

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/96212/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Kun-Rodrigues, Celia, Ross, Owen A., Orme, Tatiana, Shepherd, Claire, Parkkinen, Laura, Darwent, Lee, Hernandez, Dena, Ansorge, Olaf, Clark, Lorraine N., Honig, Lawrence S., Marder, Karen, Lemstra, Afina, Scheltens, Philippe, van der Flier, Wiesje, Louwersheimer, Eva, Holstege, Henne, Rogaeva, Ekaterina, St. George-Hyslop, Peter, Londos, Elisabet, Zetterberg, Henrik, Barber, Imelda, Braae, Anne, Brown, Kristelle, Morgan, Kevin, Maetzler, Walter, Berg, Daniela, Troakes, Claire, Al-Sarraj, Safa, Lashley, Tammarn, Holton, Janice, Compta, Yaroslau, Van Deerlin, Viviana, Trojanowski, John Q., Serrano, Geidy E., Beach, Thomas G., Clarimon, Jordi, Lleó, Alberto, Morenas-Rodríguez, Estrella, Lesage, Suzanne, Galasko, Douglas, Masliah, Eliezer, Santana, Isabel, Diez, Monica, Pastor, Pau, Tienari, Pentti J., Myllykangas, Liisa, Oinas, Minna, Revesz, Tamas, Lees, Andrew, Boeve, Brad F., Petersen, Ronald C., Ferman, Tanis J., Escott-Price, Valentina, Graff-Radford, Neill, Cairns, Nigel J., Morris, John C., Stone, David J., Pickering-Brown, Stuart, Mann, David, Dickson, Dennis W., Halliday, Glenda M., Singleton, Andrew, Guerreiro, Rita and Bras, Jose 2017. Analysis of C9orf72 repeat expansions in a large international cohort of dementia with Lewy bodies. *Neurobiology of Aging* 49 , 214.e13-214.e15. 10.1016/j.neurobiolaging.2016.08.023

Publishers page: <http://dx.doi.org/10.1016/j.neurobiolaging.2016.08...>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



***Analysis of C9orf72 repeat expansions in a large international cohort of Dementia with
Lewy Bodies***

Celia Kun-Rodrigues¹, Owen A. Ross², Tatiana Orme¹, Claire Shepherd³, Laura Parkkinen⁴, Lee Darwent¹, Dena Hernandez⁵, Olaf Ansorge⁴, Lorraine N. Clark⁶, Lawrence S. Honig⁶, Karen Marder⁶, Afina Lemstra⁷, Philippe Scheltens⁷, Wiesje van der Flier⁷, Eva Louwersheimer⁷, Henne Holstege⁷, Ekaterina Rogaeva⁸, Peter St. George-Hyslop⁸, Elisabet Londos⁹, Henrik Zetterberg¹⁰, Imelda Barber¹¹, Anne Braae¹¹, Kristelle Brown¹¹, Kevin Morgan¹¹, Walter Maetzler¹², Daniela Berg¹², Claire Troakes¹³, Safa Al-Sarraj¹³, Tammarnyn Lashley¹⁴, Janice Holton¹⁴, Yaroslau Compta¹⁵, Vivianna Van Deerlin¹⁶, John Q. Trojanowski¹⁶, Geidy E. Serrano¹⁷, Thomas G. Beach¹⁷, Jordi Clarimon¹⁸, Alberto Lleó¹⁸, Estrella Morenas-Rodríguez¹⁸, Suzanne Lesage¹⁹, Douglas Galasko²⁰, Eliezer Masliah²⁰, Isabel Santana²¹, Monica Diez²², Pau Pastor²², Pentti J. Tienari²³, Liisa Myllykangas²⁴, Minna Oinas²⁵, Tamas Revesz¹⁴, Andrew Lees¹⁴, Brad F. Boeve²⁶, Ronald C. Petersen²⁶, Tanis J. Ferman²⁷, Valentina Escott-Price²⁸, Neill Graff-Radford²⁹, Nigel Cairns³⁰, John C. Morris³⁰, David Stone³¹, Stuart Pickering-Brown³², David Mann³², Dennis W. Dickson², Glenda M. Halliday³, Andrew Singleton⁵, Rita Guerreiro³³, Jose Bras^{33,*}

