ORIGINAL ARTICLE

C9orf72 Repeat Expansions in Rapid Eye Movement Sleep Behaviour Disorder

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ABSTRACT: ***Background***: A large hexanucleotide repeat expansion in *C9orf72* has been identified as the most common genetic cause in familial amyotrophic lateral sclerosis and frontotemporal dementia. Rapid Eye Movement Sleep Behavior Disorder (RBD) is a sleep disorder that has been strongly linked to synuclein-mediated neurodegeneration. The aim of this study was to evaluate the role of the *C9orf72* expansions in the pathogenesis of RBD. ***Methods:*** We amplified the *C9orf72* repeat expansion in 344 patients with RBD by a repeat-primed polymerase chain reaction assay. ***Results***: We identified two RBD patients carrying the *C9orf72* repeat expansion. Most interestingly, these patients have the same *C9orf72* associated-risk haplotype identified in 9p21-linked amyotrophic lateral sclerosis and frontotemporal dementia families. ***Conclusions***: Our study enlarges the phenotypic spectrum associated with the *C9orf72* hexanucleotide repeat expansions and suggests that, although rare, this expansion may play a role in the pathogenesis of RBD.

RÉSUMÉ: Expansion des répétitions de C9orf72 dans le trouble des conduites du sommeil paradoxal. ***Contexte:*** Une importante expansion de la répétition de l'hexanucléotide *C9orf72* a été identifiée comme la cause génétique la plus fréquente de la sclérose latérale amyotrophique familiale et de la démence fronto-temporale. Le trouble des conduites du sommeil paradoxal est un trouble du sommeil qui a été fortement associé à une neurodégénérescence médiée par la synucléine. L'objectif de cette étude était d'évaluer le rôle des expansions de *C9orf72* dans la pathogénie des troubles du sommeil paradoxal. ***Méthodes:*** Nous avons amplifié l'expansion des répétitions de *C9orf72* chez 344 patients atteints de troubles du sommeil paradoxal au moyen d'une réaction en chaîne de la polymérase amorcée par la répétition. ***Résultats:*** Nous avons identifié deux patients atteints de troubles du sommeil paradoxal porteurs de l'expansion des répétitions de *C9orf72*. Il a été extrêmement intéressant de constater que ces patients présentaient le même haplotype de risque associé à *C9orf72* dans les familles atteintes de sclérose latérale amyotrophique et de démence fronto-temporale liées à 9p21. ***Conclusions:*** Notre étude élargit le spectre des phénotypes associés à l'expansion des répétitions de l'hexanucléotide *C9orf72* et suggère que, bien que rare, cette expansion pourrait jouer un rôle dans la pathogénie des troubles du sommeil paradoxal.

Keywords: *C9orf72*, repeat expansion, RBD, synucleinopathies

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Rapid eye movement (REM) sleep behavior disorder (RBD) is a sleep disorder which primarily involves loss of muscle atonia during REM sleep, leading to dream enactment behaviour (i.e. patients move in response to the content of their dreams).[1] Although RBD is not life threatening, recent studies indicate that it is a well-established risk factor for neurodegenerative disease.[2] Indeed, studies from sleep centres revealed that 40-65% of patients with idiopathic RBD will develop a neurodegenerative disease over a period of 10 years. In the large majority of cases this will be a synucleinopathy (Parkinson's disease (PD), Dementia with Lewy Bodies (DLB) or Multiple System Atrophy (MSA)).[3] A recent case report also described an individual diagnosed with frontotemporal dementia (FTD) three years after he started to complain of sleep disturbance that polysomnography examinations revealed to be RBD.[4]

Recently, a non-coding hexanucleotide repeat expansion in the *C9orf72* gene has been identified as the most common genetic cause of familial amyotrophic lateral sclerosis (FALS) and

