

# C9orf72 Hexanucleotide Repeat Expansions in Clinical Alzheimer Disease

Matthew Harms, MD; Bruno A. Benitez, MD; Nigel Cairns, PhD; Breanna Cooper, BS; Paul Cooper, BS; Kevin Mayo, BA; David Carrell, BS; Kelley Faber, MS; Jennifer Williamson, MS; Tom Bird, MD; Ramon Diaz-Arrastia, MD; Tatiana M. Foroud, PhD; Bradley F. Boeve, MD; Neill R. Graff-Radford, MBChB; Richard Mayeux, MD, MS; Sumitra Chakraverty, MS; Alison M. Goate, PhD; Carlos Cruchaga, PhD; for the NIA-LOAD/NCRAD Family Study Consortium

**Importance:** Hexanucleotide repeat expansions in the chromosome 9 open reading frame 72 (*C9orf72*) gene underlie a significant fraction of frontotemporal dementia and amyotrophic lateral sclerosis.

**Objective:** To investigate the frequency of *C9orf72* repeat expansions in clinically diagnosed late-onset Alzheimer disease (AD).

**Design, Setting, and Patients:** This case-control study genotyped the *C9orf72* repeat expansion in 872 unrelated familial AD cases and 888 control subjects recruited as part of the National Institute on Aging Late-Onset Alzheimer Disease Family Study cohort, a multisite collaboration studying 1000 families with 2 or more individuals clinically diagnosed as having late-onset AD.

**Main Outcomes and Measures:** We determined the presence or absence of the *C9orf72* repeat expansion by repeat-primed polymerase chain reaction, the length of the longest nonexpanded allele, segregation of the geno-

type with disease, and clinical features of repeat expansion carriers.

**Results:** Three families showed large *C9orf72* hexanucleotide repeat expansions. Two additional families carried more than 30 repeats. Segregation with disease could be demonstrated in 3 families. One affected expansion carrier had neuropathology compatible with AD. In the National Institute on Aging Late-Onset Alzheimer Disease Family Study series, the *C9orf72* repeat expansions constituted the second most common pathogenic mutation, just behind the *PSEN1* A79V mutation, highlighting the heterogeneity of clinical presentations associated with repeat expansions.

**Conclusions and Relevance:** *C9orf72* repeat expansions explain a small proportion of patients with a clinical presentation indistinguishable from AD, and they highlight the necessity of screening frontotemporal dementia genes in clinical AD cases with strong family history.

*JAMA Neurol.* 2013;70(6):736-741. Published online April 15, 2013. doi:10.1001/2013.jamaneurol.537

**A**LZHEIMER DISEASE (AD) constitutes the most common cause of dementia in clinical practice.<sup>1</sup> Alzheimer disease manifests clinically with progressive cognitive dysfunction with prominent memory loss, while its neuropathology is characterized by the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles.<sup>1</sup> Familial AD can be caused by mutations in amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*).<sup>2-6</sup> Interestingly, mutations in genes typically associated with frontotemporal dementia (FTD), including microtubule-associated protein tau (*MAPT*) and granulin (*GRN*), have also been reported in clinical AD cases,<sup>6-13</sup> highlighting the overlapping phenotypes of AD and amnesic FTD.

Recently, an intronic hexanucleotide repeat (GGGGCC) in the chromosome 9 open

reading frame 72 (*C9orf72*) gene was identified as a frequent cause of sporadic and familial FTD and amyotrophic lateral sclerosis (ALS).<sup>14,15</sup> Because some individuals with clinical AD carry mutations in FTD genes,<sup>6-13,16,17</sup> 2 studies investigated the role of *C9orf72* expansions in AD, with conflicting results. The first report identified *C9orf72* repeat expansions in 3 of 342 cases with familial AD and 6 of 711 cases with sporadic AD. Interestingly, reanalysis of autopsy material from 2 expansion carriers showed FTD pathology,<sup>18</sup> suggesting that both patients had amnesic presentations of FTD rather than AD. The second study identified no repeat expansions in 568 patients with probable AD by clinical criteria and concluded that *C9orf72* repeats are specific for FTD.<sup>19</sup>

To clarify the role of *C9orf72* hexanucleotide repeat expansions in familial late-onset AD, this study screened 872 un-

Author Affiliations are listed at the end of this article.

related AD cases and 888 unrelated control subjects and investigated whether expanded repeats were associated with age at dementia onset. Furthermore, we addressed whether a larger, but nonexpanded, GGGGCC allele was associated with the risk for AD or age at onset.

