

Repeat expansions in the C9ORF72 gene contribute to Alzheimer's disease in Caucasians

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ARTICLE INFO

Article history:

Received 14 September 2012

Received in revised form 1 October 2012

Accepted 2 October 2012

Available online 27 October 2012

Keywords:

C9ORF72

Repeat expansion

Alzheimer disease

Genetic association

Repeat-primed PCR

Spectrum of neurodegenerative phenotypes

ABSTRACT

Recently, a hexanucleotide repeat expansion in the *C9ORF72* gene has been identified to account for a significant portion of Caucasian families affected by frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Given the clinical overlap of FTD with Alzheimer's disease (AD), we hypothesized that *C9ORF72* expansions might contribute to AD. In Caucasians, we found *C9ORF72* expansions in the pathogenic range of FTD/ALS (>30 repeats) at a proportion of 0.76% in AD cases versus 0 in control subjects ($p = 3.3E-03$; 1182 cases, 1039 controls). In contrast, no large expansions were detected in individuals of African American ethnicity (291 cases, 620 controls). However, in the range of normal variation of *C9ORF72* expansions (0–23 repeat copies), we detected significant differences in distribution and mean repeat counts between Caucasians and African Americans. Clinical and pathological re-evaluation of identified *C9ORF72* expansion carriers revealed 9 clinical and/or autopsy confirmed AD and 2 FTD final diagnoses. Thus, our results support the notion that large *C9ORF72* expansions lead to a phenotypic spectrum of neurodegenerative disease including AD.

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1. Introduction

Despite clinical and genetic heterogeneity, a number of molecular commonalities have long been recognized for disorders as diverse as Alzheimer's disease (AD), Parkinson disease (PD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). These diseases show extracellular and/or intracellular deposition of abnormal proteins, which has been attributed to a number of pathways, foremost the misfolded protein hypothesis (Taylor et al., 2002). Shared molecular mechanisms are usually the result of overlapping genetic factors. This has indeed been documented by the Alzheimer Disease & Frontotemporal Dementia and Parkinson

Disease Mutation Databases (www.molgen.ua.ac.be/ADMutations and www.molgen.ua.ac.be/FTDMutations for the AD&FTLD Mutation Database, and www.molgen.ua.ac.be/PDmutDB for the PD mutations database), which curate mutations in the 15 established monogenetic disease genes for AD, FTD, and PD: with the exception of the PD genes *PARK2* and *PINK1*, all other genes, for instance the AD gene *PSEN2* or the FTD gene *MAPT*, are associated with more than 1 clinical diagnosis or characteristics thereof (Cruts et al., 2012). Further characterization of the phenotypic spectrum of genes involved in neurodegeneration does improve clinical and molecular disease models.

Genetic linkage with FTD and/or ALS was established to chromosome 9p21 (Luty et al., 2008; Morita et al., 2006; Vance et al., 2006) and subsequently an intronic hexanucleotide (GGGGCC)_n repeat expansion in the *C9ORF72* gene (NM_145005) was identified as the underlying mutation (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Expansions measured by repeat-primed polymerase

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chain reaction (PCR) showing more than 30 repeats are considered pathogenic for FTD/ALS. Large samples of population control subjects have been reported with repeat counts ranging from 2 to 23 units (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Studies of large Caucasian patient samples have shown that the C9ORF72 repeat expansion accounts for up to 50% of ALS/FTD families, 7%–12% of FTD families, and 2%–5% of sporadic FTD (Boeve et al., 2012; Mahoney et al., 2012; Simon-Sanchez et al., 2012; Snowden et al., 2012). Similar frequencies have been observed in ALS families and sporadic cases (Chio et al., 2012; Cooper-Knock et al., 2012).

Considering the clinical, pathologic, and genetic characteristics of FTD, we hypothesized that C9ORF72 expansions might also confer risk to the most common neurodegenerative disease, AD. Clinically, both dementias show progressive impairment in cognition, memory, language, behavior, and motor functions (Goedert et al., 2012). Pathologically, atrophy in the frontal and temporal lobe overlap and a tau positive pathology is typical for AD and forms of FTD (Heutink 2000). Genetically, mutations in the tau protein (MAPT) cause autosomal dominant inherited FTD and heterogeneous clinical phenotypes resembling AD (Goedert et al., 2012; Rademakers et al., 2003). In fact, Majounie et al. (2012a) recently reported C9ORF72 repeat expansions in 3 out of 342 screened families from an AD collection. Based on postmortem brain studies that revealed FTD rather than AD in 1 family, the authors concluded that the most likely explanation is misclassification, rather than C9ORF72 being a direct cause for AD (Majounie et al., 2012a). Another screen for C9ORF72 expansions in 568 AD cases did not detect any expansion carriers (Rollinson et al., 2012).