1 - Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK

2 - Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA

3 - Neuroscience Research Australia, Sydney, Australia and School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, Australia

4 - Nuffield Department of Clinical Neurosciences, Oxford Parkinson's Disease Centre, University of Oxford, Oxford, UK

5 - Laboratory of Neurogenetics, National Institutes on Aging, NIH, Bethesda, MD, USA

6 - Taub Institute for Alzheimer Disease and the Aging Brain and Department of Pathology and Cell Biology, Columbia University, New York, NY, USA

7 - Department of Neurology and Alzheimer Center, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, the Netherlands

8 - Department of Medicine, Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada

9 - Clinical Memory Research Unit, Institution of Clinical Sciences Malmö, Lund University, Sweden

10 - Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK and Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

11 - Translation Cell Sciences - Human Genetics, School of Life Sciences, Queens Medical Centre, University of Nottingham, Nottingham, UK

12 - Hertie Institute for Clinical Brain Research, Department of Neurodegeneration, Center of Neurology, University of Tuebingen, Tuebingen, and Department of Neurology, Christian-Albrechts University of Kiel, Germany

13 - Department of Basic and Clinical Neuroscience and Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

14 - Queen Square Brain Bank, Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK

15 - Queen Square Brain Bank, Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK and Movement Disorders Unit, Neurology Service, Clinical Neuroscience Institute (ICN), Hospital Clínic, University of Barcelona, IDIBAPS, Barcelona, Spain

16 - Department of Pathology and Laboratory Medicine, Center for Neurodegenerative Disease Research, Perelman School of Medicine at the University of Pennsylvania, 3600 Spruce Street, Philadelphia, USA

17 - Banner Sun Health Research Institute, 10515 W Santa Fe Drive, Sun City, AZ 85351, USA

18 - Department of Neurology, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

19 - Sorbonne Université, Université Pierre et Marie Curie-Paris 06, Inserm, Centre National de la Recherche Scientifique, and Institute du Cerveau et de la Moelle épinière, Paris, France; Assistance Publique Hôpitaux de Paris, Hôpital de la Salpêtrière, Département de Génétique et Cytogénétique, Paris, France

20 - Department of Neurosciences, University of California, San Diego, La Jolla, CA, United States; Veterans Affairs San Diego Healthcare System, La Jolla, CA, United States

21 - Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
22 - Memory Unit, Department of Neurology, University Hospital Mútua de Terrassa, and Foundation Mútua de Terrassa, Barcelona, Spain and Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain
23 - Molecular Neurology, Research Programs Unit, University of Helsinki, Department of Neurology, Helsinki University Hospital, Helsinki, Finland
24 - Department of Pathology, Haartman Institute, University of Helsinki and HUSLAB
25 - Department of Neuropathology and Neurosurgery, Helsinki University Hospital and University of Helsinki, Helsinki, Finland
26 - Neurology Department, Mayo Clinic, Rochester, MN, USA
27 - Department of Psychiatry and Department of Psychology, Mayo Clinic, Jacksonville, FL, USA
28 - MRC Centre for Neuropsychiatric Genetics and Genomics, School of Medicine, Cardiff University, Cardiff, UK
29 - Department of Neurology, Mayo Clinic, Jacksonville, FL, USA
30 - Knight Alzheimer's Disease Research Center, Department of Neurology, Washington University School of Medicine, Saint Louis, MO, USA
31 - Genetics and Pharmacogenomics, Merck Research Laboratories, West Point, Pennsylvania, USA
32 - Institute of Brain, Behaviour and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK
33 - Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK and Department of Medical Sciences and Institute of Biomedicine – iBiMED, University of Aveiro, 3810-193 Aveiro, Portugal

*** Corresponding author:**

Jose Bras, PhD
Department of Molecular Neuroscience
1 Wakefield Street (1st Floor)
London WC1N 1PJ
England
Phone: +44 (0) 207 679 4256
Email: j.bras@ucl.ac.uk

Keywords: *C9orf72*, Dementia with Lewy Bodies (DLB), genetic screen

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.