Neural transplantation in Huntington disease

Long-term grafts in two patients

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ABSTRACT Objective: Clinical trials of fetal neural tissue transplantation for Huntington disease (HD) have been conducted with variable clinical results. However, no long-term analysis of graft survival and integration has been published. Here, we report the pathologic findings in two patients with HD who died 74 and 79 months after transplantation. **Methods:** Methods used were pathologic examination, histochemistry, and immunohistochemistry. **Results:** Neostriatum from both patients showed typical neuropathologic changes of advanced HD. Surviving grafts were identified in both patients (6/6 sites and 7/8 sites, respectively) as well-demarcated nests within host neostriatum with associated needle tracts. Grafted neurons adopted either dominant calbindin/parvalbumin or calretinin immunoreactivity (IR). Few neurofilament, MAP-2, DARPP-32, tyrosine hydroxylase, or calbindin IR processes traversed the host parenchyma–graft interface despite minimal junctional gliosis. Immunohistochemistry for CD68 showed microgliosis that was more pronounced in host striatum than graft. Scattered CD45 and CD3 IR cells were present within grafts and host parenchyma. No ubiquitin IR neuronal intranuclear inclusions were identified in graft neurons, although these were prevalent in host cells. **Conclusions:** These two autopsies confirm previous findings of neuronal differentiation and survival of transplanted fetal tissue from the ganglionic eminence and also demonstrate viability of neurons from fetal transplants in human neostriatum for more than 6 years. Despite prolonged survival, these grafts had poor integration with host striatum that is likely responsible for lack of clear clinical improvement in these patients. **NEUROLOGY 2007;68:2093–2098**

Huntington disease (HD) is a progressive neurodegenerative condition caused by expansion of the trinucleotide repeat sequence of exon 1 of the gene encoding the huntingtin protein. HD is characterized clinically by motor, cognitive, and psychiatric impairment. Pathologically, HD is characterized initially by atrophy of the caudate nucleus and putamen due primarily to loss of γ-aminobutyric acid–mediated medium spiny neurons (MSNs), with secondary degeneration of globus pallidus, frontal cortex, thalamus, locus ceruleus, and subthalamic nucleus due to loss of presynaptic and postsynaptic neurons. Current treatments for HD are limited and have little impact on the long-term outcome of the affected patient.

Numerous preclinical studies in animal models of HD have demonstrated survival and connectivity of fetal neural transplants derived from the primordial striatum, located in the ganglionic eminence (for review, see references 1 and 2). Thereafter, clinical trials investigating the safety and efficacy of fetal neural transplantation in HD patients have been undertaken; clinical results have been mixed.[3-10]

Autopsy findings from only one transplanted patient with HD have been reported to date[11]; this patient received fetal neural grafts 18 months before death and had surviving grafts in 6 of 10 sites. Here, we report autopsy findings of two patients with HD who received bilateral transplantation of human embryonic lateral ganglionic eminence. These patients survived at least 74 months before succumbing to pneumonia.

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Table 1 Patient characteristics

	Sex	CAG repeat length	Age at onset, y	Age at transplantation, y	No. of grafts	Latency to death, mo	Cause of death	Immunosuppression
Patient 1	M	45	40	47	6	79	Pneumonia	CsA (18 mo)
Patient 2	F	52	26	34	8	74	Pneumonia	CsA (35 mo)

CsA = cyclosporine A.

METHODS **Patients who came to autopsy.** Both patients were part of a study that has previously been described by Kopyov et al.[5] Details on inclusion and exclusion criteria, tissue procurement and preparation for transplantation, and the surgical procedure are described in detail in this reference. In brief, fetal lateral ganglionic eminence (LGE) was collected and processed according to NIH guidelines and the California Anatomic Gift Act. All donor blood and brain tissue was examined for bacterial and viral infection. Pieces of donor LGE, 0.8 to 1.0 mm^3, were transplanted stereotactically in bilateral caudate nucleus and putamen. The number of grafts was customized for each patient based on the degree of basal ganglia atrophy and accessibility (see Case histories in the Results section for specific details on each patient). Patients were evaluated before surgery, and those not lost to follow-up participated in longitudinal postoperative clinical protocols for up to 2 years using the Core Assessment Program for Intracerebral Transplantation in Huntington's Disease and adjusted neuropsychological protocols.[12]

Patient tissue. Brain-only autopsies were performed on both patients after appropriate consent was obtained. The entire caudate and putamen were removed bilaterally and, along with other tissue blocks from multiple brain regions, were embedded in paraffin. In blocks other than striatum, 5-μm sections were stained with hematoxylin, eosin, and Luxol fast blue (H-E/LFB). Blocks of striatum were serially sectioned at 5 μm with H-E/LFB staining of every 20th section until graft was identified or the block was exhausted. Intervening sections were used for immunohistochemistry (IHC). Striatal tissue from two adults without neurologic disease who underwent contemporaneous autopsies was used for control tissue to optimize IHC protocols; however, all comparisons were made between graft and host tissue within the same striatum.

