

Investigation of *C9orf72* in 4 Neurodegenerative Disorders

Zhengru Xi, PhD; Lorne Zinman, MD; Yakov Grinberg, PhD; Danielle Moreno, BSc; Christine Sato, MSc; Juan M. Bilbao, MD; Mahdi Ghani, MD; Isabel Hernández, MD; Agustín Ruiz, MD, PhD; Mercè Boada, MD, PhD; Francisco J. Morón, PhD; Anthony E. Lang, MD; Connie Marras, MD, PhD; Amalia Bruni, MD; Rosanna Colao, MD; Raffaele G. Maletta, MD; Gianfranco Puccio, MD; Innocenzo Rainero, MD, PhD; Lorenzo Pinessi, MD; Daniela Galimberti, PhD; Karen E. Morrison, PhD; Catriona Moorby, BSc; Joanne D. Stockton, BSc; Mario Masellis, MD; Sandra E. Black, MD; Lili-Naz Hazrati, MD; Yan Liang, MD; Jan van Haersma de With, BSc; Luis Fornazzari, MD; Roque Villagra, MD; Ricardo Rojas-Garcia, MD, PhD; Jordi Clarimón, PhD; Richard Mayeux, MD; Janice Robertson, PhD; Peter St George-Hyslop, MD, FRCP(C); Ekaterina Rogaeva, PhD

Objective: To estimate the allele frequency of *C9orf72* (G_4C_2) repeats in amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer disease (AD), and Parkinson disease (PD).

Design: The number of repeats was estimated by a 2-step genotyping strategy. For expansion carriers, we sequenced the repeat flanking regions and obtained *APOE* genotypes and *MAPT* H1/H2 haplotypes.

Setting: Hospitals specializing in neurodegenerative disorders.

Subjects: We analyzed 520 patients with FTLD, 389 patients with ALS, 424 patients with AD, 289 patients with PD, 602 controls, 18 families, and 29 patients with PD with the *LRRK2* G2019S mutation.

Main Outcome Measure: The expansion frequency.

Results: Based on a prior cutoff (>30 repeats), the expansion was detected in 9.3% of patients with ALS, 5.2% of patients with FTLD, and 0.7% of patients with PD but not in controls or patients with AD. It was significantly

associated with family history of ALS or FTLD and age at onset of FTLD. Phenotype variation (ALS vs FTLD) was not associated with *MAPT*, *APOE*, or variability in the repeat flanking regions. Two patients with PD were carriers of 39 and 32 repeats with questionable pathological significance, since the 39-repeat allele does not segregate with PD. No expansion or intermediate alleles (20-29 repeats) were found among the G2019S carriers and AD cases with TAR DNA-binding protein 43-positive inclusions. Surprisingly, the frequency of the 10-repeat allele was marginally increased in all 4 neurodegenerative diseases compared with controls, indicating the presence of an unknown risk variation in the *C9orf72* locus.

Conclusions: The *C9orf72* expansion is a common cause of ALS and FTLD, but not of AD or PD. Our study raises concern about a reliable cutoff for the pathological repeat number, which is important in the utility of genetic screening.

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AMYOTROPHIC LATERAL SCLEROSIS (ALS) and frontotemporal lobar degeneration (FTLD) are fatal neurodegenerative syndromes that belong to the same clinicopathological spectrum.^{1,2} Frontotemporal lobar degeneration is a primary dementia characterized by early behavioral, language, and extrapyramidal changes, while symptoms of ALS are the result of the degeneration of motor neurons. Both syndromes may occur within the same family or even the same patient.

Previously, linkage analyses revealed a 3.7-Mb region on 9p21 associated with familial ALS/FTLD,³⁻¹⁰ and genome-wide as-

sociation studies suggested a major risk factor in the same locus for sporadic ALS and FTLD.¹¹⁻¹⁵ Recently, 2 research groups independently explained this locus by a pathological noncoding hexanucleotide (G_4C_2)₃₀₋₁₆₀₀ repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene of unknown function.^{16,17} Based on the allele frequencies in cases vs controls, the first studies suggested that expansions with more than 30 repeats should be considered pathological, while alleles with less than 20 repeats are wild type.¹⁶ However, a reliable cutoff for the pathological alleles remains to be established by additional studies (eg, segregation, neuropathological, or functional studies). Fur-

Author Affiliations are listed at the end of this article.