Here we present a study of C9ORF72 repeat expansions in AD, comprising 1184 unrelated cases and 1039 controls of European descent. We also studied 291 African American AD cases and 620 controls to investigate repeat expansion distributions between ethnicities. Each individual classified as an AD case met the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable or definite AD (McKhann et al., 1984). The results of this study likely expand the genetic basis of AD and contribute to the understanding of the phenotypic spectrum of C9ORF72 repeat expansions.

2. Methods

2.1. Study samples

Written informed consents were obtained from all participants, in accordance with Institutional Review Board protocols at each study center. The study included 1184 unrelated European and 291 unrelated African American individuals with a clinical diagnosis of AD and 1039 European and 620 African American cognitive controls. Supplementary Table 1 provides descriptive characteristics about these study samples. All cases and control subjects were clinically ascertained through the University of Miami at the John P. Hussman Institute for Human Genomics, the Vanderbilt University Center for Human Genetics Research, the Department of Biology at North Carolina Agricultural and Technical State University, and were sampled from pathologically confirmed donors to the University of Miami Brain Endowment Bank. Each individual classified as an AD case met the NINCDS-ADRDA criteria for probable or definite AD (McKhann et al., 1984). Where possible, clinically ascertained and deceased participants had AD confirmed via autopsy. Individual's pathologic diagnoses most critical to the interpretation of this study outcome were re-evaluated by 2 independent neuropathologists with long-standing experience in AD, Drs Carol Petito (University of Miami) and Christine Hulette (Duke

University). Age at onset was estimated as date of first onset of symptoms as reported by the patient, their informant, or abstracted from the patient's medical records. Control subjects had Mini Mental State Examination scores ≥ 27 at an age at exam ≥ 60 years. Genome-wide single nucleotide polymorphism (SNP) data were available for the studied individuals, allowing principal components analysis on a sample of 20,000 SNPs to remove population outliers by using the top 10 principal components over 5 iterations with a threshold of 6 standard deviations (Naj et al., 2010). The top 3 principal component loadings were used to validate the ethnic background of self-reported ethnicity. The pedigrees presented in Fig. 1 have been partially altered and masked to hide identifying information. DNA samples of 3 ALS patients, provided by M. Benatar, served as positive controls for large C9ORF72 repeat expansions (>50 repeats) (Renton et al., 2011). Apolipoprotein E (APOE) genotype data were available on all participants and were determined as previously described (Naj et al., 2011).

2.2. Repeat-primed PCR assay

DNA samples of cases and controls were subjected to repeat-primed PCR to determine the number of hexanucleotide (GGGGCC)_n repeat expansions in the C9ORF72 gene on chromosome 9p21. We used a previously described repeat-primed PCR assay (Renton et al., 2011) by applying the same reaction mix protocol and primers, but optimized the PCR cycling program to achieve robust results. Fragment length analysis was performed on an ABI 3730xl genetic analyzer (ABI), and data were analyzed using GeneMapper software (version 4, ABI). Our assay limits for the maximal number of detectable repeats was between 60 and 70 repeats which is in accordance with previous studies (Kobayashi et al., 2011). To determine the assay's precision in counting out repeat copies in the range of normal variation (≤ 4 –23 repeats observed in our control subjects), we used synthetic ultramer DNA oligos (Integrated DNA Technologies, Inc) with incorporated 8 and 16 repeat units in addition to the 3 units present in the human reference sequence. Moreover, in this range, we determined resampling reproducibility of repeat counts of the assay by running repeated measurements ($n = 260$) of 100 randomly selected samples.

Each of the expansion-carrying samples was genotyped by Sanger sequencing for the SNP rs3849942, which serves as a surrogate marker for expanded C9ORF72 haplotypes in European individuals (DeJesus-Hernandez et al., 2011).

2.3. Statistical methods

We tested for an overrepresentation of large repeat expansions (>30 repeats) in the pathogenic range for ALS/FTD (Renton et al., 2011) in AD cases versus cognitively normal elderly controls (age at exam ≥ 60 years) by conducting 1-sided Fisher's exact tests in Caucasian and African American individuals. In the normal range of repeat copy variation (≤ 4 –23 in this study's control subjects), we tested for case-control and ethnic differences in repeat count distributions and means by applying the Kolmogorov–Smirnov test and a standard *t* test, respectively. All analyses were conducted using R software version 2.13.0 (<http://www.r-project.org/>).

