C9ORF72 hexanucleotide repeat expansion in ALS patients from the Central European Russia population

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Abstract

Cohorts of amyotrophic lateral sclerosis (ALS) patients and control individuals of Caucasian origin from the Central European Russia (Moscow city and region) were analysed for the presence of hexanucleotide repeat GGGGCC expansion within the first intron of the *C9ORF72* gene. The presence of a large (>40) repeat expansion was found in 15% of familial ALS cases (3 out of 20 unrelated familial cases) and 2.5% of sporadic ALS cases (6 out of 238) but in none of control cases. These results suggest that the frequency of *C9ORF72* hexanucleotide repeats expansions in the Central European Russian ALS patients is significantly lower than in Western European or Northern American ALS patients of Caucasian origin but higher than in Asian ALS patients.

Key words: amyotrophic lateral sclerosis/mutation/repeat expansion

1. Introduction

Hexanucleotide repeat GGGGCC expansion within the first intron of the *C9ORF72* gene located on chromosome 9p21 is associated with ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This is the most common genetic defect accounting for approximately 40% of all familial cases of ALS and 30% of all familial cases of FTD in European and North American populations as well as about 8 % of sporadic ALS in the same populations (Majounie et al., 2012). Unaffected individuals commonly have less than 25 GGGGCC repeats in each of their *C9ORF72* alleles and more than half of them have only two repeats (Rutherford et al., 2012). The length of pathogenically expanded repeat loci is highly variable due to a wide range, from tens to thousands, of the repeat copy numbers detected in ALS/FTD patients. This might explain substantial phenotypic heterogeneity in patients with these expansions although exact correlations between the repeat lengths and such clinical characteristics of the disease as the onset, progression rate or prevailing symptoms have yet to be firmly established. (Byrne et al., 2012; Cooper-Knock et al., 2012; Cooper-Knock et al., 2014; Majounie et al., 2012; Simon-Sanchez et al., 2012)

There is no consensus about the origin of this genetic modification with both a single founder (Mok et al., 2012; Smith et al., 2012) and multiple events (Fratta et al., 2014) have been proposed. Geographical and population diversity of the repeat expansions in ALS and FTD is substantial. For example, the overall average frequency of expansions in Western European FTD patients was approximately 10%, however this figure was above 20% for Finland (~29%), Spain (~25%) and Sweden (~21%) but was under 5% for Germany (van der Zee et al., 2013). The presence of the repeat expansions is extremely rare in studied Asian populations (Jang et al., 2013; Majounie et al., 2012; Ogaki et al., 2012; Soong et al., 2014; Tsai et al., 2012; Zou et al., 2013).

Considering geographical position of Russian Federation between populations with such substantial difference in the frequency of the repeat expansion, information about association of this genetic modification with ALS/FTD in various populations throughout this large country can be of particular interest. Here we analyzed the *C9ORF72* hexanucleotide repeat expansion in ALS patients from the Central European Russia region.

2. Material and methods

2.1. Patients

All ALS patients were of Caucasian origin from two specialized Moscow clinics that accept patients from the Moscow city and region. These patients did not exhibit symptoms of cognitive impairment. Positive family history was registered in 21 patients from 20 unrelated families. A control group included age and sex matched Caucasians from the same region not diagnosed with any chronic diseases at the time of blood collection. All participating patients gave their informed consent, and the study was approved by the Local Ethics Committee of the Research Center of Neurology.

2.2. DNA analysis

Genomic DNA was extracted from blood samples using Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer instructions. The presence of expanded GGGGCC hexanucleotide repeats in *C9ORF72* was detected using amplified fragment length polymorphism (AFLP) analysis and repeat-primed polymerase chain reaction (PCR). AFLP analysis was performed using fluorescently-labeled primers as described previously (DeJesus-Hernandez et al., 2011). Repeat primed PCR (Warner et al., 1996) was carried out according to previously published protocol for detection of the *C9ORF72* hexanucleotide repeats (DeJesus-Hernandez et al., 2011). Characteristic stutter amplification pattern on the electrophoregram was considered as an evidence of a pathogenic repeat expansion. Automated ABI Prism 3130 Genetic Analyzer (ABI) and GeneMapper software (version 4, ABI) for data analysis were used for both applications.

Southern hybridization analysis of genomic DNA digested with restriction endonucleases XbaI and EcoRI was performed using ^{32}P-labelled pCh9.1 probe as described previously (Buchman et al., 2013).

3. Results and Discussion

The AFLP analysis was carried out for 258 ALS cases and 223 age and sex matching individuals from the same geographical region. The samples appearing as "homozygous" in this assay were further analyzed using a repeat-primed PCR method to identify the presence of the expansion within one of the *C9ORF72* alleles.

