as a possible treatment for PD.

Methods: An exhaustive literature search was conducted in Medline, CINAHL, Web of Science, Google Scholar, and EBMRmultifile, using the search terms *gene therapy* and *Parkinson's disease* in combination and alone as well as terms known to be synonymous. The search was limited to the English language, clinical trials and double-blind, randomized, controlled trials.

Results: Two studies were reviewed based on the inclusion and exclusion criteria delineated in the methods section. Both studies were double-blind, randomized, controlled trials and utilized sham surgery for comparison. Marks et al showed adeno-associated type-2 vector (AAV2)-neurturin delivery in the putamen was not superior to sham surgery. LeWitt et al showed AAV2-glutamic acid decarboxylase (GAD) delivery in the subthalamic nucleus was superior to sham surgery.

Conclusion: This systematic review shows gene therapy may prove to be a treatment option for patients with advanced Parkinson's disease in the future. More research and development of gene therapy are needed.

Degree Type

Capstone Project

Degree Name

Master of Science in Physician Assistant Studies

First Advisor

Annjanette Sommers MS, PA-C

Keywords

Parkinson's disease, gene therapy

Subject Categories

Medicine and Health Sciences

NOTICE TO READERS

This work is not a peer-reviewed publication. The Master's Candidate author of this work has made every effort to provide accurate information and to rely on authoritative sources in the completion of this work. However, neither the author nor the faculty advisor(s) warrants the completeness, accuracy or usefulness of the information provided in this work. This work should not be considered authoritative or comprehensive in and of itself and the author and advisor(s) disclaim all responsibility for the results obtained from use of the information contained in this work. Knowledge and practice change constantly, and readers are advised to confirm the information found in this work with other more current and/or comprehensive sources.

The student author attests that this work is completely his/her original authorship and that no material in this work has been plagiarized, fabricated or incorrectly attributed.

Gene Therapy for Parkinson's Disease: A Systematic Review

Kari Andersen

A Clinical Graduate Project Submitted to the Faculty of the

School of Physician Assistant Studies

Pacific University

Hillsboro, OR

For the Masters of Science Degree, August 11, 2012

Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS

When someone makes a decision, he is really diving into a strong current that will carry him to places he had never dreamed of when he first made the decision.

-Paulo Coelho, The Alchemist

Abstract

Background: Parkinson's disease (PD) is a neurodegenerative disorder of unknown cause. The characteristic motor impairments of PD including resting tremor, rigidity, slowed movement, decreased dexterity, small handwriting, flexed posture, gait disorder, and imbalance predominantly arise from the loss of neurons in the substantia nigra region of the midbrain that produce the neurotransmitter dopamine. Dopamine replacement therapy provides temporary relief of motor symptoms, but chronic use leads to serious side effects and cannot prevent disease progression. This systematic review will focus upon gene therapy as a possible treatment for PD.

Methods: An exhaustive literature search was conducted in Medline, CINAHL, Web of Science, Google Scholar, and EBMRmultifile, using the search terms *gene therapy* and *Parkinson's disease* in combination and alone as well as terms known to be synonymous. The search was limited to the English language, clinical trials and double-blind, randomized, controlled trials.

Results: Two studies were reviewed based on the inclusion and exclusion criteria delineated in the methods section. Both studies were double-blind, randomized, controlled trials and utilized sham surgery for comparison. Marks et al showed adeno-associated type-2 vector (AAV2)-neurturin delivery in the putamen was not superior to sham surgery. LeWitt et al showed AAV2-glutamic acid decarboxylase (GAD) delivery in the subthalamic nucleus was superior to sham surgery.

Conclusion: This systematic review shows gene therapy may prove to be a treatment option for patients with advanced Parkinson's disease in the future. More research and development of gene therapy are needed.

Keywords: *Parkinson's disease, gene therapy, adeno-associated type-2 vector.*

Acknowledgements

[Information redacted for privacy]

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AADC…….L–amino acid decarboxylase
AAV2…….adeno-associated type-2 vector
BPRS……...brief parkinsonism rating scale
COMT…….…….....catechol O-methyltransferase
CT.....computed tomography
DA……...dopamine
DBS…….……..…deep brain stimulation
GABA……...gama-aminobutyric acid
GAD…….……...glutamic acid decarboxylase
GCH1…….……GTP-cyclohydrolase-1
GDNF……...glial cell-derived neurotrophic factor
L-dopa…….……...… L-3,4-dihydroxyphenylalanine
NIH…….National Institutes of Health
MRI.....magnetic resonance imaging
PD……..…….Parkinson's disease
PET……..positron emission tomography
SNPC.....substantia nigra pars compacta
TH……tyrosine hydroxylase
UPDRS…….....unified Parkinson's disease rating scale

Gene Therapy for Parkinson's Disease: A Systematic Review

BACKGROUND

Parkinson's disease (PD) was named after Doctor James Parkinson, who first described the shaking palsy in 1817.[1] In the United States, 50 000-60 000 cases of PD are diagnosed annually,[2] adding to the one million people already living with the disease.[3] PD is a neurodegenerative disorder, second only to Alzheimer's disease;[4] the complications from PD are the 14th leading cause of death in the United States.[5] Worldwide, it is estimated that 4.1 million people suffer from PD,[2] a number that is predicted to more than double to 8.7 million by 2030 [6]

PD poses only a slight reduction in life expectancy[7] and most patients require therapy for at least 20 years. The combined direct and indirect financial burden of PD, including treatment, social security payments and lost income due to inability to work, is estimated to be $25 billion per year in the United States.[3] This estimate is set to dramatically increase due to rising health care costs and the growing senior population.