## METHODS

### PATIENTS

Individuals from 872 unrelated families with at least 2 individuals affected by AD and 888 unrelated unaffected control subjects from the National Institute on Aging Late-Onset Alzheimer Disease (NIA-LOAD) Family Study were included. All AD cases had been diagnosed as having dementia of the Alzheimer type using criteria equivalent to the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer Disease and Related Disorders Association for probable AD.<sup>20,21</sup> All patients had a family history of AD but not other types of dementia or other neurodegenerative diseases. Probands were required to have a diagnosis of definite or probable late-onset AD (>60 years) and a sibling with definite, probable, or possible late-onset AD with a similar age at onset. A third biologically related family member (first, second, or third degree) was also required, regardless of affected status. If unaffected, this individual had to be 60 years of age or older, but 50 years of age or older if diagnosed as having late-onset AD or mild cognitive impairment.<sup>22</sup> Within each pedigree, we selected a single individual to screen by identifying the youngest affected family member with the most definitive diagnosis (ie, individuals with autopsy confirmation were chosen over those with clinical diagnosis only). A summary of the demographics of all subjects is shown in **Table 1**. Written informed consent was obtained from all participants, and the study was approved by local institutional review board committees.

### GENETIC ANALYSIS

#### Repeat-Primed Polymerase Chain Reaction

The presence of the expanded hexanucleotide repeat and the number of repeat units in the longest allele were determined using previously reported methods for repeat-primed polymerase chain reaction (PCR) and fluorescence-based fragment size analysis.<sup>14</sup> Briefly, repeat-primed PCR was performed in a total reaction volume of 28  $\mu$ L containing 100 ng genomic DNA, 1x Fast-Start PCR Master Mix (Roche Applied Science), 3.5% dimethyl sulfoxide, 1x Q solution (Quiagen), and 0.18 mM of deaza-dGTP (New England Biolabs). Primer concentrations and sequences (chr9:27563580F and chr9:27563465R) were the same as previously reported.<sup>14</sup> Polymerase chain reaction products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems) and analyzed using GeneMapper. Consistent with standards used in prior studies, a sample was considered to have a repeat expansion when assay replicates demonstrated more than 30 peaks and a decremting saw-tooth pattern with 6 base-pair periodicity (eFigure, <http://www.jamaneuro.com>). A normal repeat allele was defined as having 30 or fewer peaks.<sup>14,15,18,19</sup>

#### Cross-Repeat Polymerase Chain Reaction

In all cases, repeat expansions identified with repeat-primed PCR were confirmed by attempting to perform PCR across the GGGGCC repeat using fluorescence-based fragment size analysis (cross-repeat PCR), as reported with minor modifications.<sup>14</sup> Briefly, using previously reported primers with fluorescent la-

**Table 1. Cohort Demographics**

	AD Cases	Control Subjects
No.	872	888
Age, mean (SD) [range], y <sup>a</sup>	75.8 (8.9) [42-101]	71.41 (6.7) [48-98]
Male, %	33.9	39.7
APOE 4+, % <sup>b</sup>	76.2	30.6
<i>C9orf72</i> , No. (%)	5 (0.57)	1 (0.11)

Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E; *C9orf72*, chromosome 9 open reading frame 72.

<sup>a</sup>For AD cases, age indicates onset of symptoms but refers to age at last assessment for control subjects.

<sup>b</sup>APOE 4+ refers to those carrying at least 1 APOE 4 allele.

bels, PCR was performed on 100 ng genomic DNA with 1x Phusion High-Fidelity DNA Polymerase Master mix (Thermo Fisher Scientific), 5% dimethyl sulfoxide, 1.25 M betaine, and 0.2 mM deaza-dGTP. Products were run on a 2% agarose gel for visual inspection and then analyzed for fragment size determination, as described for repeat-primed PCR. Based on our analysis, cross-repeat PCR can amplify alleles with 35 or fewer GGGGCC repeats but not the 700 to 1600 repeats reported for pathologic expansions.<sup>14</sup> To investigate repeat expansion segregation, we also genotyped all family members (n = 491) from every pedigree with 10 or more hexanucleotide repeats.

### Southern Blot

Southern blot hybridization analysis was conducted using previously described methods and probe sequences<sup>14</sup> to estimate the number of repeat units in expansion carriers.

### Risk Haplotype Genotyping

We also analyzed all repeat expansion carriers for the 24 single nucleotide polymorphism (SNP) at-risk haplotypes that have been associated with pathologically expanded *C9orf72* repeats.<sup>23</sup> All samples were genotyped with the Illumina Human 610 Beadchip, with direct genotyping of all analyzed SNPs. Stringent quality control criteria were applied to remove low-quality SNPs.<sup>22</sup> We used the entire NIA-LOAD genomewide association study data set and the HapMap CEU population as reference populations. Mach software<sup>24</sup> was used to phase the 24 SNPs.

