Pathogenic Huntingtin Repeat Expansions in Patients with Frontotemporal Dementia and/or Amyotrophic Lateral Sclerosis


1. Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA
2. Computational Biology Core, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA
3. Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY 10032, USA

4. Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA 2118, USA

5. Boston University Alzheimer’s Disease Center, Boston University School of Medicine, Boston, MA 2118, USA

6. Research and Development Service, Veterans Affairs Boston Healthcare System, Boston, MA 2130, USA

7. Department of Veterans Affairs Medical Center, Bedford, MA 1730, USA

8. Neurodegenerative Diseases Research Unit, Laboratory of Neurogenetics, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA


10. Department of Neurology, University of Massachusetts Medical School, Worcester, MA 1655, USA

11. see supplementary appendix for consortium members

12. Department of Molecular Neuroscience, Institute of Neurology, University College London, London, WC1B 5EH, UK

13. Department of Anatomy, Physiology and Genetics, Physiology and Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

14. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322, USA

15. Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA
16. Department of Neuroscience & Department of Clinical Genomics, Mayo Clinic, Jacksonville, FL 32224, USA
17. The Scripps Translational Science Institute, The Scripps Research Institute, La Jolla, CA 92037, USA
18. Longitudinal Studies Section, National Institute on Aging, Baltimore, MD 21224, USA
19. Laboratory of Behavioral Neuroscience, National Institute on Aging, Baltimore, MD 21224, USA
20. Division of Neuroscience and Experimental Psychology, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, M13 9PT, UK
21. Program in Behavioral Neuroscience, Department of Neurology, Boston University School of Medicine, Boston, MA 2118, USA
22. Department of Neurology, Veterans Affairs Boston Healthcare System, Boston, MA 2130, USA
23. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
24. Department of Neurology, Columbia University Irving Medical Center, New York, NY 10032, USA
25. Department of Clinical and Movement Neuroscience, Royal Free Campus UCL Institute of Neurology, University College London, London, NW3 2PF, UK
26. Rita Levi Montalcini Department of Neuroscience, University of Turin, Turin, 10126, Italy
27. Institute of Cognitive Sciences and Technologies, C.N.R., Rome, 185, Italy
28. Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, 10126, Italy
29. Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

30. The American Genome Center, Collaborative Health Initiative Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

31. Department of Neurology, Johns Hopkins University Medical Center, Baltimore, MD 21287, USA

* contributed equally to this manuscript

Corresponding authors: Bryan J. Traynor, M.D., Ph.D., Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, 35 Convent Drive, Room 1A-213, Bethesda, MD 20892-3707. Email: traynorb@mail.nih.gov

Phone: (301) 451-7606
Abstract

Background

Although repeat expansions are a significant cause of neurodegenerative disease, systematic evaluation of this mutation type in non-Alzheimer’s dementias has been limited. Recent advances in whole-genome sequencing offer novel opportunities to map unstable DNA repeats and examine their role in the pathogenesis of complex dementias.

Methods

We performed repeat sizing of ten pathogenic genetic loci previously implicated in human disease using whole-genome sequence data from 2,442 patients clinically and/or pathologically diagnosed with frontotemporal dementia (FTD) and/or amyotrophic lateral sclerosis (ALS), 2,599 patients diagnosed with Lewy body dementia (LBD), and 3,158 neurologically healthy subjects.

Results

Pathogenic expansions (range: 40 to 64 CAG repeats) in the huntingtin \((HTT)\) gene were found in three (0.2%) of patients diagnosed with pure FTD/ALS syndromes but were not present in the LBD or healthy cohorts. We replicated our findings in an independent cohort, identifying five (0.13%) out of 3,674 patients with FTD/ALS spectrum disorders. None of the FTD/ALS patients carrying the pathogenic \(HTT\) expansion had choreoathetosis or a family history of Huntington’s disease. Postmortem evaluations of two patients revealed huntingtin-positive, as well as TDP43- and ubiquitin-positive aggregates, predominantly in the frontal cortex. There was no atrophy of the neostriatum, the pathological hallmark of Huntington’s disease, thereby ruling out mimic syndromes.
Conclusions

Our findings confirm an etiological relationship between $HTT$ repeat expansions and FTD/ALS syndromes. As antisense oligonucleotide therapies targeting this pathogenic mutation have already progressed to clinical trials, genetic screening of patients presenting with FTD/ALS for $HTT$ repeat expansions should be considered.
Frontotemporal dementia (FTD, OMIM #600274) and amyotrophic lateral sclerosis (ALS, OMIM #105400) are progressive neurodegenerative disorders that are characterized clinically by cognitive deficits, language abnormalities, and muscle weakness.\textsuperscript{1,2} These aggressive illnesses typically occur between the ages of 40 and 70, leading to death within three to eight years of symptom onset.\textsuperscript{2,3} Approximately 15,000 individuals die of FTD and ALS in the United States annually\textsuperscript{4}, and there are no treatments that can halt the degenerative progression. Clinical, genetic, and neuropathologic data demonstrate that FTD and ALS are closely related conditions that exist along a spectrum of neurological disease.\textsuperscript{5}

