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C9orf72 and intracerebral hemorrhage

Isabel C. Hostettler [a,b], Manuel Bernal-Quiros [b], Andrew Wong [c], Nikhil Sharma [d,e], Duncan Wilson [a], David J. Seiffge [a,f,g], Clare Shakeshaft [a], Hans R. Jäger [h], Hannah Cohen [i], Tarek Yousry [h], Rustam Al-Shahi Salman [j], Gregory Y.H. Lip [k,l], Martin M. Brown [a], Keith W. Muir [m], David J. Werring [a], Henry Houlden [b,*], on behalf of the CROMIS-2 collaborators

[a] *Stroke Research Centre, University College London, Institute of Neurology, London, UK*
[b] *Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery and UCL Institute of Neurology, London, UK*
[c] *MRC Unit for Lifelong Health and Ageing at UCL, London, UK*
[d] *Department of Neurology, The National Hospital of Neurology and Neurosurgery, London, UK*
[e] *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK*
[f] *Stroke Centre and Institute of Neurology, University Hospital and University Basel, Basel, Switzerland*
[g] *Department of Neurology and Stroke Center, Inselspital, University Hospital Bern, Bern, Switzerland*
[h] *Neuroradiological Academic Unit, Department of Brain Repair & Rehabilitation, University College London Institute of Neurology, London, UK*
[i] *Haemostasis Research Unit, Department of Haematology, University College London, London, UK*
[j] *Centre for Clinical Brain Sciences, School of Clinical Sciences, University of Edinburgh, Edinburgh, UK*
[k] *Liverpool Centre for Cardiovascular Science, University of Liverpool and Liverpool Heart & Chest Hospital, Liverpool, UK*
[l] *Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark*
[m] *Institute of Neuroscience & Psychology, University of Glasgow, Queen Elizabeth University Hospital, Glasgow, UK*

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ABSTRACT

The chromosome 9 open reading frame 72 (*C9orf72*) GGGGCC repeat expansion has been associated with several diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. It has also been associated with increased white matter changes in frontotemporal dementia and risk of cognitive impairment in ALS. Dementia is common both before and after intracerebral hemorrhage (ICH). Because the mechanisms of cognitive impairment in patients with ICH are uncertain, we investigated whether *C9orf72* could influence dementia risk in this patient group. Therefore, we genotyped 1010 clinically characterized ICH cases and 2147 population controls in comparison with prior data of dementia and ALS cases. We did not find any association between *C9orf72* repeat expansion and repeat size with ICH compared with controls or with dementia when assessing ICH patients only. The frequency of *C9orf72* expansions in our series of individuals born in 1946 (2/2147) and other U.K. controls was age dependent, decreasing with increasing age, highlighting the high age-dependent penetrance of this expansion.

1. Introduction

The chromosome 9 open reading frame 72 (*C9orf72*) GGGGCC repeat expansion has been associated with several diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). The number of repeats to discriminate between normal repeat and pathogenic expansion is unknown although the cutoff has been suggested at 30 (Van Mossevelde et al., 2017). In patients with a pathogenic repeat expansion, the number of hexanucleotide copies can be in the thousands (DeJesus-Hernandez et al., 2011; Renton et al., 2011). *C9orf72* repeat expansions have previously been associated with white matter hyperintensities in FTD and cognitive impairment in patients with ALS (Chio et al., 2016; Mahoney et al., 2015). Brain tissues of patients with FTD have demonstrated amyloid beta plaques in some cases (2/51) (Simon-Sanchez et al., 2012). *C9orf72* repeat expansion may therefore influence pre-ICH dementia or an increased rate of dementia after the ICH event through similar mechanisms by acting on cerebrovascular factors such as small vessel diseases (SVDs) and cerebral amyloid angiopathy (CAA) (Cordonnier et al., 2010; Moulin et al., 2016; Pendlebury and Rothwell, 2009; Xiong et al., 2016). We hypothesized that increasing numbers of GGGGCC repeats in the

* Corresponding author at: Head of the Laboratory of Neurogenetics, The National Hospital of Neurology and Neurosurgery and UCL Institute of Neurology, University College London, Queen Square, WC1N 3BG London, UK. Tel.: +44 20 3448 3141.
E-mail address: h.houlden@ucl.ac.uk (H. Houlden).

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absence of a pathogenic repeat expansion may be associated with or influence pre-ICH dementia in patients with ICH through a mechanism that influences SVD and CAA. To address this hypothesis, we genotyped 1010 clinically characterized ICH cases and 2147 controls and compared them with prior data of dementia and ALS cases.

2. Methods

2.1. Study cohort

The Clinical Relevance of Microbleeds in Stroke study was approved by the National Research Ethics Service (reference: 10/H0716/64). We included patients with available DNA who had been recruited to the Clinical Relevance of Microbleeds in Stroke (NCT02513316) ICH study (Charidimou et al., 2015). Population controls were from the Medical Research Council National Survey of Health and Development (NSHD), a socially stratified cohort of 5362 singleton births that occurred during 1 week in March 1946 in England, Scotland, and Wales (Kuh et al., 2016; Rousseau et al., 2006).

