Mutant Huntingtin is Present in Neuronal Grafts in Huntington Disease Patients

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Objective: Huntington disease (HD) is caused by a genetically encoded pathological protein (mutant huntingtin [mHtt]), which is thought to exert its effects in a cell-autonomous manner. Here, we tested the hypothesis that mHtt is capable of spreading within cerebral tissue by examining genetically unrelated fetal neural allografts within the brains of patients with advancing HD.

Methods: The presence of mHtt aggregates within the grafted tissue was confirmed using 3 different types of microscopy (bright-field, fluorescence, and electron), 2 additional techniques consisting of Western immunoblotting and infrared spectroscopy, and 4 distinct antibodies targeting different epitopes of mHtt aggregates.

Results: We describe the presence of mHtt aggregates within intracerebral allografts of striatal tissue in 3 HD patients who received their transplants approximately 1 decade earlier and then died secondary to the progression of their disease. The mHtt aggregates were observed in the extracellular matrix of the transplanted tissue, whereas in the host brain they were seen in neurons, neuropil, extracellular matrix, and blood vessels.

Interpretation: This is the first demonstration of the presence of mHtt in genetically normal and unrelated allografted neural tissue transplanted into the brain of affected HD patients. These observations raise questions on protein spread in monogenic neurodegenerative disorders of the central nervous system characterized by the formation of mutant protein oligomers/aggregates.

ANN NEUROL 2014;76:31–42

Huntington disease (HD) is an autosomal dominant genetic disorder characterized by a clinical triad of a movement disorder, cognitive dysfunction, and psychiatric problems1 combined with a pathological CAG expansion in exon 1 of the huntingtin gene leading to the production of mutant huntingtin protein (mHtt).1,2 Wild-type Htt is a soluble protein that is ubiquitously expressed but is present in higher concentrations especially in the brain. This cytoplasmic and nuclear protein is associated with several organelles, microtubules, and vesicular membranes, pointing to a role in intracellular trafficking, exocytosis, endocytosis, and therefore synaptic functions.3 In HD, like all genetic trinucleotide disorders of the central nervous system, it has been suggested that the abnormal mutant protein causes cellular dysfunction through a cell-autonomous process in which only genotypically mutant cells exhibit the mutant phenotype. This results in mHtt aggregation, inclusion body formation,
and cell death, although how these events relate to each other is still debated.4

HD is incurable, and different experimental therapeutic strategies have been tested, including transplantation of fetal striatal tissue.5,6 This approach was predicated on the grounds that the primary pathology involves the striatum and that replacing it with unaffected allografts of fetal striatal tissue would be of benefit, as has been shown experimentally in non-transgenic animal models of HD.7–10 To date, these transplants have generally produced transient or no clinical benefits despite evidence that they survive in the short-term.11 This failure of clinical response to such targeted grafts may relate to the widespread pathology now recognized in HD from an early disease stage, but may also relate to the finding that these grafts survive poorly in HD patients and degenerate in a disease-like manner, as we have described previously.12 In this respect, we now show for the first time that mHtt aggregates can be found within the genetically normal transplanted tissue in 3 HD patients in whom there was long-term graft survival (the same patients were reported previously11–14).
Patients and Methods

**Patient Information**

Three of the original 7 HD patients grafted as part of the transplant trial conducted at the University of South Florida were analyzed. They all had 42 CAG repeats and were transplanted at 58, 64, and 59 years of age, respectively. An additional 28-year-old patient with 53 CAG repeats was also studied. All patients had manifest disease (ranging between 6 and 17 years of symptom duration) at the time that they received bilateral fetal striatal transplants. They all showed mild clinical improvements lasting at most 1 year, except for Patient 5, whose Unified Huntington’s Disease Rating Scale score worsened following grafting. They died between 9 and 12 years post-transplantation (Fig 1). All postmortem analyses of human brain tissue were approved by Ethical Research Committee of the Centre Hospitalier Universitaire de Québec (#A13-02-113).