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FTD.[5,6] A recent study estimated that 37.6% of FALS patients and 25.1% of familial FTD patients carry the *C9orf72* expansion, respectively.[7] The pathogenic expansion was found to be non-penetrant in individuals younger than 35 years, 50% penetrant by 58 years, and almost fully penetrant by 80 years. However, large expansions (>400 repeats) were recently found in 0.15% of controls from the United Kingdom, suggesting that the penetrance may be incomplete on a population scale.[8] Given the presence of common pathological features between ALS, FTD and other neurodegenerative diseases, such as ubiquitinated TDP-43 positive inclusion bodies in the central nervous system,[9,10] the *C9orf72* repeat expansion has since been tested in a panel of neurodegenerative diseases which revealed a wide phenotypic spectrum in the expansion carriers.[11-15]

Taken together and, given the clinical and pathological link between ALS/FTD and PD and the link between RBD and PD, we conducted a genetic screen of the *C9orf72* hexanucleotide repeat expansion in a cohort of RBD patients to investigate its potential role in the pathogenesis of RBD.

Methods

A total of 344 patients with RBD were enrolled in this study. Clinical characteristics of these patients are summarised in Table 1. Rapid eye movement sleep behavior disorder patients were recruited through an international consortium. All cases were seen by a neurologist specialized in sleep disorders and diagnosed with definite RBD according to the International Classification of Sleep Disorder criteria (ICSD-2). Written informed consent was given by all participants and the study was approved by the ethics and review boards of the participating institutions. The analysis of the C9*orf72* hexanucleotide repeat expansion was performed by repeat-primed polymerase chain reaction (RP-PCR) and complemented by a fluorescent fragment length analysis as previously described.[5] Alleles with more than 30 repeats were considered to be expanded.

Results

Genetic screening of our cohort of 344 RBD patients revealed two carriers of the *C9orf72* hexanucleotide repeat expansion (Figure 1). Fluorescent fragment length analysis of the *C9orf72* repeat region in these two patients further confirmed the amplification of one non-expanded allele in each of them (Figure 1). Moreover, 24 single-nucleotide polymorphisms (SNP) defining the *C9orf72* associated-risk haplotype[16] and observed in 9p21-linked ALS and FTD pedigrees were genotyped in these two patients and revealed the same disease risk haplotype observed in several populations (data not shown).

The first patient is a 65 year old man with no family history of RBD or neurodegenerative diseases. He developed symptoms of idiopathic PD at the age of 45, with excellent response to dopaminergic therapy. He eventually had successful deep brain stimulation for levodopa fluctuations. He developed symptoms of RBD at the age of 58, which was confirmed on polysomnography at age 60. He has no signs of cognitive impairment and is currently being treated with ropinirole. The second patient is a 69 year old man who developed symptoms of RBD at the age of 65. This was confirmed on polysomnogram three years later. He has subsequently been documented to have hyposmia (a predictor of synuclein-mediated neurodegeneration in RBD.[17] He had noted subjective cognitive impairment but formal neuropychological testing, including frontal executive function, was normal. Magnetic Resonance Imaging was normal. He later developed symptoms of bradykinesia without clear rigidity or rest tremor. Response to trials of piribedil and levodopa was equivocal.

Table 1: Clinical characteristics of our cohort of RBD patients.

Clinical data	Total affected (N = 344)
Ethnicity	
Caucasian	267 (77.6%)
Japanese	77 (22.4%)
Gender	
Male	271 (78.7%)
Female	73 (21.3%)
Age at RBD symptom onset (years)	59.9 (15-82)
Age at RBD diagnosis	66.1 (34-86)

The average and range (minimum-maximum) is given for age at symptoms onset and age at diagnosis.

Discussion

In this study, we performed a genetic analysis of the *C9orf72* hexanucleotide repeat expansion in a cohort of patients with RBD recruited through an international consortium including many of the leading RBD research groups across the world. Two patients with definite RBD were found to carry the repeat expansion. Moreover, analysis of 24 SNPs corresponding to the *C9orf72* associated-risk haplotype revealed the same common haplotype observed in several populations, further suggesting that this mutation derived from a single ancestor.