Table 1. Sample Characteristics, Including Expansion Carriers Identified in Each Cohort

Cohort	All Samples, No. (%)	No. of Expansion Carriers ^a	Frequency of Expansion, %
ALS			
Age at onset, y, mean (SD)	57.6 (12.3)		
Female	149 (38.3)		
Total	389	36	9.3
Familial	47	18	38.3
FTLD			
Age at onset, y, mean (SD)	65.4 (10.1)		
Female	258 (49.6)		
Total	520	27	5.2
Familial	211	22	10.4
AD			
Age at onset, y, mean (SD)	72.1 (9.4)		
Female	264 (62.3)		
Total	424
Familial	167
PD			
Age at onset, y, mean (SD)	52.6 (13.0)		
Female	92 (31.8)		
Total	289	2	0.7
Familial	116	1	0.9
Controls			
Age, y, mean (SD)	70.2 (9.5)		
Female	357 (59.3)		
Total	602

Abbreviations: AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; ellipses, not detected/not applicable; FTLD, frontotemporal lobar degeneration; PD, Parkinson disease

^aThe allele was counted as an expansion allele when the number of repeats was more than 30.

Furthermore, the contribution of intermediate-size alleles (20-29 repeats) to disease pathology has not yet been evaluated.

The expansion is the most frequent cause of ALS and FTLD identified to date. In the Finnish population, 46% of patients with familial ALS, 21% of patients with sporadic ALS, and 29% of patients with sporadic FTLD have the expansion.¹⁶ DeJesus-Hernandez et al¹⁷ reported the expansion in 24% of patients with familial ALS, 4% of patients with sporadic ALS, and 12% of patients with familial FTLD. In the Flanders-Belgian cohort, the mutation was observed in 47% of patients with familial ALS, 5% of patients with sporadic ALS, and 16% of patients with familial FTLD.¹⁸ The pathological mechanism associated with the expansion is currently unknown except that the expansion leads to a 50% reduction of *C9orf72* messenger RNA expression,^{17,18} and the brain pathology in mutation carriers is associated with possibly toxic nuclear RNA foci, as well as TAR DNA-binding protein 43 (TDP-43) and p62 inclusions.^{17,19} The clinical phenotype appears to be highly heterogeneous in reported mutation carriers.²⁰

Following the discovery of this novel mutation, many questions are yet to be addressed. What is the expansion frequency in other ALS/FTLD cohorts? Are there any

clinical features that can discriminate between patients with and without the *C9orf72* expansion? What is a reliable cutoff for the pathological repeat number? What is the role of alleles with intermediate repeat sizes or variability in the region flanking the G₄C₂ repeat? Could the expansion account for other neurodegenerative diseases, such as Alzheimer disease (AD) or Parkinson disease (PD)? These questions were investigated by the current study. The expansion frequency was estimated in a comprehensive case-control sample set consisting of 2224 individuals (patients with FTLD, ALS, AD, and PD and healthy controls). To our knowledge, this is the first case-control study using a 2-step genotyping strategy that allowed for the analysis of genotype information for the alleles with less than 50 repeats. Clinical data were analyzed to understand the phenotype spectrum observed in mutation carriers.

METHODS

HUMAN SAMPLES

Informed consent was obtained from all participants in accordance with the respective ethical review boards. Sample characteristics are presented in **Table 1**. All study participants were either European (from Italy, Spain, and the United Kingdom) or North American (white individuals mainly of North European origin) recruited from hospitals specializing in neurodegenerative disorders. The investigated unrelated individuals included 520 patients with FTLD, 389 patients with ALS, 424 patients with AD, and 289 patients with PD and 602 neurologically healthy controls (>65 years). Patients were diagnosed using established clinical criteria.²¹⁻²⁴ Cases with known pathological mutations were excluded from the study. However, a previously described data set of 29 Canadian patients with PD carrying the common *LRRK2* G2019S mutation²⁵ was analyzed separately for the presence of an expansion or intermediate allele in *C9orf72* as a potential modifier of age at onset of parkinsonism. In addition, all available family members of 18 extended pedigrees were genotyped for the G₄C₂ repeats (6 FTLD, 9 ALS, 1 PD, and 2 AD families).

GENOTYPING ASSAYS

Genomic DNA was isolated from blood using a QIAGEN kit. To detect the size of the *C9orf72* alleles within the normal to intermediate range of G₄C₂ repeats (detection limit is 50 repeats) and the presence of large expansions, a 2-step genotyping strategy was used as previously described¹⁷ (eFigure 1, http://www.joygrafika.com/projects/University_of_Toronto). Briefly, in the first step, DNA samples (10 ng/polymerase chain reaction [PCR]) were amplified using primers near the repeat region (5'-FAM-caaggagggaacaaccgcagcc and 5'-gcaggcaccgcaaccgcag). The fragment-length analysis was performed on an ABI PRISM 3100 DNA analyzer and visualized by Genotyper software version 2.5 (Applied Biosystems). Since expanded alleles are not amplifiable with this set of primers, expansion carriers appear to be homozygous for a normal repeat allele (in addition to true homozygotes). The number of repeats was calculated based on the fragment size (eg, 129 base pairs [bp] represents 2 repeats, which was confirmed by sequencing 7 samples [PCR primers: 5'-cgctcatgcacatagaaaaca and 5'-ggagacagctcgggtactga]). Since published sequencing analysis demonstrated that the G₄C₂ repeats are uninterrupted,¹⁷ the number of repeats for each allele was calculated using the following formula: (amplicon length - 117)/6. Samples

