Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne *GAD* gene for Parkinson's disease: an open label, phase I trial

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Summary

Background **Dopaminergic neuronal loss in Parkinson's disease leads to changes in the circuitry of the basal ganglia, such as decreased inhibitory GABAergic input to the subthalamic nucleus. We aimed to measure the safety, tolerability, and potential efficacy of transfer of glutamic acid decarboxylase (*GAD*) gene with adeno-associated virus (AAV) into the subthalamic nucleus of patients with Parkinson's disease.**

Methods **We did an open label, safety and tolerability trial of unilateral subthalamic viral vector (AAV-GAD) injection in 11 men and 1 woman with Parkinson's disease (mean age 58·2, SD=5·7 years). Four patients received low-dose, four medium-dose, and four high-dose AAV-GAD at New York Presbyterian Hospital. Inclusion criteria consisted of Hoehn and Yahr stage 3 or greater, motor fluctuations with substantial off time, and age 70 years or less. Patients were assessed clinically both off and on medication at baseline and after 1, 3, 6, and 12 months at North Shore Hospital. Efficacy measures included the Unified Parkinson's Disease Rating Scale (UPDRS), scales of activities of daily living (ADL), neuropsychological testing, and PET imaging with ^{18}F-fluorodeoxyglucose. The trial is registered with the ClinicalTrials.gov registry, number NCT00195143.**

Findings **All patients who enrolled had surgery, and there were no dropouts or patients lost to follow-up. There were no adverse events related to gene therapy. Significant improvements in motor UPDRS scores (p=0·0015), predominantly on the side of the body that was contralateral to surgery, were seen 3 months after gene therapy and persisted up to 12 months. PET scans revealed a substantial reduction in thalamic metabolism that was restricted to the treated hemisphere, and a correlation between clinical motor scores and brain metabolism in the supplementary motor area.**

Interpretation **AAV-GAD gene therapy of the subthalamic nucleus is safe and well tolerated by patients with advanced Parkinson's disease, suggesting that in-vivo gene therapy in the adult brain might be safe for various neurodegenerative diseases.**

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Introduction

Gene therapy has yielded encouraging preclinical results for various disorders; however, safety and technical concerns have restricted successful translation into clinical therapy. In 1999, the death of a patient with ornithine transcarbamylase deficiency in a gene therapy trial with adenovirus led to a temporary suspension of gene therapy trials,[1] but technological advances and regulatory changes have renewed interest in the approach. Nonetheless, challenges remain. A recent study in patients with haemophilia B showed no clear toxic effects caused by the adeno-associated virus (AAV) vector, but after an initial promising improvement seen in patients deficient in the factor IX protein, anti-AAV immunity developed, which might have caused nearly complete loss of the therapeutic gene from transduced liver cells.[2] The lack of similar findings in animals further emphasises the importance of testing in human beings; however, safety concerns call for careful design of appropriate dose-ranging clinical trials.

The brain is an attractive organ for gene therapy, because production of biologically active molecules within the brain might circumvent poor penetration of compounds that are delivered systemically due to a tight vascular blood–brain barrier. Local gene expression might also focus therapy in specific brain regions, thereby avoiding exposure of other areas to agents that might cause undesirable effects. Several attempts have been made to use gene therapy for malignant tumours, including those in the brain, but the main aim of these studies was to destroy target cancer cells.[3] A trial aimed at correcting the genetic defect in the rare and lethal paediatric neurogenetic Canavan disease was also undertaken.[4] Furthermore, a phase I study of intracerebral transplantation of genetically-modified cells in patients with Alzheimer's disease ("ex-vivo" gene therapy) was reported.[5] However, the use of modified viruses (vectors) to introduce genetic material into endogenous neurons directly (so-called "in-vivo" gene therapy) has not been previously attempted for any adult neurodegenerative disorder.

Parkinson's disease is associated with degeneration of many brainstem, limbic, and midbrain neurons, but its hallmark is the loss of dopaminergic neurons of the substantia nigra, which leads to alterations in the activity of brain networks that control movement.[6,7] The consequence is dysregulation of interacting inhibitory and

	GAD dose	Age (years)	Disease duration (years)	Time on levodopa (years)	Baseline levodopa dose*	Baseline levodopa equivalents†
Patient 1	Low	55	8	2	0‡	250
Patient 2	Low	51	9	8	2000	2246
Patient 3	Low	62	13	11	1100	2018
Patient 4	Low	53	8	8	600	656
Patient 5	Medium	62	13	12	1250	1906
Patient 6	Medium	63	6	5	600	873
Patient 7	Medium	62	6	3	1000	1267
Patient 8	Medium	58	11	9	2000	2072
Patient 9	High	67	11	9	650	1173
Patient 10	High	51	13	13	600	461
Patient 11	High	50	7	7	1600	2300
Patient 12	High	63	6	4	1600	1600
Mean	..	58·2	9	8	1083	1402

Low dose=1×10^{11} vg/mL. Medium dose=3×10^{11} vg/mL. High dose=1×10^{12} vg/mL. *Mg daily. †100 mg levodopa is equivalent to 10 mg bromocriptine, 1 mg pergolide, 1 mg pramipexole, or 3 mg ropinirole. ‡Patient 1 was unable to tolerate levodopa before study entry, and had been off of it for several years.