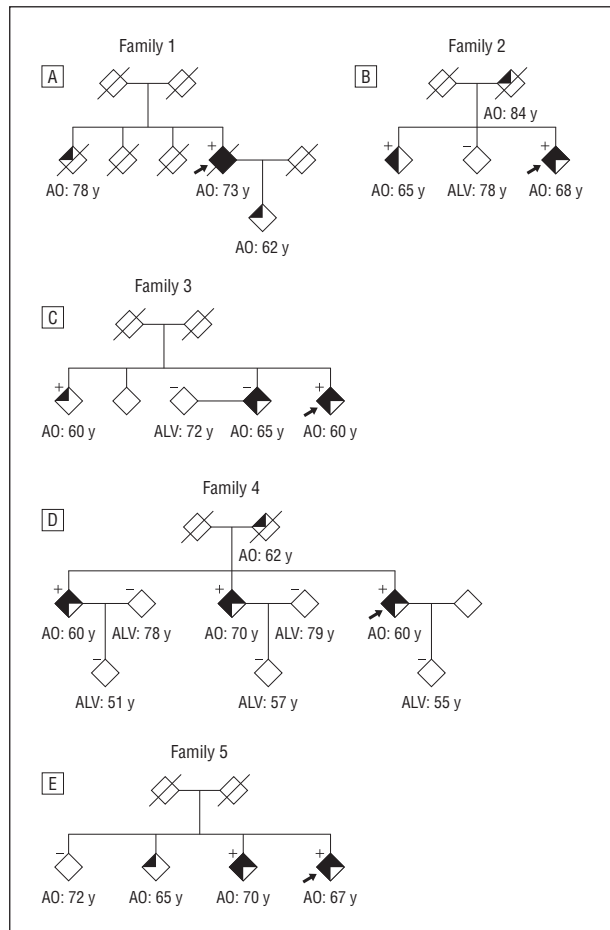
### STATISTICAL AND BIOINFORMATIC ANALYSES

A linear regression model (SAS Institute Inc) was used to test whether the number of GGGGCC repeats was associated with the risk for AD, including age, sex, and apolipoprotein E (APOE) genotype as covariates. Association with age at onset was carried out using the Kaplan-Meier method and tested for significant differences using a Cox proportional hazards model (proc PHREG, SAS Institute Inc) that included sex and APOE status.

## RESULTS

### *C9orf72* REPEAT EXPANSION FREQUENCY AND SEGREGATION IN ALZHEIMER DISEASE FAMILIES

We screened 872 unrelated familial AD cases and 888 unrelated control subjects for expansions of the hexanucleotide repeat in *C9orf72*. Five white individuals with a clinical diagnosis of AD (0.57%) and 1 normal control subject



**Figure 1.** Alzheimer disease (AD) pedigrees carrying abnormal *C9orf72* repeat expansions. Fully shaded symbols indicate autopsy-confirmed AD. Clinical diagnoses were made using National Institute of Neurological and Communication Disorders and Stroke–Alzheimer Disease and Related Disorders Association criteria, with three-quarters and one-half shading indicating probable and possible AD, respectively. + indicates the presence of repeat expansion genotypes and – indicates their absence. The absence of a genotype symbol indicates that DNA was not available for analysis. An arrow marks the proband of each pedigree. ALV indicates age at last visit; AO, age at onset.

(age, 73 years) showed an abnormal expansion with repeat-primed PCR, defined as more than 30 repeats with 6 base-pair periodicity and the expected decremting saw-tooth pattern (eFigure). To better clarify the number of repeats in these patients, we attempted to perform PCR across the GGGGCC repeat. However, in all 6 individuals, only a single peak representing the normal size allele was obtained. Southern blot of AD probands showed expansions of 1200 to 1300 GGGGCC repeats for families 1, 2, and 3, but only 35 to 100 repeat units in families 4 and 5 (data not shown). Regardless of repeat size, all 5 AD cases were found to carry the at-risk haplotype associated with pathologic repeat expansions in all patients with FTD and ALS reported to date (eTable 1).<sup>23</sup> Results from all 5 of these patients were negative for the most common mutations in *APP*, *PSEN1*, *PSEN2*, *GRN*, and *MAPT*.<sup>6</sup>