All 223 control subjects had 15 or less hexanucleotide repeats and in the ALS group 249 patients also had small number of repeats (≤16) in the *C9ORF72* locus with the longest expansion. The repeat size distribution was similar in both groups and followed previously observed (Jones et al., 2013) trimodal pattern with preferred occurrence of 2, 5 and 8 repeats (Figure 1). The remaining 9 ALS patients (3.5% of all studied disease cases) displayed a repeat-primed PCR pattern consistent with the presence of a large (>40) repeat expansion in one of their *C9ORF72* loci. In this group classified as C9ORF72-ALS the sex distribution, site of onset (see Table A.1), and the average age of onset (54 years) was similar to that in the

ALS group without pathological repeat expansion in this locus (average age of onset 52 years). Exon sequencing did not reveal mutations in several known ALS associated genes in these patients, however a combination with mutations in other genes or certain risk haplotypes as a factors contributing to the development of the disease in patients with hexanucleotide repeat expansion cannot be excluded at the moment.

Three C9ORF72-ALS patients had documented family history (see Table A.2) but only for one of these families (Figure A.1) it was possible to obtain DNA samples from the family members. A typical pattern of extended repeats was revealed for the proband (DoN-ALS-101), one of whose parents has died from ALS, using DNA repeat-primed PCR. This was confirmed by Southern hybridization analysis that showed the presence of over 2000 repeats in one *C9ORF72* allele (Figure 2). Repeat-primed PCR revealed that his only sibling and his only descendant, both healthy at the present time, are carriers of the expanded repeats, consistent with reduced penetrance at least up to the age of 42 years (current age of the sibling). Due to small size of the family, only 9 of 19 analysed SNPs in the vicinity of the repeat expansion site showed an unambiguous link with the presence of the expansion (Table A.3), eight of them were the same as in so-called Finnish haplotype (Mok et al., 2012). SNP genotyping data for two other patients with family history also at least did not contradict this haplotype (data not shown).

Taken together, the hexanucleotide repeat expansion in the *C9ORF72* locus is present in 15% of familial ALS cases (3 out of 20 unrelated familial cases) and 2.5% of sporadic ALS cases (6 out of 238) in the studied population.

In conclusion, we found that the frequency of *C9ORF72* hexanucleotide repeats expansions in the Central European Russian ALS patients is significantly lower than in Western European or Northern American ALS patients of Caucasian origin but still higher than in Asian ALS patients where its rate is almost negligible. The impact of this genetic defect to familial ALS was also found to be lower than in other studied Caucasian populations.

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5. Disclosure Statement

Authors declare no conflicts of interest

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7. Figure legends

Figure 1. Frequency of distribution of repeat sizes in 249 ALS (open bars) and 223 control (filled bars) individuals without pathological (>30) repeat expansions assessed in this study.

Figure 2. Southern blot analysis of GGGGCC repeat expansion in the C9ORF72 locus. Hybridisation of EcoRI and XbaI digested DNA extracted from the whole blood of the patient DoN-ALS-101 and an individual without pathological repeat expansion (control) as well as from two cultured lymphoblastoid cell lines of patients positive for GGGGCC repeat expansion.

Figure A.1. The family tree for the proband 101 (DoN-ALS-101). Carriers of the repeat expansion in the C9ORF72 locus are shown in black squares (males) or circles (females). Numbers designate only those family members for whom DNA samples were available for analysis of SNPs shown in Table A.2.

Appendix

Table A.1. Clinical characteristics of ALS patients with C9ORF72 GGGGCC repeat expansions assessed in this study				
ID	***Sex***	***Age of onset (years)***	***Site of onset***	***Family history of ALS/FTD***
CN-ALS-062	M	42	Lumbar	No
CN-ALS-073	M	53	Bulbar	No
CN-ALS-088	M	63	Lumbar	No
CN-ALS-108	F	30	Bulbar	No
CN-ALS-220	F	65	Lumbar	No
CN-ALS-262	F	65	Thoracic	Yes
CN-ALS-270	M	65	Thoracic	Yes
DoN-ALS-101	M	45	Thoracic	Yes
DoN-ALS-102	M	58	Bulbar	No

Table A.2. Evidence of family history of ALS for patients with C9ORF72 GGGGCC repeat expansions assessed in this study					
Patient ID	***Relative***	***Age of onset (years)***	***Age of death (years)***	***Site of onset***	***Symptoms of FTD***
CN-ALS-262	Brother	60	62	Lumbar	Not reported
	Mother	60	65	Lumbar*	Not reported
CN-ALS-270	Father	61	65	Lumbar	Not reported
DoN-ALS-101	Father	Not documented	53	Lumbar*	Not reported

*Formal diagnosis of ALS has not been established but patients suffered from the gait abnormalities characteristic for the lumbar form of the disease.