Though progress has been made, much remains unclear about the genetic etiology of FTD and ALS. Approximately 50\% of FTD cases are familial, and causative mutations have been identified in several genes, most notably \textit{MAPT}, \textit{GRN}, \textit{C9orf72}, and \textit{VCP}.\textsuperscript{6} In ALS, 10\% of patients report a family history of the disease. The genetic etiology is known for approximately two-thirds of these familial cases, whereas the underlying gene is known in 10\% of sporadic cases.\textsuperscript{7} The intronic repeat expansion of the \textit{C9orf72} gene is the most common cause of both FTD and ALS.\textsuperscript{8} Other repeat expansions have been implicated in neurological diseases. These include polyglutamine repeats observed in Huntington’s disease\textsuperscript{9} and spinobulbar muscular atrophy\textsuperscript{10}, and more complex expansions in the \textit{RFC1} gene that was recently associated with autosomal recessive cerebellar ataxia.\textsuperscript{11} Together, these data suggest that repeat expansions play a critical role in the pathogenesis of neurodegenerative diseases. This type of mutation may be amenable to antisense oligonucleotide therapy, adding further incentive to their identification.\textsuperscript{12}

The discovery of new genetic causes of FTD and ALS provides insights into the cellular mechanisms underlying neurodegeneration.\textsuperscript{7} From a clinical perspective, the molecular characterization of the genetic causes of disease in a patient assists in establishing an accurate
diagnosis and the genetic counseling of the patients and their family. It is also a necessary first step in preparation for future precision medicine. To explore the genetic architecture of FTD and ALS, we performed whole-genome sequencing in large cohorts of patients diagnosed with FTD/ALS spectrum disorders and neurologically healthy individuals. We used these data to systematically assess the role of previously identified, disease-causing repeat expansions in the pathogenesis of FTD and ALS.

Methods

Study subjects

The workflow of this study is depicted in Fig. S1. The discovery cohort included (i) 1,377 patients diagnosed with FTD spectrum disorders including the known subtypes of behavioral variant FTD, primary progressive aphasia, and progressive supranuclear palsy (PSP), (ii) 1,065 patients diagnosed with ALS, (iii) 2,599 individuals diagnosed with Lewy body dementia (LBD), and (iv) 3,158 neurologically healthy participants. Patients with FTD were diagnosed according to the Neary criteria\textsuperscript{13} or the Movement Disorders Society criteria for PSP.\textsuperscript{14} Patients with ALS were diagnosed according to the El Escorial criteria\textsuperscript{15}, whereas the LBD cases were diagnosed with pathologically definite or clinically probable disease according to consensus criteria.\textsuperscript{16,17} The LBD cases were included in this study as diseased control subjects. All participants included in the aged, healthy control cohort were free of neurological disease based on history and neurological examination (mean age = 77.0 years of age at collection, interquartile range = 69.0–86.0). All study participants were of European ancestry. Table S1 lists demographic characteristics of the cohorts.
For replication, we used DNA obtained from 1,009 patients diagnosed with FTD, 2,665 patients diagnosed with ALS, and 210 neurologically healthy individuals. The institutional review boards of participating institutions approved the study, and informed consent was obtained from all subjects or their surrogate decision-makers, according to the Declaration of Helsinki.

Whole-genome sequencing and repeat expansion analysis

Sequencing was performed on an HiSeq X Ten sequencer using PCR-free library preparations and 150-base-pair, paired-end cycles (version 2.5 chemistry, Illumina). The alignment is described in the supplementary appendix. ExpansionHunter - Targeted software (version 3.0.1) was used to estimate repeat lengths of ten known, disease-causing expansions in samples that had undergone whole-genome sequencing. This algorithm has been validated using experimentally-confirmed samples carrying expansions, including HTT. Fully-penetrant pathogenic alleles in the huntingtin (HTT) gene were defined as those containing 40 or more CAG repeats according to the American College of Medical Genetics diagnostic criteria. The number of repeats was validated using a repeat-primed PCR assay for each sample with greater than 35 HTT CAG repeats.

Repeat-primed PCR assay

The CAG trinucleotide repeat length in HTT was quantified using a previously validated repeat-primed PCR method (see Table S2). The chromatograms were used to estimate somatic mosaicism by generating an instability index for each sample.
Brain immunohistochemistry

Primary antibodies and staining methods are listed in Table S3 and the supplementary materials. The huntingtin 2B4 antibody targets the N’-end of the protein and stains soluble huntingtin and insoluble aggregates.

Statistical analyses and data availability

Trinucleotide repeat frequencies were compared between cohorts using a Fisher’s exact test, with a significance threshold of 0.005 (0.05 divided by ten repeat expansions). Genotype data defining the common haplotypes in the HTT locus were extracted from the whole-genome sequence data using PLINK (version 2.0). The ExpansionHunter - Targeted output for the CAG repeat-length was merged with the genotype information, and phasing was performed using Eagle (version 2.4). Individual-level genotype data for the discovery genomes are available on dbGaP (phs001963.v1.p1).

Results

Assessment of repeat expansions

After quality control, whole-genome sequence data from 2,442 patients diagnosed with FTD/ALS, 2,599 LBD patients, and 3,158 neurologically healthy individuals were available for analysis. We assessed ten repeat expansion motifs that have been previously associated with neurological disease using the ExpansionHunter - Targeted tool (Table S4).