**Immunohistochemistry**

Brains were processed using methods previously published. Autolysis time was 5, 4, 5, and 2 hours for Patients 1, 3, 5, and 7 respectively. Standard histology was undertaken to identify the grafts macroscopically. Additional postmortem analyses included double immunohistochemical staining for neuronal elements using an antibody against neuronal nuclei (NeuN; anti-mouse, MAB377; EMD Millipore, Billerica, MA; 1:2,500) as well as the anti-mHtt antibodies EM48 (mouse anti-human huntingtin clone EM48, MAB5374; EMD Millipore; 1:2,000) and MW7 (mouse anti-human; obtained from the Developmental Studies Hybridoma Bank, Iowa City, IA; 1:100) to identify mHtt aggregates. Photomicrographs were taken using Picture Frame software (Microbrightfield, Williston, VT) linked to an E800 Nikon microscope (Nikon Instruments, Melville, NY).

**Stereology for EM48 Aggregate Count**

The density of EM48 mHtt deposits, reported as the number of aggregates/mm² of tissue, was assessed in the cortex and putamen of the host brains as well as in p-zones and non-p-zones of the grafts using standard techniques (see Cisbani et al). All sections were observed at 60× and imaged on a Fluoview FV1000 confocal microscope system equipped with 559nm and 635nm laser diodes and an Ar 488nm laser (Olympus Canada, Richmond Hill, Ontario, Canada).

**Statistical Analyses**

To calculate the density of aggregates, a negative binomial distribution was used. Step-down Bonferroni correction was further employed to ensure that the overall significance level of the multiple comparison tests was 0.05. One-way analysis of variance was used to compare aggregate size. All statistical analyses were performed using the MIXED and GENMOD procedures of SAS (v9.2; SAS Institute, Cary, NC).

**Immunofluorescence**

A series of double and triple immunofluorescent staining was also performed to localize mHtt, using either EM48 (1:200) or MW7 (1:100; Supplementary Table). The following antibodies were used for the detection of specific cell populations: astrocytes (rabbit anti–glial fibrillary acidic protein (GFAP), Z0334; Dako Corporation, Burlington, CA; 1:500), microglia (rabbit anti-ionized calcium-binding adapter molecule 1 (iba1), 019–19741; Wako Chemicals, Richmond, VA; 1:800), neurons (rabbit anti–microtubule-associated protein 2 (MAP2), 17490-1-AP; Proteintech Group, Chicago, IL; 1:500) or neuronal cytoskeleton (chicken anti-neurofilament H (NFL-H), AB5539; EMD Millipore; 1:400), oligodendrocytes (rabbit anti–CAII, 1:2,000), perivascular macrophages (rabbit anti–CD163, NBP1–30148; Novus Biologicals, Littleton, CO), vascular endothelium (rabbit anti-laminin, Z0097; Dako Corporation; 1:500), and extracellular matrix (rat anti-phosphocan, MAB2688; R&D Systems, Minneapolis, MN; 1:100). Ubiquitinated aggregates were also identified using an anti-rabbit ubiquitin antibody (Z0458, Dako Corporation; 1:100). Some of these antibodies required an additional post-fixation with paraformaldehyde 4% pH 7.4 (Sigma-Aldrich, St Louis, MO) for 1 hour prior to staining (see Supplementary Table). Sections were stained using standard procedures (see Cisbani et al). All sections were observed at ×60 and imaged on a Fluoview FV1000 confocal microscope system equipped with 559nm and 635nm laser diodes and an Ar 488nm laser (Olympus Canada, Richmond Hill, Ontario, Canada).

**Sequential Method for Chromogenic Immunohistochemistry**

We undertook an additional series of experiments to investigate the colocalization of EM48 with MW7 and ubiquitin. To do this, we applied a sequential staining protocol using the chromogen 3-amino-9-ethylcarbazole. To confirm the complete removal of the primary antibody, the sections were also incubated solely in the secondary antibody. Photomicrographs were taken by Picture Frame software (Microbrightfield) linked to an E800 Nikon microscope.