3. Results

3.1. A subset of Caucasian AD patients presents with C9ORF72 repeat expansions

We identified C9ORF72 expansions in the reported pathogenic range of FTD/ALS (>30 repeats) in cases that met the NINCDS-ADRDA criteria for probable or definite AD from European

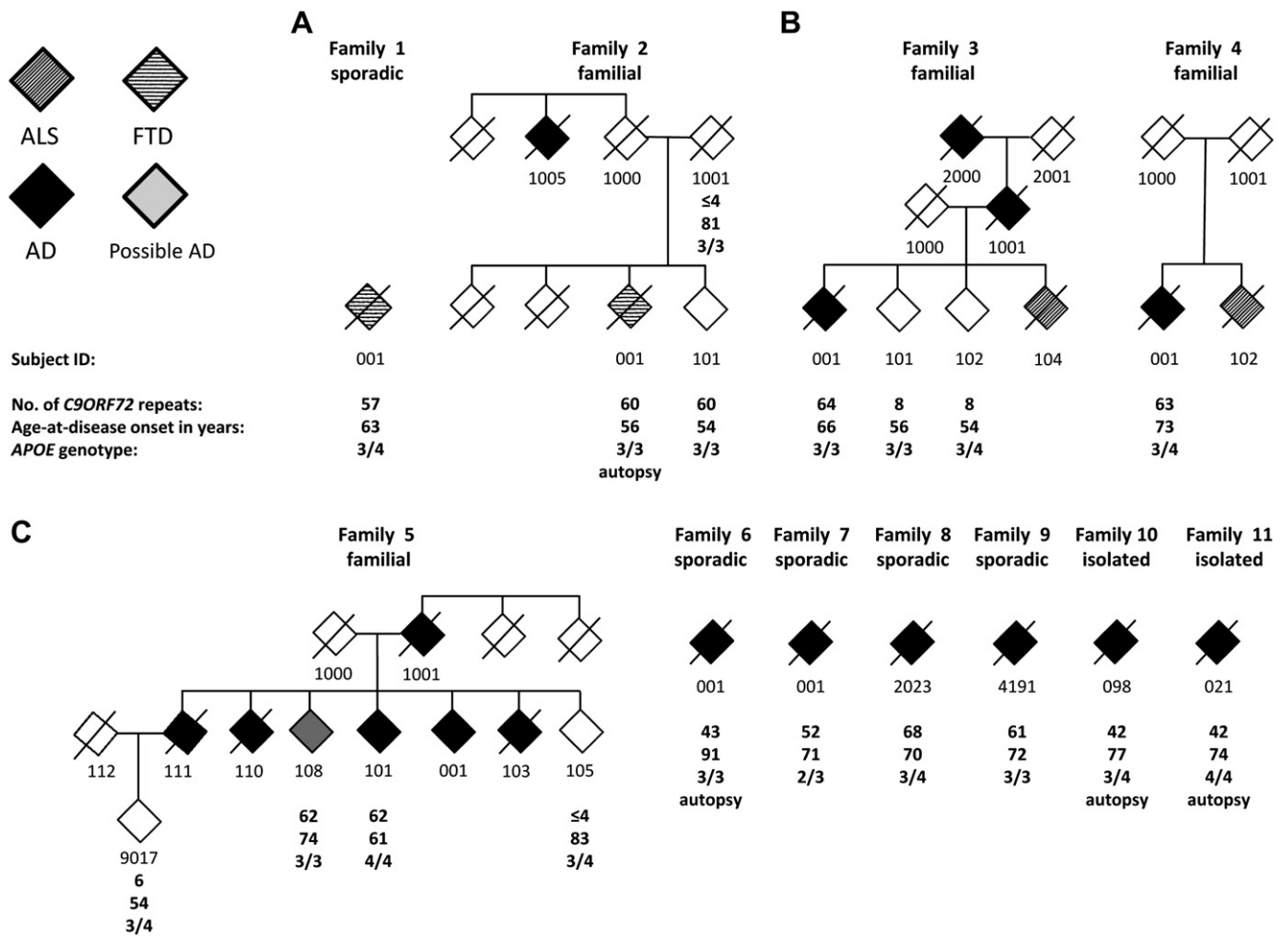


Fig. 1. Overview of families and sporadic individuals affected by *C9ORF72* expansions. (A) Frontotemporal dementia (FTD) reclassified index case/family, (B) Alzheimer's disease (AD)/amyotrophic lateral sclerosis (ALS) families, (C) AD family and sporadic/isolated AD cases. Sporadic is defined as a single case with recorded family history, and an isolated case did not have family history available. Sex is not provided for privacy reasons.

descent at a rate of 0.9% (11 out of 1184 unrelated cases, Table 1). In contrast, no expansions were identified in 1039 dementia-free elderly control subjects from the same ethnicity (1-sided Fisher exact test $p = 9.6E-04$; Table 2A). This case-control association remained significant after exclusion of 2 *C9ORF72* expansion carriers that were reclassified with autopsy-confirmed FTD and possible clinical FTD ($p = 3.3E-03$; Table 2A).

In African American individuals *C9ORF72* expansions were not present (291 cases, 620 controls). Considering the frequency of expansion carriers observed in samples of European descent we would have expected to detect 2.7 expansion carriers in the set of 291 African American cases. Formally, *C9ORF72* expansions were significantly overrepresented in Europeans compared with African Americans (1-sided Fisher exact $p = 0.023$; Table 2B).