Table A.3. Genotypes of SNPs around the C9ORF72 repeat expansion area for members of the family shown in Figure A.1.						
SNP	#101 (proband)	#202 (+ve)	#205 (-ve)	#201 (+ve)	linked allele	Consensus 19 SNP "Finnish" haplotype
rs1822723	C/T	C/T	C/T	C/T	-	C
rs4879515	C/T	C/T	C/T	C/T	-	T
rs868856	C/T	C/T	C/T	C/T	-	T
rs7046653	A/G	A/G	A/G	A/G	-	A
rs1977661	C/C	C/C	C/C	C/C	C	C
rs903603	C/T	C/T	C/T	C/T	-	C
rs10812610	C/A	C/C	A/A	C/A	C	C
rs2814707	A/G	A/G	A/G	A/G	-	A
rs3849942	A/G	A/G	A/G	G/G	G	A
rs10122902	G/A	G/G	G/A	G/A	G	G
rs10757665	T/C	T/C	T/C	T/C	-	T
rs1565948	G/A	G/A	G/A	G/A	-	G
rs774359	T/C	T/C	T/C	C/C	C	C
rs2282241	G/T	G/G	G/T	G/G	G	G
rs1948522	C/C	C/C	C/C	C/C	C	C
rs1982915	A/G	A/G	A/G	G/G	G	G
rs2453556	A/G	A/G	A/G	A/G	-	G
rs702231	C/A	C/A	C/A	C/A	-	A
rs696826	A/G	G/G	A/G	A/G	G	G

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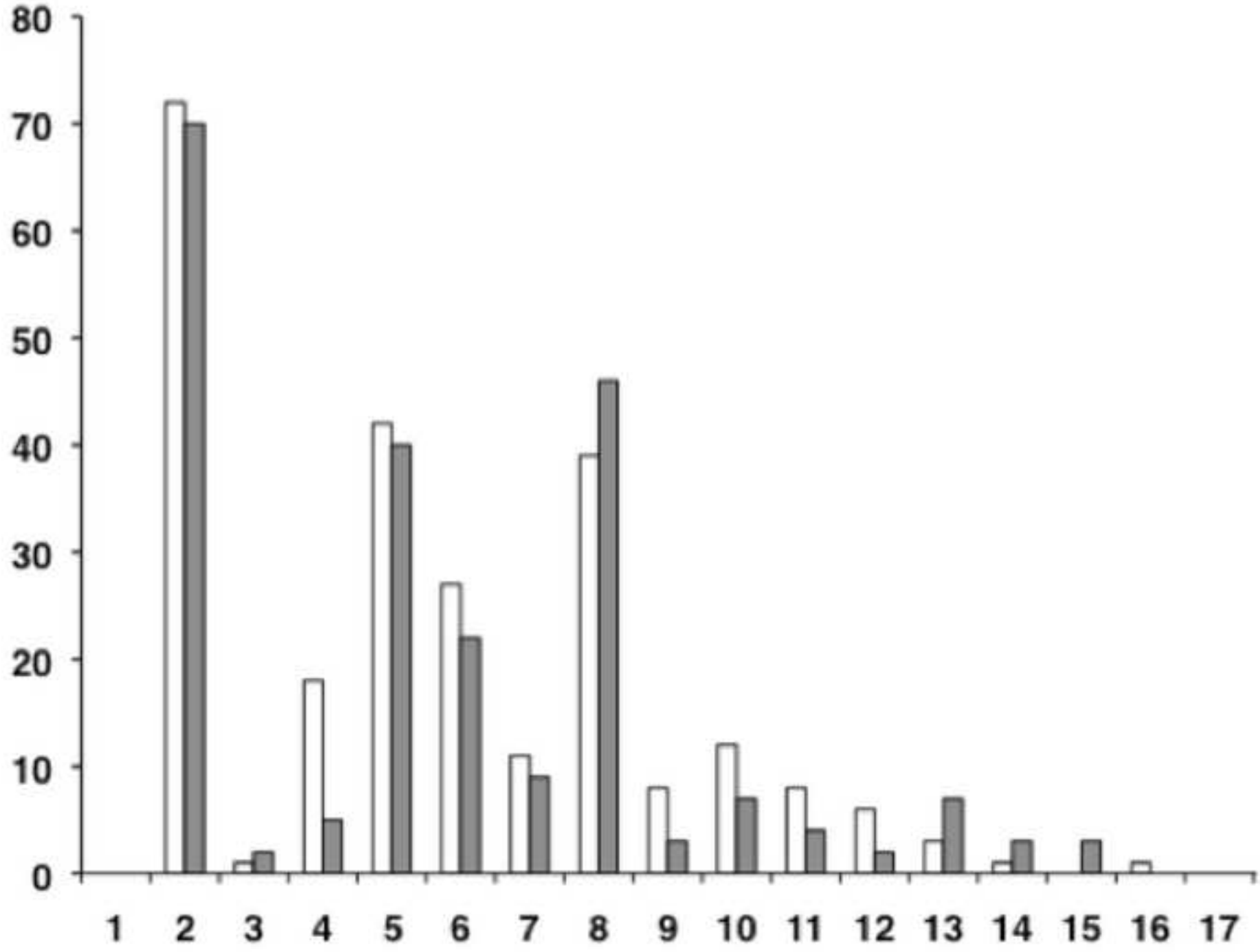