Pedigrees for the 5 families with abnormal (>30) *C9orf72* repeats are shown in **Figure 1** and clinically summarized in **Table 2**. All additional affected individuals had been diagnosed as having probable AD based on the National Institute of Neurological and Commu-

nication Disorders and Stroke–Alzheimer Disease and Related Disorders Association criteria. DNA for additional family members was available for all but family 1. Family 2 showed complete segregation of the large repeat expansion and risk haplotype with disease status (Figure 1 and eTable 2). Family 3, in which all the affected individuals were APOE 3/3 homozygous, showed segregation of the expanded repeat and risk haplotype except for a single individual who developed dementia with only 7 repeat units. Additionally, this individual did not carry the risk haplotype and had dementia onset at a later age than relatives with repeat expansions. In families 4 and 5 (repeat expansions between 35 and 100 repeats), the characteristic repeat-primed chromatogram pattern and the risk haplotype segregated perfectly with disease status. Overall, in these 5 families, 10 of 11 affected individuals for whom DNA was available carried abnormal GGGGCC expansions and the risk haplotype. It is important to note that all the individuals with more than 30 repeats also carried the risk haplotype.

The age at onset for individuals with more than 30 repeats was earlier than cases with normal repeat alleles (mean [SD], 65.6 [5.5] vs 71.4 [6.8] years;  $P = .04$ ; **Figure 2**). This association retained statistical significance even after APOE genotypes were included in the model ( $P = .03$ ).

#### CLINICAL CASE AND NEUROPATHOLOGY DESCRIPTIONS

The proband of family 1 (1200-1300 repeats), an APOE 2/3 carrier, was diagnosed as having dementia, Alzheimer type, at age 73 years. Formal neurocognitive testing was not available, but prominent memory loss with repetitive questioning and wandering behavior had developed by age 78 years. On death 12 years after diagnosis, a brain autopsy was carried out by the Brain Bank at McLean Hospital in Belmont, Massachusetts, in 1999. Microscopic examination found extensive plaque and tangle pathology. Neurofibrillary tangles (>40 plaques per 100X field) were present throughout the neocortex, hippocampus, amygdala, and nucleus basalis of Meynert. Scattered neocortical Lewy bodies were seen. Severe neuronal loss in the substantia nigra pars compacta was accompanied by a few Lewy bodies. This case would be diagnosed as having NIA-Reagan high likelihood criteria for AD and coexisting neocortical-predominant Lewy body dementia using current criteria.<sup>25,26</sup> Tissue was no longer available to carry out additional relevant staining (eg, tau, ubiquitin, TDP-43, or p62), preventing an assessment for possible FTD pathology.

Aside from age at symptom onset and meeting clinical criteria for probable AD, limited clinical information was available for the remaining index cases. The proband of family 2 (1200-1300 repeats) was diagnosed clinically as having probable AD at the age of 71 years, 3 years after disorientation and memory loss began. By the time of study inclusion at age 74 years, the patient was nonverbal and too impaired to participate in cognitive testing. The proband of family 3 (1200-1300 repeats) was diagnosed as having probable AD at the age of 60 years. Three years later, the Clinical Dementia Rating score was 0.5, which progressed over 3 years to 1.0. Review of limited caregiver re-

**Table 2. Segregation Analysis of Pedigrees With Repeat Expansions**

<i>C9orf72</i> Repeat Expansion	Affected With AD		Unaffected	
	Positive	Negative	Positive	Negative
Overall				
No. of individuals	10	1	0	8
Age, mean (SD) [range], y <sup>a</sup>	65.3 (4.9) [60-73]	65	60	69.2 (11.3) [55-79]
Family 1				
No. of individuals	1	0	0	0
Age, y	73	NA	NA	NA
Family 2				
No. of individuals	2	0	0	1
Age, y	65, 68	NA	NA	78
Family 3				
No. of individuals	2	1	0	1
Age, y	60	65	NA	72
Family 4				
No. of individuals	3	0	0	5
Age, y	60, 60, 70	NA	NA	55-79
Family 5				
No. of individuals	2	0	0	1
Age, y	67, 70	NA	NA	72

Abbreviations: AD, Alzheimer disease; *C9orf72*, chromosome 9 open reading frame 72; NA, not applicable.

<sup>a</sup>For patients with AD, age refers to age at symptom onset; for unaffected individuals, age refers to the age at last assessment.

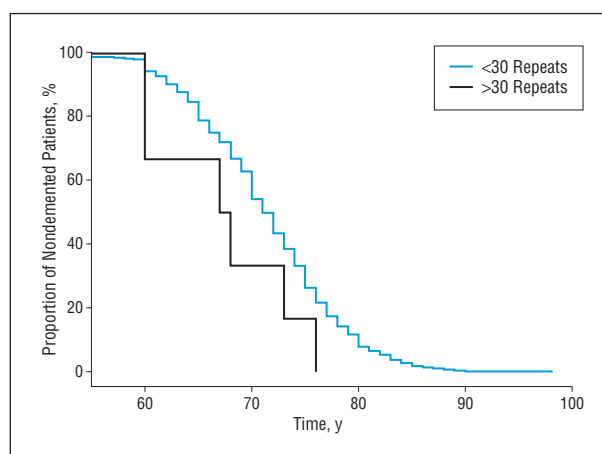
ports suggested that difficulties with disinhibition and anxiety were out of proportion to memory impairment. No additional clinical information was available for members of families 4 and 5 (35-100 repeats in each).