Four index patients with expansions had family members with diagnoses of dementia or ALS, 5 were sporadic dementia cases with known family history, and 2 were isolated cases (no family history data available). Three of these families included 2–7 dementia cases with affected individuals in multiple generations (families 2, 3, 5; Fig. 1, Table 1). The *C9ORF72* expansions cosegregated in these large families with the phenotype (Fig. 1).

The SNP marker rs389942 accurately tags the Northern European-derived haplotype on which large *C9ORF72* repeat expansions have been reported (DeJesus-Hernandez et al., 2011). We confirmed the

presence of the risk-haplotype associated A-allele in all of our expanded samples.

3.2. Clinical presentations of patients with expanded repeats

Clinical data was available for each of the 11 index patients with *C9ORF72* expansions (>30 repeats, see Supplementary data for detailed clinical reports). Four index patients obtained autopsy-confirmed diagnoses: 2-001, 6-001, 10-098, and 11-021; Figs. 1 and 2, Table 1). For 2 of these cases histological slides were available for re-evaluation of autopsy diagnoses (2-001, 6-001, see Supplementary data for autopsy reports). We identified a clinical spectrum of phenotypes associated with *C9ORF72* expansions in these families and patients (Fig. 1). In brief, 2 families were characterized by index patients, for which re-evaluation of clinical and neuropathologic data resulted in their reclassification with FTD (1-001 and 2-001). Two additional families with *C9ORF72* expansions presented with index patients with clinical AD who also had first-degree relatives with ALS (3 and 4). Finally, the large family number 5 consisted of an index patient and family members ($n = 5$) that were exclusively diagnosed with clinical AD. Furthermore, we identified 4 sporadic and 2 isolated AD index patients with clinical diagnoses of AD (families 6–11) of which 3 had autopsy-confirmed diagnoses of AD (6-001, 10-098, and 11-021).

Table 1
Final diagnoses and further characterization of *C9ORF72* expansion carriers and unaffected family members

Phenotype	Family ID	Individual ID	Repeats	Final diagnosis	Relatedness	AAO	AAE	APOE
FTD, sporadic	1	001	57	Clinical FTD	Index patient	63	65	3/4
FTD/AD	2	001	60	Autopsy confirmed FTLD-U	Index patient	56	59	3/3
		101	60	Unaffected	Sibling	-	54	3/3
		1001	≤4	Unaffected	Sibling	-	81	3/3
		1005	-	Clinical AD	Paternal uncle	-	-	-
AD/ALS	3	001	64	Clinical AD	Index patient	66	74	3/3
		101	8	Unaffected	Sibling	-	56	3/3
		102	8	Unaffected	Sibling	-	54	3/4
		104	-	ALS	Sibling	-	-	-
AD/ALS	4	001	63	Clinical AD	Index patient	73	78	3/4
		102	-	ALS	Sibling	-	-	-
AD	5	101	62	Clinical AD	Index patient	61	66	4/4
		105	≤4	Unaffected	Sibling	-	83	3/4
		108	62	Possible AD	Sibling	-	74	3/3
		9017	8	Unaffected	Nephew	-	54	3/4
AD, sporadic	6	001	43	Autopsy confirmed AD	Index patient	91	93	3/3
AD, sporadic	7	001	52	Clinical AD	Index patient	71	74	2/3
AD, sporadic	8	2023	68	Clinical AD	Index patient	70	72	3/4
AD, sporadic	9	4191	61	Clinical AD	Index patient	72	72	3/3
AD, isolated	10	098	42	Autopsy confirmed AD	Index patient	77	82	3/4
AD, isolated	11	021	42	Autopsy confirmed AD	Index patient	74	80	4/4

Key: AAE, age at exam; AAO, age at onset of disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APOE, apolipoprotein E Alzheimer's disease risk genotype; FTD, frontotemporal dementia; FTLD-U, fronto-temporal lobe degeneration with ubiquitin-only inclusions.

3.3. *C9ORF72* repeat expansions in the normal range of variation do not contribute to AD risk

The range of *C9ORF72* repeat copies varied between ≤4 and 23 repeat units in our combined samples of 1464 cases and 1659 control subjects after exclusion of cases with repeat expansions in the pathogenic range (>30 copies; Fig. 3). In the normal range of *C9ORF72* repeat expansions, the repeat-primed PCR assay applied to determine *C9ORF72* repeat copy numbers was highly accurate and reproducible: the average resampling error rate was 0.16 repeat copies per measurement with a maximal observed deviation of 2 repeat units. We addressed the hypothesis of whether *C9ORF72* repeats especially in the upper normal range of variation might already interfere with *C9ORF72* gene function leading to AD risk. We tested for differences in mean repeat counts and repeat count distributions between AD cases and control subjects. Statistical test results are given in Table 3A and histograms of repeat distributions are shown in Fig. 3A and B. We did not observe a statistically significant difference of repeat count distribution between cases and control subjects in African American, Caucasian, or the combined data sets. Case-control comparison of mean repeat copy numbers in Caucasian

subjects only reached nominal significance, but with a slightly higher mean in repeat copies in control subjects compared with cases.