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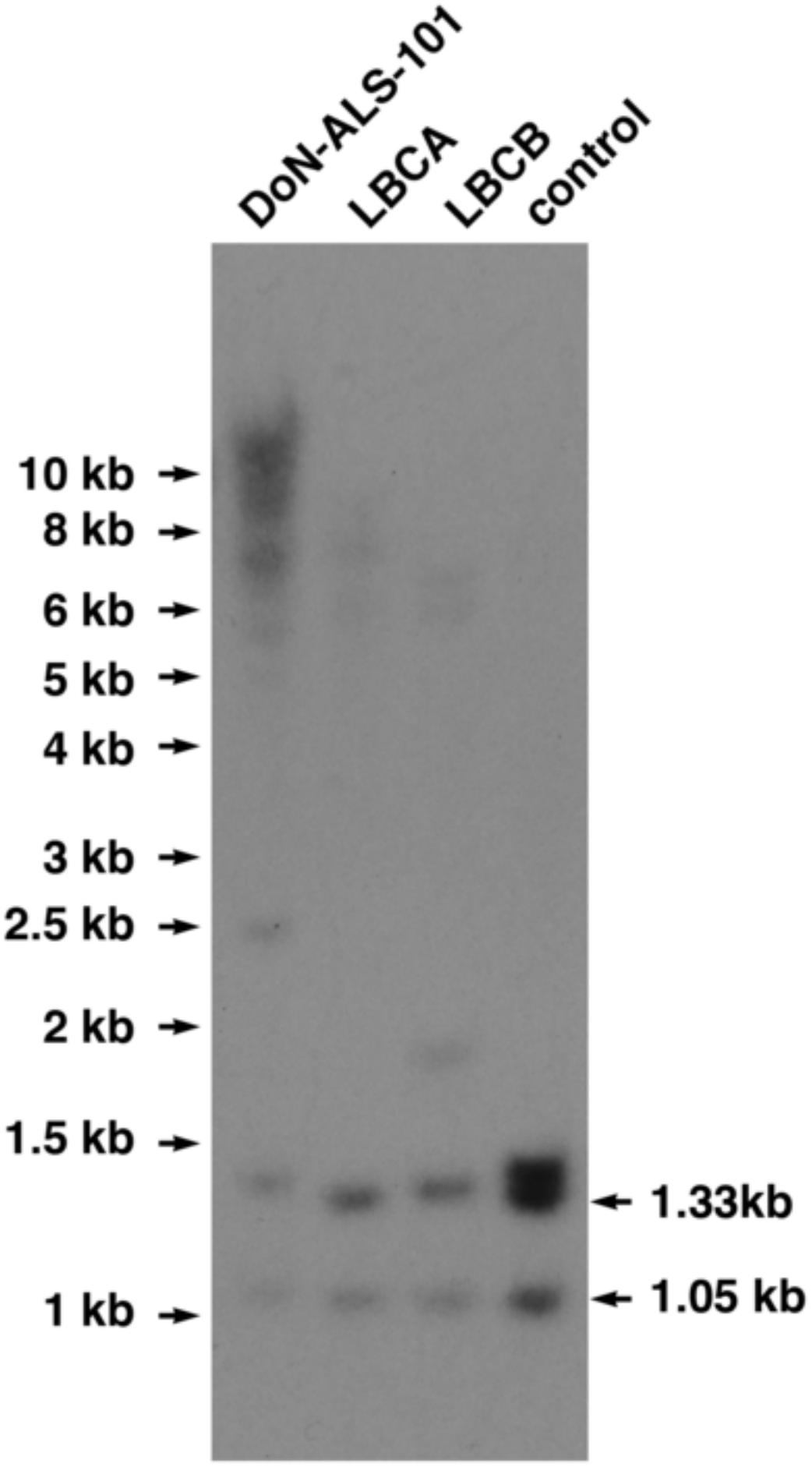

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