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Parkinson Disease is not associated with *C9ORF72* repeat expansions

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Abstract

Hexanucleotide expansions in the *C9ORF72* gene are frequently found in patients with amyotrophic lateral sclerosis and/or frontotemporal dementia, some of whom exhibit concurrent extrapyramidal symptoms. To determine if repeat expansions are a cause of Parkinson Disease (PD), we used repeat-primed PCR to investigate the frequency of *C9ORF72* repeat expansions in cohort of 478 patients with PD and 662 control subjects. While 3 control subjects were found to be expansion carriers, no expansions were found among patients, suggesting that *C9ORF72* expansions are not a common cause of PD.

Keywords

Parkinson Disease; genetics; *C9ORF72*; hexanucleotide repeat

INTRODUCTION

Expansions of an intronic hexanucleotide repeat (GGGGCC) in the *C9ORF72* gene are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)^{1, 2}. Due to a shared European founder, repeat expansions are most common in Caucasian populations where they explain 37% of familial ALS, 21% of familial FTD, and 6% of cases with sporadic ALS or FTD³. Expansions have also been reported in patients with an expanding spectrum of neurodegenerative syndromes, including Alzheimer's

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DISCLOSURE STATEMENT

The authors disclose no conflicts of interest.

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dementia, corticobasal syndrome, and olivopontocerebellar degeneration^{4–7}. Because some repeat expansion carriers have parkinsonism^{8, 9}, we sought to determine if *C9ORF72* repeat expansions cause PD by screening a large clinic cohort.

METHODS

Study Subjects

The PD cohort was comprised of 478 patients evaluated at the Washington University in Saint Louis Movement Disorder Clinic and diagnosed with definite PD according to the UK Brain Bank criteria¹⁰. 662 healthy control subjects without PD, amyotrophic lateral sclerosis, or dementia were drawn from several ongoing studies at Washington University. All participants had agreed by signed informed consent to participate in genetic studies approved by our Institutional Review Board.

Repeat Expansion Genotyping

C9ORF72 hexanucleotide repeat sizes were genotyped on blood-derived genomic DNA using repeat-primed PCR as previously published¹. Samples were considered positive for an expanded repeat if assay replicates showed the expected decrementing saw-tooth pattern with 6bp periodicity and >30 peaks (Supplementary Figure 1). In patients without expansions, this assay was also used to determine the number of repeat units in an individual's longest allele. To validate our assay, we genotyped 210 samples obtained from the NINDS Coriell Repository for whom genotypes have been published¹¹. Our results were identical in 208 of 210 samples (99.1%). One discrepancy resulted from a failed assay in our laboratory and in the remaining case, replicate assays have confirmed our allele call.

Statistics

Comparisons of *C9ORF72* allele lengths used independent sample T or Mann-Whitney U tests. Associations between longest alleles and age at PD symptom onset were investigated using the Kaplan-Meier method and tested for significant differences using a Cox proportional hazards model (proc PHREG, SAS), including gender as covariate. All statistics were two-tailed and significance was set at $p < 0.05$.

RESULTS

Demographic information and *C9ORF72* repeat genotyping for 478 participants with Parkinson Disease and 662 normal controls is summarized in Table. All PD cases had alleles with 27 repeats (Supplemental Figure 2) while three controls had 50 repeats based on repeat-primed PCR. These controls were neurologically normal and non-demented at last contact (ages 50, 67 and 71), but additional clinical data we obtained showed that two of these individuals had a first or second-degree relative with ALS. The mean number of repeat units in an individual's longest allele did not differ between PD cases and controls (7.2 ± 4.5 vs 7.6 ± 4.5 , $p = 0.14$), nor was the distribution of longest alleles different between the groups ($p = 0.09$, Supplementary Figure 2). Within PD cases, the length of an individual's longest repeat allele was not correlated with age at symptom onset ($p = 0.11$, Supplementary Figure 3).

DISCUSSION

Our study did not find any large *C9ORF72* repeat expansions in a large group of patients with PD, confirming recent reports from independent cohorts^{12–14}. Across these studies, 1673 patients with PD (29% familial) have been screened without finding a single large *C9ORF72* expansion. Xi et al reported two PD patients with marginally increased repeat

numbers (32 and 39) but demonstrated that the 39 repeats did not segregate with disease¹³. The absence of large repeat expansions in a cohort of this size is especially notable because of the 0.2–0.4% carrier frequency found in Caucasian controls^{2, 7}. Even if the combined genotyping efforts had found *C9ORF72* repeat expansions in 3–6 patients with PD, this would only equal the background carrier rate among Caucasians and would suggest an incidental rather than causative finding. Statistical differentiation from this background rate by Fischer's exact test would require more than 6–17 carriers. In summary, *C9ORF72* repeat expansions are common in ALS and fronto-temporal dementia, rare in clinical Alzheimer's disease, but current evidence is sparse for an association with Parkinson Disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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