Table 1: **Baseline demographic data**

excitatory pathways, leading to a movement disorder that is characterised by difficulty initiating movements, muscular rigidity, and tremor.[8,9] Pharmacological facilitation of dopaminergic neurotransmission benefits most patients initially, but those with advanced Parkinson's disease often develop unacceptable drug-related complications such as dyskinesia and motor fluctuations. Once these complications have begun, interventions that directly increase dopaminergic neurotransmission might simply worsen dyskinesia and other dopamine-related complications such as hallucinations. Hence, we explored non-dopaminergic strategies that might provide substantial benefit without these side-effects. On the basis of the hypothesis that re-establishment of normal brain activity within motor circuits might reverse motor deficits of Parkinson's disease, we developed a gene therapy approach to deliver the glutamic acid decarboxylase (*GAD*) gene directly into neurons of the human subthalamic nucleus with an AAV vector. GAD catalyses synthesis of GABA, the major inhibitory neurotransmitter in the brain; in patients with Parkinson's disease, activity of the subthalamic nucleus is increased mainly because of reduced GABAergic input from the globus pallidus.[6,7,10] Studies in human beings have shown that reduction of activity of the subthalamic nucleus by electrical stimulation, lesioning, or GABA infusion could ameliorate signs of advanced Parkinson's disease,[11] whereas studies in animals indicate that AAV-GAD seems to improve brain function and signs of the disease without toxic effects.[12–15]

Our aim was to assess the safety and tolerability of AAV-GAD gene therapy for patients with Parkinson's disease over a period of one year, using a single-arm, open label, dose-escalation design. Here, we report the clinical results of the completed 1 year follow-up in all study patients.

Methods

Patients

12 patients (11 men and 1 woman; age 58·2±5·7 years) with idiopathic Parkinson's disease were enrolled in the study. Entry criteria included: age between 25 and 70 years, disease duration of at least 5 years, Hoehn and Yahr stage 3 or more,[16] score of 30 or more on the motor section (part III) of the Unified Parkinson's Disease Rating Scale (UPDRS) in the off medication state,[17] motor complications of therapy with levodopa, and a stable antiparkinsonian medication regimen for at least 3 months before the baseline visit. Exclusion criteria included substantial cognitive dysfunction on neuropsychological testing, medical contraindication to surgery, secondary or atypical parkinsonism, and substantial psychiatric illness. Table 1 shows baseline demographic data.

Baseline neuropsychological assessment revealed an estimated intelligence quotient of 111·2 (SD=10·2), no evidence of dementia (dementia rating scale=137·5, SD=3·1), and minor signs of depression (Beck depression inventory=8·0, SD=4·0). The study was reviewed by the advisory committee of the US National Institutes of Health on recombinant DNA, and was approved by the US Food and Drug Administration (FDA), and the institutional review boards and institutional biosafety committees at Weill Cornell Medical College and the North Shore-Long Island Jewish Health System. The trial was monitored by a medically qualified monitor, an external monitoring board for data and safety, and the monitoring board for data and safety of Weill Cornell Medical College. Informed consent for the study was separately obtained at both participating institutions. The trial is registered with the ClinicalTrials.gov registry with the number NCT00195143.

Procedures

Patients underwent clinical screening within 1 month before surgery, at baseline (within 1 week of surgery), and 1, 3, 6, and 12 months after surgery. Participants were contacted by telephone between the time of surgery and the 1 month visit to inquire about adverse events. Additionally, adverse events were assessed at every visit. Part III (motor section) of the UPDRS was done at baseline, and at 1, 3, 6, and 12 months in the practically defined off state 12 h after withdrawal of all antiparkinsonian medications, and in the on state 1 h after administration of the usual morning dose of medication. We also rated patients for dyskinesia according to part IV of the UPDRS (complications of therapy).[17] Activities of daily living were rated at each timepoint according to the Schwab and England scale.[18] Neuropsychological tests were completed before surgery and after 12 months, according to a model that we previously used to assess the effects of deep brain stimulation of the subthalamic nucleus.[19] Dopaminergic drug dosages were assessed at every visit and expressed as levodopa equivalents.[20] Efforts were made to restrict

changes in medication dose throughout the course of the study, but changes were allowed if medically required.

PET studies with ^{18}F-fluorodeoxyglucose were done before gene therapy and repeated 12 months after surgery. The details of these studies have been provided elsewhere.[19] All antiparkinsonian drugs were withheld for at least 12 h before the imaging sessions. Images from patients who had AAV-GAD infusion in the subthalamic nucleus on the right side were reversed so that all operated hemispheres appeared on the left. Changes in regional metabolism associated with AAV-GAD infusion in the subthalamic nucleus were detected on a voxel basis by comparing the scans at 12 months to those at baseline with statistical parametric mapping (SMP99, Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK). We also identified brain regions in which changes in metabolic activity after gene therapy correlated with clinical outcome. The results were regarded as significant if $p<0·001$, and were uncorrected for multiple regional comparisons.

Viral vector

To create AAV-GAD vectors, AAV-GAD plasmids were generated that contained DNA encoding the open reading frame of human *GAD65* or *GAD67* under regulation of the cytomegalovirus enhancer–chicken β-actin promoter and woodchuck post-transcriptional regulatory element. Recombinant AAV vectors were packaged in human embryonic kidney (HEK) 293 cells and purified by heparin affinity chromatography, according to standard procedures and as previously described.[13,15] The final formulation buffer was 1× phosphate-buffered saline solution. The genomic vector titres were measured by absolute quantification with the ABI7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The viruses encoding GAD65 or GAD67 were mixed in a 1-to-1 ratio and diluted to 1×10^{11} viral genomes (vg)/mL (low dose), 3×10^{11} vg/mL (medium dose), and 1×10^{12} vg/mL (high dose) with 1× phosphate-buffered saline solution. The bulk harvest and final formulated products were rigorously examined with lot-release testing, as per FDA guidelines. Biosafety testing for mycoplasma, endotoxin, sterility, and adventitious viruses, and a general safety test were undertaken (AppTec Laboratory Services, Philadelphia, USA).