**Electron Microscopy**

Specimen areas of a 30µm nickel-intensified DAB-stained brain section were prepared by flat embedding in epoxy resin (M. A. Hayat). The embedded samples were then serially sectioned in parallel at 60nm with a diamond knife (Delaware Diamond Knives, Wilmington, DE) on an ultramicrotome (Ultracut E; Reichert-Jung, Wien, Austria). The sections were placed onto a single-hole copper/Formvar grid and stained with 3% aqueous uranyl acetate for 5 minutes. After drying, observation was performed using a Jeol JEM-1400 electron microscope.
performed with an electron microscope, JEM-1230 (JEOL, Tokyo, Japan) set at 80kV.12

**Protein Extraction from Fixed Tissue**

Brain sections were selected and dissected to isolate pieces of the cortex in both HD and control patients, as well as the grafted tissue. This was performed on 15 sections for each condition. The dissected tissue was collected into Eppendorf tubes, weighed, frozen on dry ice, homogenized in 4 volumes of extraction buffer (50mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 150mM NaCl, 10mM ethylenediaminetetraacetic acid [EDTA], 1% triton X-100 [Sigma-Aldrich], 0.5% deoxycholate [Sigma-Aldrich], 0.1% bovine serum albumin [Bishop Canada, Burlington, Ontario, Canada], 200mM dithiothreitol [DTT], 2% sodium dodecyl sulfate [SDS; Sigma-Aldrich], 20mM Tris HCl pH 8.8 [Sigma-Aldrich],19,20 containing a cocktail of protease [Roche, Basel, Switzerland] and phosphatase inhibitors [Sigma-Aldrich], and sonicated. The homogenate was then incubated at 100°C for 20 minutes followed by an incubation of 1 hour at 80°C on a shaker (80rpm). Finally, the tubes were spun for 20 minutes at 4°C at 13,000rpm, and the supernatant was collected and stored at −80°C until use. Total protein concentration was determined using the Pierce 660nm protein assay (Thermo Fisher Scientific, Waltham, MA).

**Western Blotting**

All reagents and chemicals used for immunoblotting were purchased from Sigma-Aldrich, unless otherwise specified. Fifteen micrograms of protein was prepared in the sample buffer (250mM Tris-HCl, 2% wt/vol lithium dodecyl sulfate, 100mM DTT, 0.4mM EDTA, 10% vol/vol glycerol (Thermo Fisher Scientific), 0.2mM bromophenol red, 0.2mM Brilliant Blue G, pH 8.5) and heated for 10 minutes at 70°C. The proteins were then separated for 5 hours at 150V on a 3 to 8% gradient hand-cast polyacrylamide gel (37.5:1 acrylamide:bisacrylamide scale bars: A = 300µm, B–E = 20µm). The proteins were electroblotted onto 0.45µm Immobilon polyvinylidene difluoride membranes (EMD Millipore) overnight at 15V in the transfer buffer (20% methanol [Thermo Fisher Scientific], 25mM bicine, 25mM bis-Tris, 1mM EDTA, 1.3mM sodium bisulfite, pH 7.2). After being blocked in a solution of 5% skimmed milk, 0.5% bovine serum albumin (BioShop Canada, Burlington, Ontario, Canada) in phosphate-buffered saline, 0.1% Tween-20 (Thermo Fisher Scientific), the membranes were immunoblotted with MW1 (anti-mouse antibody, 1:1,000) overnight at 4°C followed with horseradish peroxidase-conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch, West Grove, PA; 1:30,000) and detected by the addition of chemiluminescence reagents (Luminata Forte; EMD Millipore). The membranes were then stripped and reprobed for total Htt (anti-mouse antibody, clone: 1HU-4C8, MAB2166; EMD Millipore: 1:1,000), glyceraldehyde 3-phosphate dehydrogenase (anti-mouse GAPDH, G041; Applied Biological Materials, Vancouver British Columbia, Canada; 1:7,500), and NeuN (anti-mouse, MAB377, EMD Millipore, 1:1000). Images of the membranes were acquired using myEcl Imager (Thermo Fisher Scientific). Band intensities were quantified using Carestream Molecular Imaging Software (v4.0.5f7; Eastman Kodak, Rochester, NY). The graphs were generated using the Prism software (v4.0; GraphPad Software, San Diego, CA) and assembled in Illustrator CS5 (Adobe Systems, San Jose, CA).

