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Lewy body–like pathology in long-term embryonic nigral transplants in Parkinson's disease

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Fourteen years after transplantation into the striatum of an individual with Parkinson's disease, grafted nigral neurons were found to have Lewy body–like inclusions that stained positively for α-synuclein and ubiquitin and to have reduced immunostaining for dopamine transporter. These pathological changes suggest that Parkinson's disease is an ongoing process that can affect grafted cells in the striatum in a manner similar to host dopamine neurons in the substantia nigra. These findings have implications for cell-based therapies and for understanding the cause of Parkinson's disease.

In an attempt to mitigate the loss of striatal dopamine, some people with Parkinson's disease have received fetal ventral mesencephalic transplants. Double-blind, sham-controlled studies did not establish clinical benefit, although significant improvement was observed in subpopulations of subjects upon *post-hoc* analysis[1,2]. Postmortem studies performed about 18 months after transplantation showed robust survival of grafted neurons, with healthy-seeming implanted cells suggesting that grafted neurons were not affected by Parkinson's disease[3–8]. However, Parkinson's disease pathology progresses over decades, and it is possible that these cases did not survive long enough for Parkinson's disease pathology to develop in grafted cells. Additionally, new techniques for identifying Parkinson's disease pathology, such as the abnormal expression of α-synuclein[9], were established subsequently to these reports. Here, we examined the expression of Parkinson's disease–associated pathological markers in a person with Parkinson's disease who died 14 years after transplantation, the longest survival after transplantation that has been reported to date, and compared the neuroanatomical findings from this case to those from two other people who died 4 years after grafting.

This individual was a 61-year-old woman with a 22-year history of Parkinson's disease when she underwent transplantation in 1993. She was transplanted bilaterally with solid pieces of human ventral mesencephalon derived from 4 embryos aged 6.5–9 weeks post-conception into each side of the post-commissural putamen[1]. Prior to surgery, she experienced motor disability complicated by motor fluctuations and dyskinesias that could not be controlled with pharmacotherapy. After transplantation, she experienced improvement in measures of Parkinson's disease function, including unified Parkinson's disease rating scale (UPDRS) motor 'OFF' (off medication) scores, OFF time and dyskinesias, and required substantially lower doses of antiparkinsonian medications. By 1997, she had essentially no OFF time and only mild dyskinesias, and her UPDRS motor OFF scores remained substantially improved. By 2004, she experienced progressive worsening of Parkinson's disease features and was experiencing difficulty in gait, balance and falling that could not be controlled with medication. She continued to deteriorate until her death from cardiac arrest in 2007. After her death, the brain was removed and placed in a 4% Zamboni's fixative after an 11-h postmortem interval. This brain and two comparator brains from individuals who had undergone a similar fetal cell transplant procedure[1] and died approximately 4 years after grafting were processed