The subthalamic nucleus was localised with the Leksell stereotactic frame and MRI image guidance. Standard intraoperative microelectrode recording was done with patients awake to verify the precise location of the subthalamic nucleus.[11] The tip of the microelectrode was then withdrawn to what was believed to be the centre of the subthalamic nucleus. 20 µL of 20% mannitol followed by 45 µL of vector solution at the appropriate dose concentration were drawn into a 100 µL Hamilton syringe. A 165-µm diameter vitreous silica infusion cannula was attached to the syringe and the system was flushed until fluid was seen from the cannula tip. The syringe was inserted into a Harvard PicoPlus pump (Harvard, Holliston, MA, USA), which was briefly run at 2 µL/min to assess flow. The tungsten microwire was withdrawn from the centre of the bipolar microelectrode and the infusion cannula was inserted, placing the tip at the same point in the centre of the subthalamic nucleus. Infusions were done for 100 min at 0·5 µL/min. After completion, the catheter was left in place for 5 min to reduce reflux. The catheter and outer tube were then withdrawn to place the catheter tip at the dorsal edge of the subthalamic nucleus, which was left in place for a further 5 min. The guide tube and infusion catheter were then removed together to establish the integrity of the system, and then the pump was run again to verify patency and flow after completion.

Patients were divided into three equal groups (low, medium, or high dose), and all received the same final injection volume of 50 µL. To retain one intact hemisphere should an unexpected serious adverse event happen, AAV-GAD vectors were infused unilaterally into the subthalamic nucleus of the more symptomatic hemisphere, as requested by government reviewers. This procedure also enabled the untreated side to serve as a control for comparison with the AAV-GAD-injected hemispheres. The small microinfusion volume and rate combined with a single penetration of 200 µm or less in the subthalamic nucleus of every patient reduced the risk that damage to the subthalamic nucleus might result in confounding persistent clinical or physiological improvement.

To measure titres of anti-AAV antibodies in peripheral blood, an ELISA assay was developed with standard methods. ELISA plates (Costar 96 wells, Corning, Acton, MA, USA) were coated with 1×10^{9} AAV serotype 2 (AVV2) particles per well in 50 µL coating buffer (15 mmol/L Na_2CO_3, 10·5 mmol/L $NaHCO_3$, pH 9·6). After application of serum or blank controls, affinity-purified goat anti-human IgA, IgG, and IgM conjugated to horseradish peroxidise (1 in 2000; Sigma-Aldrich, St Louis, MO, USA) or blanks were applied to appropriate wells. After washing, plates were incubated with 3,3´,5,5´-tetramethylbenzidine substrate (Pierce, Rockford, IL, USA). The optical densities at 450 nm were read on a plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Titres for neutralising antibodies were also measured. HEK293 cells were plated at a concentration of 4000 cells per well in a 96-well plate, 16–24 h before addition of AAV and serum samples. Duplicate serial 5-fold dilutions from one in 20 to one in 62 500 were prepared for every patient serum sample, anti-AAV (intact particle) positive control antibody (Progen, Heidelberg, Germany), or blank negative controls in a final volume of 30 µL culture media containing $2·25\times10^{6}$ vg of AAV vector expressing the luciferase reporter gene. After 1 h incubation at 37°C, 25 µL of every sample was added to the appropriate well of the plate. After 48 h, luciferase transgene expression

was measured with Bright-Glo (Promega, Madison, WI, USA) and a Turner BioSystems luminometer (Sunnyvale, CA, USA).

Anti-GAD65 antibodies from patient serum samples were quantified with an ELISA kit (W-12; Kronus, Boise ID, USA), according to the manufacturer's instructions. Healthy blood-donor serum samples have less than 5 U/mL of anti-GAD65 antibodies, with positive controls in the range of 42–62 U/mL. A comparable assay for anti-GAD67 antibodies is not commercially available, therefore an immunoblotting-based method was developed. HEK293 cell lysates, which were transfected with either GAD67 or plasmids expressing enhanced green fluorescent protein, were run on a sodium dodecyl sulfate polyacrylamide gel, which was blotted and probed with dilutions of an anti-GAD antibody (AB1511, Chemicon, Temecula, CA, USA) with the patient serum samples. The commercial anti-GAD antibody detected the 67 kDa GAD protein at a sensitivity of one in 32 000. Analysis of serum samples for immune reaction was done by Neurologix.

Statistical analysis

Adverse events were tabulated and rated for severity (mild, moderate, and severe) and for relation to the study intervention (unlikely, possibly, probably, and likely).

