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Negative results

Analysis of C9orf72 repeat expansions in a large international cohort of dementia with Lewy bodies

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ABSTRACT

C9orf72 repeat expansions are a common cause of amyotrophic lateral sclerosis and frontotemporal dementia. To date, no large-scale study of dementia with Lewy bodies (DLB) has been undertaken to assess the role of *C9orf72* repeat expansions in the disease. Here, we investigated the prevalence of *C9orf72* repeat expansions in a large cohort of DLB cases and identified no pathogenic repeat expansions in neuropathologically or clinically defined cases, showing that *C9orf72* repeat expansions are not causally associated with DLB.

1. Introduction

Hexanucleotide repeat expansions (HREs) in a noncoding region of *C9orf72* are recognized as the most common genetic cause of familial and sporadic amyotrophic lateral sclerosis, frontotemporal dementia (FTD), amyotrophic lateral sclerosis-FTD, and Huntington disease phenocopies (Beck et al., 2013; Boeve et al., 2012; Hensman Moss et al., 2014; Majounie et al., 2012c; Simon-Sanchez et al., 2012; van der Zee et al., 2013).

A normal repeat expansion shows 1 to 23 GGGGCC repeats located between exons 1a and 1b of *C9orf72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011). HREs identified in several neurodegenerative syndromes were found to range from 500 to 4400 repeats, but on a repeat-primed polymerase chain reaction (PCR), more than 32 repeats are often considered a pathogenic genotype (Beck et al., 2013).

C9orf72 HREs have been identified in nonmotor neurodegenerative phenotypes including Alzheimer's disease (AD) at frequencies of ~1% (Beck et al., 2013; Harms et al., 2013; Kohli et al., 2013; Majounie et al., 2012b), although conflicting reports exist in the literature (Rollinson et al., 2012; Xi et al., 2012).

Dementia with Lewy bodies (DLB) accounts for 15%–25% of all dementia cases (Heidebrink, 2002). Its core features encompass cognitive impairment, fluctuating attention, parkinsonism, and recurrent visual hallucinations (Weisman and McKeith, 2007). Neuropathological diagnosis of DLB is achieved when the presence of Lewy bodies is confirmed in the cortex and the brainstem (McKeith et al., 2005). Little is known about the genetics of DLB, although molecular studies seem to point toward genetic overlaps with other neurodegenerative diseases, mainly with AD and Parkinson's disease (PD) (Bras et al., 2014; Guerreiro et al., 2016; Keogh et al., 2016; Meeus et al., 2012).

So far, the *C9orf72* repeat expansion has only been genotyped in small cohorts of ~100 DLB cases or less (Geiger et al., 2016; Lesage et al., 2013; Robinson et al., 2014; Snowden et al., 2012; Yeh et al., 2013). We have recently shown in a large cohort that *C9orf72* repeat expansions are not a common cause of DLB in pathologically diagnosed cases (Guerreiro et al., 2015). Here, we expand on these findings using a cohort of 1524 DLB cases.

2. Material and methods

Samples consisted of an international cohort of 1398 neuropathologically diagnosed DLB cases and 126 clinically diagnosed DLB cases (Supplementary Table 1). DNA was extracted from brain tissue for the neuropathologically diagnosed samples and from blood for the clinical diagnosed samples using standard procedures. We performed repeat-primed PCR according to Renton et al. (2011). Genotypes were assessed using Peak Scanner v2.0 (Applied Biosystems) with repeat expansions displaying a characteristic saw tooth pattern with a 6 base pair periodicity on analysis.

3. Results

Repeat mean number was 5.17 (±4.30 standard deviation) ranging from 1 to 58. All except 5 samples presented less than 23 repeats in the repeat-primed PCR (Supplementary Fig. 1). Two neuropathologically diagnosed DLB samples showed 32 repeats and 1 showed 33 repeats; and 2 clinically diagnosed samples exhibited 33 and 58 repeats. These last 2 samples had been previously analyzed as part of the cohort published by Snowden et al. (2012).

4. Discussion

This is the first study genotyping the *C9orf72* HREs in a large cohort of mainly neuropathologically diagnosed DLB samples. Within the neuropathologically defined DLB cases, we did not find any HREs above the typical threshold for pathogenicity (~32 repeats). This is concordant with previous studies that found no repeat expansions in 34 clinically diagnosed cases of a Taiwanese cohort or in 111 pathological DLB cases (Geiger et al., 2016; Yeh et al., 2013). Snowden et al. (2012) found 2 cases with HREs greater than 30 repeats in a study that was comprised of 102 "probable DLB" blood samples. When the same group restricted

their analysis to include only pathologically diagnosed samples, no pathogenic repeat expansions were identified (Robinson et al., 2014).

DLB is considered to be part of a spectrum between AD and PD (Weisman and McKeith, 2007) where large *C9orf72* HREs are not frequent. In AD, it was suggested that pathogenic repeat expansions may only be associated with late onset AD (Kohli et al., 2013) or that amnesic FTD (which is easily misdiagnosed as AD) could be responsible for the low frequencies observed for AD (Majounie et al., 2012b). In PD, there is no evidence for a role of *C9orf72* pathogenic repeat expansions (Majounie et al., 2012a; Xi et al., 2012).

Clinical symptoms in DLB can vary substantially from patient to patient and some can even overlap with less typical forms of FTD (Claassen et al., 2008), which could account for the pathogenic repeat expansions found in misdiagnosed DLB clinical cases. Furthermore, recent data suggest that the threshold for pathogenicity of HREs should be higher than the initially proposed 30 repeats (Xi et al., 2015).

In our cohort of neuropathologically diagnosed DLB samples, we found 3 cases with likely benign 32 and 33 repeats. Excluding the clinically diagnosed cases, we found no evidence of pathogenic repeat expansions. Even including the clinically diagnosed cohort, no extended repeat expansions were identified; with the longest allele exhibiting 58 repeats.

Our study shows that *C9orf72* pathogenic repeat expansions are not a common cause of DLB.

Disclosure statement

Ronald C. Petersen reports consultancies with Roche, Inc, Merck, Inc, Genentech, Inc, Biogen, Inc, and Eli Lilly. Brad F. Boeve reports GE Healthcare, FORUM Pharmaceuticals, and C2N Diagnostics as research support and advisory board member of the Tau Consortium. The remaining authors report no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neurobiolaging.2016.08.023.