**Infrared Spectroscopy**

Infrared (IR) spectroscopy was used to detect structural protein changes such as the β-sheets of mHtt within the HD host brain and grafted tissue of unstained sections.22,23 Fourier transform IR focal plane array maps were acquired using a 32 × 32 detector with a pixel size of 5.5µm, giving 1,024 spectra/map. The IR spectra were collected on a Nicolet Magna-IR 550 FT spectrometer (Thermo Nicolet, Madison, WI) equipped with a deuterated triglycine sulfate detector and a germanium-coated KBr beam splitter. IR data were treated with Omnic E.S.P. 5.1 (Thermo Nicolet) and Grams 32 (Spectral Notebase, v4.11; Galactic Industries Corporation, Salem, NH).
All the images were prepared using Adobe Photoshop CS5, and when necessary, brightness and contrast adjustments were made. All panels were assembled using Adobe Illustrator CS5.

Results
Surviving neuronal transplants with a compartmentalized organization were observed in 3 of 4 transplant recipient brains that we have reported previously (Figs 1 and 2). Within the surviving transplants of all 3 patients, we observed patches of striatal-like neuronal tissue referred to as p-zones, as well as areas where there was no expression of striatal phenotypes (non p-zones).

In the fourth patient (Patient 3 [M.C.] from our original series), no surviving transplants were found.

Aggregated mHtt protein was observed in the genetically unrelated transplanted tissue in all 3 HD patients with surviving grafts (see Figs 1 and 2). Aggregates
FIGURE 4: Localization of MW7\textsuperscript{+} mutant huntingtin protein (mHtt) aggregates in grafts and in the Huntington disease (HD) host cortex. As observed for EM48\textsuperscript{+} staining, MW7\textsuperscript{+} aggregates identified within the grafts did not colocalize with neurons (MAP2; see Fig 3B), microglia (Iba1; A), astrocytes (GFAP; B), and perivascular macrophages (CD163; C). In the HD host cortex, MW7\textsuperscript{+} aggregates (similar to EM48\textsuperscript{+} aggregates) were localized within neuronal elements (MAP2; D) but were not found within microglia (Iba1; E), astrocytes (GFAP; F), or perivascular macrophages (CD163; G). Scale bars: A–D = 20\textmu m, E–G = 50\textmu m.

FIGURE 5: Localization of EM48\textsuperscript{+} mutant huntingtin protein (mHtt) aggregates in the Huntington disease (HD) host cortex. (A–E) Double immunofluorescence for MAP2 (red) and EM48 (green) demonstrating the colocalization of mHtt\textsuperscript{+} aggregates in dendrites (A, C) and soma (D) of cortical cells, as visualized in the brains of HD transplanted patients. The presence of EM48\textsuperscript{+} aggregates within dendrites of cortical cells was further demonstrated using a double immunofluorescent staining with neurofilament H (NFL-H) (B). A significant number of EM48\textsuperscript{+} aggregates were also found within the extracellular matrix of the host cortex, as demonstrated with the marker phosphocan (cyan; E). (F) EM48\textsuperscript{+} mHtt aggregates were also found in the basal lamina of blood vessels. Inset depicts an EM48\textsuperscript{+} inclusion within the nucleus of a cell type associated with a blood vessel (4',6-diamidino-2-phenylindole [DAPI] staining in blue). (G–J) EM48\textsuperscript{+} mHtt aggregates were not found in microglia (Iba1; G), astrocytes (GFAP; H), perivascular macrophages (CD163; I), or oligodendrocytes (CAII; J). Scale bars: A–J = 20\textmu m.
identified with either EM48 or MW7 were frequently found within both the p-zones (see Figs 1A-C, F-I, 2A-C) and non p-zones of the grafts (see Figs 1A, C-D, F, H-I, 2A, C-D). The size of the aggregates was similar in both the grafts and surrounding non-transplanted putamen. Despite similar morphology, the number of aggregates was slightly less in the graft compartments than in the host putamen. In the host cortex, mHtt deposits were the largest and the most numerous (Fig 1, bar graphs).