#### ASSOCIATIONS OF *C9orf72* REPEAT LENGTH IN ALZHEIMER DISEASE

Previous studies in ALS and FTD have clearly demonstrated that *C9orf72* repeat expansions are causative for disease.<sup>14</sup> However, the minimum repeat number required for disease has not been established. Furthermore, it is not known whether higher repeat numbers (but still within the normal range) are associated with the risk for ALS or FTD. To address this question in AD, we compared the longest nonexpanded allele in cases and control subjects. The average longest allele was not statistically different from control subjects (mean [SD], 6.5 [4.1] vs 4.48 [3.7] repeat units;  $P = .10$ ), and the distribution of longest allele lengths were similar. Thus within the normal range, higher repeat numbers did not appear to be a risk factor for AD in this population. Furthermore, we found no association between the length of the longest nonexpanded allele and age at onset ( $P = .52$ ), nor evidence for an interaction with APOE genotype.

#### COMPARISON OF THE *C9orf72* REPEAT EXPANSION FREQUENCY WITH OTHER PATHOGENIC GENE MUTATIONS

In a previous study, we sequenced *APP*, *PSEN1*, *PSEN2*, *MAPT*, and *GRN* genes in a discovery series comprising 439 cases included in this study.<sup>6</sup> The most common pathogenic mutation identified by sequencing in the discovery series (*PSEN1* A79V) was then genotyped in the entire cohort (follow-up series). Overall, the A79V mutation was found in 4 of the 872 cases (0.46%)<sup>6</sup> compared with the 5



**Figure 2.** *C9orf72* repeat expansions correlate with an earlier age at onset. Age at onset was analyzed for association with repeat expansion genotypes by the Kaplan-Meier method and tested for significant differences using a proportional hazards model (proc PHREG; SAS Institute Inc). Repeat expansion carriers show an earlier age at onset than noncarriers (mean [SD], 68.25 [5.8] vs 71.4 [6.8] years;  $P = .04$ ).

pedigrees where abnormal *C9orf72* repeat expansions were found. Furthermore, we analyzed the overall frequency of AD gene mutations (*APP*, *PSEN1*, and *PSEN2*) vs FTD gene mutations (*MAPT*, *GRN*, and *C9orf72*) and found that 1.82% of probands carried a pathogenic, or very likely pathogenic, mutation in *APP*, *PSEN1*, and *PSEN2*, while a slightly larger number (1.94%) had mutations in *MAPT*, *GRN*, or *C9orf72*.

#### COMMENT

This study assessed *C9orf72* hexanucleotide expansions in familial late-onset AD cases and normal control subjects, identifying 5 AD families carrying abnormal *C9orf72* hexanucleotide repeat expansions. This frequency is very



similar to that found in an independent AD series,<sup>15</sup> but significantly lower than in FTD or ALS.

Three families with clinical AD (0.34%) were found to have repeat expansions in the range reported for FTD and ALS (>1 000). In a previous report documenting expansions in clinically diagnosed AD, reevaluation of autopsy material demonstrated FTD pathology and suggested that AD cases with *C9orf72* repeat expansions represent amnesic variants of FTD.<sup>9,14,15,27,28</sup> We were unable to perform an equivalent analysis because autopsies had not been performed or tissue was no longer available. Therefore, even in the proband from family 1, where the neuropathology showed coexisting AD and Lewy body dementia pathology, we cannot rule out the possibility that the families identified in this study also represented amnesic presentations of FTD rather than AD. It is notable that of the many *C9orf72* FTD cases reported, several showed concurrent AD pathology<sup>9,14,15,27,28</sup> and an additional expansion carrier with FTD+ALS demonstrated enough plaque and tangle pathology to meet diagnostic criteria for AD.<sup>27</sup> These cases suggest that some individuals with *C9orf72* repeat expansions could present with clinical symptoms of AD and have a high enough burden of AD neuropathology that biomarker analysis (cerebrospinal tau or  $\beta$ -amyloid, and/or Pittsburgh Compound B–positron emission tomography neuroimaging) would also support an AD diagnosis. This hypothesis is supported by 3 recently reported individuals with early-onset AD, cerebrospinal fluid profiles typical of AD,<sup>29</sup> who were found to carry *C9orf72* repeat expansions. In this setting, the correct diagnosis (amnesic FTD) would presumably only be reached by neuropathologic studies or genetic testing. Our cases and previous studies reinforce the heterogeneous clinical and neuropathologic presentations of *C9orf72* repeat expansions (ALS, frontotemporal lobar degeneration, frontotemporal lobar degeneration–ALS, and clinical AD).