The underlying cause of PD is unknown. PD is clinically characterized by the cardinal features of resting tremor, bradykinesia, rigidity, and postural instability[8, 9] (cumulatively termed parkinsonism)[8]. Pathologically, PD is characterized by the progressive loss of midbrain neurons in the substantia nigra pars compacta.[4] These neurons project to the putamen and caudate (striatum) and release the

neurotransmitter dopamine (DA).[10] Symptoms of parkinsonism are evidenced when more than half of the DA nerve terminals in the striatum have degenerated.[10]

DA cannot cross the blood brain barrier, so L-3,4-dihydroxyphenylalanine (L-dopa) is utilized.[11] L–amino acid decarboxylase (AADC) converts L-dopa (drug name levodopa) to DA in the central and peripheral nervous systems.[12] This drug therapy provides substantial relief during the early stages of PD, although levodopa-induced motor response fluctuations and dyskinesias (involuntary movements) are often seen with long-term treatment.[13] Further, chronic levodopa treatment is linked to motor complications and does not control for disabling features such as falling and dementia.[14] Adverse events present in more than 80% of patients within the first ten years of levodopa medication; some patients demonstrate signs of dyskinesias during the first year.[15] DA agonists, amantadine, catechol O-methyltransferase (COMT) inhibitors and other drugs can reduce fluctuations in plasma L-dopa levels, which improve mobility and reduce dyskinesias. Yet, these drugs tend to fail after several years[16] and side effects of DA agonists include impulse control disorders.[17]

Deep brain stimulation (DBS) delivers electrical impulses to a specific region, or regions of the brain and has become the "gold standard" surgical treatment for PD.[11] Loss of striatal dopaminergic innervation leads to overactivity in the internal segment of the globus pallidus and subthalamic nucleus. DBS works

by seizing the overactivity in the subthalamic nucleus and has proven to be more effective than pharmacotherapy.[14] However, serious adverse events are more common in patients receiving DBS, including fatal intracerebral hemorrhage.[14] In addition, similar to dopaminergic therapy, DBS can fail to treat the parkinsonian features of freezing of gait, imbalance, dysphagia, cognitive and psychiatric disorders and speech difficulties and does not prevent neurodegeneration.[15]

Nonetheless, the success of deep brain stimulation (DBS) by diminishing the activity of the subthalamic nucleus inspired researchers to further explore methods to modulate the basal ganglia circuitry (caudate, putamen, globus pallidus, substantia nigra and subthalmic nucleus).[14] High doses of oral levodopa precipitate negative side effects by acting upon the entire body and brain in uncontrolled concentration gradients with related extreme swings in extracellular DA levels. These insights led researchers to employ viral vector delivery of genes for local and continuous therapy into the basal ganglia.[2] Gene therapy theoretically permits lower therapeutic doses with localized effects, thereby reducing adverse side effects, while providing diffuse, long-term expression of a therapeutic protein.[16-19]

Gene therapy treatment potentially serves as a single intervention resulting in stable and long-term[20] expression of a therapeutic molecule in the brain. Reduction in the medication and its associated adverse side effects might mitigate the financial burden of PD treatment.[12] The success of gene therapy treatments

tested in in-vitro and animal models of PD paved the way for their continued investigation in clinical trials. During phase I clinical trials, researchers test a new treatment in a small group of people to evaluate safety, safe dosage range, and identify possible side effects.[21] In phase II clinical trials, the treatment is provided to a larger group to further evaluate safety and determine effectiveness.[21] For phase III clinical trials, treatment is given to large groups in order to confirm effectiveness, record effects, compare to past treatments, and collect information; which would allow for the treatment to be used safely.[21] Phase IV clinical trials are performed after the treatment has been marketed in order to gather data on the treatment's effect in various populations and possible adverse side effects linked to long-term use.[21]

Three unique gene therapy treatments for PD have advanced to clinical trials. Each gene therapy treatment utilizes a virus, which has been genetically engineered to act as a vehicle through which molecules are introduced to the central nervous system. One approach uses the lentivirus to supply the three enzymes required for DA synthesis: tyrosine hydroxylase (TH), L-amino acid decarboxylase (AADC) and GTP-cyclohydrolase-1 (GCH1).[12, 22] On April 16th, 2012, Oxford Biomedica announced completion of their phase I/II clinical trial of Prosavin®, a lentivirus encoding TH, AADC and GCH1. This triple enzyme approach promotes DA expression within the striatal GABAergic neurons in a continuous manner. Oxford Biomedica reported in a press release that Prosavin®-

treated PD patients experienced an average motor function improvement of 30%, which is a statistically significant improvement in the unified Parkinson's disease rating scale (UPDRS) motor scores.[23] The published results of this phase I/II trial are anxiously awaited.

Another gene therapy method employs the adeno-associated virus serotype 2 vector (AAV2), which has been genetically engineered to deliver novel genes into both dividing and non-dividing cells, which are then expressed by the body's cellular machinery.[24-29] The AAV2 vector does not cause an inflammatory reaction, demonstrates an excellent safety and efficacy profile in clinical trials, and provides long-term transgene expression without inducing dyskinesias.[30] AAV2-neurturin and AAV2-glutamic acid decarboxylase (GAD) are two gene therapy treatments for PD, which have been investigated in clinical trials.