scored as homozygous were included in the second step to detect large expansions (>50 repeats) by a repeat-primed PCR. DNA samples (100 ng/PCR) were amplified as described previously using 3 primers (MRX-F: FAM-tgtaaacgacgcccagtcaggagggaacaaccgcagcc, MRX-M13R: caggaaacagctatgacc, and MRX-R1: caggaaacagctatgaccgggccccgaccacgccccggccccggccccgg),¹⁷ except the primer ratio was modified to increase PCR efficiency (MRX-F/MRX-M13R/MRX-R1 = 5/5/1). Data were analyzed using GeneScan software version 3.1 (Applied Biosystems).

To search for sequence variability in regions flanking the G₄C₂ repeat, 2 PCR products were sequenced in 50 patients (44 expansion carriers and 6 noncarriers) (eFigure 1B). The 5' flanking region was amplified with primers 5'-ccctaccagggtttcagc and 5'-cgactctgagttccagagc (616 bp). The 3' flanking region was amplified with primers 5'-tgcggttgcggtgcctgc and 5'-gaatggggagcacaccgacttc (625 bp). The APOE polymorphism defining the ε2 to ε4 alleles and the 238-bp insertion/deletion in intron 9 of MAPT defining the H1/H2 haplotypes was genotyped as described previously in all patients with FTLD and ALS with a pathological expansion in C9orf72, as well as in their family members.²⁶

STATISTICAL ANALYSES

Differences in sample characteristics (eg, sex, age at onset, and familial history between cases and controls) were analyzed using the χ^2 test, Fisher exact test, or independent-samples *t* test as appropriate. Allele frequencies within the normal to intermediate range of repeats were calculated after excluding patients who carry the pathological expanded allele, defined as a repeat number more than 30 as previously suggested.¹⁶

The association between disease and alleles with less than 30 repeats was assessed using CLUMP software that is based on Monte Carlo tests for the evaluation of highly polymorphic loci.²⁷ Empirical *P* values for T1 through T4 analyses were obtained after 2000 simulations. Further analyses to obtain *P* values for each allele were also carried out as follows: allele counts from cases and controls were tested for significance using the χ^2 test after combining rare alleles. When any cell in the contingency table had an expected value less than 5, the corresponding allele was pooled with the neighboring allele (this process was repeated until no cell had an expected value <5). Each allele group was compared in turn with the rest of the alleles pooled together to calculate the χ^2 statistic. The pooling enabled the calculation of odds ratios and 95% CIs.²⁸ All the statistical analyses were done using SPSS (version 20; IBM SPSS). Statistical significance was taken to be *P* < .05 (Bonferroni correction for multiple testing was applied).

RESULTS

We used a previously suggested cutoff (>30 repeats) to distinguish the pathogenic expansion from the normal allele.¹⁶ None of the patients in our series were homozygous for the expansion allele, since all samples were successfully amplified in the first step. Samples homozygous in the first step (33%) were evaluated for the large expansion (>50 repeats). Based on the electropherogram with sawtooth peaks (eFigure 1), 65 unrelated patients were identified to be expansion carriers: 9.3% of patients with ALS (36 of 389), 5.2% of patients with FTLD (27 of 520), and 0.7% of patients with PD (2 of 289), but no expansions were detected in 424 patients with AD and 602 controls (Table 1).

Details of the clinical data for expansion carriers vs noncarriers are presented in **Table 2**. In addition, 14 case reports on expansion carriers are available in the online-only material. The expansion allele was significantly associated with family history for both ALS and FTLD (*P* < .001). The frequency of the expansion was 10.5% in patients with familial FTLD and 38.3% in patients with familial ALS. The average age at onset of FTLD was 6 years younger in expansion carriers than those without (*P* = .003). The disease subcategory of FTLD with motor neuron disease was significantly enriched in expansion carriers (*P* < .001). However, there was no significant association with any evaluated clinical characteristic of ALS.

In expansion carriers, we did not observe an intermediate or pathological number of repeats for the second allele (2-11 repeats). Also, sequencing analysis of 44 expansion carriers and 6 noncarriers did not reveal variability in the regions immediately flanking the G₄C₂ repeat that could be responsible for repeat instability, including a short tandem repeat (CGG)₈ located 294 bp downstream of the G₄C₂ repeat (eFigure 1B). Hence, it is unlikely that the number of repeats of the second allele or polymorphisms in the flanking region contribute significantly to the disease phenotype. In addition, the MAPT H1/H2 and APOE genotypes were examined in 63 expansion carriers (36 patients with ALS and 27 patients with FTLD) and their family members, since both genes are well-known risk factors for several neurodegenerative disorders including FTLD. However, statistical analysis did not reveal a significant link between these genes and disease presentation (ALS vs FTLD) (eTable 1 and eFigure 2).