We also identified 2 families carrying smaller, but abnormal repeats (>35, but < 100 units). Despite segregating with disease status, it remains unclear whether these smaller repeat expansions cause disease, increase the risk for dementia, or are incidental. Future studies correlating quantified repeat sizes with disease status will be required to answer this question.

Although the frequency of large *C9orf72* repeat expansions was low in our cohort, it was the second most common pathogenic mutation (3 of 872), just behind *PSEN1* A79V (4 of 872). In addition, mutations in FTD genes were as common as mutations in AD genes (1.94% vs 1.87%). Our results confirm that the clinical phenotype of mutations in FTD genes, including *GRN*, *MAPT*, and *C9orf72*, can be clinically indistinguishable from typical AD.<sup>30–34</sup> This fact has important implications for clinicians, who should consider both FTD and AD genes when evaluating families with strong histories of AD.

Accepted for Publication: August 27, 2012.

Published Online: April 15, 2013. doi:10.1001/2013.jamaneurol.537

**Author Affiliations:** Departments of Neurology (Drs Harms and Cairns, and Mr P. Cooper), Psychiatry (Drs Benitez, Goate, and Cruchaga, Mss B. Cooper and Chakraverty, and Messrs Mayo and Carrell), and Genetics (Dr Goate), Knight

Alzheimer's Disease Research Center (Drs Cairns and Goate), and Hope Center for Neurological Disorders (Drs Cairns, Goate, and Cruchaga), Washington University School of Medicine, St Louis, Missouri; Department of Medical and Molecular Genetics, Indiana University, Indianapolis (Ms Faber and Dr Foroud); Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, New York (Ms Williamson and Dr Mayeux); VA Medical Center and Departments of Neurology and Medicine, University of Washington, Seattle (Dr Bird); Department of Neurology, University of Texas Southwestern Medical Center, Dallas (Dr Diaz-Arrastia); Department of Neurology, Mayo Clinic, Rochester, Minnesota (Dr Boeve); and Department of Neurology, Mayo Clinic, Jacksonville, Florida (Mr Graff-Radford).

**Correspondence:** Carlos Cruchaga, PhD, Department of Psychiatry, Washington University School of Medicine, 660 S Euclid Ave, B8134, St Louis, MO 63110 (cruchaga@psychiatry.wustl.edu).

**Author Contributions:** Drs Harms and Cruchaga contributed equally to this work. *Study concept and design:* Harms, Mayeux, Goate, and Cruchaga. *Acquisition of data:* Harms, Cairns, B. Cooper, P. Cooper, Mayo, Carrell, Faber, Williamson, Bird, Diaz-Arrastia, Foroud, Graff-Radford, Mayeux, and Chakraverty. *Analysis and interpretation of data:* Harms, Benitez, Cairns, P. Cooper, Boeve, and Cruchaga. *Drafting of the manuscript:* Harms, Benitez, and Cruchaga. *Critical revision of the manuscript for important intellectual content:* Harms, Cairns, B. Cooper, P. Cooper, Mayo, Carrell, Faber, Williamson, Bird, Diaz-Arrastia, Foroud, Boeve, Graff-Radford, Mayeux, Chakraverty, Goate, and Cruchaga. *Statistical analysis:* Cruchaga. *Obtained funding:* Mayeux, Goate, and Cruchaga. *Administrative, technical, and material support:* Harms, Cairns, B. Cooper, P. Cooper, Mayo, Carrell, Faber, Williamson, Diaz-Arrastia, Boeve, Mayeux, and Chakraverty. *Study supervision:* Williamson, Diaz-Arrastia, and Foroud.

**Conflict of Interest Disclosures:** Dr Bird receives licensing fees from Athena Diagnostics Inc. Dr Boeve has served as an investigator for clinical trials sponsored by Cephalon Inc, Allon Pharmaceuticals, and GE Healthcare. He receives royalties from the publication of a book titled *Behavioral Neurology of Dementia* (Cambridge Medicine, 2009). He has received honoraria from the American Academy of Neurology, and he receives research support from the National Institute on Aging (P50 AG16574 [co-investigator], U01 AG06786 [co-investigator], and RO1 AG32306 [co-investigator]) and the Mangurian Foundation. Dr Goate has received honoraria from AstraZeneca for consultancy services; Howrey & Associates, Finnegan HC, and Dickstein Shapiro for expert testimony; and Pfizer, AstraZeneca, and Genentech for lectures; as well as royalties from Taconic.