The diagnosis of PD was made in one case and the second eventually developed equivocal signs of parkinsonism, with a clear diagnosis not yet established. Although there are reports of RBD occurring in a broad array of neurological conditions,[1] RBD is considered to be a very strong sign of a synuclein-mediated neurodegenerative process. A very recent autopsy study concluded that 80 of 82 neurodegenerative disease patients with polysomnogram (PSG)-proven RBD had synuclein deposition on autopsy. In the total cohort of 172 patients (i.e. including non-PSG confirmed cases), none had a primary diagnosis of frontotemporal dementia and none had deposition of TDP-43.[18] Among our two patients, one had very strong clinical evidence of synucleinopathy (particularly with levodopa fluctuations and positive response to deep brain stimulation), whereas the second patient's clinical diagnosis had not yet become clear (although the hyposmia and equivocal parkinsonism provided additional evidence for neurodegenerative synucleinopathy). Therefore, our study suggests that *C9orf72* mutations can occur in clinical synucleinopathies, including RBD. Recent studies of the *C9orf72* expansions in cohorts of PD patients across several populations revealed no major role in PD pathogenesis.[19-21] In addition, evidence is sparse for the contribution of *C9orf72* expansions in DLB although this expansion was recently identified in 2 out of 102 patients who

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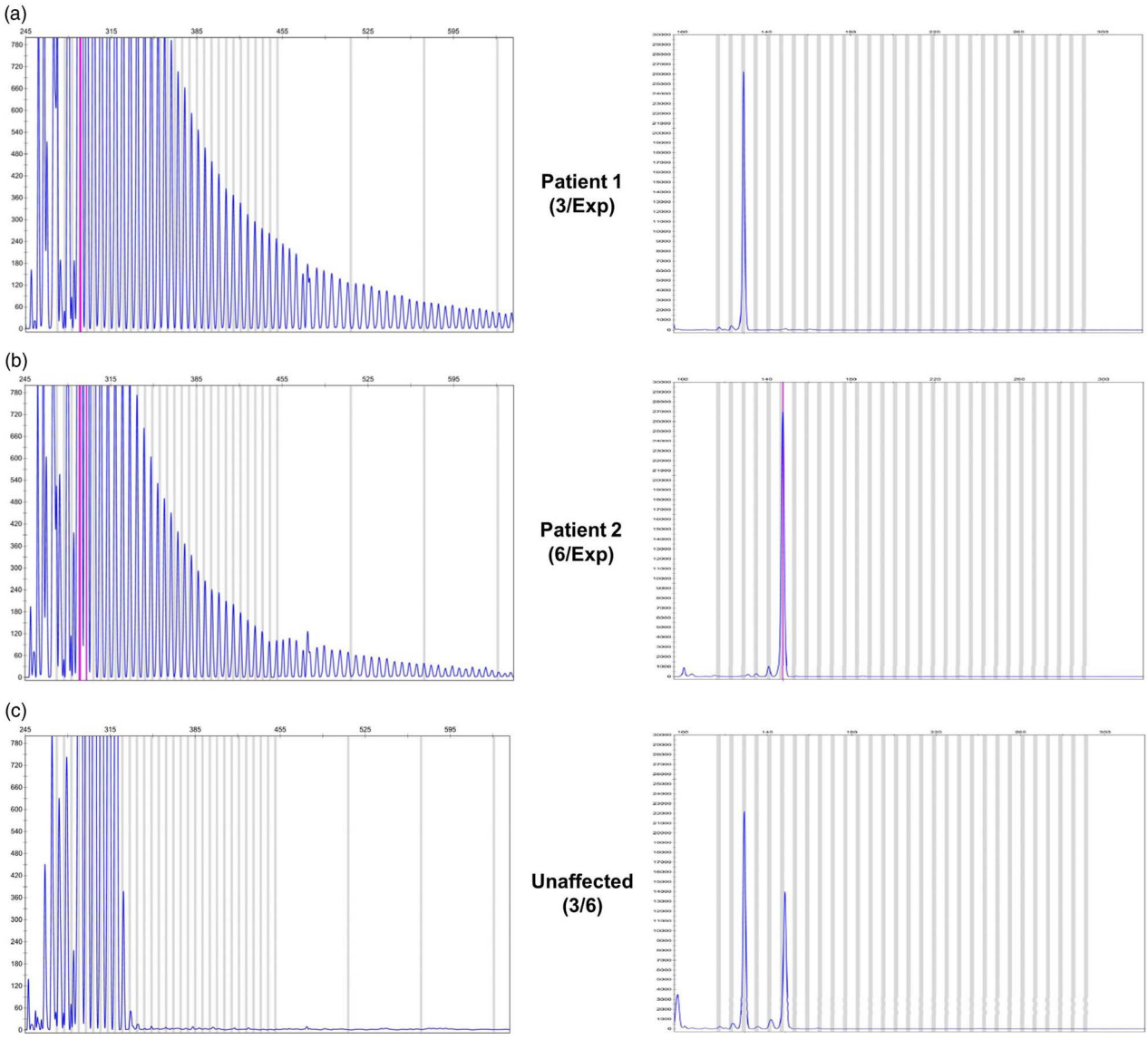