We identified three FTD patients who carried full-penetrance pathogenic repeat expansions (≥ 40) in the HTT gene, representing 0.2% of the discovery cohort (n = 1,377, Table 1). In contrast, none of the LBD cases or control subjects carried pathogenic HTT expansions.
The lengths of the repeat expansions were confirmed using a repeat-primed PCR assay (Figure 1A). We did not observe a higher rate of intermediate and low-penetrance \textit{HTT} repeat expansions (36–39 CAG repeats) among patients diagnosed with FTD/ALS or LBD compared to control subjects. None of the other repeat expansions tested by the ExpansionHunter - Targeted algorithm displayed a similar pattern of being present in cases and absent in control subjects (see Table S4). For this reason, we focused our efforts on the \textit{HTT} repeat expansion.

To replicate our findings, we assessed the \textit{HTT} CAG repeat length in an independent cohort of 3,674 patients diagnosed with FTD/ALS spectrum disorders and 210 healthy control participants. Published data of the occurrence of \textit{HTT} repeat expansions among the general population were included as part of the replication (n = 10 of 31,463 individuals had ≥ 40 repeats).\textsuperscript{23,24} We detected an additional five patients diagnosed with FTD/ALS in this replication cohort that carried pathogenic \textit{HTT} repeat expansions.

Overall, the carrier rate among patients diagnosed with FTD/ALS spectrum disorders was 4.4 times higher than that observed among healthy individuals (Fisher’s test p-value = 2.68x10^{-3}, odds ratio = 4.55, 95% CI = 1.56–12.80, Table 1). All of the patients found to carry the \textit{HTT} expansion had no additional disease-causing mutations in other genes implicated in neurodegeneration (see supplementary appendix).

\textit{Haplotype analysis and somatic mosaicism}

The FTD/ALS patients carrying the \textit{HTT} repeat expansions harbored several different haplotypes that have previously been associated with this locus (Figure S6). The presence of multiple haplotypes indicated diverse ancestral sources among our samples, making it unlikely that another genetic variant outside of the expansion was causing disease in these patients.
Furthermore, we did not detect interruptions within the *HTT* repeat expansion in any of the patients, and only detected the loss of interruption in the CAA-CAG trailing sequence in a single individual (patient #8, Figure 1D).

Similar to patients with Huntington’s disease, we observed a tendency towards CAG repeat length contraction among our patients diagnosed with FTD/ALS (instability index = -1.54, range -0.73 to -2.94). Additionally, we detected the presence of somatic mosaicism across multiple brain regions in a patient diagnosed with ALS (Fig. S4).

**Clinicopathological description**

The clinical details of the eight patients carrying the full-penetrance pathogenic *HTT* repeat expansions are summarized in Table 2. None of the patients reported choreoathetosis. Two patients had a family history of either ALS or FTD, but none of the carriers described a family history of Huntington’s disease.

We further examined postmortem brains obtained from two of our patients harboring full-penetrance *HTT* CAG repeats. The first case was a woman carrying 40 *HTT* CAG repeats, who developed symptoms of ALS at age 56 and died eleven years later of respiratory failure after a typical course of motor neuron disease (Table 2, patient #5). Postmortem examination showed mild atrophy of the precentral gyrus and thinning of the anterior roots of the spinal cord.

Microscopic examination revealed loss of the anterior horn neurons of the spinal cord and hypoglossal neurons (Figure 2A-B). Staining with TDP-43 antibodies showed rare neurons with translocation from the nucleus to the cytoplasm, and occasional neuropil skeins confined to the frontal cortex (Figure 2C). The dentate gyrus was normal. Dual staining of the prefrontal cortex
and striatum using huntingtin/p62 antibodies showed intranuclear and extranuclear aggregates of huntingtin and p62 with the highest density in the infragranular layers of the prefrontal cortex (Figure 2D). Staining of the prefrontal cortex (BA9) obtained from three neurological control subjects did not show this pattern of huntingtin/p62 staining. Ubiquitin-positive inclusions were found in the tail of the caudate and the frontal cortex. However, there was no neuronal loss or active gliosis in the striatum (Figure 2F, 2H-I).

The second autopsy involved a man carrying 41 CAG repeats in HTT, who presented with right foot weakness at age 61. He was diagnosed with ALS based on disease progression and electromyography, and he died from respiratory failure nine years after symptom onset following a typical course of motor neuron disease (Table 2, patient #8). Postmortem examination showed mild atrophy of the precentral gyrus and degeneration of the anterior spinal roots. There was otherwise no atrophy of the cerebral cortex or striatum (Figure 3A), or evidence of neuronal loss or gliosis in the striatum on microscopic examination (Figure 3B). Staining with ubiquitin (Figure 3C) and 1c2 for polyglutamine showed scattered intranuclear and extranuclear aggregates within the striatum (Figure 3D) and peri-Rolandic cortex (Figure 3E). Polyglutamine aggregates were not observed in the spinal cord. There was marked loss of anterior horn cells (Figure 3F), accompanied by degeneration of the corticospinal tracts, including the medulla and lateral spinal cord. Staining with TDP-43 antibodies showed ALS-type TDP-43 cytoplasmic inclusions within some of the remaining motor neurons (Figure 3F inset).