**NIA-LOAD/NCRAD Family Study Consortium Investigators:** Robert Green, Neil Kowall, Lindsay Farrer, Boston University, Massachusetts; Jennifer Williamson, Vincent Santana, Columbia University, New York, New York; Donald Schmechel, Perry Gaskell, Kathleen Welsh-Bohmer, Margaret Pericak-Vance, Duke University, Durham, North Carolina; Bernardino Ghetti, Martin R. Farlow, Kelly Horner, Indiana University, Bloomington; John

H. Growdon, Deborah Blacker, Rudolph E. Tanzi, Bradley T. Hyman, Massachusetts General Hospital, Boston; Bradley Boeve, Karen Kuntz, Lindsay Norgaard, Nathan Larson, Mayo Clinic, Rochester, Minnesota; Dana Kistler, Fracine Parfitt, Jenny Haddow, Mayo Clinic, Jacksonville, Florida; Jeremy Silverman, Michal Schnaider Beeri, Mary Sano, Joy Wang, Rachel Lally, Mount Sinai School of Medicine, New York, New York; Nancy Johnson, Marcel Mesulum, Sandra Weintraub, Eileen Bigio, Northwestern University, Evanston, Illinois; Jeffery Kaye, Patricia Kramer, Jessica Payne-Murphy, Oregon Health and Science University, Portland; David Bennett, Holli Jacobs, Jeen-Soo Chang, Danielle Arends, Rush University, Chicago, Illinois; Lindy Harrell, University of Alabama at Birmingham; George Bartzokis, Jeffery Cummings, Po H. Lu, Usha Toland, University of California, Los Angeles; William Markesbery, Charles Smith, Alise Brickhouse, University of Kentucky, Lexington; John Trojanowski, Vivianna Van Deerlin, Elisabeth McCarty Wood, University of Pennsylvania, Philadelphia; Steven DeKosky, Robert Sweet, Elise Weamer, University of Pittsburgh, Pennsylvania; I. Helena Chui, Arousiak Varpertian, University of Southern California, Los Angeles; Ramon Diaz-Arrastia, Roger Rosenberg, Barbara Davis, University of Texas Southwestern, Dallas; Thomas Bird, Malia Rumbaugh, Gerard D. Schellenberg, Murray Raskind, University of Washington, Seattle; Alison Goate, John Morris, Joanne Norton, Denise Levitch, Betsy Grant, Mary Coats, Washington University at St Louis, Missouri.

**Funding/Support:** This study was supported by grants P30-NS069329-01, R01-AG035083, and K08-NS075094 from the National Institutes of Health. Samples from the National Cell Repository for Alzheimer's Disease, which receives government support under cooperative agreement grant U24 AG21886, were used in this study.

**Online-Only Material:** The eTables and eFigure are available at <http://www.jamaneuro.com>.

**Additional Contributions:** We thank contributors, including the Alzheimer's Disease Centers and the Washington University Neuromuscular Genetics Project, which collected samples used in this study, as well as patients and their families, whose help and participation made this work possible.