Figure 1: *C9orf72 hexanucleotide repeat expansion analysis in RBD patients using the GeneMapper software (Applied Biosystems, Carlsbad, CA, USA). Repeat-primed PCR fragment revealed the expanded pattern with a 6 base pair periodicity in patients 1 and 2 compared to the pattern observed in the unaffected individual (a-c, left). Fluorescent fragment length analysis of a PCR fragment containing the C9orf72 repeat expansion showed the amplification of one non-expanded (wild-type) allele in patients 1 and 2 and two wild-type alleles in the unaffected individual with no C9orf72 expansion (a-c, right).*

fulfilled the criteria for probable DLB (although diagnosis was not confirmed pathologically).[22]

Since both patients in this study are Caucasians and both had developed symptoms of neurodegenerative disease, one question arises from this observation: can the *C9orf72* repeat expansion be considered as pathologically linked to RBD or it is in fact an underlying genetic cause of the more severe neurodegenerative subgroup of patients? The simplest way to answer this question would be to analyze a larger cohort of patients, including those who have had longstanding RBD without progression to other neurodegenerative diseases. Such a comparison will allow direct assessments of the role of *C9orf72* mutations in prediction of outcome. Moreover, the *C9orf72* expansion appears to be much more frequent in Caucasian ALS patients (37.6% in FALS) compared to the Japanese ALS population (3.4% in FALS).[23] Consequently, the combination of Japanese and Caucasian RBD patients in this study could have led to an underestimation of the *C9orf72* expansion frequency in the Caucasian subjects or its overestimation in the Japanese subjects.

We cannot exclude that the co-occurrence of PD or DLB with RBD in the same patient could be coincidental but, given the low prevalence of these conditions in the general population and the fact that RBD is strongly linked to synucleinopathies, a causal relationship between these conditions is supported.

The examination of a genetic defect originally linked to ALS and FTD in a cohort of RBD cases, a disease strongly linked to synucleinopathy, suggests common pathways involved in several neurodegenerative disorders.[24] Interestingly, a recent

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study showed an association between RBD and familial ALS in two siblings with a p.L84F missense mutation in the *SOD1* gene.[25]

In summary, we report here two patients with a clinical diagnosis of RBD carrying the *C9orf72* repeat expansion. To the best of our knowledge, this is the first report of a potential genetic risk factor for RBD. Despite their novelty, our results have to be interpreted with caution and will need to be independently replicated by other groups to firmly confirm a causative role of the *C9orf72* repeat expansion in the pathogenesis of RBD.

