AAV2-*GAD* gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial

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Summary

Background Gene transfer of glutamic acid decarboxylase (*GAD*) and other methods that modulate production of GABA in the subthalamic nucleus improve basal ganglia function in parkinsonism in animal models. We aimed to assess the effect of bilateral delivery of AAV2-*GAD* in the subthalamic nucleus compared with sham surgery in patients with advanced Parkinson's disease.

Methods Patients aged 30–75 years who had progressive levodopa-responsive Parkinson's disease and an overnight off-medication unified Parkinson's disease rating scale (UPDRS) motor score of 25 or more were enrolled into this double-blind, phase 2, randomised controlled trial, which took place at seven centres in the USA between Nov 17, 2008, and May 11, 2010. Infusion failure or catheter tip location beyond a predefined target zone led to exclusion of patients before unmasking for the efficacy analysis. The primary outcome measure was the 6-month change from baseline in double-blind assessment of off-medication UPDRS motor scores. This trial is registered with ClinicalTrials.gov, NCT00643890.

Findings Of 66 patients assessed for eligibility, 23 were randomly assigned to sham surgery and 22 to AAV2-*GAD* infusions; of those, 21 and 16, respectively, were analysed. At the 6-month endpoint, UPDRS score for the AAV2-*GAD* group decreased by 8·1 points (SD 1·7, 23·1%; p<0·0001) and by 4·7 points in the sham group (1·5, 12·7%; p=0·003). The AAV2-*GAD* group showed a significantly greater improvement from baseline in UPDRS scores compared with the sham group over the 6-month course of the study (RMANOVA, p=0·04). One serious adverse event occurred within 6 months of surgery; this case of bowel obstruction occurred in the AAV2-*GAD* group, was not attributed to treatment or the surgical procedure, and fully resolved. Other adverse events were mild or moderate, likely related to surgery and resolved; the most common were headache (seven patients in the AAV2-*GAD* group *vs* two in the sham group) and nausea (six *vs* two).

Interpretation The efficacy and safety of bilateral infusion of AAV2-*GAD* in the subthalamic nucleus supports its further development for Parkinson's disease and shows the promise for gene therapy for neurological disorders.

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Introduction

Neurodegeneration of dopaminergic neurons underlies the motor manifestations of Parkinson's disease. When mild, Parkinson's disease is generally well controlled by drugs; however, as the disease progresses, pharmacotherapy often fails to provide adequate symptom relief and sometimes causes disabling complications, such as motor fluctuations.[1,2] Additional treatment approaches, such as deep brain stimulation (DBS) and pharmacological interventions at sites beyond the nigrostriatal dopaminergic pathway have been used to manage problems of advanced Parkinson's disease.[3] In vivo gene therapy is a new approach. Despite promising results in animal models of parkinsonism and in several open-label clinical investigations,[4–7] the efficacy of gene therapy has yet to be confirmed in a randomised double-blind clinical trial.[8]

In Parkinson's disease, loss of nigrostriatal dopaminergic neurons alters striato-pallidal circuitry such that decreased GABA input to the subthalamic nucleus renders this structure disinhibited.[9] Treatments that diminish or modulate the activity of the subthalamic nucleus, such as subthalamotomy and DBS, can help with some parkinsonian symptoms.[10,11] Like dopaminergic treatment, however, DBS can fail to improve some parkinsonian features such as freezing of gait, imbalance, dysphagia, cognitive and psychiatric problems, and speech difficulties.[12–14] Furthermore, this technique necessitates implantation of devices and much effort to adjust electrical stimulation variables.

Gene therapy consisting of insertion of the glutamic acid decarboxylase gene (*GAD*) into the subthalamic nucleus may offer an alternative therapeutic strategy. GAD is the rate-limiting enzyme for GABA production, and the activity of both GABA efferents to the subthalamic nucleus and its targets within the basal ganglia circuitry are affected in Parkinson's disease. During DBS surgery in patients with this disease, an infusion of the GABAergic agonist muscimol into the subthalamic nucleus suppressed its neuronal firing rates and temporarily improved parkinsonian symptoms,[15] suggesting that

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improvement of GABA transmission within the subthalamic nucleus could be beneficial in Parkinson's disease. Similar results in animal models of parkinsonism were achieved with gene transfer of *GAD*.[16,17] This strategy uses an adeno-associated viral vector (AAV2) to deliver *GAD* to the subthalamic nucleus to both restore local GABA transmission within the nucleus and to normalise output from the nucleus (by adding an inhibitory GABA outflow, thereby reducing excessive excitatory glutamate output to key targets such as the globus pallidus interna and the substantia nigra reticulata). An open-label clinical trial[5] of AAV2-*GAD* injected unilaterally into the subthalamic nucleus showed this procedure to be safe and associated with improvements of parkinsonism. Although studies of other gene, cell, and biological therapies in patients with Parkinson's disease have also shown promise in small, open-label studies, subsequent randomised double-blind clinical trials have not substantiated their initial findings.[8] Consequently, progress in the assessment of CNS gene therapy needs careful attention to all aspects of study methods, including sham procedures, effective blinding, and successful delivery of the experimental therapy to the intended targets. This trial was done to assess the effect of bilateral delivery of AAV2-*GAD* into the subthalamic nucleus compared with bilateral sham surgery in patients with advanced Parkinson's disease.

Methods

Patients

This double-blind, randomised trial was done at seven centres in the USA specialising in Parkinson's disease care and functional neurosurgery. All patients had progressive, levodopa-responsive Parkinson's disease as defined by the UK Parkinson's Disease Society criteria.[18] Besides levodopa, other drugs for this disorder were allowed if no change in dose or drug type was made for 4 weeks or more before enrolment. An overnight off-medication unified Parkinson's disease rating scale (UPDRS)[19] part 3 summed subscore (motor score) of 25 or more was required. Additional inclusion criteria were age 30–75 years, duration of symptoms of Parkinson's disease for at least 5 years, and levodopa responsiveness for at least 12 months. Patients could not have had previous brain surgery, used dopamine-receptor-blocking drugs, had focal neurological deficits, or had abnormal cranial MRI; ^{18}F-fluorodeoxyglucose PET scans needed to be compatible with Parkinson's disease, according to criteria for a metabolic brain pattern specific to Parkinson's disease that excluded patients with atypical parkinsonism or indeterminate patterns.[20,21] Patients were also excluded for cognitive impairment as defined by a Mattis dementia rating scale score of less than 130. ^{18}F-fluorodeoxyglucose PET was repeated at 6 months and is planned for all patients after 12 months. The results of this secondary imaging outcome measure will be reported separately. Study protocols and consent forms were approved by the institutional review boards of all participating institutions. Written consent was obtained from every patient after detailed explanation of the procedures.