The loss of nigrostriatal dopaminergic neurons alters striato-pallidal circuitry so that decreased gamma-aminobutyric acid (GABA) input renders the subthalamic nucleus disinhibited, which disrupts output to the basal ganglia circuitry.[24] Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme for GABA production; delivering GAD to the subthalamic nucleus would replenish GABA transmission within the nucleus and normalize output to key targets in the basal ganglia.[15,24] Exciting data from preclinical and open-label, phase I trials demonstrated AAV2-GAD gene therapy improved motor function,[31] was neuroprotective, and slowed degeneration of dopaminergic neurons.[32]

Neurturin belongs to the glial cell derived neurotrophic factor (GDNF) family[33] and is a potent neurotrophic survival factor for several populations of central and peripheral neurons.[34] Neurturin encodes for the tyrosine kinase receptor and enhances survival and function of DA neurons in animal models of PD.[35-38]AAV2-neurturin gene therapy shows potential for delaying degeneration of dopaminergic neurons in PD.[39-41] An open-label, phase I trial of intraputaminal AAV2-neurturin in PD patients demonstrated improved motor function and treatment safety.[42] This systematic review will assess the efficacy of AAV2 gene therapy treatment for PD.

METHODS

An exhaustive literature search without restrictions was conducted in Medline, CINAHL, Web of Science, Google Scholar, and EBMRmultifile using the search terms *Parkinson's disease* and *gene therapy* in combination and alone. Scientific terms that were synonymous with these terms, including *PD*, *Parkinson disease*, *Parkinson's*, *primary parkinsonism*, *paralysis agitans*, *adeno-associated type-2 vector*, *AAV2-GAD* and *AAV2-neurturin*, were searched to prevent the omission of any relevant articles.

Inclusion criteria were then set for articles published in the English language, clinical trials (human subjects) and double-blind, randomized, controlled trials. Phase I open-labeled clinical trials, perspectives and research proposals were excluded, duplicates were removed, and titles and abstracts were

screened for relevancy. The references of selected articles were screened for the presence of any articles not produced in the original literature search.

The articles reviewed were critically appraised using GRADE,[43] an approach to evaluate quality of evidence. Each article was placed into a category based on the quality of evidence: high, medium, low and very low. Study limitations for randomized trials included lack of allocation concealment, lack of blinding, incomplete accounting for patients and outcome events, selective outcome reporting bias and stopping trial early for benefit and use of invalidated outcome measures.[41] There were no study limitations in Marks et al.[44]or LeWitt et al.[15]

RESULTS

The initial search identified 419 articles. Based upon inclusion and exclusion criteria, two double-blind, randomized, controlled trials assessing gene therapy for PD were deemed appropriate for this systematic review. See Figure 1. Both studies were phase II clinical trials using sham surgery as their control, and used change from baseline in off-medication UPDRS motor scores as their primary outcome measures. The UPDRS motor score (part 3) ranges from 0 to 108, with higher scores indicating more severe disease. Clinically significant differences in the UPDRS motor score are estimated to be 2.5 points for minimal, 5.2 for moderate, and 10.8 for large effects. [45]

Marks et al.[44] found the efficacy of AAV2-neurturin gene delivery in the putamen was not superior to sham surgery when assessed using the UPDRS motor

score at 12 months (p=0.91). Results were statistically significant at 18 months, though modest (p=0.023). LeWitt et al.[15] found the efficacy of AAV2-GAD gene therapy in the subthalamic nucleus was superior to sham surgery when assessed by UPDRS at 6 months to an extremely significant degree (p<.0001).

Marks et al.[44] conducted a multicenter, double-blind, phase II, sham-surgery controlled trial to assess AAV2-neurturin gene therapy for PD. Enrolled in the study were men and women of any ethnicity, 35-75 years of age, with PD according to the UK Brain Bank Criteria, and with disease duration of at least five years. Inclusion criteria were levodopa responsiveness and levodopa-induced motor complications unsatisfactorily controlled with medical therapy; at least two hours per day of off time (poor motor function), score of at least 30 on the motor section (part 3) of UPDRS in the off state, and stable doses of anti-parkinsonian drugs for a minimum of one month prior to enrollment. The protocol was approved by the institutional review boards at each of the participating institutions as well as the recombinant DNA advisory committee of the National Institutes of Health (NIH). Exclusion criteria were atypical or secondary PD; mini-mental state exam of 26 or less; previous neurosurgical treatment for PD; and significant medical, psychiatric, or laboratory abnormalities. Between December 2006 and November 2008, 58 eligible patients from nine sites in the United States participated. There were no notable differences in demographics and baseline characteristics between groups. Of the 38 patients randomly assigned to AAV2-

neurturin and 20 to sham surgery (2:1), 37 and 20 were included in primary analysis, respectively. One patient in the AAV2-neurturin group suffered a myocardial infarction and did not complete the study. All patients and study personnel, with the exception of the neurosurgical team, were blinded to treatment assignment.[44]

Stereotactic surgery was performed via neuroimaging to plan injection trajectories. After participants were anesthetized with propofol sedation, a treatment kit was opened, which determined intervention assignments. Patients assigned to active treatment received administration of AAV2-neurturin to the putamen, bilaterally through frontal burr holes. Patients assigned to sham surgery underwent a similar procedure, with the exception that the burr holes did not penetrate the inner table of the skull and intracranial injections of AAV2-neurturin were not performed.[44]

Serious adverse events occurred in 13 of the 38 patients treated with AAV2-neurturin and four of the 20 patients assigned to sham surgery. Three patients in the AAV2-neurturin group and two in the control group developed tumors. Two patients in the AAV2-neurturin group died (one myocardial infarction and one pulmonary embolism); however, the events were judged as unrelated to AAV2-neurturin. Using quantitative PCR assay, biopsied tumors occurring in the AAV2-neurturin group (one glioblastoma, one esophageal adenocarcinoma and one adenocarcinoma of the prostate) were not found to contain AAV2-neurturin. In

addition, upon further examination, the glioblastoma had been present on MRI prior to study entry.[44]