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References

1. Gagnon JF, Postuma RB, Mazza S, Doyon J, Montplaisir J. Rapid-eye-movement sleep behaviour disorder and neurodegenerative diseases. Lancet Neurol. 2006;5:424-32.
2. Postuma RB, Gagnon JF, Montplaisir JY. REM sleep behavior disorder: from dreams to neurodegeneration. Neurobiol Dis. 2012;46:553-8.
3. Postuma RB, Gagnon JF, Montplaisir J. Rapid eye movement sleep behavior disorder as a biomarker for neurodegeneration: the past 10 years. Sleep medicine. 2013;14:763-7.
4. Lo Coco D, Cupidi C, Mattaliano A, Baiamonte V, Realmuto S, Cannizzaro E. REM sleep behavior disorder in a patient with frontotemporal dementia. Neurol Sci. 2012;33:371-3.
5. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72:245-56.
6. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72:257-68.
7. Majounie E, Renton AE, Mok K, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 2012;11:323-30.
8. Beck J, Poulter M, Hensman D, et al. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. Am J Hum Genet. 2013;92:345-53.
9. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006;314:130-3.
10. Nakashima-Yasuda H, Uryu K, Robinson J, et al. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol. 2007;114:221-9.
11. Le Ber I, Camuzat A, Guillot-Noel L, et al. C9ORF72 repeat expansions in the frontotemporal dementias spectrum of diseases: a flow-chart for genetic testing. J Alzheimers Dis. 2013;34:485-99.
12. Kohli MA, John-Williams K, Rajbhandary R, et al. Repeat expansions in the C9ORF72 gene contribute to Alzheimer's disease in Caucasians. Neurobiol Aging. 2013;34(5):1519e5-12.
13. Cacace R, Van Cauwenberghe C, Bettens K, et al. C9orf72 G4C2 repeat expansions in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging. 2013;34(1712):e1711-7.
14. Wojtas A, Heggeli KA, Finch N, et al. C9ORF72 repeat expansions and other FTD gene mutations in a clinical AD patient series from Mayo Clinic. Am J Neurodegener Dis. 2012;1:107-18.
15. Xi Z, Zinman L, Grinberg Y, et al. Investigation of C9orf72 in 4 Neurodegenerative Disorders. Arch Neurol. 2012:1-8.
16. Mok K, Traynor BJ, Schymick J, et al. Chromosome 9 ALS and FTD locus is probably derived from a single founder. Neurobiol Aging. 2012;33(1):209e3-8.
17. Postuma RB, Gagnon JF, Vendette M, Desjardins C, Montplaisir JY. Olfaction and color vision identify impending neurodegeneration in rapid eye movement sleep behavior disorder. Ann Neurol. 2011;69:811-8.
18. Boeve BF, Silber MH, Ferman TJ, et al. Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. Sleep medicine. 2013;14:754-62.
19. Daoud H, Noreau A, Rochefort D, et al. Investigation of C9orf72 repeat expansions in Parkinson's disease. Neurobiol Aging. 2013;34(6):1710e7-9.
20. Akimoto C, Forsgren L, Linder J, et al. No GGGGCC-hexanucleotide repeat expansion in C9ORF72 in parkinsonism patients in Sweden. Amyotroph Lateral Scler Frontotemp Degen. 2013;14:26-9.
21. Majounie E, Abramzon Y, Renton AE, Keller MF, Traynor BJ, Singleton AB. Large C9orf72 repeat expansions are not a common cause of Parkinson's disease. Neurobiol Aging. 2012;33(10):2527e1-2.
22. Snowden JS, Rollinson S, Lafon C, et al. Psychosis, C9ORF72 and dementia with Lewy bodies. J Neurol Neurosurg Psychiatry. 2012;83:1031-2.
23. Konno T, Shiga A, Tsujino A, et al. Japanese amyotrophic lateral sclerosis patients with GGGGCC hexanucleotide repeat expansion in C9ORF72. J Neurol Neurosurg Psychiatry. 2013;84:398-401.
24. Shulman JM, De Jager PL. Evidence for a common pathway linking neurodegenerative diseases. Nat Genet. 2009;41:1261-2.
25. Ebben MR, Shahbazi M, Lange DJ, Krieger AC. REM behavior disorder associated with familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2012;13:473-4.