Immunohistochemistry. IHC was performed according to standard ABC-immunoperoxidase protocols using diamino benzidine tetrachloride as chromogen substrate. Primary antibodies were to microtubule-associated protein 2 (MAP-2; BMB, 1:500), neurofilament (NF211; DAKO, 1:1,700), glial fibrillary acidic protein (GFAP; DAKO, 1:500), synaptophysin (Chemicon, 1:400), ubiquitin (Chemicon, 1:20,000), calbindin-D28K (Chemicon, 1:1,000), calretinin (Chemicon, 1:500), parvalbumin (Sigma, 1:500), tyrosine hydroxylase (TH; Chemicon, 1:500), dopamine and cyclic AMP–associated receptor phosphoprotein (DARPP-32; Sigma, 1:10), CD45 (DAKO, 1:500), CD3 (Novacastra, 1:200), CD20 (DAKO, 1:300), and CD68 (DAKO, 1:3,000).

RESULTS **Case histories.** Characteristics of both patients are presented in table 1. Both patients had well-documented family histories of HD. Patient 1 initially exhibited personality changes, lack of motivation, and depression. At the time of transplantation surgery, he was unable to work because of choreiform movements as well as balance and gait difficulties. He received six grafts: two in the right putamen, three in the left putamen, and one in the left caudate. Postoperatively, he returned to his state of residence and had chronic headaches. Two months later, bilateral subdural hematomas were identified on brain MRI. His chronic headaches gradually improved after surgical intervention, but he never returned for follow-up examination as part of the transplantation clinical trial. Limited clinical reports from his local health care providers describe gradual progression of his disease ultimately resulting in long-term admission to a nursing home, where he died of pneumonia at age 54 years.

Patient 2 first noticed decreased motor control, then difficulties with speech and swallowing with occasional choking, and then cognitive difficulties. At the time of transplantation surgery, she had moderately severe choreiform movements of her trunk and limbs as well as slurred speech. She re-

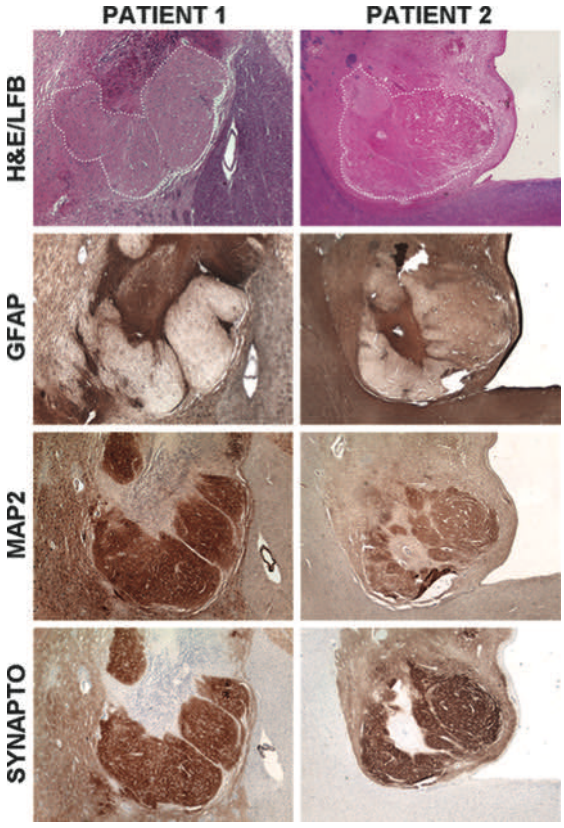
Table 2 Pretransplant and posttransplant clinical measurements in Patient 2

Patient 2	Walk,* s	Read,† sec	Months of year,‡ s	UHDRS motor	UHDRS behavioral	UHDRS functional assessment	UHDRS functional capacity	Independence scale
Preop 1 y	9	16	106	49	12	30.5	10	65
Preop 6 mo	9	25	69	59.5	12	37	11	60
Postop 1 y	12	27	26	33	4	31	9.0	70

Patient 1 lost to follow-up.
* Distance to walk was 20 ft.
† Length of paragraph was 88 words.
‡ Denotes time to recite 12 months of the year backward.
UHDRS = Unified Huntington's Disease Rating Scale.