For the segregation analysis, we genotyped all available family members of probands with the expansion (6 FTLD and 9 ALS families) and detected 30 additional mutation carriers. The expansion allele showed perfect cosegregation with disease, and for those expansion carriers who were asymptomatic (mean [SD] age, 46.3 [10.7] years; range, 24-64 years), follow-up is ongoing (**Table 3**, **Figure 1A**, and eFigure 2A and B). Within the pedigrees, there was no evidence of instability in the repeat size for the normal alleles (Figure 1A and eFigure 2). The method used in the study did not allow the same question to be addressed for the expanded allele, and the DNA quality/quantity was not sufficient to conduct Southern blotting to estimate the size of the expanded allele. However, in the 2 families with expansion carriers in 2 generations, a younger age at onset in the subsequent generation was observed (48 vs 74 years in ALS15 pedigree and 45-47 years vs 60s in the FTLD TOR73 pedigree), indicating genetic anticipation (eFigure 2A).

Two patients with PD (without signs of ALS or dementia) were categorized as expansion carriers (39 and 32 repeats). Family history of PD was known only for 1 patient; however, the 39-repeat allele does not segregate with PD, since the affected sibling did not inherit it (Figure 1B). The role of intermediate alleles (20-29 repeats) in neurodegenerative diseases is currently unknown. The clinical characteristics of the patients with PD with the expansion and intermediate alleles are presented in eTable 2. Intermediate alleles were observed only in 4 PD cases, 2 of which had parkinsonism fol-

Table 2. Comparison of the Clinical Statistics Between Expansion Carriers and Noncarriers

Cohort	No. (%)		P Value ^a	OR ^b (95% CI)
	Noncarriers	Expansion Carriers		
ALS Cases				
No. of ALS cases	353	36		
Age at onset, y, mean (SD)	57.6 (12.6)	57.8 (9.1)	.92	...
Female	134 (38.0)	15 (41.7)	.68	...
Familial cases	29 (8.2)	18 (50.0)	2.31×10^{-9}	11.2 (5.2-23.8)
Cases with FTLD	41 (11.6)	4 (11.1)	.93	...
Site of onset				
Limb	235 (66.6)	24 (66.7)	.99	...
Bulbar	93 (26.3)	10 (28.7)	.85	...
Mixed	7 (2.0)
Unknown	18 (5.1)	2 (5.6)
FTLD Cases				
No. of FTLD cases	493	27		
Age at onset, y, mean (SD)	65.7 (10.1)	59.6 (7.6)	.003	...
Female	248 (50.5)	10 (37.0)	.17	...
Familial cases	188 (38.1)	22 (81.5)	7.8×10^{-6}	7.1 (2.7-19.2)
Diagnosis subcategory				
bvFTD	218 (44.2)	11 (40.7)	.72	...
FTLD unspecified	131 (26.6)	8 (29.6)	.73	...
SD	60 (12.2)
PNFA	59 (12.0)
FTLD-MND	20 (4.1)	8 (29.6)	2.8×10^{-5}	10.0 ^c (3.9-25.5)
Other	5 (1.0)

Abbreviations: ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant of frontotemporal dementia; ellipses, not detected/not applicable; FTLT, frontotemporal lobar degeneration; FTLT-MND, frontotemporal lobar degeneration with motor neuron disease; OR, odds ratio; PNFA, progressive nonfluent aphasia; SD, semantic dementia.

^aA *t* test was used to compare the continuous variables. Categorical variables were compared by Pearson χ^2 test or Fisher exact test (when expected value <5).

^bThe OR was calculated only for variables showing $P < .05$.

^cThe OR was obtained by comparing FTLT-MND with the rest of the subcategories.

Table 3. Expansion Carriers Identified in the ALS and FTLT Pedigrees

Status	Sample Size		
	All	Expansion Carriers	Noncarriers
6 FTLT pedigrees	48	20	28
FTLT	8	8	...
FTLT-MND	1	1	...
bvFTD	2	2	...
Relatives	30	9	21
Spouses	7	...	7
9 ALS pedigrees	34	25	9
ALS	7	7	...
ALS-FTLT	2	2	...
FTLT	1	1	...
Relatives	24	15	9

Abbreviations: ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant of frontotemporal dementia; ellipses, not detected; FTLT, frontotemporal lobar degeneration; FTLT-MND, frontotemporal lobar degeneration with motor neuron disease.

lowed by severe dementia. We also assessed if the presence of intermediate alleles could influence the age at onset of parkinsonism in *LRRK2* G2019S mutation carriers (onset between age 40-80 years). However, none of the 29 *LRRK2* mutation carriers had an expanded or intermediate repeat allele (<11 repeats).