	Patients	Events	Severity	Relation to study intervention
Bronchitis	1	1	Mild	Unrelated
Incisional pain	1	1	Mild	N/A
Fatigue	1	1	Mild	Unrelated
Bursitis	1	1	Mild	Unrelated
Worsening PD	1	1	Severe (SAE)	Unrelated
Rash	1	1	Mild	Unrelated
Painful dyskinesias	1	1	Moderate	Unrelated
Falls	4	5	Mild	Unrelated
Rotator cuff injury	1	1	Mild	Unrelated
*Pneumonia	2	1 1	Moderate/ Severe (SAE)	Unrelated Unrelated
Insomnia	1	1	Mild	Unrelated
Oedema	2	2	Mild	Unrelated
Toothache	1	1	Mild	Unrelated
Headache	1	1	Mild	Unrelated
↑White blood cells	1	1	Mild	Unrelated
↑Glucose	1	1	Mild	Unrelated
Urinary frequency	2	3	Mild	Unrelated
Urinary tract infection	1	1	Mild	Unrelated
Depression	1	1	Mild	Unrelated
Thumb fracture	1	1	Mild	Unrelated

Data are numbers. PD=Parkinson's disease. N/A=not determined. SAE=severe adverse event. ↑=raised. *One patient had pneumonia, but did not need hospital admission because pneumonia resolved with oral antibiotics, and one patient had SAE.

Table 2: **Adverse events**

Secondary effectiveness measures (UPDRS and activity of daily living) were analysed by one-way repeated measures analysis of variance (RMANOVA) with all five timepoints (baseline, 1, 3, 6, and 12 months), with posthoc Bonferroni's test to assess the statistical significance of changes at follow-up timepoints with respect to baseline. Changes in regional metabolic activity between baseline and 12 months were analysed by two-way RMANOVA, with hemisphere (treated and untreated) and time (baseline and 12 months) as within-subject repeated measures. Changes from baseline to 12 months in neuropsychological test performance and dopaminergic drug use were assessed by paired *t* tests.

Role of the funding source

The funding source had no role in study design, monitoring, or clinical data collection or analysis. This study was done according to an investigator-initiated FDA Investigational New Drug (IND) application held by MJD. MGK, AF, and MJD had full access to all data in the study. MJD had final responsibility for the decision to submit for publication.

Results

All patients who enrolled had surgery; during the study, no dropouts or loss of patient follow-up occurred, and there were no adverse events related to the gene therapy. No MRI evidence of haemorrhage or oedema was seen at the infusion site after surgery, and no abnormalities were noted on any of the postsurgical MRIs up to 1 year, with the exception of expected routine signal changes, which were seen on T2 images along the penetration tract. All patients were discharged from the hospital 2 days after surgery. No fever or medically relevant alteration in routine blood and physiological indices were seen during this time. Various adverse events took place that were rated as mild and unrelated to the study intervention (table 2).

Three serious adverse events (defined as events that need hospitalisation) took place, but they were unrelated to the study intervention. One patient who received low-dose AAV-GAD was treated in hospital for 1 night, 5 months after surgery, for a severe freezing episode in the off state after discontinuing entecapone. 200 mg entecapone was resumed, and the patient was discharged the day after, with no subsequent adverse events. One patient who received medium-dose AAV-GAD was treated in hospital for exacerbation of a pre-existing chronic obstructive pulmonary disease, 6 months after receiving gene therapy. The patient recovered to baseline and had no further such episodes. Finally, one patient who received high-dose AAV-GAD needed an arthroscopic knee procedure 9 months after surgery, with no subsequent complications. No deaths and no new neurological deficits were reported in any patient during the planned 1 year course of the study. At present, three patients have had subthalamic nucleus AAV-GAD surgery 3 or more years previously, four 2–3 years previously, and

the remaining five at least 1·5 years previously. No deaths or reports of new unexpected neurological complications were recorded in any patients during these extended periods, although patients' examinations were not routinely done beyond 1 year.

Clinical outcome

Figures 1 and 2 show that unilateral AAV-GAD treatment of the subthalamic nucleus leads to a substantial improvement in UPDRS motor ratings in both the off and on states ($F[4,44]=5·23$, $p=0·0015$ for the off state; $F[4,44]=3·78$, $p=0·01$ for the on state). Posthoc testing showed no significant change in the clinical ratings at 1 month after surgery compared with those at baseline. However, both the off and on state ratings improved at 3 months (19%, $p=0·0244$; and 25%, $p=0·0182$), 6 months (28%, $p=0·0006$; and 26%, $p=0·0126$), and 12 months (24%, $p=0·0038$; and 27%, $p=0·0098$).

Ten of the 12 patients showed improvement in UPDRS off scores at 12 months. Four patients improved between 0% and 20%; two improved between 20% and 40%; and four improved more than 40% in whole body off period motor UPDRS with this unilateral intervention. Table 3 shows off and on state UPDRS scores for all participants at all timepoints.

Analysis of UPDRS ratings by body side showed a consistent benefit in limbs contralateral to the gene therapy (figures 1 and 2). A significant effect of time was noted for the body side opposite the treated hemisphere (off state: $p=0·0035$; on state: $p=0·0007$; RMANOVA). Off state UPDRS scores on the treated side improved by 33% ($p=0·0012$), and 29% ($p=0·0057$) at 6 and 12 months, respectively. There was no substantial effect of time on the untreated body side.

Improvement in the ADL scores (off and on states) were not significant during the course of the study. However, there was a trend towards improvement in the off state ratings at 12 months (18%, $p=0·06$; paired *t* test). There was no significant change with time in UPDRS dyskinesia ratings, although a trend towards improvement was noted at 12 months compared with that at baseline ($p=0·0558$, paired *t* test). Importantly, the amount of antiparkinsonian medication per day did not change significantly during the course of the study (figure 3). There were also no substantial changes in any of the neuropsychological tests.

