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***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 (ALS) and frontotemporal dementia (FTD). To date, no large-scale study of dementia with Lewy bodies (DLB) has been undertaken in order 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 non-coding region of *C9orf72* are recognized as the most common genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), ALS-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 PCR more than 32 repeats is often considered a pathogenic genotype (Beck et al., 2013).

C9orf72 HREs have been identified in non-motor 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).

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 & 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 towards 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 SD) ranging from 1 to 58. All except five samples presented less than 23 repeats in the repeat-primed PCR (**Supplementary Fig. 1**). Two neuropathologically diagnosed DLB samples showed 32 repeats and one showed 33 repeats; and two clinically diagnosed samples exhibited 33 and 58 repeats. These last two samples had been previously analysed 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 & 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 suggests 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 three 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.