The primary endpoint was change from baseline to 12 months in the motor subscore of the UPDRS in the practically defined off state. From baseline to 12 months, the AAV2-neurturin group decreased 7.21 points, while the sham surgery decreased 6.91 points, for an average difference of 0.31 points; a statistically insignificant amount (p=0.91). Between baseline and 18 months, eight patients in the AAV2-neurturin group were followed up and showed an average decrease of 11.96 points, while the six patients in sham surgery that were followed up decreased by an average of 4.34 points, for an average difference of 7.61 points between the groups; a modest but significant benefit (p=0.023). Multiple secondary endpoints (UPDRS part 1 and 2, home diary assessments, timed walking test, Purdue pegboard test, dyskinesia rating scale, PDQ-39 index score, physical health composite score, severity of illness and global improvement) favored AAV2-neurturin at 12 and 18 months, but none favored the sham surgery at either time point. Still, these data are not the primary endpoint, and the sample size was small.[44]

LeWitt et al.[15] assessed AAV2-GAD gene therapy for PD by conducting a double-blind, phase II, randomized, sham-surgery controlled trial, which took place at seven centers in the United States between November 2008 and May 2010. All patients had progressive, levodopa-responsive PD as defined by UK Parkinson's

Disease Society criteria.[46] Levodopa and other PD drugs were allowed if no change in dose or drug type was made for at least one month prior to enrollment. An overnight off-medication UPDRS[47] part 3 score of 25 or more was required. Further inclusion criteria were age 30-75 years, symptom duration of PD for at least five years, and levodopa responsiveness for at least 12 months. Exclusion criteria were previous brain surgery, use of DA receptor-blocking drugs, focal neurological deficits, abnormal cranial magnetic resonance imaging (MRI), or cognitive impairment by Mattis dementia rating scale less than 130. ^{18}F-fluorodeoxyglucose positron emission tomography (PET) scans were required for diagnostic confirmation of PD and excluded patients with atypical parkinsonism or indeterminate patterns.[31, 48] Study protocols and consent forms were approved by institutional review boards at each participating institution.[15]

A statistician and a programmer -each with no further role in the study - created the computer-generated random treatment assignment. Twenty-three patients were randomly assigned to sham surgery and 22 to AAV2-GAD infusions in the subthalamic nucleus; of those, 21 and 16, respectively, were analyzed. Six patients from the AAV2-GAD group and two patients from the sham surgery group were excluded from analysis because they did not receive the allocated intervention. This was due to unilateral infusion, catheter malfunction, missed surgical target or catheter misplacement, or unblinding. Patients, caregivers and investigators were masked to treatment assignment. For sham-surgery

participants, the neurosurgeon and operating room team simulated a bilateral stereotaxic procedure identical to the AAV2-GAD group. Sham-surgery patients received partial-thickness burr holes after a stereotaxic frame was placed. Simulation included sounds of microelectrode recording; pumps and catheters infusing normal saline into the burr hole sites were used identically as for patients treated with AAV2-GAD. For participants receiving AAV2-GAD, a frame-based targeting and intraoperative microelectrode recording specified the boundaries of the subthalamic nucleus,[49, 50] and microelectrode centering within the nucleus defined by measurement and fluoroscopy. A catheter was placed and attached to the pump, and an infusion of vector genomes AAV-GAD65 and AAV-GAD67 was delivered at .23 uL/min over 2.5 hours in the subthalamic nucleus on both sides of the brain. Following infusions, a fine-cut head computed tomography (CT) scan showed catheter tip locations. A neurosurgeon with expertise on the subthalamic nucleus—who remained masked to study data—determined if the catheter tip location was in the subthalamic nucleus. In one participant, the postoperative CT showed that both catheter tips had been unintentionally placed in the same subthalamic nucleus, so this patient was unmasked after surgery and removed from group analysis.[15]

One serious adverse event - bowel obstruction - occurred within six months of surgery in an AAV2-GAD participant, but was not attributed to the treatment or surgical procedure, and fully resolved. Other adverse events were mild or

moderate and considered likely related to surgery; the most common being headache (7 patients in the AAV2-neurturin group versus 2 in the sham surgery group) and nausea (6 versus 2).[15]

One movement-disorder specialist who was masked to treatment allocation judged UPDRS motor examinations and brief parkinsonism rating scales (BPRS) at baseline, and at one, three and six months following treatment. All randomly assigned patients were followed up and assessed through the six-month trial. Authors defined a clinically meaningful response to be more than a 9.0-point reduction in UPDRS motor score, which corresponds to a moderate to large clinically important difference reported in an analysis for PD treatments.[45] The primary endpoint was change from baseline to six months in UPDRS motor score. AAV2-GAD treatment led to a reduction of 8.1 points ($p<0.0001$) in off-medication UPDRS motor score at the study endpoint of six months, compared to a reduction of 4.7 points ($p=0.003$) for sham surgery.[15] See Table 1.

DISCUSSION

The demonstrated safety, tolerability and efficacy of certain gene therapy techniques for PD are quite exciting, and these results justify continued investigation and development. Numerous questions remain about what constitutes optimal gene therapy for PD, and which area of the brain would be best served by such treatment. Oxford Biomedica[23] delivered gene therapy to the striatum and reported both clinically and statistically significant improvements in UPDRS motor scores. Marks et al.[44] discovered that AAV2-neurturin delivered

to the putamen was not beneficial at 12 months but demonstrated a modest improvement at 18 months. It's possible that the putamen is not an ideal target site for AAV2-neurturin. The benefit of neurturin depends upon transport of the protein (or DNA) from the striatum to the substantia nigra. The degeneration of the nigrostriatal pathway of PD patients could have impaired transport of neurturin, blunting its therapeutic benefit. LeWitt et al.[15] delivered AAV2-GAD to the subthalamic nucleus and published statistically significant improvement in UPDRS motor scores.