3.4. *C9ORF72* repeat distributions vary between European and African American ethnicities

As we reported above, carriers of large *C9ORF72* repeat expansions (>30 repeats) were exclusively found in AD cases of European descent, but not in African American cases. Interestingly, we did observe significant differences in mean repeat copies and repeat distributions between samples of African American and European descent in the normal range of *C9ORF72* repeat copy variation (between ≤4 and 23 repeats; Table 3B). The mean repeat length was slightly greater in European versus African American control subjects and in the combined case and control samples (6.97 vs. 6.72 repeat copies, *t* test *p* = 0.023). The Kolmogorov–Smirnov test showed highly significant differences in the distribution of repeat counts in African American versus European samples. This was observed in control subjects, cases, and the combined samples (*p* = 5.9E-07; Table 3B). The histograms in Fig. 3C and D show that specific repeat copy number classes are more common in either African American or European samples.

Table 2
C9ORF72 repeat mean copy count and distribution comparisons in the range of normal repeat copy variation (≤4–23 repeats)

Comparisons	<i>n</i>	Average repeats		KS test		<i>t</i> test	
		Case	Control	<i>D_n</i>	<i>p</i>	<i>t</i>	<i>p</i>
(A) Case-control status ^a							
African American	292	620	6.64	6.76	0.07	0.220	-0.68 0.495
European descent	1257	1039	6.81	7.08	0.05	0.141	-2.00 0.046
All (AA and ED)	1549	1659	6.78	6.96	0.05	0.073	-1.71 0.087
(B) AA vs. ED ethnicity ^a							
Control	620	1039	6.76	7.08	0.11	1.9E-04	-2.17 0.0305
Case	292	1257	6.64	6.81	0.10	0.015	-1.02 0.3080
All (cases and controls)	912	2296	6.72	6.93	0.11	1.5E-07	-1.96 0.0498

Key: AA, African American; ED, European descent.

^a Samples with >30 repeats were excluded.

4. Discussion

4.1. *C9ORF72* repeat expansions underlie a spectrum of neurodegenerative phenotypes, including AD

Our study represents a sizable screen for *C9ORF72* expansions in AD comprising more than 3100 unrelated individuals from European and African American descent. We detected *C9ORF72* expansion carriers at a proportion of 0.9% exclusively among Caucasian individuals with an initial clinical diagnosis of AD meeting NINCDS-ADRDA criteria for probable or definite AD (McKhann et al., 1984). This case-control association remained significant after removal of 2 cases that were reclassified as FTD (*p* = 3.3E-03; 1-001 and 2-001, see Supplementary data). These findings are in concordance with a recently reported *C9ORF72* repeat expansion screen in another AD cohort, reporting 1 misclassified FTD family based on autopsy