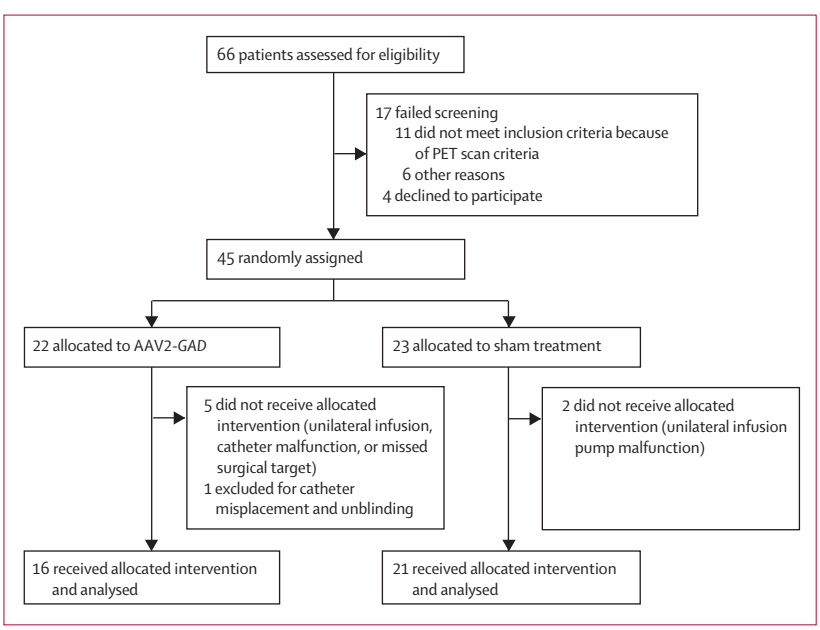

Figure 1: **Trial profile**
AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase.

Randomisation and masking

A statistician and a programmer at PharmaNet Inc (each with no further role in the study) generated the randomisation code. When patients arrived at the operating theatre, the neurosurgeon opened an envelope with the computer-generated random treatment assignment (ratio 1:1) for either AAV2-*GAD* or the sham procedure. Patients, caregivers, and investigators were masked to treatment assignment. For sham-assigned patients, the operating room team enacted a previously rehearsed plan for simulating a bilateral stereotaxic procedure identical to that done for the AAV2-*GAD* group. Those treated with sham received partial-thickness burr holes after a stereotaxic frame was placed. The simulation included sounds of microelectrode electrophysiological recording; infusion pumps and external catheters infusing normal saline into the burr hole site were used exactly as for patients receiving AAV2-*GAD* infusion.

Masking was carefully planned for all information about treatment assignment, and no deviations occurred at any site. All raters were masked to treatment allocation and had no access to sequestered postoperative images and surgical records. On the third day after surgery and at all subsequent visits, patients were questioned for opinions about treatment assignments. In this Article, we report only the 3-day questionnaire results, because these are most likely to portray the effectiveness of the masking efforts undertaken at operation and afterwards.

After discharge from the hospital, individuals had no contact with the unmasked surgical team, with the exception of a single visit to the primary surgeon for staple removal.

Procedures

Similar to the pilot study,[5] the clinical product was a mix of vector genomes (vg) of AAV-*GAD65* and AAV-*GAD67* (1:1 vg/vg), at a final vector concentration of 1×10^{12} vg/mL in 2×PBS/1 mmol/L $MgCl_2$ diluent, stored at –70°C. Sham treatment was with sterile saline. In preclinical studies of hemiparkinsonian rats, the combination of AAV-*GAD65* and AAV-*GAD67* was more effective in restoring a normal behavioural phenotype than either GAD isoform alone (unpublished data). Additionally, monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) had improved locomotor scores with *GAD67* versus *GAD65* (unpublished data), whereas *GAD65* showed stronger neuroprotective efficacy than *GAD67* in the acute 6-hydroxydopamine (6-OHDA) rodent model of Parkinson's disease.[17] Together with the finding that most GABAergic neurons in the brain express both isoforms and that 30% of *GAD* exists as heterodimers of the two isoforms, we felt that the combination provided the most physiological and optimum approach for clinical translation. Biological measures of safety, such as antibodies to the viral vector, were measured as part of this study, and these results will be reported with the 1-year open-label data in a future report.

For patients receiving AAV2-*GAD*, frame-based targeting and intraoperative microelectrode recording defined the borders of the subthalamic nucleus by conventional methods.[22–25] Microelectrode centring in the nucleus was noted by measurement and fluoroscopy. After microelectrode removal, a guide tube was inserted to 10 mm above the centre of the nucleus. A catheter with a 10 mm tip of 200 µm in diameter was flushed with AAV2-*GAD* infusate and was inserted into the putative centre of the nucleus. The catheter was locked in place with a cap containing a rip-cord tethering the catheter for post-procedure release at bedside. Unlike our phase 1 study,[5] in which we used a custom made infusion system, here we used a new infusion system that was devised to simplify the process for use in a multicentre clinical trial setting and that could form the basis for an eventual commercial product. Owing to concerns about catheter occlusion, the scalp was closed after placement of the first catheter, which was attached to the pump infusing at 0·23 µL/min for 2·5 h. The procedure was repeated on the other side. Following completion of infusions, a fine-cut head CT scan showed catheter tip locations. Post-catheter CT and MRI scans were done on two occasions 24–48 h later for all patients.

In one individual, the postoperative CT scan showed that both catheter tips had been unintentionally placed in the same subthalamic nucleus. This occurred because the arc was rotated to the contralateral side after the first side was

	AAV2-*GAD* (n=16)	Sham (n=21)
Mean age in years (SD, range)	61·8 (7·0, 43–71)	60·6 (7·4, 47–75)
Sex		
Male	12	15
Female	4	6
Years since diagnosis (SD, range)	10·6 (4·3, 5–19)	12·0 (5·0, 5–22)
Baseline modified Hoehn and Yahr rating (number of patients)		
Stage 2	3	4
Stage 2·5	8	5
Stage 3	3	10
Stage 4	2	0
Stage 5	0	2
Mean total daily levodopa dose taken with carbidopa (mg/day, SD)		
Baseline	800·0 (429·3)	961·9 (592·0)
6 months	789·1 (425·3)	916·7 (581·2)
Number of patients receiving dopaminergic agonists		
Baseline	8 pramipexole, 2 ropinirole	6 pramipexole, 8 ropinirole,1 rotigotine
6 months	7 pramipexole, 3 ropinirole	6 pramipexole, 8 ropinirole, 1 rotigotine
Number of patients receiving other medications		
Amantadine		
Baseline	7	7
6 months	6	8
Anticholinergic medication (trihexyphenidyl)		
Baseline	0	2
6 months	0	2
Monoamine oxidase-B inhibitor (rasagiline or selegiline)		
Baseline	6	5
6 months	6	6
Catechol-O-methyltransferase inhibitor (entacapone or tolcapone)		
Baseline	5	6
6 months	5	5
Mean baseline UPDRS part 2 total score (SD)	16·4 (5·6)	18·9 (6·1)
Mean baseline UPDRS part 3 total score (overnight practically-defined off state; SD)	34·8 (6·6)	39·0 (8·7)
Number of patients reporting at baseline		
Consistent medication effect	3	2
Wearing-off responses	10	13
On-off effects	9	8
Freezing of gait	8	12
Number of patients per site		
Henry Ford Health System*	3	3
Massachusetts General Hospital	4	4
Ohio State College of Medicine	3	4
Stanford University School of Medicine	2	2
University of Colorado School of Medicine	6	6
University of Rochester School of Medicine*	1	0
Wake Forest University School of Medicine	3	4

AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase. UPDRS=unified Parkinson's disease rating scale. *Operations were done by the same neurosurgeon.

