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C9orf72 Hexanucleotide Repeat Analysis in Cases with Pathologically Confirmed Dementia with Lewy Bodies

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Abstract

Background—Dementia with Lewy bodies (DLB) is the second most common neurodegenerative dementia affecting the elderly. The GGGGCC hexanucleotide expansion mutation at the *C9orf72* locus has been identified as a major cause of amyotrophic lateral sclerosis and frontotemporal dementia, raising the question of whether this mutation is a factor in DLB. Furthermore, a small number of clinically diagnosed DLB patients have previously been reported to carry the pathologic *C9orf72* hexanucleotide repeat expansion.

Objective—To explore whether the *C9orf72* mutation is present in pathologically confirmed DLB patients.

Methods—We screened a cohort of 111 definite DLB cases with extensive Lewy body pathology for the *C9orf72* hexanucleotide repeat expansion using the repeat-primed polymerase chain reaction assay.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Results—No pathogenic expansions of the *C9orf72* hexanucleotide repeat were found, suggesting that there is no causal relationship between *C9orf72* and DLB.

Conclusion—Our data illustrate that *C9orf72* screening of clinically diagnosed DLB patients should only be considered in cases with a family history of motor neuron disease or frontotemporal dementia to distinguish between mimic diseases.

Keywords

Dementia with Lewy bodies; amyotrophic lateral sclerosis; frontotemporal dementia; *C9orf72*; hexanucleotide repeat expansion

INTRODUCTION

Dementia with Lewy bodies (DLB) is a degenerative neurological disorder clinically characterized by a combination of progressive cognitive impairment, altered mental status, neuroleptic sensitivity, visual hallucinations, and parkinsonism.[1] DLB is the second most prevalent neurodegenerative dementia in the elderly population next to Alzheimer's disease, accounting for approximately 20 percent of cases.[2] The molecular mechanism of DLB is largely unknown, although a genetic component has been proposed.[3]

A [GGGGCC]_n hexanucleotide expansion at the *C9orf72* locus is the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia.[4, 5] This repeat expansion has also been identified in clinically diagnosed patients with Alzheimer's disease, atypical parkinsonism or psychosis.[6-8] Psychosis is a common symptom in DLB, raising the question of whether the *C9orf72* hexanucleotide expansion is a genetic factor in DLB. Previous clinical studies have considered this question[9-12], with one study reporting two out of 102 clinically diagnosed DLB patients having a pathogenic repeat expansion.[10] However, the clinical diagnosis of DLB is challenging with a high false positive rate due to mimic syndromes.[13, 14] As a consequence, the research community is increasingly relying on specimens that are pathologically confirmed as this represents the gold standard for diagnosis. To investigate if *C9orf72* repeat expansions have a role in pathologically confirmed DLB, we conducted a genetic screening study on a cohort of 111 definite DLB cases.

METHODS

A total of 111 pathologically confirmed DLB cases from the Johns Hopkins Morris K. Udall Center for Parkinson's Disease Research Center of Excellence and the Johns Hopkins Alzheimer Disease Research Center were studied. The cohort included neocortical cases (n=86) and transitional-type DLB cases (n=25) diagnosed using the McKeith criteria.[15] All subjects were Caucasian, with males representing 67 percent of cases. The average age of death was 78 (range: 57 – 94) years. The majority of patients (66%) also met pathological criteria for Alzheimer disease. Additional clinicopathological characteristics are displayed in Table 1. DNA was extracted from frozen brain sections using the Qiagen DNeasy extraction protocol (Qiagen, Hilden, Germany). The hexanucleotide repeat at the *C9orf72* locus was genotyped using the repeat-primed polymerase chain reaction assay as previously described.

[4] The resulting PCR amplicons were analyzed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Grand Island, NY, USA). A pathologic expansion was defined as over 30 GGGGCC repeats.[4]

RESULTS

We observed hexanucleotide repeats of less than 20 in our cohort, which is considered normal. None of the 111 pathologically confirmed cases carried a pathogenic *C9orf72* repeat expansion.

DISCUSSION

This study shows that the pathogenic *C9orf72* expansion is not present in pathologically confirmed Caucasian DLB patients. In a large screening study of familial Alzheimer dementia cases, Harms et al. identified an Alzheimer dementia patient with a pathological *C9orf72* repeat expansion. [16] Interestingly, this case was also found to have neocortical Lewy bodies in addition to typical Alzheimer pathology. However, tissue was not available for evaluation of pathologic changes consistent with frontotemporal dementia. It is possible the pathologic *C9orf72* mutation in this patient was coincidental and this patient could have had frontotemporal dementia in addition to Alzheimer dementia. Cases of frontotemporal lobar degeneration with motor neuron disease can present with symptoms mimicking DLB. [13] Our data suggest that the presence of a pathological *C9orf72* hexanucleotide expansion in a clinically diagnosed DLB case likely represents an atypical presentation of frontotemporal dementia. Thus, *C9orf72* expansion screening should only be considered for DLB patients with a family history of either motor neuron disease or frontotemporal dementia. In this situation genetic testing provides a method to help distinguish among intersecting clinical syndromes.

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Table 1

Clinical characteristics for 111 pathologically confirmed DLB cases

Pathologic Diagnosis	n	% Male	Age at Onset (Range)	Age at Death (Range)	Disease Duration (Range)	Family History (%)	Clinical Diagnosis							DLB subtype	
							AD	PD	AD/PD	FTD	PDD	DLB	Other	Neocortical	Limbic
Pure DLB	27	85	60 (40 - 79)	76 (60 - 84)	16 (3 - 33)	6 (22)	0	2	2	0	20	3	0	18	9
DLB/AD	73	60	66 (42 - 87)	78 (57 - 94)	12 (2 - 30)	23 (32)	12	4	1	2	37	8	9	63	10
DLB/other	11	64	68 (43 - 83)	82 (64 - 89)	14 (6 - 27)	4 (36)	1	2	0	0	8	0	0	5	6
Total	111	67	65 (40 - 87)	78 (57 - 94)	13 (2 - 33)	33 (30)	13	8	3	2	65	11	9	86	25

Clinical data of the pathology confirmed cohort is grouped by pathology type. Clinical diagnosis and extent of Lewy body pathology is presented. Family history was positive if the patient reported at least one first- or second-degree relative with dementia, cognitive impairment, parkinsonism, Dementia with Lewy bodies, Parkinson disease, or Alzheimer dementia. Abbreviations: AD, Alzheimer disease; DLB, Dementia with Lewy bodies; FTD, Frontotemporal dementia; PD, Parkinson disease; PDD, Parkinson disease dementia