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Analysis of *C9orf72* in patients with frontotemporal dementia and amyotrophic lateral sclerosis from Argentina

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ABSTRACT

Pathologic expansion of the G_4C_2 repeat in *C9orf72* is the main genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). To evaluate the frequency of the G_4C_2 expansion in a Latin American cohort of FTD and ALS patients, we used a 2-step genotyping strategy. For FTD, we observed an overall expansion frequency of 18.2% (6 of 33 unrelated cases). Moreover, the *C9orf72* expansion accounted for 37.5% of all familial FTD cases (6 of 16 families). The expansion frequency in sporadic ALS cases was 2% (1 of 47 unrelated patients), whereas we observed the expansion in 1 of 3 families with a positive history for ALS. Overall, the expansion frequency in our FTD group was similar to that reported for patients in Europe and North America, whereas the frequency in our sporadic ALS group was significantly lower. To our knowledge, this is the first report on the frequency of the *C9orf72* expansion in a Latin American population.

1. Introduction

Frontotemporal dementia (FTD; MIM: 600274) and amyotrophic lateral sclerosis (ALS; MIM: 612069) represent a clinicopathologic continuum which has been further supported by the identification of the GGGGCC (G_4C_2) expansion in *C9orf72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Studies on the frequency of the G_4C_2 expansion showed considerable variation among continents. For instance, in Europe and North America, the *C9orf72* expansion accounted for almost 40% of familial and 8% of sporadic ALS patients, as well as 25% of familial and 6% of sporadic FTD cases (Majounie et al., 2012). In contrast, the frequency was extremely rare in Asian (Jang et al., 2013; Ogaki et al., 2012) and Middle Eastern countries (Alavi et al., 2014). In the first studies, G_4C_2-repeats >30 U was suggested to be pathologic; however, this notion has recently been challenged and the exact cutoff for pathogenic repeat number is currently unknown (Xi et al., 2015).

Data on the *C9orf72* G_4C_2 repeat expansion in Latin American patients with ALS or FTD have been missing. Here, we present novel data on the *C9orf72* hexanucleotide repeat expansion in an Argentinian population.

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Table 1
Clinical and demographic features of the FTD and ALS cohorts from Argentina

	FTD patients (n = 33)		ALS patients (n = 50)	
Male:female ratio	1:1.54		1:0.92	
Median disease duration (range) in years	3 (1–15)		2 (0–13)	
Median age at DNA collection (range) in years	59 (41–80)		61 (22–83)	
Positive family history	16/33 (48.5%)		3/50 (6%)	
Clinical diagnosis subtype	C9+	C9−	C9+	C9−
bvFTD	5/6	24/27		
PPA	1/6	3/27		
Bulbar onset			1/2	6/48
Spinal onset			1/2	39/48
Flail arm syndrome			0	3/48

Key: bvFTD, behavioral variant frontotemporal dementia; C9+, C9orf72 expansion carrier; C9−, C9orf72 expansion non-carrier; PPA, primary progressive aphasia.

2. Materials and methods

2.1. Individuals

A total of 33 FTD patients were diagnosed according to established criteria (Rascovsky et al., 2011) at the Memory and Aging Clinic (FLENI, Buenos Aires, n = 32) and at the "Hospital Escuela de Agudos Dr. Ramón Madariaga" (Province of Misiones, Northern Argentina, n = 1). Fifty ALS patients were assessed either at the ALS Clinic (FLENI, n = 49) or Hospital Escuela de Agudos Dr. Ramón Madariaga (n = 1). Seventy-three age-matched control individuals, who at the time of blood collection did not exhibit any neurologic signs, were also included in this study. All individuals and/or legal guardians gave their informed consent to participate. This study was approved by the Institutional Ethics Committee. Table 1 lists clinical and demographic features of the FTD and ALS cohorts.

2.2. C9orf72 G_4C_2-repeat genotyping

Genomic DNA were obtained from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit (Promega) according to manufacturer's instructions. A 2-step algorithm was used to genotype the G_4C_2 repeat as previously described (Xi et al., 2012). Briefly, the first step involved a fluorescent fragment length analysis to assess repeat numbers of up to ~50. Samples exhibiting homozygous genotypes underwent repeat-primed polymerase chain reaction to evaluate for the presence of the repeat expansion.

3. Results and discussion

Several studies have highlighted that expansion of the G_4C_2 repeat in *C9orf72* (MIM: 614260) is the main genetic cause of both hereditary and sporadic FTD or ALS, especially in populations of Caucasian origin (Woollacott and Mead, 2014). To our knowledge, there are no reports on the frequency of the G_4C_2 expansion in Latin America. For this reason, we assessed patients affected by FTD and ALS, mostly from the Buenos Aires metropolitan area. As listed in Table 1, positive family history was present in 48.5% of FTD and 6% of ALS cases. We observed a 1:1 male-to-female ratio in the ALS group. However, 60.6% of the FTD individuals were women, similar to reports in Southern Italy, in which a clear bias toward a higher female incidence was observed (Onyike and Diehl-Schmid, 2013).