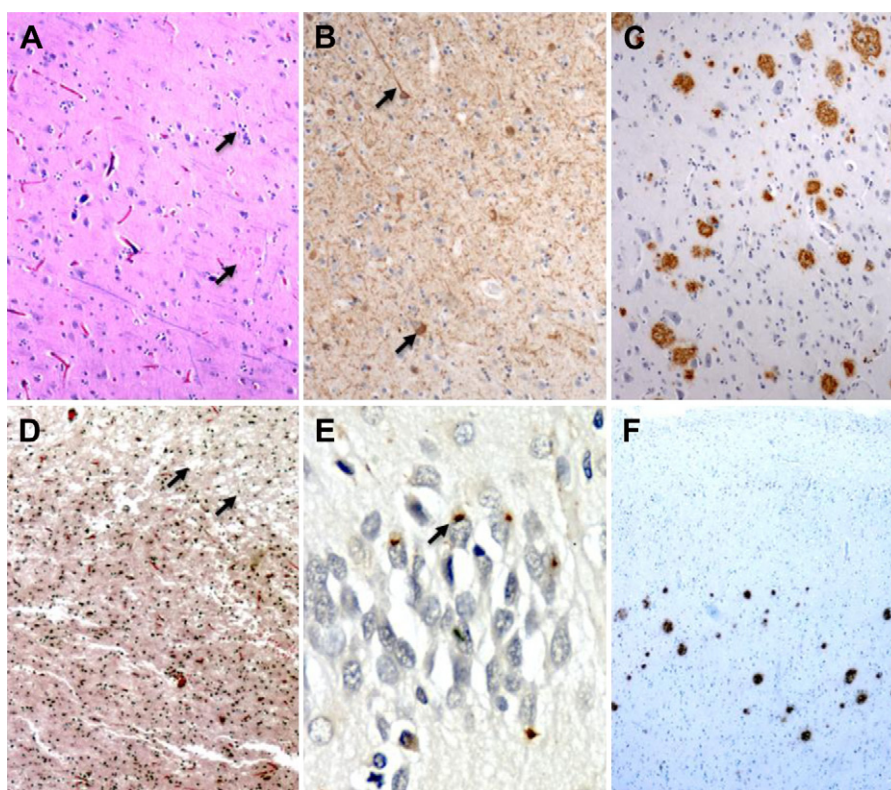


Fig. 2. Histopathologic findings in *C9ORF72* expansion carriers. (A–C) The index case in family 6 showed classic Alzheimer's disease changes including (A) neuronal loss and plaques (B) tau-positive threads and tangles, and (C) amyloid plaques throughout the neocortex and hippocampus. (D–F) The index patient from family 2 showed (D) microvacuolation in the frontal cortex, (E) ubiquitin-immunoreactive intracellular inclusions in the hippocampus, and (F) cortical extracellular amyloid plaques. For more details see [Supplementary data](#).

(Majounie et al., 2012a). However, the remaining 9 expansion carriers of this study presented with a final diagnosis of AD after careful clinical and pathologic re-evaluation of their diagnoses. Three of these index cases additionally obtained an autopsy-confirmed diagnosis of AD (6-001, 10-098, and 11-021). None of the 9 confirmed AD cases showed any signs of clinical or pathologic FTD. The autopsy-confirmed AD diagnosis of index case 6-001, for which histologic slides were available, was independently ascertained by 2 experienced neuropathologists (see [Methods](#)). Furthermore, 2 clinically confirmed AD index cases belonged to AD families with first- and/or second-degree relatives affected by AD. Importantly, repeat expansions in these families cosegregated with AD (family 3 and 5; [Fig. 1](#)). Interestingly, 2 AD index cases had family members with a diagnosis of ALS (family 3 and 4). Although we have no knowledge of the *C9ORF72* repeat status of these family members it is an intriguing observation that suggests additional environmental or genetic factors modulating the phenotypic outcome of expanded repeats.

AD is a genetically heterogeneous disease with the APOE $\epsilon 4$ allele constituting a considerable risk factor for late-onset AD (Corder et al., 1993). However, the APOE $\epsilon 4$ allele was not over-represented in *C9ORF72* expansion carriers of this study. Five expansion carriers were also carriers for the APOE $\epsilon 4$ allele and 4 were not ([Table 1](#), [Fig. 1](#)). Among the 3 *C9ORF72* expansion carriers with a pathologically confirmed diagnosis of AD, 2 carried APOE $\epsilon 4$ risk alleles ($\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ genotypes), but a third did not ($\epsilon 3/\epsilon 3$). Further, mean disease onsets were not significantly different between APOE $\epsilon 4$ carriers and noncarriers.

In *C9ORF72* FTD/ALS, average age of disease onset is in the 50s, but a very wide age range has been reported from 32 to 78 (Hodges 2012). The condition is highly penetrant with 50% of carriers affected at 58 years of age and near 100% by age 80 (Majounie et al., 2012b). The 9 *C9ORF72* carriers affected by AD in this study

presented with a seemingly higher average disease onset of 77.8 years, although their number is not large enough for an adequately powered comparison. All of them had an onset of AD at older than 60 years of age, thus representing cases of late-onset AD (Corder et al., 1993). Intriguingly, this higher disease onset observed in our index cases is consistent with findings on c9FTD/ALS cases of a previous pathological study in which the subgroup of FTD patients characterized by Mackenzie Type 1 TAR DNA-binding protein 43 (TDP-43) pathology with neuronal intranuclear inclusions and hippocampal sclerosis presented with a similarly higher disease onset, and with some of them being thought to have an Alzheimer-type dementia (Murray et al., 2011).

Although we definitely can exclude phenocopies for the 3 index AD cases that obtained an autopsy-confirmed diagnosis of AD, defined by the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles (Heutink 2000) which are absent in classic FTD, we cannot completely rule out concomitant AD causally unrelated to *C9ORF72* expansions. However, we believe that this scenario is unlikely, considering the nearly complete penetrance of *C9ORF72* FTD/ALS at the age of our index patients together with the absence of clinical or neuropathologic signs of FTD in 9 AD index cases, cosegregation of expansions in 2 AD families, and the significant case-control association between large *C9ORF72* expansions and re-evaluated AD cases compared with elderly neurologically healthy control subjects.