Table 1: **Demographic and clinical characteristics of patients at baseline and 6 months**

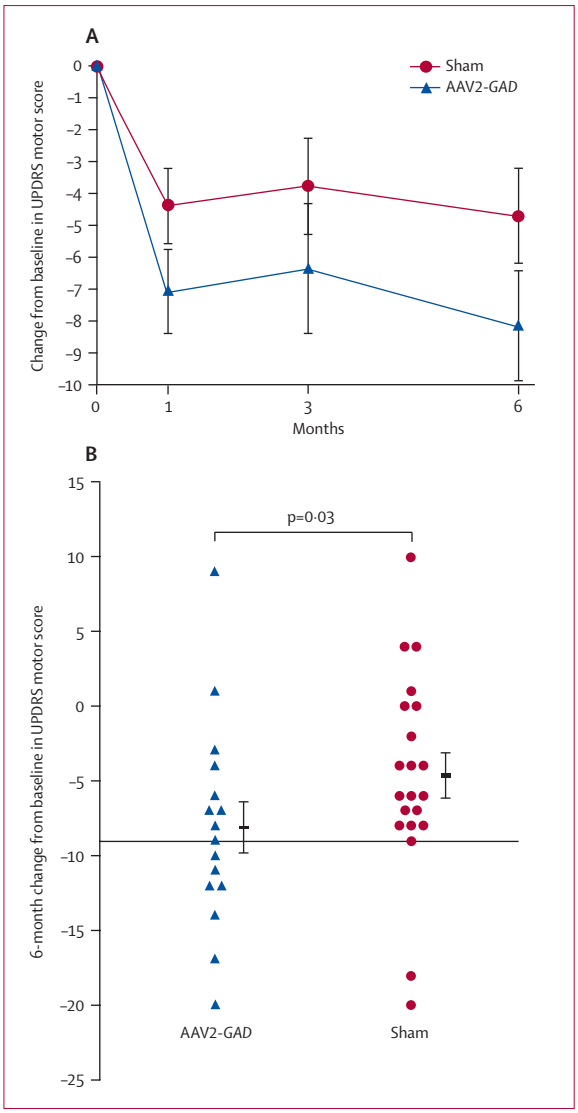

Figure 2: **Primary outcome**
(A) Mean change in off-medication UPDRS motor scores at 1, 3, and 6 months. (B) Change in off-medication raw UPDRS motor scores from baseline to the study endpoint of 6 months. The horizontal line at –9·0 is the cutpoint we chose to define a treatment response (see text); numbers of responders were compared with Fischer's exact test. Bars show mean change within groups and SE; p value based on Fisher's exact test. AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase. UPDRS=unified Parkinson's disease rating scale.

completed, but the previous target coordinates in the frame were not changed. The patient received a double-dose of AAV2-*GAD* on one side of the brain. No adverse events resulted from this surgical error, and this event was reported to all relevant institutional, study, and Federal regulatory boards. In response to this event, the protocol was amended to require a time-out before the beginning of surgery on each side of the brain, with the coordinates confirmed by the surgeon and documented in writing by a study coordinator or other surgical team member before penetration of the brain.

All patients were assessed with the UPDRS and the brief parkinsonism rating scale (BPRS) at baseline, 1, 3, and 6 months after treatment.[26] One movement-disorder specialist at each centre, who was masked to treatment allocation, completed the UPDRS motor examination after overnight medication withdrawal in fully parkinsonian (off) and full-medication effect (on) states. He also did the BPRS test. The Parkinson's disease questionnaire (PDQ)-39[27] and a questionnaire about motor fluctuations (constancy of medication effect, wearing off, on–off fluctuations, or freezing of gait) were assessed at baseline, 3, and 6 months after surgery. The Hagell-Widner dyskinesia rating scale[28] and investigator's clinical global impression were assessed at baseline and 6 months after surgery. Patients were trained to complete home diaries assessing the amount of time with emergence of parkinsonian features (off), with good control of parkinsonian features (on), or on with involuntary movements. Diaries were completed on two non-sequential days during the week before study visits. At baseline and 6 months after surgery, patients completed neuropsychological testing (Mattis dementia rating scale,[29] neuropsychiatric inventory,[30] Beck depression inventory,[31] symbol digit modality test,[32] Stroop color and word test,[33] Hopkins verbal learning test,[34] and controlled oral word association test[35]).

All randomly assigned patients were followed up and assessed through the 6-month study visit. According to a prespecified plan, and before database locking and unblinding, a neurosurgeon with expertise in DBS of the subthalamic nucleus and who remained masked to all study data except for the MRI and CT images (ARR) assessed whether catheter tip location was within the nucleus. With a surgical planning workstation (Medtronic Stealth System, FrameLink 5 software, Medtronic, Minneapolis, MN, USA), thin-cut post-operative CT scans and pre-operative MRI images for each individual were merged and morphed into standard X, Y, Z anterior and posterior commissure and mid-commissural point stereotaxic space.[22–24] The radio-opaque steel catheter tip was visualised and marked in three planes on the CT scan and the merged preoperative MRI.[23,24] For definition of the target zone of successful catheter localisation in the subthalamic nucleus, coordinates were prespecified as 9–14 mm lateral, 2 mm anterior to 5 mm posterior, and 1 mm dorsal to 7 mm ventral to the midcommissural point in stereotactic space.

Statistical analysis

The authors of this study participated in detailed review and analysis of the data, which were prepared by PharmaNet Development Group (Princeton, NJ, USA) under the direction of LVM. The statistical methods and data analyses were also reviewed by an independent academic biostatistician who confirmed the validity of the methods and the interpretation of the results.

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Before unblinding and locking of the database, the analysis groups for AAV2-*GAD* and sham treatment were determined from prespecified targeting and infusion criteria, which required catheter placement within defined boundaries of the subthalamic nucleus and delivery of at least 90% of the planned infusion in both subthalamic nuclei for patients treated with AAV2-*GAD*. For the sham group, inclusion in the analysis group required the infusion procedure to be completed without obvious pump failure. For the sham group, catheter location was not an issue because catheters did not enter the brain, but inclusion in the analysis required that the infusion be completed without obvious pump malfunction, as in the AAV2-*GAD* group. This was to avoid unmasking of the patient due to possible discussions in the operating theatre regarding the pump failure and to avoid exclusion of patients from only one group.

The sample size calculation was based on the primary outcome: change in UPDRS part 3 ratings in the off state from baseline to 6 months. Data from previous trials and published data[5] were used to estimate the effect size of clinical interest. An effect size of 1·19 was used, which corresponded to a sample size of 13 patients per group to achieve 80% power, α=0·05. To account for drop-outs and incompletely treated patients (owing to poor catheter tip location or pump failure), we planned to enrol 20 patients in each treatment group.

The primary outcome measure was the difference in off medication state UPDRS motor ratings between the sham and AAV2-*GAD*-treated groups. Repeated measures analysis of variance (RMANOVA) was done on the basis of a mixed model with terms for treatment, visit, and treatment-by-visit. To account for individual baseline differences in UPDRS motor scores, we calculated the ratios of scores at each of the three post-operative timepoints to baseline scores. Since the residuals from the RMANOVA on the ratio data were non-normally distributed, these ratios were natural logarithm-transformed and further analysed with RMANOVA. The residuals from this analysis were normally distributed, and the RMANOVA results are reported. In both treatment groups, a secondary post-hoc analysis was also done for 6-month responder data. This analysis was done on the raw (ie, not adjusted for baseline scores, and not natural logarithm-transformed) UPDRS motor score data. We defined a clinically meaningful response as more than 9·0-point improvement in UPDRS motor score, which corresponds to the mean improvement of 25% in the initial AAV2-*GAD* study[5] and to a moderate-to-large clinically important difference [36] reported in an analysis of treatments for Parkinson's disease. Fisher's exact test was used to examine differences in the proportion of responders between the AAV2-*GAD* and sham groups. To analyse endpoints with a single measure, Student's two-sample *t*-test was used. In every instance, significance was defined as p lower than 0·05. This trial is registered with ClinicalTrials.gov, NCT00643890.