An unbiased blinded voxel-based comparison of the PET scans at 12 months and baseline showed a substantial reduction in glucose metabolism of the thalamus in the operated hemisphere ($p<0·001$, uncorrected; SPM paired *t* test) (figure 4). This change was not present in the mirror-image region on the non-operated side. Changes in regional metabolism were different for the two hemispheres (interaction effect: $p=0·0096$, two-way RMANOVA). There was a significant decline in metabolic activity on the treated side ($p=0·007$, posthoc test with Bonferroni's correction). No change was evident on the untreated side. Improvements in motor UPDRS ratings at 12 months were highly correlated with localised metabolic increases in the supplementary motor area of the operated hemisphere ($p<0·001$, uncorrected; SPM correlation analysis) (figure 5). No correlation was detected between clinical outcome with gene therapy and metabolic changes in the mirror-image cortical region of the untreated hemisphere.

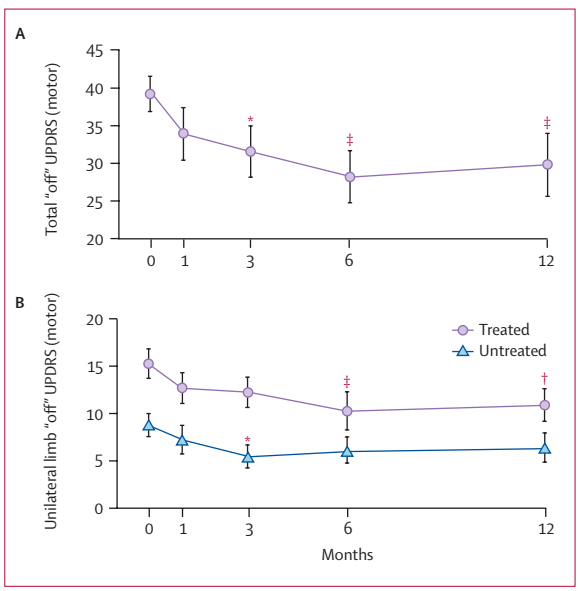

Figure 1: **Clinical improvement in motor ratings**
At each timepoint, the motor component of the UPDRS was measured 12 h after discontinuation of oral medications (off state). (A) Time-dependent improvement in motor ratings. (B) Changes in motor ratings for both body sides. *p<0·05; †p<0·01; ‡p<0·005.

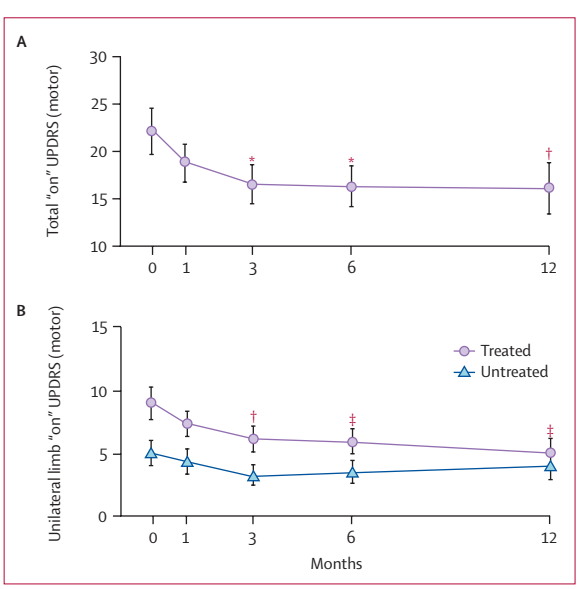

Figure 2: **Clinical improvement in motor ratings**
At each timepoint, the motor component of the UPDRS was measured 1 h after administration of the usual morning dose of medication (on state). (A) Time-dependent improvement in motor ratings. (B) Changes in motor ratings for both body sides. *p<0·05; †p<0·01; ‡p<0·005.

	Dose	Baseline	1 month*	3 months*	6 months*	12 months*
Off states						
Patient 1	Low	35	26 (–26%)	18 (–49%)	25 (–29%)	15 (–57%)
Patient 2	Low	48	52 (8%)	45 (–6%)	48 (0%)	52 (8%)
Patient 3	Low	31	25 (–19%)	39 (26%)	22 (–29%)	27 (–13%)
Patient 4	Low	46	49 (7%)	44 (–4%)	52 (13%)	42 (–9%)
Patient 5	Medium	37	35 (–5%)	30 (–19%)	28 (–24%)	36 (–3%)
Patient 6	Medium	30	33 (10%)	30 (0%)	15 (–50%)	18 (–40%)
Patient 7	Medium	33	22 (–33%)	18 (–45%)	34 (3%)	22 (–33%)
Patient 8	Medium	38	19 (–50%)	17 (–55%)	18 (–53%)	16 (–58%)
Patient 9	High	36	43 (19%)	43 (19%)	33 (–8%)	49 (36%)
Patient 10	High	56	51 (–9%)	48 (–14%)	30 (–46%)	46 (–18%)
Patient 11	High	45	29 (–36%)	22 (–51%)	18 (–60%)	16 (–64%)
Patient 12	High	35	23 (–34%)	25 (–29%)	16 (–54%)	19 (–46%)
Mean	..	39·2	33·9	31·6	28·3	29·8
Change in group means relative to baseline			–13·4%	–19·4%	–27·9%	–23·8%
On states						
Patient 1	Low	19	17 (–11%)	13 (–32%)	17 (–11%)	12 (–37%)
Patient 2	Low	29	25 (–14%)	22 (–24%)	29 (0%)	22 (–24%)
Patient 3	Low	9	14 (56%)	16 (78%)	15 (67%)	16 (78%)
Patient 4	Low	27	22 (–19%)	16 (–41%)	20 (–26%)	20 (–26%)
Patient 5	Medium	27	24 (–11%)	22 (–19%)	24 (–11%)	28 (4%)
Patient 6	Medium	19	18 (–5%)	16 (–16%)	7 (–63%)	9 (–53%)
Patient 7	Medium	24	17 (–29%)	12 (–50%)	20 (–17%)	10 (–58%)
Patient 8	Medium	12	14 (17%)	13 (8%)	10 (–17%)	8 (–33)
Patient 9	High	15	16 (7%)	20 (33%)	16 (7%)	26 (73%)
Patient 10	High	35	35 (0%)	33 (–6%)	24 (–31%)	32 (–9%)
Patient 11	High	33	15 (–55%)	9 (–73%)	8 (–76%)	5 (–85%)
Patient 12	High	16	9 (–44%)	6 (–63%)	5 (–69%)	5 (–69%)
Mean	..	22·1	18·8	16·5	16·3	16·1
Change in group means relative to baseline			–14·7%	–25·3%	–26·4%	–27·2%