Hematoxylin, eosin, and Luxol fast blue (H-E/LFB)–stained sections show nodular, well-circumscribed grafts in both patients. The graft–host interface is distinct, with part of the graft–host border occupied by densely gliotic needle tract. Grafts are delineated by dotted lines in H-E/LFB–stained photomicrographs. Glial fibrillary acidic protein (GFAP)–stained sections confirm densely gliotic needle tracts abutting the transplants, which exhibit less gliosis than surrounding host parenchyma. Other than the needle tract, there are no significant gliotic borders between donor and host cells. Microtubule-associated protein 2 (MAP-2)–stained sections show dense labeling within the grafts, increased over adjacent host parenchyma, and the host–graft border is well delineated because of paucity of MAP-2–positive projections spanning the interface. The grafts are densely immunoreactive for synaptophysin.

Figure 1 Serial sections through representative grafts in both patients



ceived eight grafts: three in each putamen and one in each caudate nucleus. Her postoperative course was uncomplicated, and she was seen for follow-up at 6 months and 1 year after transplantation, with mixed results (table 2). She reported improved ambulation 3 months after transplantation. However, her speech continued to deteriorate. At 9 months, she maintained optimism with regard to the procedure, but reported frequent falls and, on clinical examination, exhibited marked chorea. At 2 years, the frequency of falls continued to increase, and she had more anger outbursts and was more dysarthric. At 3 years, she was in a wheelchair, and at 4 years after transplantation, she was wearing a protective helmet, was taking haloperidol, and was disoriented. She died of pneumonia in a nursing home at age 41 years.

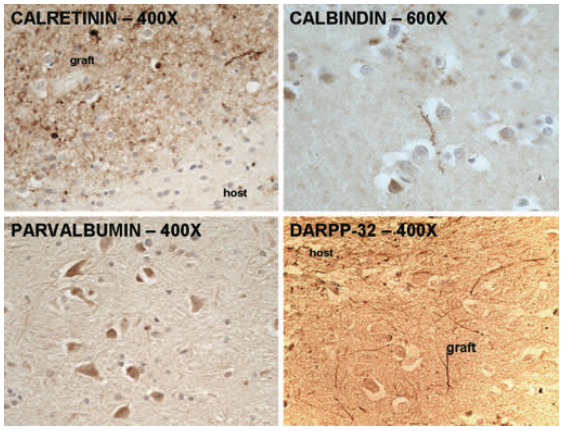
Although the rate of progression of disability from HD in these two patients was not followed by protocol over the full 7 years after transplantation, it was the impression of physicians who cared for both patients that the overall course of their illnesses was typical for HD despite the initial apparent slower progression in Patient 2.

General autopsy findings. Total brain weights were 1,190 and 1,145 g for Patient 1 and Patient 2. No evidence of chronic subdural hematoma was identified in either patient. Both patients had mild to moderate frontal cortical atrophy, severe ventricular dilatation, and severe atrophy of the caudate and putamen bilaterally. Microscopically, the striatum of Patients 1 and 2 showed expected changes of HD: marked astrogliosis and microgliosis of anterior caudate and putamen with severe reduction in neuron number and common neuronal intranuclear ubiquitin IR inclusions.

Grafts. All six grafts were identified in Patient 1, whereas seven of the eight grafts were identified in Patient 2 (figure 1); the left caudate graft was not found in Patient 2. All grafts were well circumscribed and readily identified histologically. Grafts contained cells with the morphologic features of neurons and astrocytes as well as dense neuropil. All transplants were associated with a tract of intense astrogliosis highlighted by GFAP IR that partially abutted the grafts; this was interpreted as scar secondary to cannulation. There was no evidence of neoplasia, and no contaminating leptomeningeal tissue was present. Away from needle tract gliosis, the borders between host and graft tissue did not display significantly increased astrocytic reaction (figure 1). Indeed, fewer GFAP positive cells were present in the grafts than in the surrounding parenchyma with only rare reactive astrocytes within grafts. Grafts were most easily identified by uniform and intense MAP-2 IR, neurofilament protein, and synaptophysin IR.