	AAV2-*GAD* (n=16)	Sham (n=21)	Difference at 6 months (95% CI)	p value
UPDRS part 3 total score (off state)				
Baseline	34·8 (1·6)	39·0 (1·9)	..	..
6 months	26·6 (2·0)	34·3 (2·5)	..	0·04*
UPDRS part 3 total score (off state) responder analysis (number of patients, %)				
6 months	8 (50·0%)	3 (14·3%)	35·7% (7·4 to 64·6)	0·03†
Clinical global impression: severity of illness				
6 months	3·4 (0·1)	3·9 (0·1)	−0·4 (−0·8 to −0·1)	0·02‡
BPRS global rating of parkinsonism (off state)				
Baseline	−3·1(0·2)	−3·0 (0·2)	0·4 (−0·2 to 0·9)	..
6 months	−2·6 (0·2)	−3·0 (0·2)	0·4 (−0·2 to 0·9)	0·02§
Motor fluctuation impact scale (number of patients, %; descriptive data)				
Consistent medication effect				
Baseline	3 (18·8%)	2 (9·5%)	..	..
6 months	5 (31·3%)	2 (9·5%)	..	..
Wearing-off responses				
Baseline	10 (62·5%)	13 (61·9%)	..	..
6 months	9 (56·3%)	14 (66·7%)	..	..
On-off effects				
Baseline	9 (56·3%)	8 (38·1%)	..	..
6 months	6 (37·5%)	10 (47·6%)	..	..
Freezing of gait				
Baseline	8 (50·0%)	12 (57·1%)	..	..
6 months	5 (31·3%)	13 (61·9%)	..	..

Data are mean (SE) unless otherwise indicated. AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase. UPDRS=unified Parkinson's disease rating scale. BPRS=brief parkinsonism rating scale. *p value based on repeated measures analysis of log transformed ratios (postoperative scores to baseline scores) by use of a mixed model with terms for treatment, visit, and treatment-by-visit. †p value based on Fisher's exact test. ‡p value based on 2-sample *t* test of AAV2-*GAD vs* sham at 6 months. §p value based on repeated measures analysis of change from baseline by use of a mixed model with terms for treatment, visit, and treatment by visit.

Table 2: **Results for primary efficacy in patients treated with AAV2-*GAD* and sham**

Role of the funding source

The funding source for the study (Neurologix) took part in study design, data interpretation, and writing of the report, but did not contribute to data collection or data analysis. A writing committee composed of PAL, ARR, LVM, CVS, MJD, MGK, and AF had full access to the data and had final responsibility for decision to submit the report for publication.

Results

Potential participants were screened and underwent ^{18}F-fluorodeoxyglucose PET scanning for diagnostic confirmation of Parkinson's disease. Although meeting all clinical criteria, four were classified as having probable atypical parkinsonism and seven were classified as having an indeterminate diagnosis with an established protocol for PET scan data analysis[27] (figure 1). The remaining patients with probable Parkinson's disease were randomly assigned at seven clinical sites; the first patient was enrolled on Nov 17, 2008, and the last completed the study on May 11, 2010. The patient who received two AAV2-*GAD* infusions into the same subthalamic nucleus was unmasked after surgery and

removed from further group analysis. Because our hypothesis was that infusion of the gene product into the subthalamic nucleus would improve off-medication UPDRS motor score, the study prespecified criteria for exclusion of patients from analysis before unblinding of data. Patients were excluded on the basis of infusion catheter location outside the predetermined target zone (because attempts to reposition catheters would have unmasked patients) or failures of catheter or infusion pump performance. Table 1 shows baseline clinical characteristics and demographics for the 37 patients assessed for efficacy.

See Online for webappendix

AAV2-*GAD* treatment led to an improvement of 8·1 (SE 1·7, 23·1%; p<0·0001) in off-medication UPDRS motor score at the study endpoint of 6 months, compared with 4·7 (1·5, 12·7%; p=0·003) with sham treatment (figure 2 and table 2). The change of UPDRS scores from baseline differed significantly between treatment groups across all three postoperative timepoints (RMANOVA of natural-logarithm-transformed ratios of follow-up scores to baseline scores p=0·04; treatment × visit interaction: p=0·97; figure 2). The cutpoint of improvement by 9·0 points identified a greater responder rate in the AAV2-*GAD* group (eight of 16, 50%) than in the sham group (three of 21, 14%; figure 2). In patients excluded from the analysis because of poor catheter tip location or infusion pump failure, UPDRS motor scores either increased or did not change in four of five patients during the 6-month blinded analysis, although one had a 21% decrease at 6 months (see webappendix p 1). All the excluded patients who received AAV2-*GAD* did have a complete infusion in one hemisphere, which perhaps explains the mixed results in these patients. Inclusion of all these inadequately treated patients in an intention-to-treat analysis showed no significant difference between groups (data not shown).

Some secondary measures supported an improved outcome for patients given AAV2-*GAD* (table 2). For example, the investigator's clinical global impression of severity of Parkinson's disease showed significant improvement at 6 months compared with baseline (3·4, SE 0·1 for AAV2-*GAD vs* 3·9, 0·1 for sham; p=0·02, two-sample *t* test). The off-medication global rating of parkinsonism provided by a masked rater at every study visit (question 5 of the BPRS) showed differences between groups in changes from baseline at 1, 3, and 6 months (p=0·02, RMANOVA; figure 3). Additionally, the percentage of patients treated with AAV2-*GAD* who