*Data are numbers (percentage change from baseline).

Table 3: **Off and on state UPDRS scores**

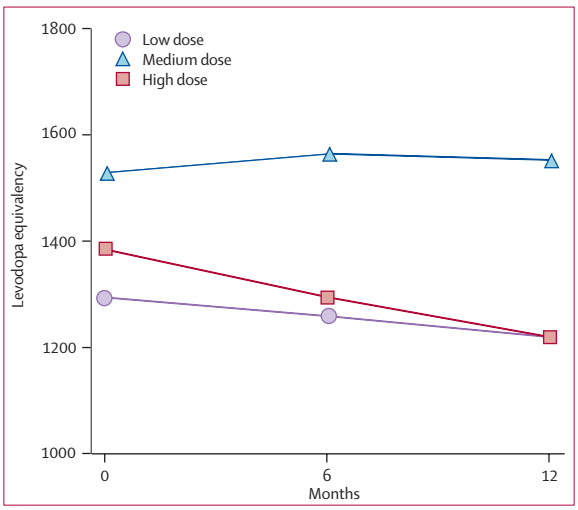

Figure 3: **Changes of daily dose of dopaminergic medication**

At baseline, two patients showed evidence of substantial anti-AAV2 immunity, with antibody titres of one in 6400 and one in 1600, respectively. All other patients had titres of one in 200 or lower, with five below the lowest dilution of one in 50. None of these titres changed in any patient at any of the postsurgical timepoints tested (1, 3, 6, and 12 months). There were no changes in IgA or IgM concentrations in any patient over time, except for a small IgM spike at 6 months in one patient, which was not accompanied by a subsequent IgG spike and which fell at 12 months. This range of baseline immunoglobulin titres is very similar to what has been previously seen in healthy populations,[21] and the absence of change with time suggests that the vector infusion into the brain did not induce immunity against AAV2.

The presence of high titres of antibodies, such as those seen in two patients, would not necessarily preclude AAV-mediated gene transfer unless their binding can prevent AAV from entering cells and delivering genetic material (ie, neutralising AAV transduction). Serum samples were therefore also tested for the presence of neutralising antibodies. With this assay, the same two patients with high baseline IgG titres had titres of neutralising antibodies of one in 12 500 and one in 2560, respectively, in their presurgical serum samples, and the titres remained constant at all postsurgery timepoints. All serum samples from the remaining ten patients were negative for neutralising antibodies at the lowest dilution of one in 20 at baseline and throughout the study. Although the number of patients was very small, a correlation between pre-existing immunity and clinical outcome did not seem to exist. The patient with a titre of neutralising antibodies of one in 12 500 had an 8·7% improvement in off period motor UPDRS at 12 months (patient 4, table 3), whereas the patient with a titre of neutralising antibodies of one in 2560 had a 46% improvement at 6 months and 18% at 12 months, which is greater than that in four patients who had very low titres of neutralising antibodies (patient 10, table 3). Finally, there was no evidence of pre-existing anti-GAD65 or anti-GAD67 autoantibodies, and no induction of such antibodies at any timepoint during the 1 year of study in any patient.

Discussion

Our results show that AAV-mediated gene transfer can be done safely in the human brain, with no evidence of substantial toxic effects or adverse events in the perioperative period and for at least 1 year after treatment. Most patients have been followed up for more than 2 years after surgery, with some for more than 3 years. No deaths and no evidence of substantial adverse events were reported.

The promise of gene therapy has yet to be fulfilled; however, the ability to alter cellular function genetically remains a powerful potential opportunity for treatment of