While each of these studies reports improvement in motor function by delivering gene therapy to one specific area of the brain, PD affects multiple regions of the brain. There is little scientific basis for reasoning that localized delivery of therapeutic proteins, genes or enzymes would significantly and/or clinically improve the non-dopaminergic features of PD.[51] Even so, the neurological dysfunction brought about by progressive DA degeneration is a major pathological feature of PD. AAV2-neurturin and AAV2-GAD exhibit potential to restore DA function without the dyskinesias from levodopa therapy or the adverse events of DBS.[52]

Gene therapy treatments for PD are in the beginning phases of clinical research: phase II clinical trials serve as proof of concept in a smaller population. The next step will be phase III/IV clinical trials aiming to demonstrate robust results in larger populations, determine if any long-term adverse side effects exist,

and better assess if gene therapy for PD is a practical option for general clinical use. More in-depth research may elucidate the required predictability of the immediate and long-term actions of each therapeutic protein involved gene therapy treatment. Better understanding, combined with refined and reliable technique, more sophisticated mechanics and possibly a blend of optimal gene therapies - may one day prove to be an effective, alternative treatment for PD. In clinical practice, patients wishing to participate in clinical trials of gene therapy for PD would potentially receive such treatment. At present, gene therapy for PD provides additional insight into the disease and offers hope for an alternative treatment. It may take decades before gene therapy treatment modalities become readily available for patients. A summary of findings is presented in Table 1.

Gene therapy treatments for PD are currently being investigated in patients with advanced PD, who are likely to be an older subset of the population. Gene therapy needs to be delivered to specific regions of the brain via surgery warrants extreme caution. Risks from surgery are often augmented in an elderly population. For Marks et al.,[44] most of the adverse events in both the AAV2-neurturin and sham surgery groups were associated with surgery. For LeWitt et al.,[15] the adverse events were mainly headache and nausea and were attributed to intracranial surgery in both the AAV2-GAD group and the sham surgery group. No infection, reaction to anesthesia, seizure, stroke, coma, or memory loss occurred in either study and other serious adverse events were not attributed to the gene therapy or

the surgical procedure. Yet, these serious adverse events remain a concern when performing intracranial surgery, especially in an older population.

Both articles are phase II clinical trials, which are the earlier stage of the drug development process. The absence of obvious safety concerns in these phase II trials is heartening but not a definitive proof of safety, and follow-up of larger numbers of patients over many years is required. Immune responses to gene therapy remain unknown. Continually expressing a therapeutic gene, protein or enzyme within the brain via a viral vector remains a novel idea with unpredictable short-term and long-term effects. Once gene therapy is injected it is impossible to control or reverse gene expression. The tumors occurring in the AAV2-neurturin group in Marks et al.[44] were not thought to be related to the treatment, yet the potential for a trophic factor to induce and/or accelerate tumor growth is possible, and patients should remain in follow-up for longer periods of time.

Double-blind, randomized, controlled studies provide a high level of evidence and according to GRADE [43], there were no major deficiencies in study design found. Major deficiencies include very large loss to follow-up, inadequacy of allocation concealment, and/or an unblinded study with subjective outcomes highly susceptible to bias.[41] Nonetheless, study limitations were found. Each study used relatively few patients, which can lead to sparse and imprecise data (wide confidence intervals).[41] Yet, it is expected that small, phase II trials are likely underpowered for some of the clinical outcomes.

Both studies received funding from drug companies responsible for producing the investigated gene therapy, which introduces the risk of bias. For LeWitt et al.[15] Neurologix took part in the study design, data interpretation and writing of the report, but did not contribute to data collection or data analysis.[15] For Marks et al.,[44] Ceregene funded the study and was responsible for supplying AAV2-neurturin, collecting data, and monitoring the clinical and statistical analysis. The authors were responsible for study design, data analysis, data interpretation, writing of the report and submittal for publication. Variability in results was seen between Marks et al.[44] and LeWitt et al.[15]. This may have been anticipated since the investigators were employing unique gene therapy treatments into separate regions of the brain.

Lastly, the authors made no mention of cost. CT, MRI and PET imaging combined with pre-, intra- and post-operation care, surgery, a hospital stay and the price of a newly marketed drug could prove to be financially prohibitive for patients, and insurance companies are slow to cover the cost of new therapies.

Recommendations for further study include multiple, phase I-IV clinical trials, with an increased number of participants, longer-term follow up for adverse events and funding by a government agency. See Table 2.

CONCLUSIONS

The continued research and development of gene therapy treatments for PD are warranted, and may offer an alternative to traditional, subpar

pharmacological or surgical treatment. Intraputaminal AAV2-neurturin is not superior to sham surgery when assessed using the UPDRS motor score at 12 months, but showed improvement at 18 months.[44] AAV2-GAD in the subthalamic nucleus showed significantly greater improvement from baseline in UPDRS motor scores compared with sham surgery over six months.[15]

Gene therapy remains a relatively unpredictable and novel treatment modality, and these trials are still in the early stages of research. For these reasons, more studies, involving hundreds of patients with long-term follow up are needed to confirm that gene therapy for PD is safe and effective. The modest benefit of AAV2-neurturin in the putamin, the published efficacy of AAV2-GAD in the subthalamic nucleus [15] and reported results of Prosavin® therapy [22] offer a glimpse of gene therapy's current potential and the future possibilities.