Furthermore, our results do not suggest that the intermediate *C9orf72* alleles significantly contribute to AD risk, since the frequency of such alleles was similar between patients with AD and controls (about 1%), and the alleles with 23 and 21 repeats did not segregate with AD in 2 families (eFigure 2C). Importantly, 15 of the 31 autopsied AD cases had TDP-43 inclusions (a frequent co-pathology in AD); however, no intermediate allele carriers were found among these cases. This is of note because ALS/FTLT caused by the expansion is associated with TDP-43 brain pathology.^{17,20,29,30} Hence, the TDP-43 pathology in AD could not be explained by an increased number of repeats in *C9orf72*.

Finally, a case-control association analysis was carried out using Monte Carlo tests. All carriers of alleles with more than 30 repeats were excluded from this analysis to avoid association due to the pathological expansion. Analyses T1 and T2 revealed association between normal repeat alleles (<30) with FTLT and ALS (eTable 4). Analysis T3 showed that the 10-repeat allele was significantly associated with FTLT. To get statistics for each allele (eg, *P* value or odds ratio), we assessed the distribution of normal alleles in 4 disease groups vs controls (**Figure 2** and eTable 3). In each group, the most frequent were the 2-, 5-, and 8-repeat alleles, which account for about 75% of all observed alleles. A marginal protective effect of the 5-repeat allele was detected in FTLT, ALS, and PD, as well as of the 11-repeat allele in ALS and AD (nominal $P < .05$). The frequency of the 10-

