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C9orf72 and intracerebral haemorrhage

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Intracerebral haemorrhage, C9orf72, genetics, dementia, white matter changes, imaging markers

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Abstract

The *C9orf72* GGGGCC repeat expansion has been associated with several diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD). It has also been associated with increased white matter changes in FTD as well as risk of cognitive impairment in ALS. Dementia is common both before and after intracerebral haemorrhage (ICH). Since 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 characterised 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 or repeat size with ICH compared to 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 UK controls was age-dependent decreasing with increasing age, highlighting the high age-dependant penetrance of this expansion.

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 cut-off 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 as well as cognitive impairment in ALS patients(Chio et al., 2016; Mahoney et al., 2015). Brain tissue of FTD patients 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 and cerebral amyloid angiopathy(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 absence of a pathogenic repeat expansion may be associated with or influence

1 pre-ICH dementia in ICH patients through a mechanism that influences small vessel disease (SVD)
2 and cerebral amyloid angiopathy (CAA). To address this hypothesis, we genotyped 1010 clinically
3 characterised ICH cases and 2147 controls and compared them with prior data of dementia and ALS
4 cases.

5 **Methods: Study cohort:** The CROMIS-2 study was approved by the National Research Ethics Service
6 (reference: 10/H0716/64). We included patients with available DNA who had been recruited to the
7 CROMIS-2 (NCT02513316) ICH study(Charidimou et al., 2015). Population controls were from the
8 Medical Research Council National Survey of Health and Development (NSHD), a socially stratified
9 cohort of 5362 singleton births that occurred during 1 week in March 1946 in England, Scotland, and
10 Wales(Kuh et al., 2016; Rousseau et al., 2006).

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

18 **Outcome measures and procedures:** Outcome variables in patients with ICH were history of
19 dementia, SVD burden and CAA. We analysed SVD by assessing the overall burden of SVD on CT
20 and possible and probable CAA using the modified Boston criteria(Arba et al., 2017; Linn et al.,
21 2010). **Statistics:** Continuous variables are presented as means and SD if normally distributed, median
22 and IQR if not normally distributed. Categorical variables are presented as number and percentage. In
23 a second step we adjusted the regression model for age and sex. For possible and probable CAA, we
24 adjusted for sex only. We performed all statistical analysis in STATA 15 (StataCorp. 2017. Stata
25 Statistical Software: Release 15. College Station, TX: StataCorp).

26 **Results**

1 We analysed *C9orf72* in 3157 individuals: 1010 cases and 2147 controls (Supplementary Figure 1).
2 The frequency of ICH patients heterozygous for *C9orf72* alleles was higher compared to controls
3 (Supplementary Table 1 and Figure 2). See Supplementary Table 2 for an overview of allele counts.
4 When considering allele count, we did not observe a significant difference between cases and controls
5 (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).
6 However, when considering GGGGCC repeats as a binary variable (dichotomized into <20 or ≥ 20
7 repeats), controls more frequently had ≥ 20 repeats ($p=0.01$, supplementary Table 1). In fact, only 4
8 ICH patients had a repeat ≥ 20 (0.4%) compared to 34 controls (1.6%), none of these 4 ICH patients
9 had a history of dementia. Two individuals had a pathogenic expansion (with >80 repeats), both of
10 whom were from our control cohort (0.09% of NSHD population controls, supplementary Table 3 and
11 Figure 3). No pathogenic expansion was found in the ICH cohort. In the ICH cohort a history of pre-
12 ICH dementia was associated with age but not with repeats (supplementary Table 4) measured by
13 dominant and additive repeat variable and homozygosity. We did not observe association between the
14 dominant repeat variant and SVD burden (OR 0.99 per point increase, 95%CI 0.95-1.02, $p=0.42$) nor
15 possible or probable CAA. None of the patients with ≥ 20 repeats had cognitive deficits, history of
16 dementia or imaging markers.

17 Discussion

18 This is the first study to evaluate the influence of *C9orf72* alleles in an ICH cohort where we do not
19 report pathogenic *C9orf72* repeat expansions. Controls had a higher frequency of higher repeats
20 compared to ICH patients. We could not demonstrate an effect of number of repeats on history of
21 dementia, SVD burden, nor CAA. We observed differences between ICH patients and controls in
22 homozygous frequency, ICH patients more frequently heterozygous. Two controls had a pathogenic
23 expansion (0.09%). Our findings suggest the expansion in population controls is lower than previously
24 suggested. The absence of *C9orf72* repeat expansions in the ICH cohort is likely expected due to
25 individuals with pathogenic expansions most likely having died by the time an individual suffers from
26 an ICH (age of ALS onset ranges around 58 year of age for carrier of the *C9orf72* repeat
27 expansion)(Chio et al., 2015; Murphy et al., 2017; Westeneng et al., 2018; Wijesekera and Leigh,

1 2009). The *C9orf72* expansion cut-off has been suggested at 30. Although cases and controls were
2 different with regards to homozygous frequency and number of repeats, the percentage of individuals
3 with ≥ 20 repeats was lower in both cohorts: 0.4% in ICH patients and 1.6% in population controls
4 compared to previous results. The control data also highlights the lower frequency of *C9orf72*
5 expansions in our NSHD controls(Kuh et al., 2016; Rousseau et al., 2006). Blood samples for the
6 NSHD controls were taken when they were 53.5 years of age, therefore early death due to pathogenic
7 expansion is unlikely causing the lower percentage of expansions (Supplemental Table 3 and Figure
8 3). ICH individuals with ≥ 20 repeats did not have an increased rate of history of pre-ICH dementia. In
9 conclusion, *C9orf72* repeat are not associated with history of pre-ICH dementia, SVD markers or CAA
10 in the ICH population. The prevalence of *C9orf72* GGGGCC repeat expansion in the normal
11 population is lower than previously reported(Rutherford et al., 2012).

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