	Marks et al[44]		LeWitt et al[15]	
Study Type	Therapy Study Double blind, randomized, sham surgery controlled, multicentre trial		Therapy Study Double blind, randomized, sham surgery controlled, multicentre trial	
Population	Men and women, age 35–75, with idiopathic, levodopa-responsive PD according to the UK Brain Bank Criteria with disease duration at least 5 years		Men and women, age 30–75, with progressive, levodopa-responsive PD defined by the UK PD Society criteria with disease duration at least 5 years	
	UPDRS motor score change: baseline to 12 months		UPDRS motor score change: baseline to 6 months	
	AAV2-neurturin therapy -7.19 points*	Sham Surgery -6.95 points[3]	AAV2-GAD therapy -8.1 points[^]	Sham Surgery -4.7 points[ϕ]

Primary Endpoint Results	UPDRS motor score change: baseline to 18 months		UPDRS motor score change: baseline to 12 months	
	AAV2-neurturin therapy -11.96 points[δ]	Sham Surgery -4.34 points[Ω]	AAV2-GAD therapy To be published in separate paper	Sham Surgery To be published in separate paper
Conclusions	Intraputaminal AAV2-neurturin is not superior to sham surgery when assessed using the UPDRS motor score at 12 months. Modest, but significant motor improvement was seen at 18 months.		AAV2-GAD in the subthalamic nucleus showed significantly greater improvement from baseline in UPDRS motor scores compared with sham surgery over six months.	

Table 1. Summary of Findings

UPDRS=unified Parkinson's disease rating scale. Range 0-108, higher number indicates more severe impairment. Marks et al[44], data are in least squares mean (SE, 95% Confidence Intervals) * 1.56, -10.34 to -4.09. [ϡ] 2.12, -11.16 to -2.66. A difference of -0.31 (2.63, -5.58 to 4.97) p=0.91 [δ] 1.87, -15.8 to -8.7. [Ω] 2.48, -9.52 to 0.75. A difference of -7.61 (3.16, -14.1 to -1.13) p=0.023
^SD1.7, 23.1%; p<0.0001. [ϕ] SD1.5, 12.7%; p=0.003

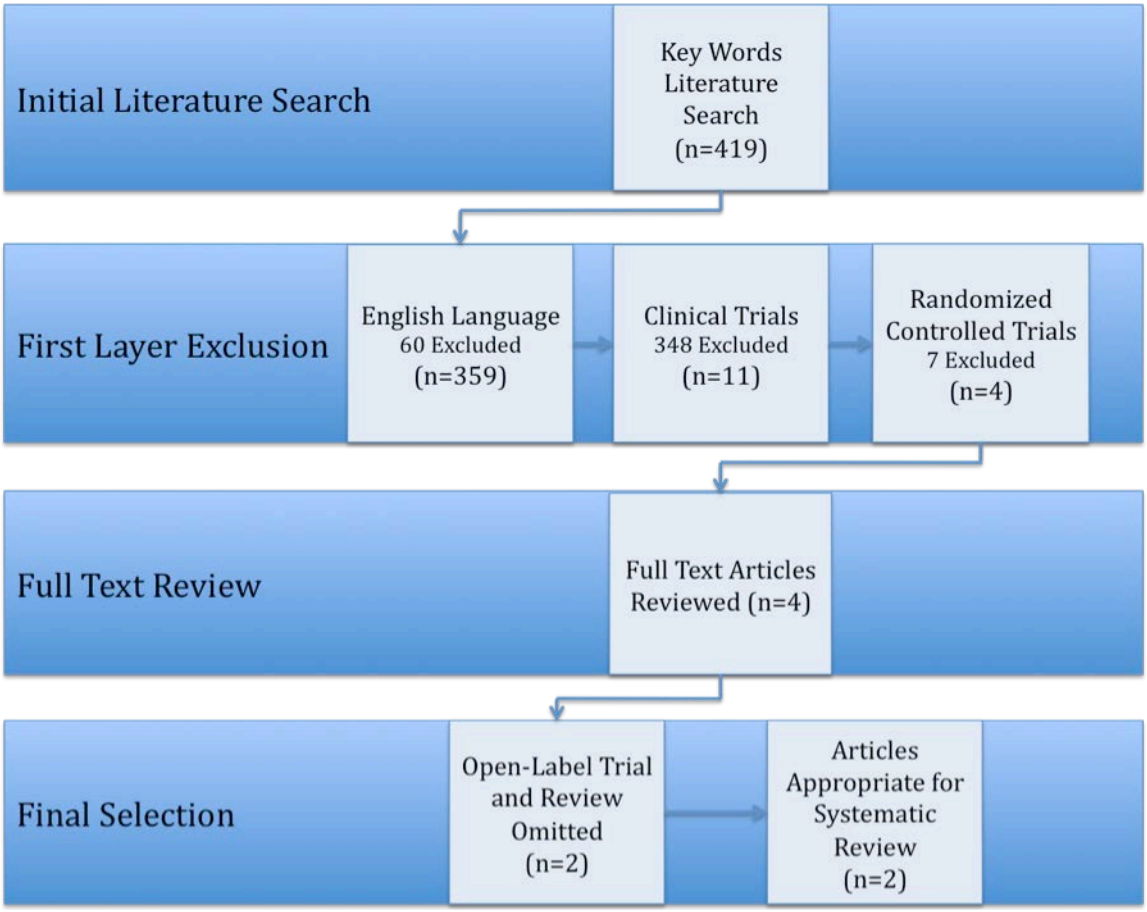
Table 2. Quality of Evidence

QUALITY ASSESSMENT								
Study	Study Design	Limitations	Publication Bias	Inconsistency	Indirectness	Imprecision	Sample Size	Quality
Marks et al[44]	Therapy Study – Double blind, randomized controlled trial	No study limitations	Ceregene funded study	No inconsistencies	No areas of indirectness	No serious imprecision [∇]	53	High