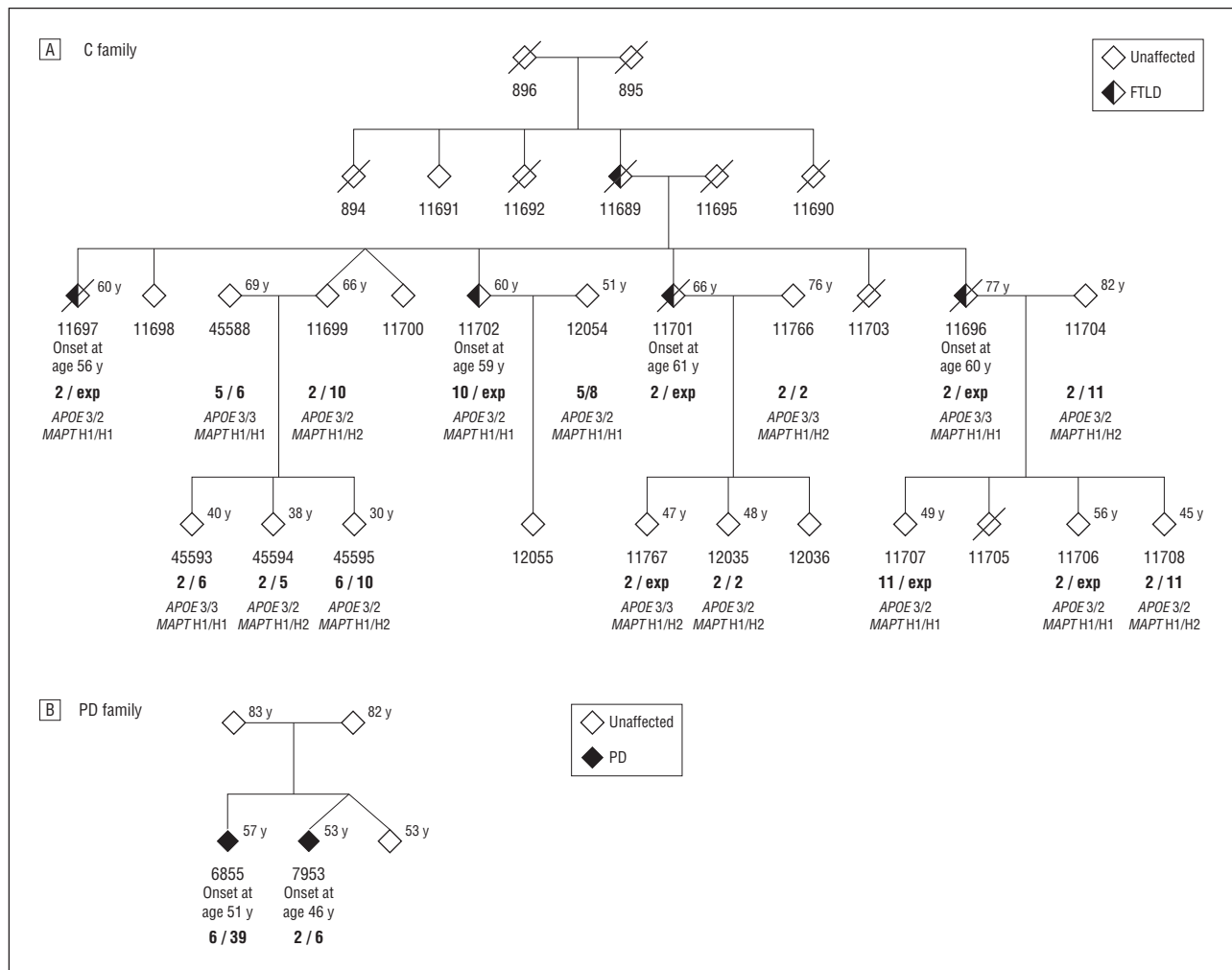


Figure 1. Families with the G₄C₂ repeat expansion. A, Frontotemporal lobar degeneration (FTLD) pedigree. B, Parkinson disease (PD) pedigree. Individual genotypes are shown beneath the corresponding diamond, including “at-risk” currently asymptomatic individuals. Arabic numbers indicate the repeat units (exp indicates expansion). The age at the time of examination is shown in the upper right corner. Age at onset is indicated for patients below the ID number. Sex of the family members is masked to protect privacy.

repeat allele was marginally increased in all 4 neurodegenerative diseases vs the control group (nominal $P < .05$). The risk associated with this allele remained significant in the FTLD data set even after Bonferroni correction ($P = .03$; odds ratio, 2.14) (eTable 3).

COMMENT

Our findings further support the expansion in *C9orf72* as a common cause of ALS and FTLD, but not AD or PD. In contrast to the 1-step genotyping used in published case-control studies of *C9orf72*, 2-step genotyping allowed us to obtain the exact genotype for each individual (except >50 repeats). No homozygous expansion carriers were found in the current study, suggesting that such a genotype could be lethal. A heterozygous expansion (>30 repeats) was detected in 9.3% of patients with ALS and 5.2% of patients with FTLD and was significantly more frequent in cases with a family history of ALS or FTLD. The frequency was doubled in our familial FTLD cases (10.4%), which is similar to that reported by DeJesus-Hernandez et al¹⁷ (11.7%).

We did not detect significant association between the presence of the expansion and clinical characteristics of patients with ALS, while in the FTLD series, the expansion was associated with a younger age at onset and the FTLD with motor neuron disease phenotype. These findings are in line with previous observations.^{18,31} Notably, none of our FTLD cases with the expansion were diagnosed with primary progressive aphasia, which is similar to the Mayo clinic cohort³² and different from the Manchester cohort wherein 4 primary progressive aphasia cases had the expansion.³¹ Wide clinical variability in expansion carriers has been previously described (eg, age at onset and disease duration),^{20,33} and future characterization of such data in a large cohort of carriers could generate a clinically useful algorithm to prioritize patients for mutation analysis of *C9orf72*.³⁴

In our study, a variable phenotype in expansion carriers (ALS vs FTLD) was not associated with the *APOE* alleles or the extended *MAPT* H1/H2 haplotypes. However, only an evaluation of a large data set could reach a solid conclusion about *APOE* and *MAPT* as phenotype modifiers. It is also possible that *C9orf72* itself is respon-