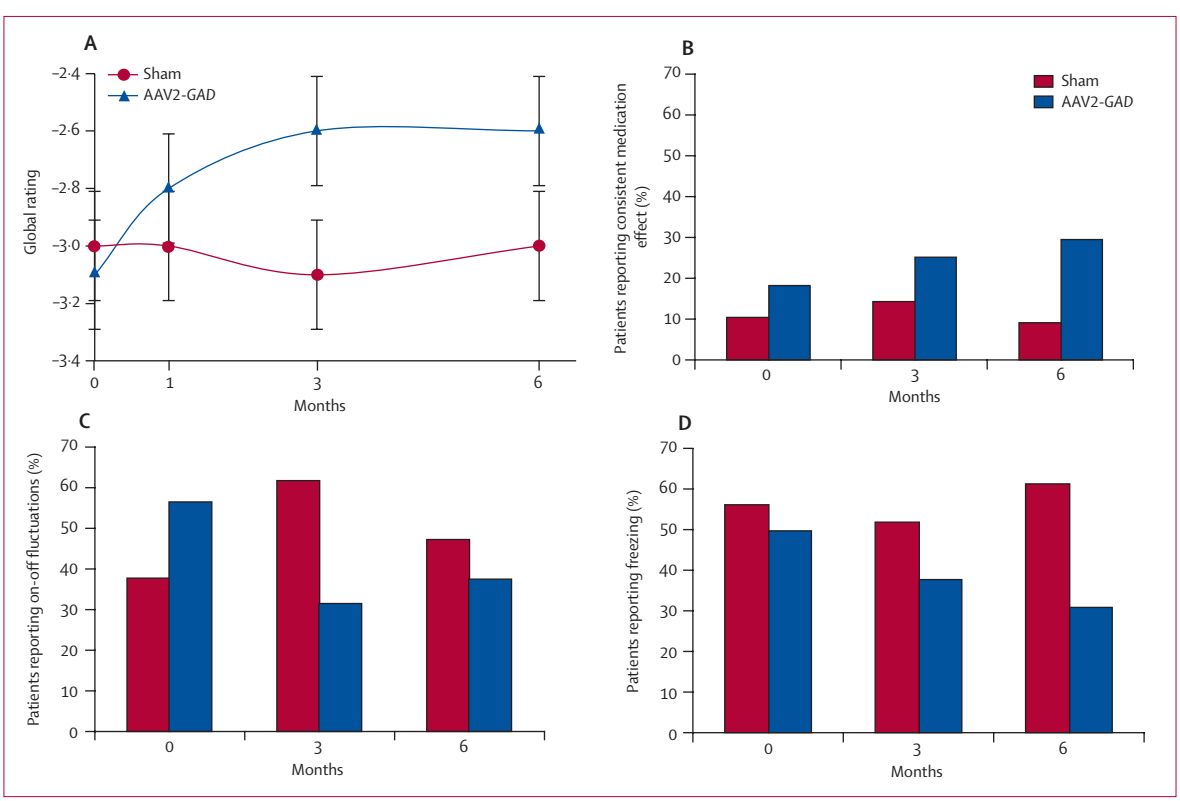

Figure 3: **Selected secondary endpoints**
(A) Global rating of parkinsonism (a question from the brief parkinsonism rating scale) showed a significant difference between groups (p=0·02, RMANOVA). Although not compared using statistical tests, more patients in the AAV2-*GAD* group seemed to report favourable clinical outcomes than did patients in the sham group in the questionnaire to assess motor fluctuations at 6 months: (B) two patients of 21 in the sham group *vs* five of 16 in the AAV2-*GAD* group reported a consistent medication effect; (C) ten of 21 in the sham group *vs* six of 16 in the AAV2-*GAD* group reported on–off fluctuations; and (D) 13 of 21 in the sham group *vs* five of 16 in the AAV2-*GAD* reported freezing gait. AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase.

	AAV2-*GAD* (n=16)	Sham (n=21)	p value*
UPDRS			
Part 1 total score			
Baseline	2·1 (0·4)	2·6 (0·4)	..
6 months	2·0 (0·6)	2·5 (0·5)	0·97
Part 2 total score			
Baseline	16·4 (1·4)	18·9 (1·3)	..
6 months	13·8 (1·2)	16·2 (1·5)	0·75
Part 4 total score			
Baseline	6·9 (0·9)	6·2 (0·6)	..
6 months	5·0 (0·7)	5·1 (0·5)	0·19
Hoehan and Yahr stage			
Baseline	2·7 (0·2)	2·9 (0·2)	..
6 months	2·5 (0·2)	2·8 (0·2)	0·25
Schwab and England ADL scale			
Baseline	78·1 (3·4)	68·6 (4·6)	..
6 months	81·3 (3·2)	69·5 (4·4)	0·96
UPDRS part 3 total score (on state)			
Baseline	18·7 (2·2)	20·0 (2·0)	..
6 months	15·9 (1·7)	16·7 (1·7)	0·81
BPRS			
Number of taps in 60 s (off state)			
Baseline	108·5 (19·8)	95·5 (6·5)	..
6 months	121·1 (7·6)	103·5 (5·9)	0·25
Timed walking (off state)			
Baseline	20·5 (3·3)	25·8 (3·5)	..
6 months	20·1 (4·2)	26·9 (4·5)	0·39
Global rating of dyskinesia or dystonia (off state)			
Baseline	0·1 (0·1)	0·2 (0·1)	..
6 months	0·1 (0·1)	0·2 (0·1)	0·58
Number of taps in 60 s (on state)			
Baseline	156·1 (32·1)	118·6 (5·1)	..
6 months	151·0 (10·8)	125·9 (5·9)	0·97
Timed walking (on state)			
Baseline	13·6 (0·7)	15·4 (1·7)	..
6 months	14·9 (3·1)	13·7 (0·7)	0·51
(Continues in next column)			

	AAV2-*GAD* (n=16)	Sham (n=21)	p value*
(Continued from previous column)			
Global rating of parkinsonism (on state)			
Baseline	–1·7 (0·2)	–1·7 (0·2)	..
6 months	–1·6 (0·2)	–1·6 (0·2)	0·92
Global rating of dyskinesia or dystonia (on state)			
Baseline	1·2 (0·3)	1·4 (0·3)	..
6 months	0·8 (0·3)	1·4 (0·2)	0·26
Dyskinesia rating scale			
Hyperkinesias (total score)			
Baseline	2·2 (0·7)	2·9 (0·7)	..
6 months	2·2 (0·8)	3·9 (0·6)	0·37
Dystonia total score			
Baseline	0·3 (0·2)	1·2 (0·4)	..
6 months	0·2 (0·1)	1·1 (0·4)	0·90
Clinical global impression			
Global improvement			
6 months	3·5 (0·3)	3·3 (0·2)	0·63
Efficacy index			
6 months	10·3 (0·8)	9·7 (0·7)	0·59
PDQ-39 total score			
Baseline	33·7 (3·1)	38·0 (2·8)	..
6 months	29·1 (4·4)	35·8 (3·7)	0·76
Patient's on/off diary			
Total hours in off state			
Baseline	5·8 (0·9)	5·1 (0·6)	..
6 months	5·8 (0·9)	5·1 (0·5)	0·51
Total hours in on state			
Baseline	8·3 (1·0)	9·1 (0·9)	..
6 months	8·6 (1·2)	9·7 (0·7)	0·60
Total hours in on state with dyskinesia			
Baseline	2·8 (0·9)	2·4 (0·5)	..
6 months	2·7 (1·0)	2·1 (0·4)	0·97

Data are mean (SE). AAV2-*GAD*=adeno-associated virus serotype 2-glutamic acid decarboxylase. UPDRS=unified Parkinson's disease rating scale. ADL=activities of daily living. BPRS=brief parkinsonism rating scale. PDQ-39=The Parkinson's disease questionnaire *p values based on two-sample *t* test

Table 3: **Additional results of efficacy in patients treated with AAV2-*GAD* and sham**

reported consistent medication effects increased and that of those having on–off fluctuations and severe freezing episodes decreased (figure 3), although the significance of these changes was not analysed statistically. During the 6 months after surgery, investigators were encouraged to avoid changes in medication for Parkinson's disease to lessen drug-induced variability. This goal was accomplished, because the changes in doses were minimum (table 1). No significant differences between the groups were noted in other secondary outcome measures at 6 months, including PDQ-39, UPDRS activities of daily living, dyskinesia ratings, and measurements of off-state walking and tapping rates (tables 2 and 3). No significant changes emerged from the repeated neuropsychological testing (see webappendix p 1).

In the questionnaire about masking done 3 days after surgery, nine sham-treated patients thought their treatment was AAV2-*GAD*, seven stated they did not know, and five guessed correctly. Of 16 patients given AAV2-*GAD*, ten guessed correctly and five stated that they did not know (no data were obtained for one patient). Clinical outcomes at 6 months were not related to the self-assessments of treatment assignment.