LeWitt et al[15]	Therapy Study – Double blind, randomized controlled trial	No study limitations	Neurologix funded study	No inconsistencies	No area of indirectness	No serious imprecision [δ]	45	High
Overall quality of evidence	CONCLUSION: Both studies offered high quality evidence. However, further research is likely to impact the confidence in these studies' estimates and may change the estimates.							Moderate

[∇] Small sample size, but met predetermined n=51 for 90% power to detect a difference of 10 (SD 10) points between groups
[δ] Small sample size, but met predetermined n=13 per group to achieve 80% power to detect effect size of 1.19

Figure 1. Search Methodology


Initial Literature Search
Key Words Literature Search (n=419)
First Layer Exclusion
English Language 60 Excluded (n=359)
Clinical Trials 348 Excluded (n=11)
Randomized Controlled Trials 7 Excluded (n=4)
Full Text Review
Full Text Articles Reviewed (n=4)
Final Selection
Open-Label Trial and Review Omitted (n=2)
Articles Appropriate for Systematic Review (n=2)

References

1. Feng LR, Maguire-Zeiss KA. Gene therapy in Parkinson's disease: rationale and current status. *CNS Drugs*. 2010;24:177-192.

2. National Parkinson Foundation. Parkinson's disease. Available at: http://www.parkinson.org/parkinson-s-disease.aspx. Accessed April 16, 2012.

3. Parkinson's Disease Foundation. Statistics on Parkinson's. Available at: http://www.pdf.org/en/parkinson_statistics. Accessed April 16, 2012.

4. Nussbaum RL, Ellis CE. Alzheimer's Disease and Parkinson's Disease. *N Engl J Med*. 2003;348:1356-1364.

5. Murphy SL, Xu J, Kochanek MA. Deaths: Preliminary data for 2010. *National Vital Statistics Reports.* U.S. Department of Health and Human Services. Centers for Disease Control and Prevention.; 2012;60, Number 4.

6. National Institute of Neurological Disorders and Stroke. National Institutes of Health. Parkinson's Disease. Available at: http://www.ninds.nih.gov/disorders/parkinsons_disease/parkinsons_disease.htm. Accessed April 16, 2012.

7. Ishihara LS, Cheesbrough A, Brayne C, Schrag A. Estimated life expectancy of Parkinson's patients compared with the UK population. *J Neurol Neurosurg Psychiatry*. 2007;78:1304-1309.

8. Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. *Neurology*. 1967;17:427-442.

9. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry*. 2008;79:368-376.

10. Lewitt PA. Levodopa for the treatment of Parkinson's disease. *N Engl J Med*. 2008;359:2468-2476.

11. Bjorklund T, Kordower JH. Gene therapy for Parkinson's disease. *Mov Disord*. 2010;25:S161-73.

12. Bjorklund T, Kirik D. Scientific rationale for the development of gene therapy strategies for Parkinson's disease. *Biochim Biophys Acta*. 2009;1792:703-713.

13. Lang AE, Gill S, Patel NK, et al. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol*. 2006;59:459-466.

14. Deuschl G. Schade-Brittinger C. Krack P. Volkmann J. Schafer H. Botzel K. Daniels C. Deutschlander A. Dillmann U. Eisner W. Gruber D. Hamel W. Herzog J. Hilker R. Klebe S. Kloss M. Koy J. Krause M. Kupsch A. Lorenz D. Lorenzl S. Mehdorn HM. Moringlane JR. Oertel W. Pinsker MO. Reichmann H. Reuss A. Schneider GH. Schnitzler A. Steude U. Sturm V. Timmermann L. Tronnier V. Trottenberg T. Wojtecki L. Wolf E. Poewe W. Voges J. German Parkinson Study Group,Neurostimulation Section. A randomized trial of deep-brain stimulation for Parkinson's disease. *N Engl J Med*. 2006;355:896-908.

15. LeWitt PA, Rezai AR, Leehey MA, et al. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. *Lancet neurol*. 2011;10:309-319.

16. Alexander BL, Ali RR, Alton EW, et al. Progress and prospects: gene therapy clinical trials (part 1). *Gene Ther*. 2007;14:1439-1447.

17. Kordower JH, Herzog CD, Dass B, et al. Delivery of neurturin by AAV2 (CERE-120)-mediated gene transfer provides structural and functional neuroprotection and neurorestoration in MPTP-treated monkeys. *Ann Neurol*. 2006;60:706-715.

18. Kordower JH, Emborg ME, Bloch J, et al. Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science*. 2000;290:767-773.

19. Eslamboli A, Georgievska B, Ridley RM, et al. Continuous low-level glial cell line-derived neurotrophic factor delivery using recombinant adeno-associated viral vectors provides neuroprotection and induces behavioral recovery in a primate model of Parkinson's disease. *J Neurosci*. 2005;25:769-777.

20. Eberling JL, Jagust WJ, Christine CW, et al. Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology*. 2008;70:1980-1983.

21. National Institutes of Health. Clinical Trial Phases. Available at: http://www.nlm.nih.gov/services/ctphases.html. Accessed May 1, 2012.