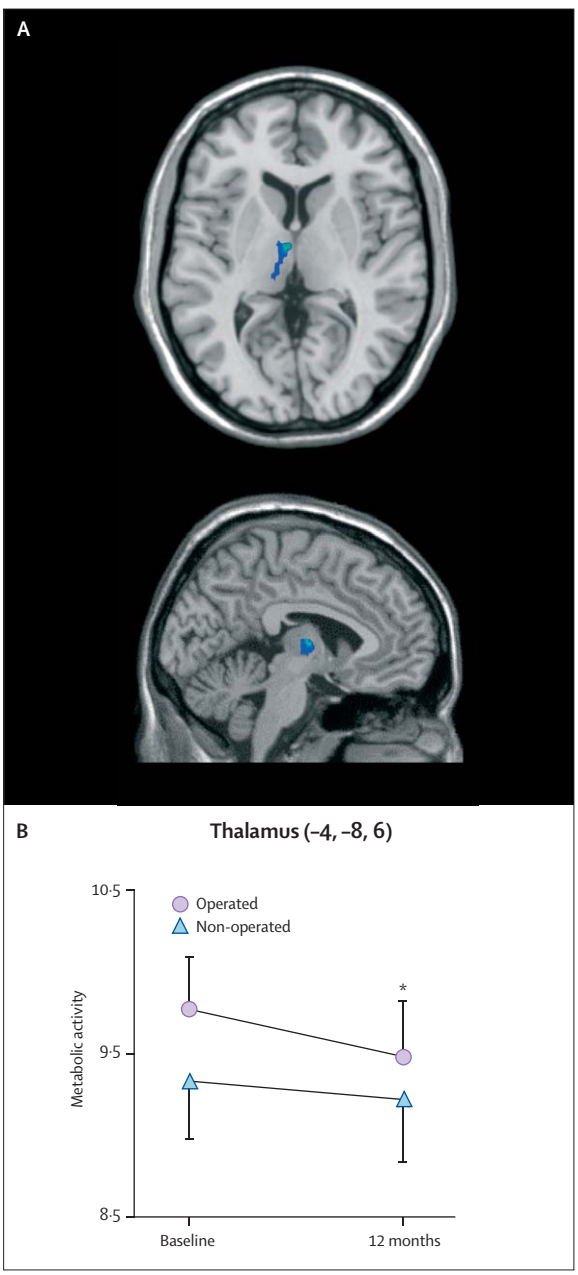

Figure 4: **Reduction of thalamic metabolic activity after gene therapy of the subthalamic nucleus**
(A) Representative axial (top) and sagittal (bottom) slices at 12 months after gene therapy. (B) Metabolic activity in this region was plotted for the operated and non-operated hemispheres at baseline and 12 months. *p<0·02.

various devastating diseases. Safety and efficacy issues continue to raise concerns, especially when gene therapy is applied to diseases such as neurological disorders, in which there is limited prior experience. Indeed, our original protocol was modified by federal reviewers to restrict treatment to only one hemisphere of the brain because of the concern that unexpected toxic effects might produce a more devastating outcome if they happened bilaterally.

After approval and initiation of our study, several additional AAV-mediated clinical gene therapy studies have been undertaken, including two approaches to gene therapy for Parkinson's disease[22] (ClinicalTrials.gov, NCT00252850), one for Alzheimer's disease (ClinicalTrial.gov, NCT00087789); and a paediatric study of Batten disease (ClinicalTrials.gov, NCT00151216). These studies emphasise the interest in further development of gene therapy for neurological diseases.

Direct introduction of genetic material into neurons is increasingly interesting for various neurological diseases. The only previous in-vivo gene therapy study for a non-neoplastic neurological disorder was done in young children affected by lethal neurogenetic Canavan disease. Immunological data indicated detectable neutralising antibodies in one of ten children before receiving a total of 1×10^{12} vector genomes; however, three of ten children showed detectable neutralising antibodies after treatment.[4] Although we reported a slightly higher rate of pre-existing neutralising antibodies (two of 12), there was no change at any timepoint after surgery. Half of the

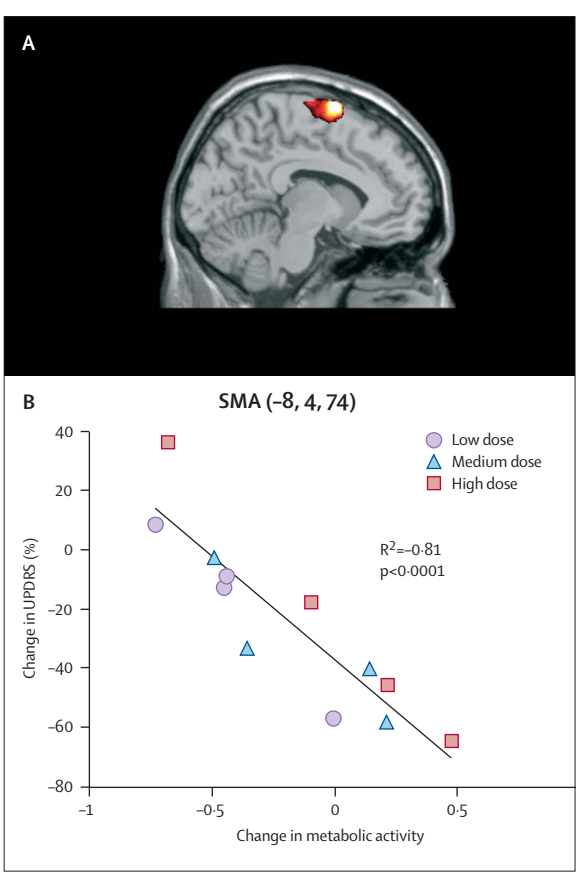

Figure 5: **Changes in cortical metabolism after gene therapy of the subthalamic nucleus**
(A) Representative sagittal slice showing a significant correlation between clinical outcome at 12 months and postoperative metabolic changes in the supplementary motor area of the operated hemisphere. (B) Regression line showing this highly significant clinical–metabolic correlation on the operated hemisphere. No correlation was evident in the untreated side.

patients with Canavan disease also had postoperative fever, and four of ten patients developed small subarachnoid haematoma, subdural haematoma, or both, after surgery, whereas these clinical signs were not reported in our study. It is likely that, in our study, the use of a lower amount of virus delivered via a single brain injection to a deep target accounts for these differences, because in the study of patients with Canavan disease many injections in the brain involving more viral vectors might have increased the risk of immune reactions and haemorrhagic events. Furthermore, the adult brain might react differently to in-vivo gene therapy compared with the brains of children. The absence of antibody responses to either the AAV vector or the *GAD* genes after infusion, even in preimmune individuals, also suggests that immune-mediated reduction of gene expression, which has been reported in a previous haemophilia gene therapy study, might be a less serious issue for gene therapy in the brains of most patients.