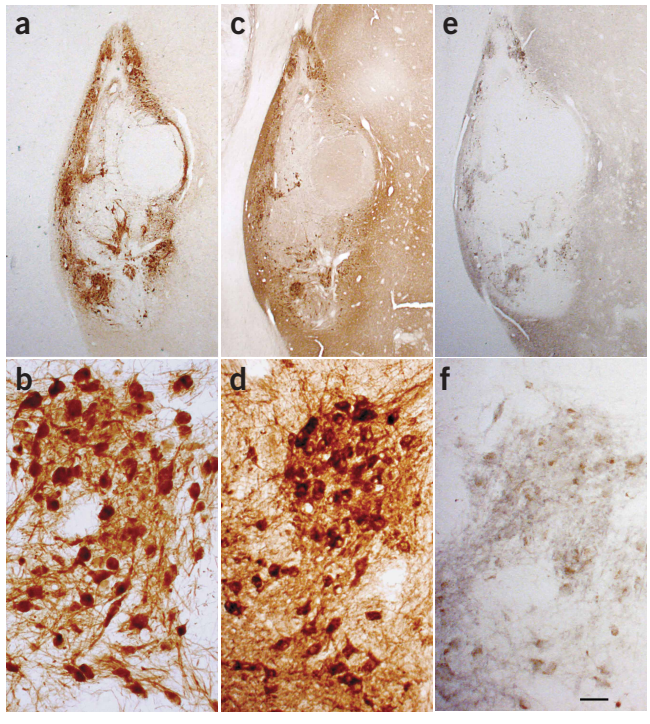

Figure 1 Long-term graft viability in Parkinson's disease. (**a**–**f**) Low- and high-power photomicrographs of a transplant located in the right post-commissural putamen from a person 14 years post-transplantation stained for tyrosine hydroxylase (**a**,**b**), VMAT2 (**c**,**d**) and DAT (**e**,**f**). These photomicrographs show that the graft is viable, as determined by tyrosine hydroxylase and VMAT2, but it lacks DAT expression. Scale bar represents 520 μm (**a**,**c**,**e**) and 80 μm (**b**,**d**,**f**).

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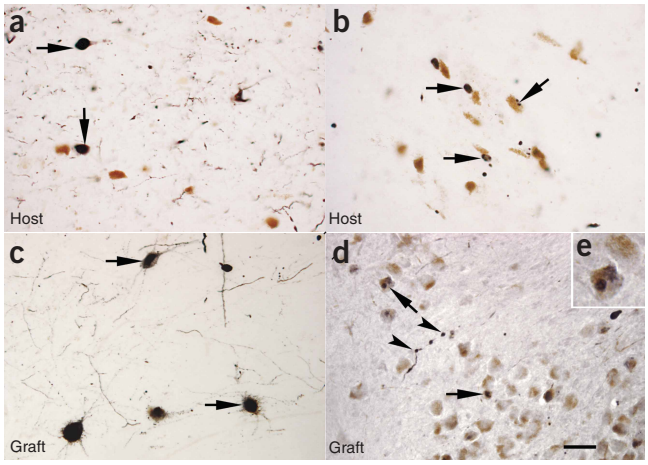

Figure 2 Parkinson's disease–like pathology in long-term nigral grafts. In the nongrafted host's nigra, typical α-synuclein (**a**) and ubiquitin (**b**) neuropathology was observed (arrows). (**c**) Extensive α-synuclein pathology was seen in grafted neurons, including cytoplasmic and aggregated α-synuclein (arrows) as well as α-synuclein neurites. (**d**,**e**) Pathological aggregates of ubiquitin were also seen in grafted neurons (arrows) and fibers (arrowheads). Scale bar, 40 μm. All people studied in this manuscript gave their informed consent to participate in transplant studies approved by the Institutional Review Board at the University of South Florida at Tampa. Proper informed consent was subsequently obtained for brain donation.

immunohistochemically using standard procedures for tyrosine hydroxylase, dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT2), α-synuclein, ubiquitin and CD45[1,5,8,10].

In the 14-year postmortem brain, tyrosine hydroxylase immunostaining revealed robust graft survival with a typical cytoarchitectonic appearance (**Fig. 1a**,**b**). These cells did contain discernible neuromelanin, a marker of nigral neurons, but at a level typical for the age of the cells. These cells provided extensive and dense tyrosine hydroxylase–immunoreactive innervation of the host striatum in a patch matrix pattern as previously described[5–7]. A similar staining pattern was seen with VMAT2 (**Fig. 1c**,**d**), another marker for dopaminergic neurons. In contrast, staining for DAT revealed very light to no staining in the graft (**Fig. 1e**,**f**), a finding that diverges from previous reports[6]. Within the host nigra of this individual, there was a considerable loss of tyrosine hydroxylase-immunoreactive and melanin-containing neurons. The remaining nigral neurons showed both cytoplasmic and aggregated α-synuclein (**Fig. 2a**) and ubiquitin (**Fig. 2b**) within nigral somata. Most surprisingly to us, we observed cytoplasmic, aggregated and neuritic α-synuclein in grafted neurons (**Fig. 2c**). The presence of abnormal protein aggregation in implanted cells was supported by the presence of ubiquitinated aggregates in grafted neurons (**Fig. 2d**), some of which had the appearance of Lewy bodies (**Fig. 2e**). The grafts in this case (**Supplementary Fig. 1a**,**b**) were filled with activated microglia to a degree that far exceeded the expression of microglia seen in the host striatum (**Supplementary Fig. 1c**) and nigra (data not shown). We also stained the two comparator brains for α-synuclein and ubiquitin. Numerous dopaminergic neurons were observed in these grafts 4 years after transplantation (**Supplementary Fig. 2a**,**b**) that showed α-synuclein staining that was mostly cytoplasmic and nonaggregated (**Supplementary Fig. 2c**). Ubiquitinated aggregates were not observed (**Supplementary Fig. 2d**).