We evaluated the G_4C_2-repeat allele frequency in 73 age-matched individuals with no neurologic disease. We observed that the most common alleles were 2, 5, and 8 repeats (Fig. 1) as previously reported (Jones et al., 2013). We did not observe any expanded allele in this group nor were there any intermediate allele sizes >18 repeats. There was no significant difference in allele frequencies between normal controls and patients with FTD or ALS (Fig. 1).

In the FTD group, most patients exhibited behavioral variant FTD (bvFTD regardless of their expansion carrier status [Table 1]). We observed an overall G_4C_2 expansion frequency of 18.2% (6 of 33 cases). A G_4C_2 expansion in *C9orf72* explained 37.5% of familial cases (6 of 16 families). Eighty-three percent (5 of 6) of expansion carriers exhibited behavioral variant FTD and a single case presented with language impairment (Table 1). Interestingly, 60% of expansion carriers (3 of 5) eventually developed signs of motor neuron disease in contrast to previous reports that showed concomitant ALS in 30% (Kaivorinne et al., 2013) or 26.9% (DeJesus-Hernandez et al., 2011) of expansion carriers. Overall, the frequency of expansion carriers in

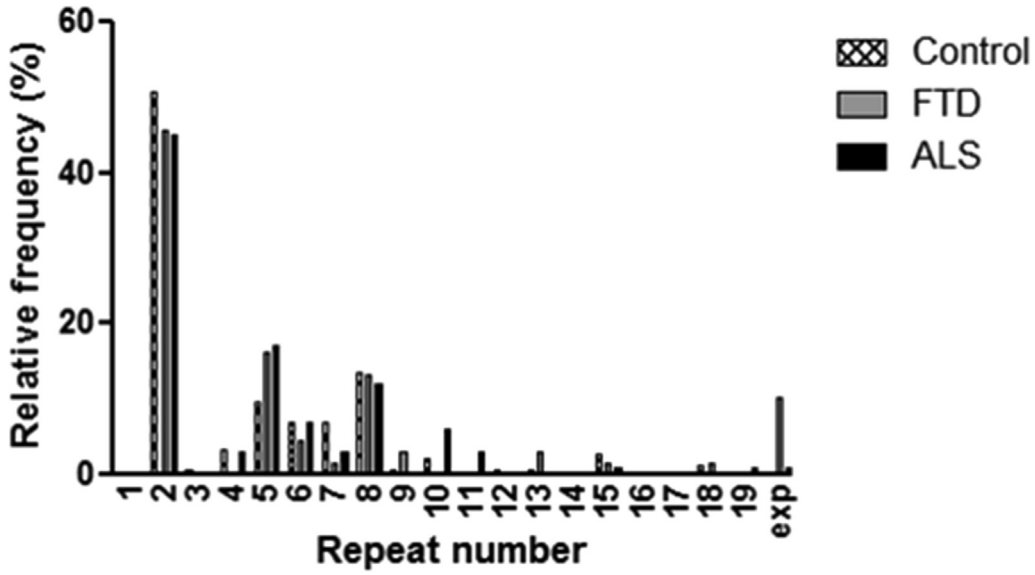

Fig. 1. Relative allele frequency of the C9orf72 G_4C_2 repeat in normal controls, and patients affected by frontotemporal dementia (FTD) or amyotrophic lateral sclerosis (ALS).

our Argentine FTD cohort was comparable with that observed in patients from Europe, North America, and Australia.

As for ALS, our group consisted mainly of sporadic cases (94%) the majority of which exhibited spinal onset (Table 1). Of the 3 cases with positive family history, 1 patient carried a G_4C_2 expansion. Notably, this 60-year-old patient with spinal onset belonged to a family in which a sibling was affected with FTD and their father and 2 paternal uncles had died of ALS. The other expansion carrier case in our cohort corresponded to a 57-year-old woman, with no documented family history, who exhibited bulbar onset. Interestingly, in sporadic ALS, we found a 2.1% (1 of 47 cases) expansion carrier frequency. This is in contrast to a previous report (Majounie et al., 2012) in which they found an 8.3% (6 of 72) expansion carrier frequency in sporadic ALS from a Hispanic population in the United States. One possible reason for this discrepancy could be due to ethnic background differences between US Hispanics and our group. In particular, a study about the genetic structure of the Argentine population has shown a large variance in European (65%–79%) and Amerindian (17%–31%) admixture and a very small African (2%–4%) contribution (Seldin et al., 2007). In contrast, Mexico and other Caribbean countries (which substantially contribute to the US Hispanic population) exhibit a larger influence of either Amerindian (51%–56% in Mexico) or African (77% in the Caribbean) origin (Salzano and Sans, 2014).

In conclusion, we present novel data on the *C9orf72* G_4C_2 expansion frequency in an Argentine cohort of FTD and ALS patients. This work warrants further studies in other Latin American populations to gain insight into the possible contribution of *C9orf72* expansion in the pathogenesis of FTD and ALS in this geographical region.

Disclosure statement

The authors declare no conflicts of interests.

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