One serious adverse event was recorded during the 6-month blinded phase of the study, in the AAV2-*GAD* group (bowel obstruction). This event resolved and was classified as unrelated to AAV2-*GAD* treatment or the surgical procedure. Other adverse events during the study (table 4) were mostly mild and resolved. The two groups differed little in the variety of events reported,

	AAV2-*GAD*					Sham				
	Patients	Events	Severity	Relation to study drug	Relation to procedure	Patients	Events	Severity	Relation to study drug	Relation to procedure
Nausea	6	1 1 1 1 2	Mild Mild Moderate Moderate Moderate	Unrelated Possibly Unrelated Possibly Possibly	Probably Probably Unrelated Unrelated Possibly	2	1 1	Mild Moderate	Unrelated Unrelated	Probably Possibly
Influenza	2	2	Mild	Unrelated	Unrelated	0	..	..	..	..
Sinusitis	0	..	..	..	..	2	2	Mild	Unrelated	Unrelated
Incision site complication	2	1 1	Mild Moderate	Unrelated Possibly	Definitely Probably	0	..	..	..	..
Muscular weakness	2	2	Mild	Possibly	Possibly	0	..	..	..	..
Headache	7	2 1 1 1 1 2	Mild Mild Mild Moderate Moderate Moderate	Unrelated Unrelated Possibly Unrelated Possibly Possibly	Possibly Probably Probably Probably Possibly Probably	2	1 1 1	Mild Mild Moderate	Unrelated Possibly Possibly	Unrelated Possibly Possibly
Hypoaesthesia	2	1 1	Mild Mild	Unrelated Unrelated	Unrelated Probably	0	..	..	..	..
Worsening parkinsonism	0	..	..	..	..	4	2 1 1	Mild Moderate Severe	Unrelated Unrelated Unrelated	Unrelated Unrelated Unrelated
Depression	4	2 2	Mild Moderate	Unrelated Unrelated	Unrelated Unrelated	2	1 1	Moderate Moderate	Unrelated Unrelated	Unrelated Probably
Insomnia	2	1 1	Mild Moderate	Unrelated Unrelated	Unrelated Unrelated	1	1	Moderate	Unrelated	Unrelated
Rash	2	2	Mild	Unrelated	Unrelated	0	..	..	..	..

Table 4: **Adverse events that occurred in at least two patients from either treatment group**

apart from those likely to have arisen from intracranial surgery, such as headache and nausea. The one unblinded patient was closely monitored and no adverse events related to the AAV2-*GAD* surgery were reported. For this patient, off-medication UPDRS scores improved throughout the 6-month study period. MRI imaging after surgery and at 6 months revealed no evidence of lesion in the subthalamic nucleus or other brain injury due to gene therapy in any patient.

UPDRS off motor scores were either unchanged or increased compared with baseline for two of the 16 AAV2-*GAD*-treated patients, and in four of the five AAV2-*GAD*-treated patients who were excluded from efficacy analysis because of missed targeting or infusion failure. Because delivery of AAV2-*GAD* to the intended target is likely to be important for optimum effect, the positions of infusion catheter placement (figure 4) were analysed in comparison with clinical outcome. This post-hoc analysis found some correlation between catheter location (eg, right Y and Z coordinates) and 6-month percentage-change in UPDRS motor score (figure 4).

Discussion

This randomised double-blind clinical trial of targeted intracerebral AAV2-*GAD* gene therapy met the primary outcome measurement of improved UPDRS motor score at 6 months. Despite concerns about the safety of CNS in vivo gene therapy related to off-target transduction, insertional mutagenesis, immune responses, and the inability to reverse gene expression, the adverse events associated with this procedure were mild and did not suggest unforeseen risks associated with bilateral infusion of AAV2-*GAD* in the subthalamic nucleus. Some other clinical assessments of parkinsonism also provided evidence for improvements after AAV2-*GAD* treatment, especially for symptoms that are often resistant to drug control in advanced Parkinson's disease. These assessments included reductions in measures of overall severity of Parkinson's disease and complications related to treatment. The safe outcome and clinical benefits from AAV2-*GAD* treatment in this trial were consistent with results from the previous open-label study[5] of unilateral AAV2-*GAD* infusion.

Potential limitations of this study included the possibility of inadequately masked procedures in the operating theatre, because the patient was awake during the surgery. However, in advance of the first experimental procedure, operating teams practised and did a carefully scripted performance to disguise the choice of treatment. Ideally, all patients in both groups would believe soon after surgery that they received the treatment, because most patients who enrolled wished to receive such treatment. The number of patients who believed they had received AAV2-*GAD* or were not certain was similar in both groups.

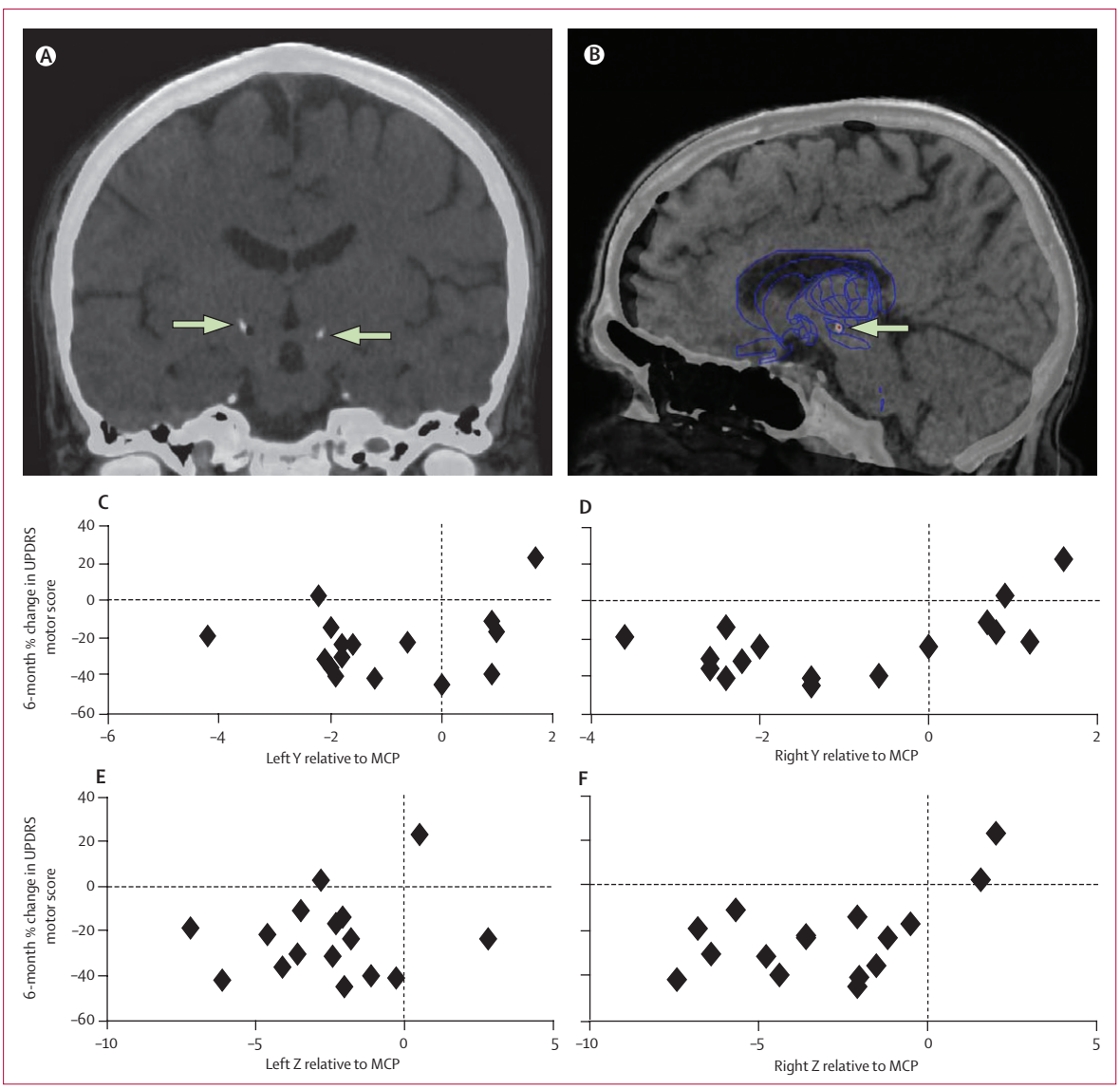

Figure 4: **Association between catheter tip location and the primary outcome for patients receiving AAV2-*GAD***