In conclusion, our data support the notion that large *C9ORF72* expansions underlie a spectrum of neurodegenerative phenotypes, likely including AD, besides well-established FTD and ALS. As shown in the Results, we detected large *C9ORF72* expansion carriers with a final diagnosis of AD at a proportion of 0.76%. Although this portion seems small, it actually is of importance, because with the exception of rare mutations in the established early-onset AD genes (*APP*, *PSEN1*,

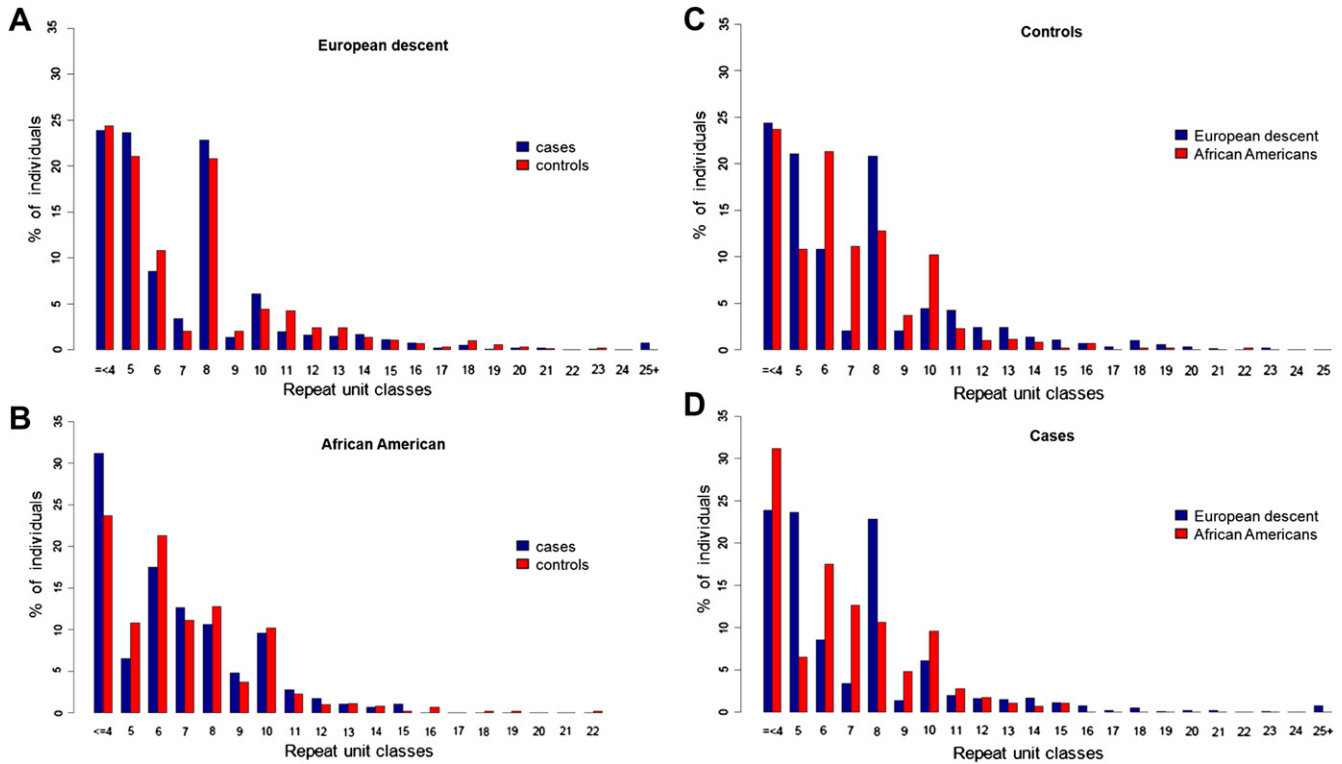


Fig. 3. (A and B) Histograms of *C9ORF72* repeat copies in Alzheimer's disease case-control collections of (A) European and (B) African American descent. (C and D) Histograms of *C9ORF72* repeat copies compared between European and African American ethnicities in (C) cases and (D) control subjects.

and *PSEN2*) (Cruchaga et al., 2012) monogenic disease genes have not yet been described for late-onset AD. Moreover, *C9ORF72* expansions in AD at the observed proportion would account for total AD cases at a similar ratio as the 3 yet described AD genes combined lead to monogenic forms of AD (Brouwers et al., 2008). However, further studies of even larger AD cohorts, preferably with neuropathologically confirmed diagnoses and segregation patterns in families are warranted to firmly establish the contribution of large *C9ORF72* expansions to AD pathology. The broad phenotypic spectrum associated with *C9ORF72* expansions offers an opportunity to further decipher the underlying molecular pathology of neurodegenerative disease.