Neuronal differentiation within grafts. MSNs within the striatal matrix compartment reliably express calbindin D28k as well as parvalbumin, whereas calretinin IR neurons are largely confined to the striosome compartment.[13-15] Because expression of these calcium binding proteins is not specific to striatum, we also used antibodies to DARPP-32, a pro-

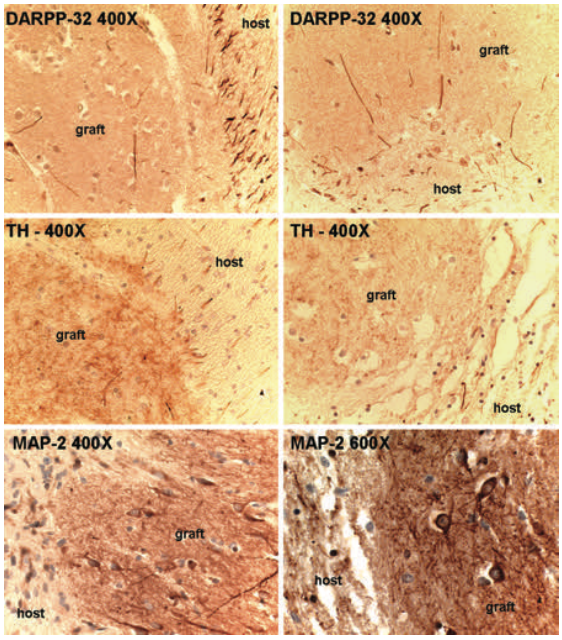
Figure 2 Sections through representative grafts showing variable differentiation of transplanted neurons



The majority of neurons within grafts either labeled with calbindin and dopamine and cyclic AMP–associated receptor phosphoprotein (DARPP-32) or calretinin. Although DARPP-32–positive projections were prominent within and outside of grafts, DARPP-32–positive neuronal somata were not labeled.

Tyrosine hydroxylase (TH)–positive axons are identified outside the grafts, but are increasingly rare within transplants and are exceedingly rare projecting across the interface. Dopamine and cyclic AMP–associated receptor phosphoprotein (DARPP-32)– and microtubule-associated protein 2 (MAP-2)–positive dendritic projections are present within and outside the grafts, but are also very infrequently identified crossing the graft–host interface.

Figure 3 Sections through representative grafts showing minimal dendritic and axonal connectivity across the graft–host interface



tein specifically expressed by MSNs. Calcium binding protein IR was variable among grafts, with some showing a mixture of focal calretinin and sparse calbindin IR in soma and processes whereas others showed mostly parvalbumin IR in soma and processes. All grafts showed DARPP-32 IR processes throughout (figure 2).

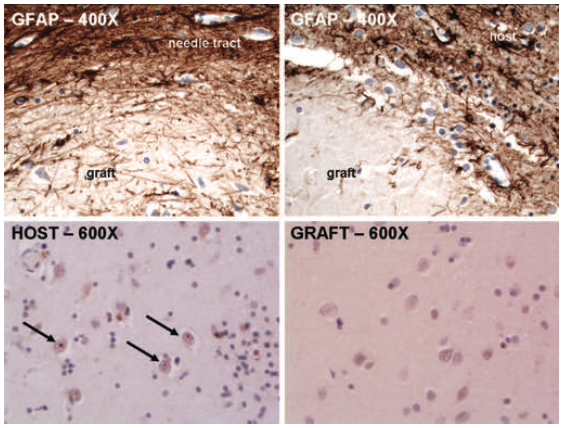
Integration of graft with host parenchyma. Only rare MAP-2 or DARPP-32 IR processes seemed to cross between host and graft parenchyma (figure 3); no convincing neurofilament IR process crossed the graft–host interface. In addition, no calbindin or parvalbumin IR fibers crossed the graft–host junction. In contrast, rare TH IR processes were present in the graft neuropil, and occasional TH IR fibers crossed the graft–host interface (figure 3).

Immune reaction to grafts. CD45, a pan-leukocyte marker, showed IR cells that were slightly increased in the host parenchyma surrounding grafts but not in grafts themselves; these marked predominantly as CD3 IR T cells, rather than CD20 IR B cells, and were commonly perivascular. CD68 IR microglia were increased in host striatum as expected; only rare CD68-positive cells were present in donor tissue (data not shown).

HD pathologic changes in grafts. The greatly reduced astrogliosis and microgliosis in grafts compared with adjacent host parenchyma suggest that grafted tissues did not succumb to the same disease process that affected the host. We evaluated this further with ubiquitin IHC. Striatal neurons in HD accumulate neuronal polyubiquitinated intranuclear inclusions; this was confirmed in both patients (figure 4). However, grafted neurons uniformly lacked ubiquitinated inclusions.

Glial fibrillary acidic protein (GFAP)–stained sections show rare reactive astrocytes within grafts. However, the host caudate and putamen have regularly spaced, stellate-appearing reactive astrocytes interspersed throughout the sampled tissues, consistent with progressive neurodegeneration of Huntington disease. Ubiquitin immunohistochemistry in host vs donor tissues. High-power view of host and grafted striatal neurons shows a high frequency of ubiquitin-positive neuronal intranuclear inclusions (arrows). However, no such inclusions are identified in donor-derived neurons.