Discussion
Our data indicate that pathogenic expansions in \textit{HTT} can give rise to FTD/ALS syndromes that are clinically distinct from the classical Huntington’s disease syndrome. A careful review of the clinical features of the eight patients carrying pathogenic \textit{HTT} expansions confirmed the diagnosis of FTD or ALS. None of the patients manifested choreoathetoid movements during their illness or reported a family history of Huntington’s disease. Furthermore, the postmortem findings of two of our patients with full-penetrance \textit{HTT} repeat expansions displayed the classical features of ALS, including loss of anterior horn cells and hypoglossal neurons, and the presence of TDP-43-positive inclusions, thereby ruling out mimic syndromes as an explanation of our findings. However, the effects of the pathogenic repeat expansions were corroborated by the occurrence of pathogenic polyglutamine/huntingtin co-pathology.

It is possible that the patients carrying the \textit{HTT} repeat expansions suffered from two separate neurodegenerative diseases by chance, and that they would have developed the classic symptoms of Huntington’s disease if they had lived long enough. We believe that this is an unlikely scenario for several reasons. First, we identified multiple patients in our discovery cohort following the same clinical pattern, and found a similar rate of occurrence in our replication cohort. In contrast, full-penetrance pathogenic \textit{HTT} expansions were not present in our LBD disease control or healthy control whole-genome sequence data. Second, the apparently normal striatum in both patients who underwent postmortem evaluation diminishes the likelihood of subclinical Huntington’s disease as an explanation for their symptoms. Choreoathetoid movements observed in Huntington’s disease originate from the striatum, and the lack of detectable neuronal loss or reactive gliosis in this region implies that the motor neuron disease was not masking these symptoms. Third, two of our eight patients lived at least nine years after the onset of their symptoms and did not manifest signs of Huntington’s disease during
this extended survival period. Fourth, the prevalence rates of FTD (22 per 100,000 population)\textsuperscript{25}, ALS (6 per 100,000)\textsuperscript{3}, and Huntington's disease (3 per 100,000)\textsuperscript{26} indicate that, by chance, there should only be three cases of disease co-occurrence in the entire United States population of 327 million. Instead, we identified eight patients among a moderately-sized cohort of FTD/ALS cases. Finally, the age of onset among our patients overlapped with the predicted age of onset of Huntington’s disease based on their CAG repeat length (Figure 1C).

Regardless of the nosological and semantic distinctions of designating \textit{HTT} repeat expansions as a genetic cause of FTD/ALS spectrum disorders, our findings have direct implications for how these diseases are considered etiologically, the clinical care of patients presenting with these neurological conditions, and the neuropathologic staging of disease. From a clinical and diagnostic perspective, our work defines a new genetic cause of both ALS and FTD. Although there have been previous reports of the coexistence of FTD/ALS and Huntington's disease\textsuperscript{27,28,31,32,33,34}, pathogenic \textit{HTT} mutations have not been described in cases of pure FTD and ALS. Even though these expansions account for less than 1\% of FTD and ALS cases, clinical practice should be adapted to include regular screening of these patient populations for this mutation, particularly in light of the antisense oligonucleotide treatments targeting the \textit{HTT} locus that are undergoing clinical trials.\textsuperscript{12}

From a neuropathologic perspective, we have identified a pathological subtype that is distinct from the classical features observed in the brains of patients diagnosed with Huntington’s disease.\textsuperscript{35} This novel pattern is characterized by abundant huntingtin-positive, ubiquitin-positive inclusions in the frontal cortex and the absence of neostriatal degeneration, with scarce TDP-43 positive co-pathology. The neuropathologic staging of Huntington’s disease, as defined by Vonsattel and colleagues in 1985\textsuperscript{35}, rests on the progressive degeneration of the
striatum. Based on our work, an addendum of the neuropathologic consensus criteria for the
diagnosis of Huntington’s disease should be considered to capture the true frequency of this
subtype among the disease population.

Our study has several limitations. Our cohorts focused on individuals of European
ancestry. Future studies should determine the importance of the HTT expansions among non-
European FTD and ALS populations. Additionally, the algorithm used in this study only
examines known disease-causing repeat expansions.19 There may be undiscovered repeat
expansions driving neuropsychiatric disease. The emergence of high-throughput, low-cost, long-
range sequencing will allow us to identify these regions among large cohorts of patients
methodically.37