22. Björklund A, Björklund T, Kirik D. Gene Therapy for Dopamine Replacement in Parkinson´s Disease. *Sci. Transl. Med*. 2009;1:2ps2.

23. Oxford Biomedica. Oxford Biomedica Announces Successful Completion of ProSavin Phase I/II Study in Parkinson's Disease-16/04/2012. Available at: http://www.oxfordbiomedica.co.uk/page.asp?pageid=59&newsid=308. Accessed April 17, 2012.

24. Bevan MD, Atherton JF, Baufreton J. Cellular principles underlying normal and pathological activity in the subthalamic nucleus. *Curr Opin Neurobiol*. 2006;16:621-628.

25. Fischer AC, Beck SE, Smith CI, et al. Successful transgene expression with serial doses of aerosolized rAAV2 vectors in rhesus macaques. *Mol Ther*. 2003;8:918-926.

26. Nicklin SA, Buening H, Dishart KL, et al. Efficient and selective AAV2-mediated gene transfer directed to human vascular endothelial cells. *Mol Ther*. 2001;4:174-181.

27. Rabinowitz JE, Xiao W, Samulski RJ. Insertional mutagenesis of AAV2 capsid and the production of recombinant virus. *Virology*. 1999;265:274-285.

28. Shi W, Bartlett JS. RGD inclusion in VP3 provides adeno-associated virus type 2 (AAV2)-based vectors with a heparan sulfate-independent cell entry mechanism. *Mol Ther*. 2003;7:515-525.

29. Wu P, Xiao W, Conlon T, et al. Mutational analysis of the adeno-associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism. *J Virol*. 2000;74:8635-8647.

30. Kaplitt MG. Parkinson disease: Another player in gene therapy for Parkinson disease. *Nat Rev Neurol*. 2010;6:7-8.

31. Feigin A, Kaplitt MG, Tang C, et al. Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. *Proc Natl Acad Sci U S A*. 2007;104:19559-19564.

32. During MJ, Kaplitt MG, Stern MB, Eidelberg D. Subthalamic GAD gene transfer in Parkinson disease patients who are candidates for deep brain stimulation. *Hum Gene Ther*. 2001;12:1589-1591.

33. Kotzbauer PT, Lampe PA, Heuckeroth RO, et al. Neurturin, a relative of glial-cell-line-derived neurotrophic factor. *Nature*. 1996;384:467-470.

34. Golden JP, DeMaro JA, Osborne PA, Milbrandt J, Johnson EM. Expression of Neurturin, GDNF, and GDNF Family-Receptor mRNA in the Developing and Mature Mouse. *Experimental Neurology*. 1999;158:504-528.

35. Gash DM, Zhang Z, Ovadia A, et al. Functional recovery in parkinsonian monkeys treated with GDNF. *Nature*. 1996;380:252-255.

36. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. 1993;260:1130-1132.

37. Hurelbrink CB, Barker RA. The potential of GDNF as a treatment for Parkinson's disease. *Exp Neurol*. 2004;185:1-6.

38. Horger BA, Nishimura MC, Armanini MP, et al. Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons. *J Neurosci*. 1998;18:4929-4937.

39. Gasmi M, Herzog CD, Brandon EP, et al. Striatal delivery of neurturin by CERE-120, an AAV2 vector for the treatment of dopaminergic neuron degeneration in Parkinson's disease. *Mol Ther*. 2007;15:62-68.

40. Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Bjorklund A. Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. *Eur J Neurosci*. 1999;11:1554-1566.

41. Freese A, Stern M, Kaplitt MG, et al. Prospects for gene therapy in Parkinson's disease. *Mov Disord*. 1996;11:469-488.

42. Marks WJ,Jr, Ostrem JL, Verhagen L, et al. Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet neurol*. 2008;7:400-408.

43. GRADE Working Group. Available at: http://www.gradeworkinggroup.org/index.htm. Accessed April 12, 2012.

44. Marks WJ,Jr, Bartus RT, Siffert J, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet neurol*. 2010;9:1164-1172.

45. Shulman LM, Gruber-Baldini AL, Anderson KE, Fishman PS, Reich SG, Weiner WJ. The clinically important difference on the unified Parkinson's disease rating scale. *Arch Neurol*. 2010;67:64-70.

46. Hughes AJ, Daniel SE, Blankson S, Lees AJ. A clinicopathologic study of 100 cases of Parkinson's disease. *Arch Neurol*. 1993;50:140-148.

47. Fahn S, Elton RL, eds. *Members of the UPDRS Development Committee, Unified Parkinson's Disease Rating Scale*. New York: MacMillan; 1987. In: Fahn S, Marsden DM, Goldstein M, Calne DB, eds., ed. Recent Developments in Parkinson's Disease; No. 153-163.

48. Tang CC, Poston KL, Eckert T, et al. Differential diagnosis of parkinsonism: a metabolic imaging study using pattern analysis. *Lancet neurol*. 2010;9:149-158.

49. Bartlett JS, Samulski RJ, McCown TJ. Selective and rapid uptake of adeno-associated virus type 2 in brain. *Hum Gene Ther*. 1998;9:1181-1186.

50. Zonenshayn M, Sterio D, Kelly PJ, Rezai AR, Beric A. Location of the active contact within the subthalamic nucleus (STN) in the treatment of idiopathic Parkinson's disease. *Surg Neurol*. 2004;62:216-225.

51. Olanow CW, Stern MB, Sethi K. Scientific and clinical basis for the treatment of Parkinson disease. *Neurology*. 2009;72:S1-136.

52. Weaver FM, Follett K, Stern M, et al. Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. *JAMA*. 2009;301:63-73.