Figure 4 Sections through representative grafts showing increased gliosis and microgliosis in host, rather than grafted, parenchyma



DISCUSSION This study is the first to examine fetal neural transplants in patients with HD several years after surgery. The findings in both patients were similar. Thirteen of 14 grafts were identified, and were characterized by nests of mostly neurons with occasional astrocytes and few microglia as well as dense neuropil. Although immunohistochemical analysis showed the grafts to express markers consistent with striatal neuronal differentiation, there was scant evidence of integration from the graft to the host, even though minimal gliosis bordered most of the graft. Some apparent innervation of graft by host TH IR processes was observed. Immune response was minimal despite years without immunosuppression. Finally, histopathologic changes characteristic of HD, including microgliosis, astrocytosis, and neuronal intranuclear polyubiquitinated inclusions, were not present in grafts.

Three clinical trials are investigating the efficacy of fetal human neural transplantation in HD, but each study has used different fetal tissues as donor sources. Whereas most MSNs are derived from the lateral ganglionic eminence, and precursors for these cells are most concentrated in the lateral portion of the lateral ganglionic eminence,[16] striatal interneurons develop in the medial ganglionic eminence and subsequently migrate to the striatum and cortex. Because MSNs are the predominant cells lost until late in HD, a logical approach is to transplant lateral ganglionic eminence. Indeed, the patients described here received whole lateral gan-

glionic eminence for donor tissue. Alternatively, Hauser et al.[9] chose to use the lateral half of the lateral ganglionic eminence for their transplant tissue. Finally, it has been proposed that the whole ganglionic eminence should be used also to recapitulate the entire striatum, including modulatory interneurons.[8,10] All three approaches have strong data indicating that the procedures are safe,[8-10] although there seems to be an increased risk of hemorrhage, specifically subdural hematoma, in patients with moderate to severe cortical atrophy.[9]

The donor tissues grafted into these patients seemed to adopt striosome or matrix phenotypes, based on differential calbindin/DARPP-32 and calretinin IR. Although the goal of this trial was to transplant MSN precursors (calbindin/DARPP-32) from the lateral ganglionic eminence, calretinin IR interneurons could have been derived in a number of ways: the embryonic donor lateral ganglionic eminence could contain migrating interneurons; interneurons could arise from transplantation of multilineage progenitor cells; and donor preparations may have contained medial ganglionic eminence because blocks of donor tissue, rather than suspensions, were used for transplantation in the patients described here. Comparison with autopsies from patients who received cell suspensions in other trials will shed additional light on these possibilities.

Another interesting comparison will be with the 12 HD patients who received porcine xenografts as part of a limited phase I clinical trial in the late 1990s.[17] Although long-term clinical performance and disease progression results have not been published yet and no autopsy data are available, it will be interesting to compare allotransplantation with xenotransplantation, particularly with regard to disease progression, host immune reaction to the grafted cells, and graft–host integration. Indeed, insights provided by these comparisons may help to elucidate mechanisms that impair graft–host communication and therefore provide options for adjuvant therapies aimed at enhancing engraftment.

We did observe apparent innervation of grafts by host TH IR processes. This finding is consistent with experiments that transplanted allogeneic mouse fetal ganglionic eminence into transgenic HD mice and showed host connectivity with the graft as determined by TH.[18] In contrast, we did not observe clear evidence for graft projection to host parenchyma. The basis for limited integration is unclear, but multiple possibilities exist. First, a gliotic scar could impair fiber migration, but this seems unlikely given the evidence for limited gliosis surrounding the grafts and the apparent innervation of grafts by TH IR processes. Second, grafts may require a more primitive or fetal environment for fiber extension and synaptogenesis. Third, difficulties may arise in integrating healthy grafted cells with tissue that is undergoing chronic degeneration. The second or third possibility could result from incompatible and disparate expression of growth factors, cell adhesion molecules, or neurotransmitters, and would promote an intrinsic graft network largely distinct from host parenchyma, a situation supported by our histologic findings. However, this seems to be at odds with studies of toxin-induced animal models of HD that have shown robust graft–host connections using adult host animals.[19-26]

These results are encouraging especially because they show greater than 6-year survival for 13 of 14 grafts with limited immunosuppression. However, highly restricted integration between graft and host parenchyma seems to be more of an obstacle to this therapeutic approach than would have been predicted from animal models and likely was the reason for the limited clinical benefit in these patients.

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