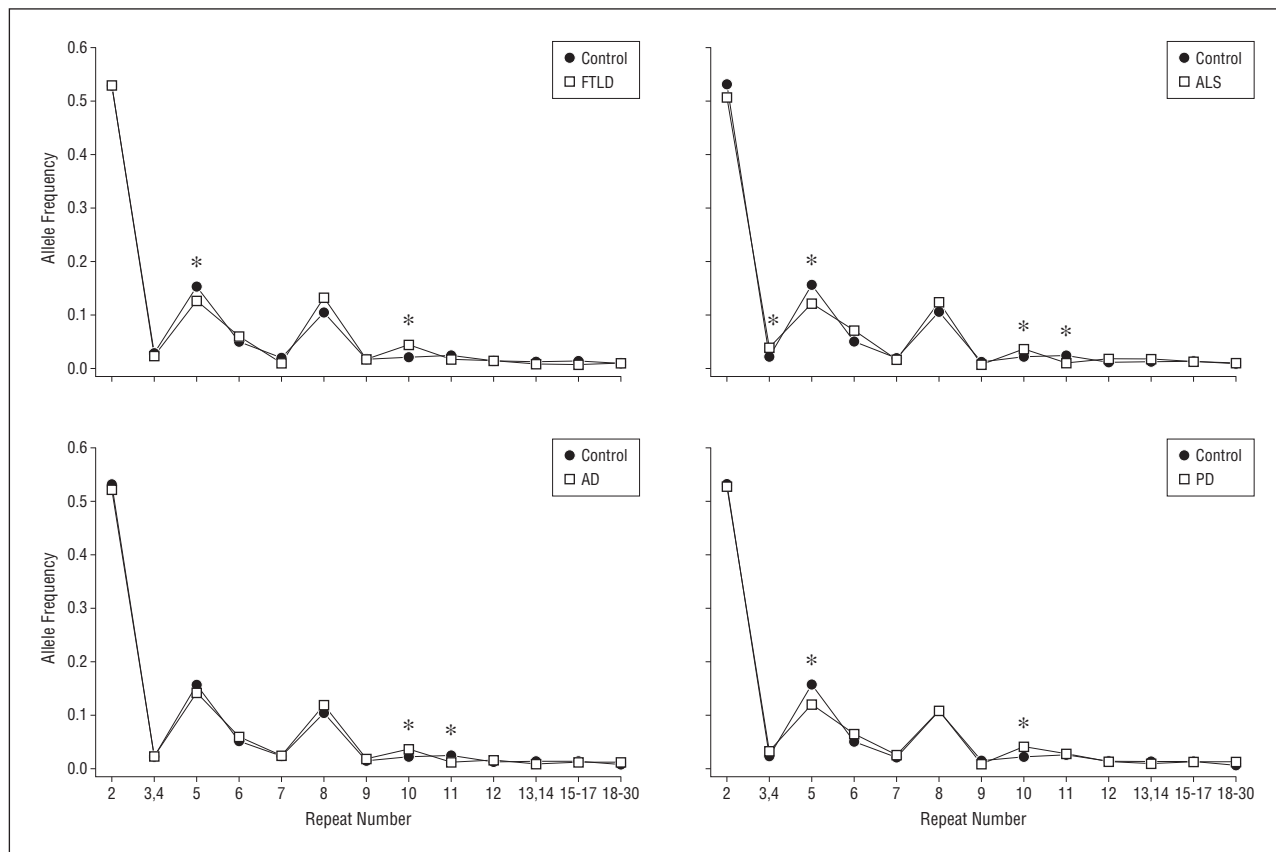


Figure 2. Comparison of the allele frequencies (repeats <30) between controls and cases in each disease cohort (AD indicates Alzheimer disease; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; and PD, Parkinson disease). Asterisks indicate a significant *P* value (nominal *P* < .05). In comparison with controls, there were 2 significant differences observed for the allele frequencies for the patients with FTLD, 4 for the patients with ALS, 2 for the patients with AD, and 2 for the patients with PD. A similar pattern was observed for all 4 diseases. The 5-repeat allele was significantly lower in FTLD, ALS, and PD than in controls and the 10-repeat allele in disease was significantly higher in all 4 disease groups than in controls. Expansion carriers were excluded from this analysis: controls, *n* = 602; FTLD, *n* = 488; ALS, *n* = 353; AD, *n* = 424; and PD, *n* = 287.

sible for the modifying effect because of coding sequence variations, repeat size, and/or repeat instability. We demonstrated that the repeat size of the second allele in expansion carriers was within the normal range (2-11 repeats) and unlikely to contribute to the variability in disease phenotype. In addition, our sequencing analysis did not reveal variations in regions flanking the G₄C₂ repeat that could be responsible for repeat instability or the variable phenotype of expansion carriers. Future studies should assess intronic and coding variations in the entire *C9orf72* locus as potential phenotype modifiers.

Instability of the G₄C₂ repeat region was suggested to be a possible mechanism for the occurrence of the expansion.¹⁶ However, a founder effect is more likely to be responsible for the incidence of the mutation, since most carriers harbor a common haplotype.^{17,29} Despite the method's limitation (the size of large expansions could not be determined), we estimated the stability of 2 to 30 repeats in the pedigrees. The number of repeats was stable across generations.

Given the clinical/pathological overlap between neurodegenerative diseases,³⁵⁻³⁸ our study determined whether the expansion plays a role in AD or PD. Importantly, the PD samples were previously collected to generate a data set enriched in genetic predisposition to PD and mainly

consisted of cases with either an early age at onset (mean [SD], 52.6 [13.04] years) and/or family history of PD (40%).³⁹ However, the frequency of expansion alleles in our PD data set was unremarkable (0.7%). Only 2 patients (without symptoms of ALS or dementia) were carriers of 32 and 39 repeats, which is much smaller than the expansion estimated by Southern blot (700-1600 repeats),¹⁷ and segregation analysis did not support the pathogenic nature of the 39-repeat allele. This raises concern about a reliable cutoff for a pathological repeat number, which is important in the utility of genetic screening in patient care. Likely, a higher cutoff or establishing a gray zone will be proposed in the future based on an increasing number of observations from case-control and neuropathological studies.^{40,41} Among our ALS and FTLD mutation carriers, only 2 had an allele with questionable pathological significance (<40 repeats). The connection between the repeat number and pathological significance has to be carefully investigated. It is possible that the pathological cutoff is disease dependent (eg, ALS vs FTLD) and could be modulated by individual genetic background. Furthermore, future studies have to test the possibility of somatic instability of the repeat region (the disease-related tissues, such as the spinal cord, could have larger expansions than blood cells from the same individual).