This open label, non-randomised phase I study was not designed to assess the effectiveness of the intervention. Nonetheless, the clinical outcomes were encouraging. Substantial improvements in both the off and on states were evident, beginning at 3 months after surgery and continuing until the end of the trial. This improvement was localised predominantly to the side of the body contralateral to the treatment. The absence of change at the earliest timepoint after surgery suggests that the improvement that was seen was probably not due to surgical lesions of the target region, which typically give rise to an immediate perioperative benefit. This result is also consistent with previous studies of AAV-mediated gene therapy in which gene expression gradually increases to a maximum in a period of weeks.[12,15,23] In this study,[2] a posthoc analysis showed a significant improvement in UPDRS scores from the visit at 1 month to those at 6 months and 12 months, further supporting a persistent effect of gene therapy rather than of a static lesion.

Despite the non-blinded nature of the clinical assessments that were done in this study, these findings were in accord with PET imaging blinded to treatment side. We reported that a substantial metabolic decline in the ipsilateral thalamus happened 12 months after unilateral gene therapy of the subthalamic nucleus. Thalamic neurons in this brain region receive inhibitory projections from the internal globus pallidus and the substantia nigra pars reticularis, which are the primary targets of output of the subthalamic nucleus. Glucose use in the thalamus is consistently correlated with pallidal neuronal activity in patients with Parkinson's disease,[24] and has been shown to fall after therapeutic lesions of the globus pallidus and the subthalamic nucleus.[25,26] Therefore, the drop in thalamic metabolism after AAV-GAD injection into the subthalamic nucleus is consistent with the changes that have been reported after effective surgical interventions for this disorder. We also showed that improvements in UPDRS motor ratings at 12 months were highly correlated with increases in the metabolism of premotor regions, as also reported after surgery of patients with Parkinson's disease.[25,27] Overall, the findings of the imaging studies suggest that the clinical improvement after gene therapy of the subthalamic nucleus is associated with objective changes in the activity of thalamocortical motor pathways, as described with other treatment strategies for this disorder.[19]

If effectiveness is confirmed in larger, more definitive studies, several potential advantages for the gene therapy approach compared with traditional deep brain stimulation exist. The absence of indwelling hardware reduces the risk of infection, and some patients with Parkinson's disease simply prefer not to have an implanted device.[28,29] Additionally, frequent visits for deep brain stimulation adjustments are not needed. Finally, gene therapy might be a more physiological method to correct basal ganglia motor circuitry. Although deep brain stimulation of the subthalamic nucleus uses a fixed voltage to regulate the activity of this area locally, AAV-GAD gene therapy might make the motor network function return to normal through activity-dependent release of GABA both locally within the subthalamic nucleus and throughout the network via connections to other hyperactive areas, as shown in our preclinical study[15] (ie, with loss of dopaminergic tone and consequent disinhibition of the subthalamic nucleus, the transduced neurons increased firing and GABA release). The locally released GABA acts as an autoregulatory negative feedback mechanism on $GABA_A$ receptors of the subthalamic nucleus to hyperpolarise and reduce neuronal firing, leading to a reduction in the ectopically-derived GABA release and providing homeostatic physiological control. Indeed, the magnitude of the improvement in the on state UPDRS, an effect not usually seen to this degree with deep brain stimulation of the subthalamic nucleus, lends support to this possibility.[29–31]

We did not see a clear effect of viral-vector dose on clinical outcome in this small study. Several factors might have contributed to the absence of such an effect. First, the small sample size per dose group is not powered to detect differences in effectiveness by the clinical outcome measures reported here. Second, Parkinson's disease can be quite heterogeneous in presentation, and it is possible that this therapy might be most effective for some rather than all of the symptoms. Because this study was not randomised, the different dose groups were not tightly matched for disease severity and symptom expression. Finally, more uniform and pronounced effects might be achieved if bilateral surgery had been done. Although the apparent laterality and time course of benefits lend support to a specific biological effect and this hypothesis is reinforced by the blinded regional hemispheric 18F-fluorodeoxyglucose PET metabolic changes in these patients, concerns regarding possible placebo effects cannot yet be completely excluded from this study.

Contributors

MGK, AF, DE, and MJD designed the study. MJD sponsored the FDA IND. Preparation and characterisation of viral vectors were done by PAL, RJB, HLF, and DY. Surgical procedures were done by MGK, and infusions were done by MGK and MJD. Pre-surgical clearance was done by MGK and KS. All UPDRS ratings, other clinical measurements, and PET studies, before surgery and at all subsequent timepoints, were undertaken by AF and DE. AF and DE also had final control over patient recruitment and entry into the study. HLF measured immunoglobulins in the serum of patients. Statistical analysis was done by CT. Data interpretation and writing of the article were primarily done by MGK, AF, DE, and MJD, with contributions from all authors. MGK and AF, and DE and MJD, contributed equally to this study.

Conflict of interest statement

MGK and MJD are founders of and consultants to Neurologix, which funded this study. They and their families have substantial ownership interest in the company. HLF and RJB are employees of Neurologix. The remaining authors, including those responsible for the assessment of study eligibility, and for the clinical measurements and statistical analyses, have no involvement in Neurologix and declare no conflict of interest.

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