There is increasing consensus that molecularly defined genetic causes of disease can
present with heterogeneous, neuropsychiatric syndromes. The polyglutamine expansion diseases
SCA2 and SCA3 typically cause ataxia but can also cause levodopa-responsive Parkinson’s
disease.36 This consideration is particularly valid for frontal lobe diseases that can present with
protean syndromes. For example, patients with mutations in the MAPT gene can present with
behavioral variant FTD, nonfluent variant primary progressive aphasia, progressive supranuclear
palsy, or corticobasal syndrome38, and the pathogenic repeat expansion in the C9orf72 gene has
united two clinically disparate neurologic diseases, FTD and ALS, into a single disease entity.8
In that regard, two of the patients identified in this study reported that elderly relatives had been
diagnosed with Alzheimer’s disease. It may be worthwhile to screen patients presenting with
psychiatric symptoms later in life or with other forms of dementia to elucidate the real
phenotypic spectrum associated with pathogenic HTT repeat expansions.
We have made the individual-level genome sequence data for our patients and control subjects publicly available on the dbGaP web portal as a resource for other researchers. Our research highlights the power of performing whole-genome sequencing in large cohorts of patients with complex neurodegenerative syndromes. We prioritized performing whole-genome sequencing in autopsy samples, as it allowed us to evaluate the neuropathologic changes associated with genetic variation quickly. As the cost of this technology decreases, the size of cohorts that can undergo whole-genome sequencing will increase, enhancing our ability to detect rare, clinically actionable genetic mutations underlying neurologic diseases.

Our work leads to an increase in diagnostic accuracy and a refinement of the phenotype characteristics associated with pathogenic HTT repeat expansions. Although our discovery accounts for a small subset of FTD/ALS patients, clinicians should be aware of this unusual presentation associated with pathogenic HTT repeat expansions. They should consider instituting testing for their FTD and ALS patients, especially as it paves the way for disease-modifying therapy in this small subset of patients.