2.2. Genotyping

C9orf72 screening was performed as previously described using sizing and repeat-primed polymerase chain reaction (DeJesus-Hernandez et al., 2011; Renton et al., 2011). In cases of suggested expansion, we performed Southern blot (DeJesus-Hernandez et al., 2011). We evaluated the number of repeats as continuous (dominant and additive) and binary variable (dichotomized into <20 and ≥ 20 repeats) (Rutherford et al., 2012; Van Mossevelde et al., 2017). To evaluate for dominant effect, we used the number of repeats of the longer of the 2 normal alleles and for additive effect the summed repeat number of both alleles (Rutherford et al., 2012).

2.3. Outcome measures and procedures

Outcome variables in patients with ICH were history of dementia, SVD burden, and CAA. We analyzed SVD by assessing the overall burden of SVD on CT and possible and probable CAA using the modified Boston criteria (Arba et al., 2017; Linn et al., 2010).

2.4. Statistics

Continuous variables are presented as means and SD if normally distributed and median and IQR if not normally distributed. Categorical variables are presented as number and percentage. In a second step, we adjusted the regression model for age and sex. For possible and probable CAA, we adjusted for sex only. We performed all statistical analysis in STATA 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp).

3. Results

We analyzed *C9orf72* in 3157 individuals: 1010 cases and 2147 controls (Supplementary Fig. 1). The frequency of patients with ICH heterozygosity for C9orf72 alleles was higher compared with controls (Supplementary Table 1and Fig. 2). See Supplementary Table 2 for an overview of allele counts. When considering allele count, we did not observe a significant difference between cases and controls (OR, 1.03; 95% CI, 0.99–1.07; $p = 0.17$ for allele 1 and OR, 0.99; 95% CI, 0.97–1.02; $p = 0.53$ for allele 2). However, when considering GGGGCC repeats as a binary variable (dichotomized into <20 or ≥ 20 repeats), controls more frequently had ≥ 20 repeats ($p = 0.01$, Supplementary Table 1). In fact, only 4 patients with ICH had a number of repeats ≥ 20 (0.4%) compared with 34 controls (1.6%); none of these 4 patients with ICH had a history of dementia. Two individuals had a pathogenic expansion (with >80 repeats), both of whom were from our control cohort (0.09% of NSHD population controls, Supplementary Table 3 and Fig. 3). No pathogenic expansion was found in the ICH cohort. In the ICH cohort, a history of pre-ICH dementia was associated with age but not with number of repeats (Supplementary Table 4) measured by dominant and additive repeat variable and homozygosity. We did not observe association between the dominant repeat variant and SVD burden (OR, 0.99 per point increase; 95% CI, 0.95–1.02; $p = 0.42$) or possible or probable CAA. None of the patients with ≥ 20 repeats had cognitive deficits, history of dementia, or imaging markers.

4. Discussion

This is the first study to evaluate the influence of *C9orf72* alleles in an ICH cohort where we do not report pathogenic *C9orf72* repeat expansions. Controls had a higher frequency of higher repeats compared to patients with ICH. We could not demonstrate an effect of number of repeats on history of dementia, SVD burden, or CAA. We observed differences between patients with ICH and controls in homozygous frequency; patients with ICH more frequently heterozygous. Two controls had a pathogenic expansion (0.09%). Our findings suggest that the frequency of expansion in population controls is lower than previously suggested. The absence of *C9orf72* repeat expansions in the ICH cohort is likely expected because of individuals with pathogenic expansions most likely having died by the time an individual suffers from an ICH (age of ALS onset ranges around 58 years of age for the carrier of the *C9orf72* repeat expansion) (Chio et al., 2015; Murphy et al., 2017; Westeneng et al., 2018; Wijesekera and Leigh, 2009). The *C9orf72* expansion cutoff has been suggested at 30. Although cases and controls were different with regard to homozygous frequency and number of repeats, the percentage of individuals with ≥ 20 repeats was lower in both cohorts than expected: 0.4% in patients with ICH and 1.6% in population controls compared with previous results. The control data also highlights the lower frequency of *C9orf72* expansions in our NSHD controls (Kuh et al., 2016; Rousseau et al., 2006). Blood samples for the NSHD controls were taken when they were 53.5 years of age; therefore, early death due to pathogenic expansion is unlikely causing the lower percentage of expansions (Supplemental Table 3 and Fig. 3). ICH individuals with ≥ 20 repeats did not have an increased rate of history of pre-ICH dementia. In conclusion, *C9orf72* repeats are not associated with history of pre-ICH dementia, SVD markers, or CAA in the ICH population. The prevalence of *C9orf72* GGGGCC repeat expansion in the normal population is lower than previously reported (Rutherford et al., 2012).

Disclosure

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.07.007.

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