This case reveals that pathological changes typical of Parkinson's disease can develop in human fetal neurons grafted into a host with Parkinson's disease. In our previously reported autopsy cases (18 months after transplant), metabolic measures and the phenotypic appearance of dopamine cells were normal, suggesting that the graft was unaffected by the disease process[5–7], perhaps because the time period after transplantation was too short to observe these pathological changes. In contrast, in the 14-year postmortem brain, numerous grafted neurons showed aggregated Lewy body–like structures that stained for α-synuclein and ubiquitin. In previous reports, we did not observe aggregated α-synuclein in nigral neurons in normal humans aged 29–102[10]. Indeed, neither cytoplasmic nor aggregated α-synuclein would be expected in the transplanted cells, as they were developmentally only 13–14 years old. Notably, we observed nonaggregated α-synuclein in grafted nigral neurons that survived for 4 years after transplantation even though cytoplasmic (nonaggregated) α-synuclein is not normally seen in nigral neurons until at least middle age[10]. Taken together, these findings suggest that abnormal Lewy body–like structures develop in implanted neurons as a result of an accelerated Parkinson's disease–like pathological process that affects grafted neurons. Further, young age may not protect transplanted neurons from the disease process, suggesting that the aging of dopamine neurons may not be the only essential factor for disease onset. We previously hypothesized that α-synuclein aggregates in sporadic Parkinson's disease result from an impaired capacity of the ubiquitin proteasome–autophagy system to clear unwanted proteins[10,11]. The present data suggest that this impairment may also be occurring in grafted cells in people with Parkinson's disease.

The presence of α-synuclein– and ubiquitin-positive aggregates in neurons 14 years after implantation into a person with Parkinson's disease has far-reaching implications. It has long been debated whether Parkinson's disease results from an acute insult that leads to progressive neurodegeneration or whether it is an ongoing pathological process that continues to affect previously healthy neurons. This study indicates that the mechanisms responsible for initiating the degenerative process are still present at this late stage of the disease and are capable of affecting grafted neurons. These data also indicate that the offending processes that destroy dopamine neurons in Parkinson's disease are not restricted to the midbrain. Finally, our findings argue that there must be either a pathogenic factor in the brain milieu that affects dopaminergic neurons or a pathological process that can spread from one cellular system to another.

It is noteworthy that the subject initially had marked improvement, but motor function gradually deteriorated. This deterioration may have been due to graft failure caused by the Parkinson's disease process or an immune reaction, as suggested by the extensive microglial infiltration of the graft, or due to disease progression affecting both dopaminergic and nondopaminergic regions. Reduced graft DAT expression despite tyrosine hydroxylase and VMAT staining was not seen in transplant subjects who had shorter survival times[1,4–6,8] and might be an early compensatory response to graft failure. It remains unclear whether the pathological sequelae seen in these grafted neurons are universal phenomena or indicative of a subset of grafted subjects. Furthermore, it is unclear whether a similar fate would befall stem cell grafts, the next generation of cell replacement procedures. Nonetheless, our results suggest that grafted cells can be affected by the disease process and thereby might limit the long-term clinical benefit of these treatment approaches.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS
J.H.K. and Y.C. performed the histological analysis. R.A.H. performed the clinical assessments. T.B.F. did brain ascertainment. J.H.K., R.A.H., T.B.F. and C.W.O. wrote the manuscript. C.W.O., T.B.F. and J.H.K. designed the original open label transplant trial. T.B.F. and C.W.O. implemented the original clinical transplant trial.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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