Our results did not suggest that the expansion or intermediate alleles are associated with AD. In contrast, there was a report of 6 expansion carriers in a familial AD cohort (<1%), 4 of whom were from the same family.⁴² However, autopsy indicated that 3 carriers actually had amnesic FTLD. Whether the remaining carriers were also clinically misdiagnosed as having AD remains to be seen. In addition to typical AD pathology, 15 of our patients with AD had TDP-43 inclusions, which are known to be associated with the brain pathology of the expansion^{17,20,29,30}; however, all of these patients with AD have genotypes within the normal range (2-12 repeats).

Our case-control studies also assessed the frequency of alleles within the normal range (<30 repeats) and observed a trend toward an association between the 10-repeat allele and risk for all 4 disorders (odds ratio, 1.72-2.14). It is tempting to speculate that this allele is in linkage disequilibrium with an unknown *C9orf72* risk variation. Further genetic work has to validate this observation, including follow-up case-control studies and sequencing of 10-repeat carriers.

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Author Affiliations: Tanz Centre for Research in Neurodegenerative Diseases (Drs Xi, Grinberg, Ghani, Hazrati, Liang, Robertson, St George-Hyslop, and Rogaeva, Mss Moreno and Sato, and Mr van Haersma de With), Division of Neurology, Department of Medicine (Drs Lang, Marras, Masellis, Black, St George-Hyslop, and Rogaeva), and Department of Psychiatry, St. Michael's Hospital (Dr Fornazzari), University of Toronto, Sunnybrook Health Sciences Centre (Drs Zinman, Bilbao, Masellis, and Black), Morton and Gloria Shulman Movement Disorders Clinic and the Edmond J. Safra Program in Parkinson's Disease, Toronto Western Hospital (Drs Lang and Marras), and LC Campbell Cognitive Neurology Research Unit, Sunnybrook Research Institute (Drs Masellis and Black), Toronto, Ontario, Canada; Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades (Drs Hernández, Ruiz, and Boada), Hospital Universitari Vall d'Hebron-Institut de Recerca, Universitat Autònoma de Barcelona (VHIR-UAB) (Dr Boada), and Neurology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona (Drs Rojas-Garcia and Clarimón), Barcelona, Department of Structural Genomics, Neocodex, Seville (Dr Morón), and Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid (Drs Rojas-Garcia and Clarimón), Spain; Regional Neurogenetic Centre, Lamezia Terme, Azienda Sanitaria Provinciale Catanzaro (Drs Bruni, Colao, Maletta, and Puccio), and Neurology II, Department of Neuroscience, University of Torino, Turin (Drs Rainero and Pinessi), and Department of Neurological Sciences, University of Milan, Centro Dino Ferrari, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan (Dr Galimberti), Italy; School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham (Dr Morrison and Mss Moorby and Stockton), and Cambridge

Institute for Medical Research and the Department of Clinical Neurosciences, University of Cambridge, Cambridge (Dr St George-Hyslop), England; Salvador Hospital, University of Chile, Santiago (Dr Villagra); and The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, The Gertrude H. Sergievsky Center, Departments of Neurology, Psychiatry, and Medicine, College of Physicians and Surgeons, Columbia University, New York, New York (Dr Mayeux).

Correspondence: Ekaterina Rogaeva, PhD, Tanz Centre for Neurodegenerative Diseases, 6 Queen's Park Crescent West, Toronto, ON M5S 3H2, Canada (ekaterina.rogaeva@utoronto.ca) or Peter St George-Hyslop, MD, FRCP(C) (p.hyslop@utoronto.ca).

Author Contributions: *Study concept and design:* Xi, St George-Hyslop, and Rogaeva. *Acquisition of data:* Xi, Zinman, Grinberg, Moreno, Sato, Bilbao, Ghani, Hernández, Ruiz, Boada, Morón, Lang, Marras, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Masellis, Black, Hazrati, Liang, van Haersma de With, Fornazzari, Villagra, Rojas-Garcia, Clarimón, Mayeux, Robertson, and Rogaeva. *Analysis and interpretation of data:* Xi, Grinberg, Marras, Masellis, and Rogaeva. *Drafting of the manuscript:* Xi and Rogaeva. *Critical revision of the manuscript for important intellectual content:* Xi, Zinman, Grinberg, Moreno, Sato, Bilbao, Ghani, Hernández, Ruiz, Boada, Morón, Lang, Marras, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Masellis, Black, Hazrati, Liang, van Haersma de With, Fornazzari, Villagra, Rojas-Garcia, Clarimón, Mayeux, Robertson, and St George-Hyslop. *Statistical analysis:* Xi. *Obtained funding:* Zinman, Grinberg, Bilbao, van Haersma de With, Mayeux, Robertson, St George-Hyslop, and Rogaeva. *Administrative, technical, and material support:* Xi, Zinman, Moreno, Sato, Ghani, Hernández, Ruiz, Boada, Morón, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Black, Hazrati, Liang, Fornazzari, Rojas-Garcia, Clarimón, and Rogaeva. *Study supervision:* Morrison, St George-Hyslop, and Rogaeva.

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