Within the transplanted tissue, mHtt aggregates were localized to the extracellular matrix of both the p-zone and non p-zone throughout the grafts, as confirmed using 2 distinct antibodies (EM48 and MW7; Fig 3A-B). EM48+ and MW7+ aggregates within transplants were not observed in neurons (see Figs 3A-B), microglia (see Figs 3C, 4A), astrocytes (see Figs 3D, 4B), perivascular macrophages (see Figs 3E, 4C), oligodendrocytes (data not shown), endothelial cells (data not shown), or blood vessels (see Fig 3F, data not shown for MW7).

In contrast, in these same patients, mHtt protein aggregates found within the non-transplanted cortex were localized to the neurons and neuropil (see Figs 4D, 5A-D), as previously described.25,26 mHtt was also frequently observed within the extracellular matrix of the host cortex (see Fig 5E), a finding that has not been previously reported. Additionally, aggregates of mHtt protein were observed within the vascular space of the HD brain (see Fig 5F), including within cells of the vascular space (see Fig 5F, inset, EM48/4′,6-diamidino-2-phenylindole staining (DAPI)). Aggregates of mHtt protein (stained either with MW7 or EM48) were not observed in host microglia (see Figs 4E, 5G), astrocytes (see Figs 4F, 5H), perivascular macrophages (see Figs 4G, 5I), or oligodendrocytes (see Fig 5J), nor within endothelial cells (data not shown).

Colocalization of EM48+ aggregates and the protein ubiquitin was confirmed within the grafted tissue (Fig 6A) and the cortex (see Fig 6B). Using a technique of chromogen stripping,15,16,18 we further demonstrated that EM48, ubiquitin, and MW7 colocalize in the cortical tissue of transplanted HD patients (Fig 7). To confirm the presence of these aggregates, we further performed electron microscopy (Fig 8), Western immunoblotting (Fig 9), and IR spectroscopy (Fig 10), all of which corroborated our original immunohistochemical findings of aggregates in the graft and host brain.

Discussion

Here we describe for the first time that mHtt can be found within genetically unrelated tissue grafted into the brains of patients with advancing HD. The mHtt was localized to
the extracellular matrix of the transplants. This differs from the localization of mHtt within the non-grafted regions of the brain of patients with HD, where the mHtt protein is primarily localized to neurons and the neuropil, as has been described before in HD.25,26

The fetal striatal allografts implanted in these patients with HD are derived from normal donors not carrying the mutant gene and thus mHtt. This raises 2 questions: (1) How did the mHtt protein from the patient become localized within the transplanted tissue and (2) Did this localization of mHtt within the transplants contribute to their compromised survival?11–13 Several possible mechanisms can be put forward to explain these findings.