## REFERENCES

- Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. *Sci Transl Med*. 2011;3(77):77sr71.
- Rogaeva EA, Fafel KC, Song YQ, et al. Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations. *Neurology*. 2001;57(4):621-625.
- Brickell KL, Leverenz JB, Steinbart EJ, et al. Clinicopathological concordance and discordance in three monozygotic twin pairs with familial Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2007;78(10):1050-1055.
- Athan ES, Williamson J, Ciappa A, et al. A founder mutation in presenilin 1 causing early-onset Alzheimer disease in unrelated Caribbean Hispanic families. *JAMA*. 2001;286(18):2257-2263.
- Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991;349(6311):704-706.
- Cruchaga C, Haller G, Chakraverty S, et al; NIA-LOAD/NCRAD Family Study Consortium. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families [published correction appears in *PLoS One*. 2012;7(5):doi/10.1371/annotation/c92e16da-7733-421d-b063-1db19488daa6]. *PLoS One*. 2012;7(2):e31039.
- Cortini F, Fenoglio C, Guidi I, et al. Novel exon 1 progranulin gene variant in Alzheimer's disease. *Eur J Neurol*. 2008;15(10):1111-1117.
- Rademakers R, Baker M, Gass J, et al. Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C-->T (Arg493X) mutation: an international initiative. *Lancet Neurol*. 2007;6(10):857-868.
- Brouwers N, Nuytemans K, van der Zee J, et al. Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. *Arch Neurol*. 2007;64(10):1436-1446.
- Lindquist SG, Holm IE, Schwartz M, et al. Alzheimer disease-like clinical phenotype in a family with FTDP-17 caused by a MAPT R406W mutation. *Eur J Neurol*. 2008;15(4):377-385.
- Rademakers R, Dermaut B, Peeters K, et al. Tau (MAPT) mutation Arg406Trp presenting clinically with Alzheimer disease does not share a common founder in Western Europe. *Hum Mutat*. 2003;22(5):409-411.
- Momeni P, Pittman A, Lashley T, et al. Clinical and pathological features of an Alzheimer's disease patient with the MAPT Delta K280 mutation. *Neurobiol Aging*. 2009;30(3):388-393.
- Ludolph AC, Kassubek J, Landwehrmeyer BG, et al; Reinsburg Working Group for Tauopathies With Parkinsonism. Tauopathies with parkinsonism: clinical spectrum, neuropathologic basis, biological markers, and treatment options. *Eur J Neurol*. 2009;16(3):297-309.
- DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*. 2011;72(2):245-256.
- Renton AE, Majounie E, Waite A, et al; ITALSGEN Consortium. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*. 2011;72(2):257-268.
- Kelley BJ, Haidar W, Boeve BF, et al. Alzheimer disease-like phenotype associated with the c.154delA mutation in progranulin. *Arch Neurol*. 2010;67(2):171-177.
- Reed LA, Grabowski TJ, Schmidt ML, et al. Autosomal dominant dementia with widespread neurofibrillary tangles. *Ann Neurol*. 1997;42(4):564-572.
- Majounie E, Abramzon Y, Renton AE, et al. Repeat expansion in C9ORF72 in Alzheimer's disease. *N Engl J Med*. 2012;366(3):283-284.
- Rollinson S, Halliwell N, Young K, et al. Analysis of the hexanucleotide repeat in C9ORF72 in Alzheimer's disease. *Neurobiol Aging*. 2012;33(8):1846.e5-e6.
- Berg L, McKeel DW Jr, Miller JP, et al. Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease. *Arch Neurol*. 1998;55(3):326-335.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939-944.
- Wijsman EM, Pankratz ND, Choi Y, et al; NIA-LOAD/NCRAD Family Study Group. Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. *PLoS Genet*. 2011;7(2):e1001308.
- Mok K, Traynor BJ, Schymick J, et al. Chromosome 9 ALS and FTD locus is probably derived from a single founder. *Neurobiol Aging*. 2012;33(1):209.e3-8.
- Li Y, Ding J, Abecasis GR. Mach 1.0: rapid haplotype reconstruction and missing genotype inference. *Am J Hum Genet*. 2006;79:S2290.
- Hansen L, Salmon D, Galasko D, et al. The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. *Neurology*. 1990;40(1):1-8.
- Förstl H, Burns A, Luthert P, Cairns N, Levy R. The Lewy-body variant of Alzheimer's disease: clinical and pathological findings. *Br J Psychiatry*. 1993;162:385-392.
- Murray ME, DeJesus-Hernandez M, Rutherford NJ, et al. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. *Acta Neuropathol*. 2011;122(6):673-690.
- Boeve BF, Boylan KB, Graff-Radford NR, et al. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. *Brain*. 2012;135(Pt 3):765-783.
- Wallon D, Rovelet-Lecrux A, Deramecourt V, et al. Definite behavioral variant of frontotemporal dementia with C9ORF72 expansions despite positive Alzheimer's disease cerebrospinal fluid biomarkers. *J Alzheimers Dis*. 2012;32(1):19-22.
- Tolboom N, Koedam EL, Schott JM, et al. Dementia mimicking Alzheimer's disease owing to a tau mutation: CSF and PET findings. *Alzheimer Dis Assoc Disord*. 2010;24(3):303-307.
- Lindquist SG, Schwartz M, Batbayli M, Waldemar G, Nielsen JE. Genetic testing in familial AD and FTD. *Clin Genet*. 2009;76(2):205-209.
- Carecchio M, Fenoglio C, De Riz M, et al. Progranulin plasma levels as potential biomarker for the identification of GRN deletion carriers. *J Neurol Sci*. 2009;287(1-2):291-293.
- Brouwers N, Sleegers K, Engelborghs S, et al. Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. *Neurology*. 2008;71(9):656-664.
- Ostojic J, Elfgren C, Passant U, et al. The tau R406W mutation causes progressive presenile dementia with bitemporal atrophy. *Dement Geriatr Cogn Disord*. 2004;17(4):298-301.