4.2. *C9ORF72* repeat count distributions vary between ethnicities of African and European origin

The prevalence of the *C9ORF72* repeat expansion has been established in large studies of ALS/FTD, but is mainly based on

Table 3
Case-control and ethnic comparisons of AD case-control collections with and without *C9ORF72* expansions in the pathogenic range of ALS/FTD (>30 repeat copies)

Comparisons	<30 repeats	≥30 repeats	<30 repeats	≥30 repeats	Fisher exact p, 1-sided
(A) Case-control	Case	Control			
African American	291	0	620	0	1.0
European descent	1173	11 (9)	1039	0	9.6E-04 (3.3E-03)
(B) AA vs. ED ethnicity	AA	ED			
Cases	291	0	1173	11 (9)	0.044 (0.069)
Cases and controls	911	0	2212	11 (9)	0.023 (0.045)

Key: AA, African American; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ED, European descent; FTD, frontotemporal dementia. Counts and corresponding *p* values in parentheses represent an analysis that excluded subsequently reclassified FTD cases.

samples of European descent. However, large expansion carriers have been reported in African American, Hispanic American, and Japanese ALS patients (Ishiura et al., 2012; Majounie et al., 2012b). To our knowledge, our study represents the largest screen for *C9ORF72* expansions in AD case-control cohorts in African American samples. We did not identify any carriers of large *C9ORF72* expansions in African American subjects. Although our study reached a significant underrepresentation of *C9ORF72* expansion carriers in African American compared with Caucasian samples, this analysis was not statistically highly powered because of the smaller number of African American samples compared with those from European descent (*n* = 292 vs. *n* = 1184) and low *C9ORF72* expansion frequency in AD. However, our results suggest a lower frequency of large *C9ORF72* expansion carriers in African American subjects which is consistent with the proposed Northern European origin of the expansion haplotype. Most expansion carriers, including non-Europeans, share the same ancestral founder haplotype (Ishiura et al., 2012; Majounie et al., 2012b; Mok et al., 2012). In Caucasian subjects, this haplotype is highly tagged by the A-allele of the surrogate marker, SNP rs3849942 (DeJesus-Hernandez et al., 2011). However, in contrast to some African populations, the A-allele frequency of rs3849942 is only slightly lower in African American (18%–20%) compared with 23.5% in Caucasian populations. Thus, *C9ORF72* expansion carrier proportions could potentially be similar, though not cohesively, because of varying linkage disequilibrium structures between both ethnicities (Gabriel et al., 2002).

Interestingly, in the normal range of repeat copy variation (≥4–23 copies), we observed significant differences in mean *C9ORF72* repeat copies and repeat copy distributions between samples of African American and European descent. Mean repeat copy numbers were significantly higher in individuals of European descent. The ethnic differences in repeat copy number distributions were most pronounced and reached significance in case and

control separate analyses with the lowest p value in the combined analysis ($p = 1.5E-07$; Table 3B). The frequency distribution of large C9ORF72 expansions throughout European populations is in concordance with a single 1-off expansion that occurred in Northern Europe approximately 1500 years ago (Majounie et al., 2012b; Mok et al., 2012). Alternatively, it has been speculated that the expansion haplotype could be more prone to repeat expansions. In fact, the later has been proposed based on a study reporting a highly significant mean C9ORF72 repeat copy number difference in Caucasian control subjects being homozygous for the A-allele of rs3849942 (mean: approximately 8 repeat copies) in relation to controls being homozygous for the alternative G-allele (mean: approximately 3 repeat copies at population average) (DeJesus-Hernandez et al., 2011). The on average already higher C9ORF72 repeat copy numbers on the 'expansion' haplotype might be at higher risk for further expansion during meiosis, a phenomenon referred to as genetic anticipation that has been reported for C9ORF72 expansions at least in the pathogenic range (Hodges 2012). In this respect, the here reported ethnic differences in C9ORF72 repeat copy number distributions in the normal range of variation becomes important, especially because these differences are strongest in repeat copy number classes ranging from 3 to 10 repeat copies (Fig. 3C and D). Thus, the rather lower abundance of large C9ORF72 expansions in African American compared with Caucasian subjects could be a consequence of different haplotype distributions between these ethnicities, including haplotypes at different risk for spontaneous expansion, consistent with their genetic history and a founder event in Northern Europe. Differences in the prevalence of C9ORF72 expansion carriers between ethnicities have the potential to increase our knowledge on the underlying mechanisms and prevent ethnically driven health disparities.

Disclosure statement

The authors report no potential conflicts of interest.

Written informed consents were obtained from all participants, in accordance with Institutional Review Board protocols at each study center.

Acknowledgements

The authors thank Drs Carol Petito (University of Miami) and Christine Hulette (Duke University) for their neuropathological expertise, and the families and staff who participated in this study. This work was supported by National Institute of Health grants R01 AG027944 (MAP-V), R01 AG019085, R01 AG028786-02 (MAP-V), RC2AG036528 (MAP-V; JLH), 5R01 AG028786-04 (MAP-V, JLH, GB), the Alzheimer's Association grant IIRG09133827 (MAP-V), and the American Health Assistance Foundation grant A2011048 (MAP-V, SZ). Michael Benatar's funding support relevant to this work are from the MDA (Muscular Dystrophy Association), the ALS Association, and the ALS Recovery Fund.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2012.10.003>.

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