Acknowledgements

We thank contributors who collected samples used in this initiative, as well as patients and families, whose help and participation made this work possible. The International Lewy Body Dementia Genomics Consortium is co-directed by Dr. Bryan Traynor and Dr. Sonja W. Scholz. This research was supported in part by the Intramural Research Program of the National Institutes of Health (National Institute on Aging, National Institute of Neurological Disorders and Stroke; project numbers: 1ZIAAG000935 [PI Bryan J. Traynor, MD PhD], 1ZIAN$003154 [PI Sonja W. Scholz, MD PhD], 1ZIAN$003033 and 1ZIAN$003034 [David S. Goldstein, MD
Drs. Sidransky, Lopez and Tayebi were supported by Intramural Research Program of the National Human Genome Research Institute. The American Genome Center is supported in part by an NHLBI grant: IAA-A-HL-007.001. The study used samples from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort (Sant Pau Hospital, Barcelona, Spain). We would like to thank the University of Toronto Brain Bank for providing DNA specimens, and we thank the Canadian Consortium on Neurodegeneration in Aging. We are grateful to the Rush Alzheimer’s Disease Center for providing brain tissue and DNA samples, which was supported by the grants P30 AG10161, R01 AG15819, R01 AG17917, U01 AG46152, U01 AG61356. We thank the Dublin Brain Bank, Oregon Health & Science University Brain Bank (with support of the ADC grant 5 P30 AG008017), King’s College Brain Bank, Manchester University Brain Bank, and Kansas University Brain Bank for contributing tissue specimens. We are grateful to Dr. Bernardino Ghetti from the Department of Pathology and Laboratory Medicine at Indiana University for providing tissue samples. The biospecimens from the University of California, Irvine ADRC used in this project were supported by the NIH/NIA grant P50 AG016573. Samples from the National Centralized Repository for Alzheimer’s Diseases and Related Dementias (NCRAD), which receives government support under a cooperative agreement grant (U24 AG021886) awarded by the National Institute on Aging (NIA), were used in this study. The National Alzheimer’s Coordinating Center (NACC) database is funded by the NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA-funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P50 AG047266 (PI Todd Golde, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P50
AG005134 (PI Bradley Hyman, MD, PhD), P30 AG062677 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Thomas Wisniewski, MD), P30 AG013854 (PI M. Marsel Mesulam, MD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P50 AG047366 (PI Victor Henderson, MD, MS), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P50 AG005131 (PI James Brewer, MD, PhD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG053760 (PI Henry Paulson, MD, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P30 AG049638 (PI Suzanne Craft, PhD), P50 AG005136 (PI Thomas Grabowski, MD), P50 AG033514 (PI Sanjay Asthana, MD, FRCP), P50 AG005681 (PI John Morris, MD), P50 AG047270 (PI Stephen Strittmatter, MD, PhD). We would like to thank the South West Dementia Brain Bank (SWDBB) for providing DNA samples for this study. The SWDBB is part of the Brains for Dementia Research Programme, jointly funded by Alzheimer’s Research UK and Alzheimer’s Society, and is supported by BRACE (Bristol Research into Alzheimer’s and Care of the Elderly) and the Medical Research Council. We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona, for the provision of human brain tissue (PI Thomas G. Beach, MD). The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson’s Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer’s Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer’s
Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson’s Disease Consortium) and the Michael J. Fox Foundation for Parkinson’s Research. This study was supported in part by an Alzheimer’s Disease Core Center grant (P30 AG013854) from the National Institute on Aging to Northwestern University, Chicago, Illinois. We gratefully acknowledge the assistance of the Neuropathology Core. The Alzheimer’s Disease Genetics Consortium supported the collection of samples used in this study through the National Institute on Aging (NIA) grants U01 AG032984 and RC2 AG036528). This study used samples from the NINDS Repository at Coriell (https://catalog.coriell.org), as well as clinical data. Samples and data from patients included in this study were provided by the Biobank Valdecilla (PD13/0010/0024), integrated into the Spanish National Biobank Network, and they were processed following standard operating procedures with the appropriate approval of the Ethics and Scientific Committees. We thank the Mayo Clinic Brain Bank for contributing DNA samples and data from patients with Lewy body dementia, which is supported by U54 NS110435 (PI Dennis W Dickson, MD), U01 NS100620 (PI Kejal Kantarci, MD) and The Mangurian Foundation Lewy Body Dementia Program. Sample collection and characterization is also supported by the Mayo Clinic Functional Genomics of LBD Program, NINDS R01 NS78086 (PI Owen Ross, PhD), Mayo Clinic Center for Individualized Medicine, and The Little Family Foundation. Mayo Clinic is an American Parkinson Disease Association (APDA) Mayo Clinic Information and Referral Center, an APDA Center for Advanced Research and the Mayo Clinic Lewy Body Dementia Association (LBDA) Research Center of Excellence. We thank the Paris Neuro-CEB Brain Bank (C. Duyckaerts) for contributing DNA samples. We would like to thank the Baltimore Longitudinal Study of Aging for providing DNA samples.
Tissue samples were provided by the Johns Hopkins Morris K. Udall Center of Excellence for Parkinson’s Disease Research (NIH P50 N38377) and the Johns Hopkins Alzheimer Disease Research Center (NIH P50 AG05146). This study used tissue samples and data provided by the Michigan Brain Bank, the Michigan Alzheimer’s Disease Center (P30 AG053760), and the Protein Folding Disorders Program. We are indebted to the IDIBAPS Biobank (Barcelona, Spain) for sample and data procurement. Tissue for this study was provided by the Newcastle Brain Tissue Resource, which is funded in part by a grant from the UK Medical Research Council (G0400074), by NIHR Newcastle Biomedical Research Centre and Unit awarded to the Newcastle upon Tyne NHS Foundation Trust and Newcastle University, and as part of the Brains for Dementia Research Programme jointly funded by Alzheimer’s Research UK and Alzheimer’s Society. We thank members of the North American Brain Expression Consortium (NABEC) for providing DNA samples derived from brain tissue. Brain tissue for the NABEC cohort was obtained from the Baltimore Longitudinal Study on Aging at the Johns Hopkins School of Medicine, and from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, USA. We would like to thank the United Kingdom Brain Expression Consortium (UKBEC) for providing DNA samples. Tissue samples and associated clinical and neuropathological data were supplied by the Parkinson's UK Brain Bank, funded by Parkinson's UK, a charity registered in England and Wales (258197) and in Scotland (SC037554). The authors would like to thank Parkinson’s UK for their continued support as well as the donors and family for their invaluable donation of brain tissue to the Parkinson’s UK Tissue Bank. This work was supported by a grant from the Luxembourg National Research Fund (Fonds National de Recherche, FNR) within the National
Centre of Excellence in Research on Parkinson’s disease (NCER-PD). This work was in part supported by the NIA grants U01AG049508 (PI Alison Goate, DPhil) and U01 AG052411 (PI Alison Goate, DPhil). We would like to thank Estelle Sandt, Integrated Biobank Luxembourg (IBBL), and Lars Geffers, Luxembourg Centre for Systems Biomedicine (LCSB) of the University of Luxembourg, for excellent project management and the whole NCER-PD Consortium. Finally, we would like to thank all participants of the Luxembourg Parkinson’s Study within the NCER-PD. This work was in part supported by the Italian Ministry of Health (Ministero della Salute, Ricerca Sanitaria Finalizzata, grant RF-2016-02362405), the European Commission’s Health Seventh Framework Programme (FP7/2007-2013 under grant agreement 259867), the Italian Ministry of Education, University and Research (Progetti di Ricerca di Rilevante Interesse Nazionale, PRIN, grant 2017SNW5MB), the Joint Programme – Neurodegenerative Disease Research (Strength and Brain-Mend projects), granted the by Italian Ministry of Education, University and Research. This study was performed under the Department of Excellence grant of the Italian Ministry of Education, University and Research to the Rita Levi Montalcini Department of Neuroscience, University of Torino, Italy. Tissue samples were supplied by The London Neurodegenerative Diseases Brain Bank, which receives funding from the MRC (grant MR/L016397/1) and as part of the Brains for Dementia Research programme, jointly funded by Alzheimer’s Research UK and Alzheimer’s Society. DNA samples were generated by funding from the Alzheimer’s Society (422; AS-URB-18-013; PI Angela Hodges, PhD). The biospecimens from the Sunnybrook Dementia Study (https://clinicaltrials.gov/ct2/show/NCT01800214) used in this project was supported by a grant from the Canadian Institutes of Health Research (MOP13129; PIs Sandra E. Black, MD, Mario
Masellis, MD, PhD) and an Early Researcher Award from the Ministry of Research, Innovation and Science (MRIS; Ontario; PI Mario Masellis, MD, PhD). “The Harvard Biomarkers Study (“HBS”); https://www.bwhparkinsoncenter.org) is a collaborative initiative of Brigham and Women’s Hospital and Massachusetts General Hospital, co-directed by Dr. Clemens Scherzer and Dr. Bradley T. Hyman. The HBS Investigators have not participated in reviewing the current manuscript. The HBS Study Investigators are: Harvard Biomarkers Study. Co-Directors:

Brigham and Women’s Hospital: Clemens R. Scherzer, Massachusetts General Hospital: Bradley T. Hyman; Investigators and Study Coordinators: Brigham and Women’s Hospital: Yuliya Kuras, Karbi Choudhury, Michael T. Hayes, Aleksandar Videnovic, Nutan Sharma, Vikram Khurana, Claudio Meleo De Gusmao, Reisa Sperling; Massachusetts General Hospital: John H. Growdon, Michael A. Schwarzschild, Albert Y. Hung, Alice W. Flaherty, Deborah Blacker, Anne-Marie Wills, Steven E. Arnold, Ann L. Hunt, Nicte I. Mejia, Anand Viswanathan, Stephen N. Gomperts, Mark W. Albers, Maria Allora-Palli, David Hsu, Alexandra Kimball, Scott McGinnis, John Becker, Randy Buckner, Thomas Byrne, Maura Copeland, Bradford Dickerson, Matthew Frosch, Theresa Gomez-Isla, Steven Greenberg, Julius Hedden, Elizabeth Hedley-Whyte, Keith Johnson, Raymond Kelleher, Aaron Koenig, Maria Marquis-Sayagues, Gad Marshall, Sergi Martinez-Ramirez, Donald McLaren, Olivia Okereke, Elena Ratti, Christopher William, Koene Van Dij, Shuko Takeda, Anat Stemmer-Rachaminov, Jessica Kloppenburg, Catherine Munro, Rachel Schmid, Sarah Wigman, Sara Wlodarczyk; Data Coordination:

Brigham and Women’s Hospital: Thomas Yi; Biobank Management Staff: Brigham and Women’s Hospital: Idil Tuncali. We thank all study participants and their families for their invaluable contributions. HBS is made possible by generous support from the Harvard
NeuroDiscovery Center, with additional contributions from the Michael J Fox Foundation, NINDS U01NS082157, U01NS100603, and the Massachusetts Alzheimer’s Disease Research Center NIA P50AG005134. This study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, Maryland, USA (http://biowulf.nih.gov). This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support. J.E.L. was supported by the National Institute of Health/NINDS (R01 NS073873) and the ALS Association National and Massachusetts Chapters.

Conflicts of interest

Author contributions
References


9. A novel gene containing a trinucleotide repeat that is expanded and unstable on
Huntington’s disease chromosomes. The Huntington's Disease Collaborative Research

10. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor
9.


12. Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, et al. Targeting Huntingtin Expression in

13. Faber R, Neary D. Frontotemporal lobar degeneration: A consensus on clinical diagnostic

supranuclear palsy: The movement disorder society criteria. Mov Disord 2017;32(6):853–
64.

15. Brooks BR. El escorial World Federation of Neurology criteria for the diagnosis of

16. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with


24. Website.


26. Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N. The incidence and


Table 1. Pathogenic *HTT* repeat expansions within the discovery and replication cohorts

<table>
<thead>
<tr>
<th></th>
<th>Discovery cohort</th>
<th>Replication cohort*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number carriers / number screened</td>
<td>Rate</td>
</tr>
<tr>
<td>FTD/ALS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTD</td>
<td>3/1,377</td>
<td>0.2%</td>
</tr>
<tr>
<td>ALS</td>
<td>0/1,065</td>
<td>0</td>
</tr>
<tr>
<td>LBD</td>
<td>0/2,599</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>0/3,158</td>
<td>0</td>
</tr>
</tbody>
</table>

* The replication cohort included 210 neurologically-healthy controls, 13,670 population controls from Gardiner et al., 2019\textsuperscript{23}, and 17,703 neurologically-healthy individuals from the UK 100K Genomes Project\textsuperscript{24}; The replication cohort included 1,236 samples that were analyzed by repeat-primed PCR and 2,648 samples analyzed by next-generation sequencing. All samples predicted to have more than 35 CAG repeats based on the whole-genome sequence data were verified by repeat-primed PCR.
Table 2. Clinical details of the eight patients carrying a full-penetrance pathogenic HTT repeat expansion