mHtt Protein Transmission, Deposition, or Diffusion into the Transplant

In another neurodegenerative disorder of the central nervous system, Parkinson disease (PD), it has been demonstrated that the pathologically associated protein α-synuclein (ie, Lewy body pathology) could spread into the allografted ventral mesencephalic tissue.27,28 This was hypothesized to occur in a prion-like fashion.27,29–32 Both in vitro and in vivo studies have subsequently demonstrated that α-synuclein can be released and taken up by neurons33,34 and seed pathology. Intracerebral inoculation of brain homogenates derived from α-synuclein transgenic mice, or injection of synthetic α-synuclein preformed fibrils, accelerates the formation of α-synuclein protein aggregates and precipitates neurological dysfunction in animals.35 A similar propagation of tau pathology has been described in a model of early Alzheimer disease (AD) and tauopathies.36,37 It is therefore possible that mHtt was transferred from the patients’ brains to the grafts via a similar mechanism. However, unlike the host brain, where mHtt aggregates were mainly seen within the neuropil, inclusions in the grafts were only observed within the extracellular matrix and not within any cellular elements. Therefore, within these case reports, we do not have direct evidence of cell-to-cell propagation of mHtt.

Findings from in vivo models of AD suggest that neurodegeneration in this disease results from tau pathology being spread via trans-synaptic connections.30,38 It is possible that mHtt could similarly be transmitted trans-synaptically to the transplants from the diseased cortex, and in this respect we have previously demonstrated synaptic connections between the diseased cortex and the grafted neurons in these HD patients.12,39 Although we did not observe mHtt within grafted neurons, the extracellular localization of aggregates within the p-zones could be derived from dying cortico-striatal synaptic terminals that innervated the transplant12 and expressed mHtt, possibly leading to graft cell lysis and degeneration with release of the aggregates within transplant neuropil. However, extracellular localization of mHtt was also observed within non p-zones of the grafts, structures that do not receive direct projections from cortical neurons. This would suggest that, at most, this mechanism is partially involved in the mHtt spread and other mechanisms of protein transport may exist.

An alternative possibility related to this is that aberrant cortical striatal neurons containing the mHtt protein may leave axonal debris within transplant extracellular spaces in the process of undergoing cell death. In support of this hypothesis, a large number of aggregates that did not colocalize with MAP2+ grafted neurons were nonetheless found in close proximity to these neuronal elements.
Regardless of the mechanism of cortical interactions with the putamen and graft (trans-synaptic transmission of mHtt vs. mHtt deposits via synaptic debris), our results suggest a time-dependent influence of the cortex on the putamen and transplant. The mHtt deposits are the most abundant and largest in the host cortex. They are smaller and less abundant in the putamen. The mHtt deposits in the grafts are the same size but less abundant than deposits in the host putamen. The transplants have been exposed to the disease process for a shorter period of time than the putamen, as the patients already had the disease at the time of transplantation. This suggests that the number of deposits correlates with the amount of time that the striatal tissue is exposed to the disease process if it originates in the cortex.

In vivo models of AD have also demonstrated that pathology may be associated with diffusion of the soluble form of Aβ in the extracellular space with uptake by cells in the vicinity. Ren et al also described that fibrillar polyglutamine peptide aggregates can be internalized by mammalian cells in vitro. It is similarly possible that the localization of mHtt in our transplants results from diffusion of mHtt from the putamen to the transplants via the extracellular matrix. Uptake of this mutant protein may then compromise the viability of the cells in the graft that have endocytosed it. However, in our postmortem samples, we have no evidence of mHtt in any cell type within the graft, which would argue against such a mechanism compromising the transplants.

**Oxidative Stress, Excitotoxicity, Inflammation, and Poor Trophic Support**

These mechanisms have been implicated in neuronal transplant pathology in both PD and HD. CAG repeat length gains may occur in non-dividing cells, independent of the DNA replication process. It is possible that oxidative stress, excitotoxicity, inflammation, or a poor trophic milieu may induce pathological polyglutamine expression in the transplants. However, Shelbourne et al and Kennedy et al noted this CAG expansion within neurons and glia, whereas we only saw the abnormal CAG in the extracellular milieu of the grafts. Furthermore, if such a mechanism was dominant, one...
would expect to see similar inclusions in other types of grafts such as ventral mesencephalon transplants in PD, which is not the case.