(A) A representative coronal head CT merged with a presurgical MRI showing the localisation of catheter tips (green arrows) that were within the predefined target zone for the subthalamic nucleus. (B) Representative sagittal section from a merged head CT-MRI scan showing a catheter tip (green arrow) correctly located in the subthalamic nucleus and with a superimposed adjusted Schaltenbrand-Wharen atlas section. (C–F) Data points plotted from each patient treated with AAV2-*GAD* indicate the change in 6-month off-medication UPDRS motor scores versus left and right catheter tip coordinates relative to the MCP in the anterior-posterior (C and E) and dorsal-ventral (D and F) orientations. For each graph, the *x*-axis is the tip distance (in mm) relative to MCP (MCP=0) and the *y*-axis is change from baseline in the 6-month off-medication UPDRS motor scores. Most datapoints from the patients treated with AAV2-*GAD* are in the lower left quadrant of each graph,suggesting an association between clinical improvement on UPDRS motor score and optimum catheter tip placement. Change in UPDRS scores correlated significantly with the right Y (r=0·58 [Pearson correlation coefficient], p=0·02) and Z (r=0·55, p=0·03) coordinates. MCP=midcommissural point. UPDRS=unified Parkinson's disease rating scale.

Although absolute success of the blinding cannot be proven, the results from the inquiry about blinding supported effective masking of the surgical procedures. Furthermore, the correlation between catheter location and outcome also suggests that the AAV2-*GAD* treatment was beneficial, because sham or unblinding effects would not be affected by location of the catheter tip relative to the known motor territory of the subthalamic nucleus. The absence of improvement in four of the five patients excluded from the efficacy analysis because of inaccurate catheter tip location, and the significant improvement only in the efficacy set and not in the total patient group, further suggest that delivery of AAV2-*GAD* as intended was necessary for the improvements recorded in this study. Finally, it is unlikely that benefits in the AAV2-*GAD* treatment group were caused by the temporary placement of catheters in the subthalamic nucleus rather than from the infusion of the gene product. Although lesioning of

Panel: Research in context

Systematic review

We searched PubMed to identify all relevant clinical trials of gene therapies in Parkinson's disease up to Dec 31, 2010, with the search terms "gene therapy", "Parkinson's disease", and "clinical trials". We have also reviewed key reports about the basic science behind the use of AAV2-*GAD* in Parkinson's disease. Additionally to AAV2-*GAD*, the search identified two other gene therapy approaches for Parkinson's disease that have been assessed in clinical trials: AAV-delivered CERE-120 (neurturin, which is structurally related to GDNF),[6,8] and viral vector delivery of L-aromatic aminoacid decarboxylase (AADC) to the putamen.[4,7] Both these approaches showed promise in phase 1 open-label clinical trials, but the initial findings for CERE-120 were not confirmed in a subsequent randomised double-blind controlled trial.[8]

Interpretation

In this double-blind sham-surgery controlled study, we used a predetermined highly selective recruitment and assessment process to show that gene therapy with AAV2-*GAD* into the subthalamic nucleus significantly improves motor function in patients with Parkinson's disease (RMANOVA, p=0·04). In this proof-of-concept study we sought to avoid potential confounding factors by carefully screening patients to assure enrolment of only patients with Parkinson's disease and not atypical parkinsonism. Furthermore, we prespecified that the primary analysis would be limited to patients who received the full assigned treatment, so individuals were excluded for pump failures or inaccurate targeting of the subthalamic nucleus. With this approach, in this small phase 2 study we found evidence of a benefit of subthalamic nucleus AAV2-*GAD* surgery versus sham surgery.

the subthalamic nucleus can improve parkinsonism, microlesion effects from DBS are generally transient, and the infusion catheter we used has a volume about 40 times smaller than a typical electrode used in DBS.[37] Furthermore, postoperative MRI of every patient treated with AAV2-*GAD* showed no evidence for lesions in the subthalamic nucleus.

Challenges are inherent in a small phase 2 trial that includes novel procedures and blinding, and several features of this study deserve special attention. We took care to enrol only patients with idiopathic Parkinson's disease on the basis of strict clinical criteria and ^{18}F-fluorodeoxyglucose PET imaging. We also limited the primary analysis to patients who successfully received bilateral treatment (ie, patients were excluded if the infusion catheter tip location did not meet predetermined criteria, or if the infusion pump failed). Although the selectivity of these criteria might limit how readily the results can be generalised, we should recognise that this clinical trial was intended to serve mainly as a proof-of-concept study. Moreover, several secondary outcome measures did not show improvement in the AAV2-*GAD* treatment group compared with the sham group. This result might indicate the absence of a benefit or relate to the fact that this small phase 2 trial was likely to be underpowered for some of the clinical outcomes that are more difficult to achieve (eg, improvement in function, activities of daily living, and quality of life measures). Nonetheless, the data obtained for study design details, such as frequency of mistargeting and mechanical failures and magnitude of clinical effects, will be valuable for optimisation of the design of a subsequent larger clinical trial. Such a trial will be needed to confirm the present results and to assess whether this treatment is practical for more widespread clinical use.

The use of somatic-cell gene transfer to alter gene expression in well characterised brain neurochemical systems offers a novel alternative to conventional pharmacological or surgical treatment. This study was the first successful randomised, double-blind gene therapy trial for a neurological disorder (panel) and it justifies the continued development of AAV2-*GAD* for treatment of Parkinson's disease.

Contributors

PAL, LVM, CVS, MJD, MGK, and AF participated in the study design; PAL, MAL, SGO, AWF, ENE, SKK, KT, AS, MSS, SBT, JMS, KLP, JMH, RMK, IHR, and AF worked on patients' assessments and procedures, collected data, and critically reviewed the report; PAL, ARR, LVM, CVS, MJD, MGK, and AF participated in data analysis; PAL, ARR, LVM, CVS, MJD, MGK, and AF wrote the report.