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Cohort</th>
<th>CAG repeats</th>
<th>Clinical diagnosis</th>
<th>Age at onset (y)</th>
<th>Gender</th>
<th>Family history</th>
<th>Presenting symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Discovery</td>
<td>41</td>
<td>PSP-FTD</td>
<td>68</td>
<td>M</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Discovery</td>
<td>40</td>
<td>bvFTD</td>
<td>56</td>
<td>F</td>
<td>Yes</td>
<td>Behavioral changes</td>
</tr>
<tr>
<td>3</td>
<td>Discovery</td>
<td>40</td>
<td>nfvPPA</td>
<td>57</td>
<td>F</td>
<td>No</td>
<td>Language disturbance</td>
</tr>
<tr>
<td>4</td>
<td>Replication</td>
<td>64</td>
<td>PSP-FTD</td>
<td>17</td>
<td>F</td>
<td>Yes</td>
<td>Academic decline, dysarthria, bradykinesia, and gait disturbance</td>
</tr>
<tr>
<td>5</td>
<td>Replication</td>
<td>40</td>
<td>ALS</td>
<td>56</td>
<td>F</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Replication</td>
<td>44</td>
<td>bvFTD</td>
<td>44</td>
<td>M</td>
<td>Yes</td>
<td>Personality changes and apathy</td>
</tr>
<tr>
<td>7</td>
<td>Replication</td>
<td>40</td>
<td>ALS</td>
<td>76</td>
<td>M</td>
<td>Yes</td>
<td>Lower limb weakness</td>
</tr>
<tr>
<td>8</td>
<td>Replication</td>
<td>41</td>
<td>ALS</td>
<td>61</td>
<td>M</td>
<td>No</td>
<td>Right foot weakness</td>
</tr>
</tbody>
</table>

Clinical diagnoses include progressive supranuclear palsy - frontotemporal dementia type (PSP-FTD), behavioral variant frontotemporal dementia (bvFTD), nonfluent variant primary progressive aphasia subtype of FTD (nfvPPA), and amyotrophic lateral sclerosis (ALS). Family history refers to family history of FTD/ALS.
Figure legends

Figure 1. *HTT* repeat expansions detected in patients diagnosed with FTD/ALS.

(A) An ideogram of chromosome 4 showing the location of the *HTT* gene at 4p16.3, the gene transcript (exon 1 in red), and representative repeat-primed PCR chromatograms depicting wild-type and *HTT* CAG repeat expansions. (B) The distributions of *HTT* CAG repeat expansions in the FTD/ALS (n = 2,442), LBD (n = 2,599), and control (n = 3,158) discovery cohorts based on analysis of whole-genome sequence data. Inset figures are zoomed views showing the number of cases carrying CAG repeat expansions >=36 repeats. (C) Ages at symptom onset among FTD/ALS patients compared to the size of their *HTT* repeat expansions. The curve represents the estimated age at onset and corresponding standard deviation based on the number of CAG repeats (as described in Langbehn et al.\textsuperscript{39}). (D) The allelic structure of samples carrying *HTT* repeat expansions. The pathogenic repeat sequence is represented by [CAG]_n, where n corresponds to the number of repeats. The trailing CAG-CAA glutamine sequence, the CCG-CCA proline sequence, and the [CCT]_n codons are also shown (modified from Ciosi et al.\textsuperscript{40}).
Figure 2. Neuropathologic changes observed in a patient diagnosed with ALS carrying a full-penetrance pathogenic HTT repeat expansion (patient #5).

(A) A representative section of cervical cord showing pallor of the lateral (*) and anterior corticospinal tracts (**) with atrophy of the ventral horns. (B) The loss of motor neurons of the anterior horns is severe. (C) Nucleocytoplasmic translocation of TDP-43 (arrows) involving the prefrontal cortex (BA9). (D) Frequent p62 (red arrow) and huntingtin (black arrow) dystrophic neurites (Insert), intranuclear huntingtin (black arrow) and p62 (red arrow) inclusions are noted within the prefrontal cortex. (E) The neostriatum is apparently normal, for example, at the level of the nucleus accumbens, and neither neuronal loss nor reactive gliosis is detectable. (F) & (G) Occasional huntingtin aggregates are seen within the neuropil of the nucleus accumbens. (H) The tail of the caudate nucleus is not atrophic, and the neuronal density is normal and without reactive gliosis. (I) & (J) Rare huntingtin aggregates involve the neuropil of the tail of the caudate nucleus (arrows). Scale bars: A: 1 mm, and C-D: 50 microns.
Figure 3. Neuropathologic changes observed in a patient diagnosed with ALS carrying a full-penetrance pathogenic \textit{HTT} repeat expansion (patient #8).

(A) Coronal section of the fresh brain shows that the caudate, putamen, and globus pallidus are intact with no evidence of atrophy. (B) Luxol fast blue/hematoxylin and eosin staining of the caudate nucleus shows no neuronal loss or gliosis. (C) Ubiquitin immunostaining of the caudate nucleus shows extranuclear aggregates (arrow) and rare intranuclear inclusions (arrowhead). (D-E) Immunohistochemistry for polyglutamine expansions shows occasional extranuclear inclusions within the caudate nucleus (D) and the peri-Rolandic cortex (E, arrows). (F) There is severe motor neuron loss within the anterior horn of the spinal cord (Luxol fast blue/hematoxylin and eosin). (Insert) A remaining motor neuron with a TDP-43 cytoplasmic inclusion. Scale bars: B: 50 microns, C-D: 20 microns, and F: 100 microns.
Figure 1.

A  Representative RP-PCR chromatograms of non-pathogenic and full-penetration pathogenic HTT CAG repeat carriers

B  Intermediate, reduced-penetration, and full-penetration repeat number distributions

C  Age at onset for full-penetration pathogenic HTT CAG repeat carriers

D  Allelic structure at the HTT exon one CAG/CAG repeat expansion locus
Figure 3.