**Hematogenous Transport of mHtt**
mHtt aggregates were occasionally observed within the HD cerebral blood vessels (see Fig 3F), within cells of these blood vessels (see Fig 3F, inset), and possibly within perivascular macrophages of the host brain (see Fig 8). These new observations raise the possibility that mHtt could be transported from the host to the transplant via blood-borne cells such as those of the immune system. Interestingly, it was recently shown that certain types of immune cells can contribute to disease progression in animal models of HD. This mechanism is further supported by the diffuse localization of mHtt within both the p-zone and non p-zone aspects of the graft. This potential mechanism of HD pathology in man has not been previously reported. If true, then the morphological and quantitative differences in mHtt depositions between the cortex, putamen and grafts would suggest that these different regions of the brain have different susceptibility to mHtt or differences in vascularity and/or blood–brain barrier permeability.

Previously, we and others have reported the absence of mHtt aggregates within transplants in HD patients. Our new observation that mHtt inclusions are present within grafts differs from these previous reports and can be explained in 2 ways. As in fetal ventral mesencephalon grafts in PD, α-synuclein depositions developed in a time-dependent manner and were only observed in grafts exposed to the disease process for >1 decade. Likewise, Freeman et al and Keene et al examined transplants in HD patients that were exposed to the disease process for comparatively short times (18 months, 6 years, and 7 years, respectively). In Cicchetti et al, 1 of the 3 patients did not show graft survival precluding histological evaluation. However, in the other 2 subjects in this paper, we did not observe mHtt aggregates using standard techniques of the time. We therefore sought to re-examine these cases in this report using several techniques that were not available at the time of the original publication,
including some techniques developed for this study, and we now can show that these grafts do contain mHtt.

This is the first demonstration in vivo of the transmission of a gene product to genetically unrelated cells. In genetic disorders such as HD, pathogenesis is thought to occur via cell-autonomous mechanisms. The localization of mHtt expression within grafted tissue suggests that non–cell-autonomous mechanisms may also play an important role in mHtt spread within the HD brain. Further research is needed to determine the scientific, clinical, and therapeutic implications of these findings for patients with HD and possibly other genetic disorders.

Acknowledgment
This work was supported by a grant from the International Organization of Glutaric Acidemia awarded to F.C. Salary support for F.C. and S.L. was provided by the Fonds de recherche du Québec en santé. G.C. was supported by the Bourse d’excellence du Centre thématique de recherche en neurosciences (Laval University) and I.S.-A. by a fellowship from a joint program of the Huntington Society of Canada and Canadian Institutes of Health Research. The Cambridge Brain bank is supported by an NIHR Biomedical Research Centre award to the Addenbrooke’s Hospital and University of Cambridge.

We thank R. Janvier for his very skillful electron microscopy sample preparation and analyses; M. Leroy, Dr P. Chevalier, L. Levesque, and Dr G. Larochelle for Fourier transform IR analysis; and G. Sutter and P. Pierce, who participated in the procurement of the brain from Patient 7. The MW7 and MW1 antibodies developed by Dr P. H. Patterson were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the Department of Biology, University of Iowa, Iowa City, IA.

Authorship
F.C. made the observation of the presence of mHtt within the grafted tissue, and was involved in experimental designs, image acquisition, and data interpretation. She supervised the project and wrote the manuscript. S.L. was involved in data interpretation and manuscript writing. G.C. performed most of the immunohistochemical and immunofluorescent staining and aggregate quantifications, and some of the image acquisition, and was responsible of assembling all figure panels. N.V. helped troubleshoot immunofluorescent protocols and performed all the confocal image acquisition. M.S.-P. performed some of the immunohistochemical and immunofluorescent staining as well as some of the image acquisition. I.S.-A. performed the Western immunoblotting. R.T. performed the IR spectroscopy. J.N.S. helped with the electron microscopy analyses/interpretation. R.A.H. ensured the clinical follow-up of the patients. D.M. provided expertise for the IR spectroscopy. R.A.B. was involved in manuscript writing. T.B.F. was involved in data interpretation and manuscript writing.

Potential Conflicts of Interest
Nothing to report.

References