Conflicts of Interest

PAL served as a paid speaker for Allergan, Boehringer-Ingelheim, Chelsea Therapeutics, Lundbeck, Novartis, and Ipsen, served as a paid consultant for Boehringer-Ingelheim, GlaxoSmithKline, Impax, Itec, Ipsen, NeuroDerm, Merck, Schering-Plough, and XenoPort; he receives grant funding (grants pending) from Allergan, Boehringer-Ingelheim, Chelsea Therapeutics, The Michael J Fox Foundation for Parkinson's Research, Merz, Novartis, Santhera, and UCB. ARR served as a paid consultant for Autonomic Technologies and Neurologix, received grant funding (grants pending) from Medtronic Neurological, served as a paid speaker for Medtronic Neurological, patents with money to institution from Autonomic Technologies and Intelect Medical, and holds stock options in Surgivision and Autonomic Technologies. MAL received grant funding (grants pending) from Schwartz Biosciences, Molecular Biometrics, IMPAX Pharmaceuticals; served as a paid speaker for Athena Diagnostics, Teva Neurosciences, American Federation of Research, The Movement Disorders Society; and received meeting expenses from Parkinson Study Group. SGO served as a paid consultant for Medtronic. SKK receives salary support from Ohio State University and Chalmers P Wiley VA Medical Center and receives grant funding (grants pending) from National Institute of Health and Huntington's Disease Society of America. KT receives grant funding (grants pending) from Teva, Acadia, UCB, Ipsen, Impax and Chelsea Pharmaceuticals. MSS received travel expenses for research-related meetings, and provisions of writing assistance, medicines, equipment, or administrative support to Wake Forest University School of Medicine from Neurologix. SBT reports grant funding (grants pending) to Wake Forest University School of Medicine from the US Department of Defense, National Institutes of Health and from the National Institutes of Health Neurological Disorders and Stroke. JMH has served on the speakers' bureau for Medtronic. RMK served as a paid consultant for Boehringer-Ingelheim and receives grant funding (grants pending) from Kyowa and Boehringer Ingelheim. IHR reports grant funding (grants pending) to University of Rochester School of Medicine from Eli Lilly and Novartis, and served as a paid speaker for Teva Pharmaceuticals. LVM, an employee of PharmaNet Development Group, Princeton, NJ, USA, served as the study biostatistician under contract from Neurologix. CVS is a paid employee of and holds stock options in Neurologix. MJD is co-founder of, paid consultant for, and holds stock options in Neurologix, and is a co-author on issued patent related to the AAV-*GAD* product. MGK is cofounder of, paid consultant for, receives grant funding (grants pending) from and holds stock options in Neurologix, patents with financial gain to Rockefeller University and Weill Cornell Medical College, royalties to Weill Cornell Medical College from Neurologix, Ceregene and Genzyme. AF served as a paid consultant for Rexahn and Alnylam Pharmaceuticals, provided expert testimony for medico-legal cases; receives grant funding (grants

pending) from National Institutes of Health, The Dana Foundation, Thomas Hartman Foundation for Parkinson's Research, Huntington's Disease Society of America; and has served on the speakers' bureau for Teva Pharmaceuticals and Allergan. The other authors declare that they have no conflicts of interest.

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At last, a gene therapy for Parkinson's disease?

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Surgical interventions to treat Parkinson's disease, specifically thalamotomy and pallidotomy, pre-date levodopa therapy. They were largely discontinued after the introduction of levodopa, but reintroduced 20 years ago as adjunctive therapy for later stages of the disease. In 1998, deep brain stimulation (DBS) of the subthalamic nucleus was shown to be an effective alternative approach to pallidotomy.[1] Since then several new surgical approaches have been proposed, although only two have been tested in randomised double-blind clinical trials: intraputamenal infusion of glial-derived neurotrophic factor (GDNF)[2] and intraputamenal gene therapy with neurturin[3] (a structural relative of GDNF). However, these surgical approaches have not shown the same level of efficacy in randomised controlled trials as in the preceding open-label studies. In the case of intraputamenal GDNF, explanations for this failure included the possibility of a large placebo effect and differences in methodology[2] and statistical underpowering (type 2 error).[4]

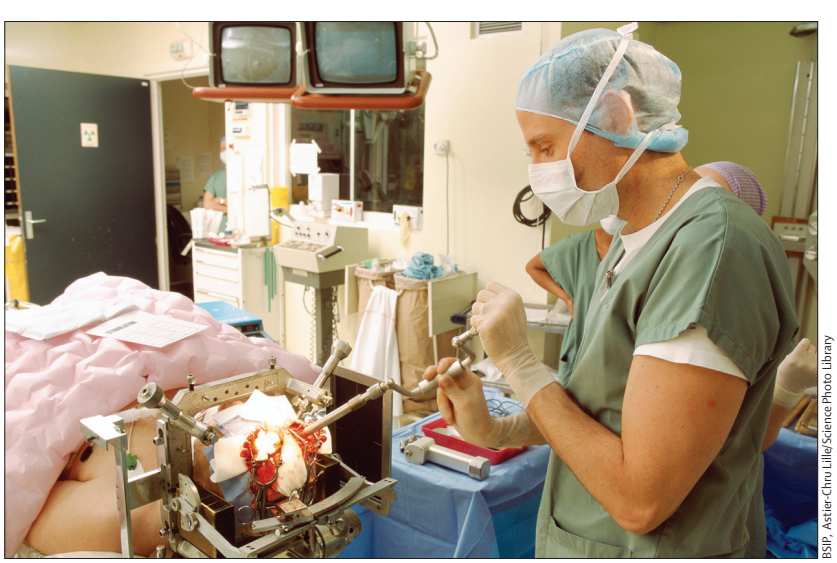
In this issue of *The Lancet Neurology*, Peter LeWitt and colleagues[5] report the first positive result for a double-blind study of a gene therapy for Parkinson's disease. The method that the investigators used involved insertion of the gene for glutamic acid decarboxylase (*GAD*) into the subthalamic nucleus by use of the adeno-associated viral vector AAV2. The enzyme GAD is the rate-limiting step in the production of GABA, the neurotransmitter in afferent terminals within the subthalamic nucleus. An increase in GABA activity in these afferents would be expected to reduce output from the subthalamic nucleus and thereby produce an effect analogous to DBS.

The main result was a mean improvement in unified Parkinson's disease rating scale (UPDRS) motor score in the treatment group of 23·1% at 6 months, compared with 12·7% in the placebo group; the difference between the groups was significant when compared across the 6-month double-blind period (p=0·04).

In view of the difficulties with previous surgical studies, the investigators included a suitably large number of participants (22 in the treatment group and 23 in the placebo group), and the study design was fastidious. For example, patients whose catheters were improperly placed were removed from the analysis. This requirement reduced the number of participants to 16 in the treatment group and 21 in the placebo group. This was a prudent strategy. In the study of intraputamenal GDNF,[2] at least two of 17 patients in the treatment group had catheters in the wrong place, yet were included in the analysis.

Several questions remain. How long will the effect last? Will untoward long-term effects arise after the introduction of viruses into the brain? Does the technique offer any advantages over DBS, for which clinical improvements seem twice as large?[1] For example, freezing of gait can be difficult to control with DBS.[6] LeWitt and colleagues indicate that freezing might improve after gene therapy, but did not report a statistical analysis of these results.

There is a hidden value to this meticulous study: it confirms that although a sustained placebo effect can be associated with surgery (12·7%), it is not of sufficient magnitude to explain the large beneficial effects detected in open-label surgical trials.[2,7] This placebo effect was sufficiently large that there was a difference of only 10·4% between treatment and sham surgery groups, although this small difference was statistically significant. In the surgical trial of intraputamenal GDNF,[2] the mean difference between the treated and placebo groups was not significant and hence the study was inconclusive. Although LeWitt and colleagues[5] used different analyses, the finding of



BSIP, Astier-Chru Lille/Science Photo Library

Surgeon drilling a hole in the skull of a patient with Parkinson's disease

a significant difference between treatment groups is a tribute to their careful study design and underscores the exceptional importance of fastidiousness in small surgical trials.

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I have no conflicts of interest.

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