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Gene Expressions Of C9orf72 And Rab7ain Human Tissues Derived From The Central Nervous System (Cns) In Amyotrophic Lateral Sclerosis (Als), Frontotemporal Dementia (Ftd) And Alzheimer's Disease (Ad)

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ABSTRACT

The non-coding hexanucleotide repeat expansions (HRE) in intron 1 of the C9ORF72 gene have been identified as the most frequent genetic cause of familial ALS and familial frontotemporal degeneration (FTD). In this report, we studied the effect of C9ORF72 HRE in C9ORF72 and RAB7A (Rab7)expressions using human postmortemtissues derived from the spinal cord of sporadic ALS and the motor cortex of other frontotemporal dementia (FTD) with or without C9ORF72 HRE and Alzheimer's disease (AD).To investigate the pathogenic effects of C9ORF72 HRE in these diseases, the gene expression approach was applied through RNA extraction, cDNA synthesis and quantitative PCR.Here, we compared the C9ORF72 isoforms a and b mRNA levels in the spinal cord from sporadic ALS cases compared to controls and there was no difference in both of C9ORF72 isoforms mRNA levels. We found that in frontal and temporal cortex, there was no difference in the C9ORF72 isoform a mRNA level in frontal and temporal cortex samples between frontotemporal (FTD) individuals in the presence or absence of C9ORF72 HRE and Alzheimer's disease (AD) compared to healthy controls. Interestingly, in frontal cortex, there was a significant upregulation in C9ORF72 isoform b mRNA expression in FTD without C9ORF72 HRE and AD cases whilst in temporal cortex, there was a significant upregulation in C9ORF72 isoform b mRNA level in FTD individuals with C9ORF72 HRE. We also observed that in Alzheimer's disease cases, Rab7 mRNA was significantly downregulated in frontal cortex whilst in temporal cortex Rab7 mRNA was significantly upregulated. C9ORF72 expression have been well established in ALS but less so in frontotemporal dementia and Alzheimer's disease. Further, the C9ORF72 HRE expressions have been investigated mainly in cerebellum and cervical spinal cord whilst our study focusing in lumbar spinal cord and motor cortex. Our results suggested that different C9ORF72 isoforms may play different roles in the pathogenesis of ALS and Alzheimer's disease (AD).

Keywords: Amyotrophic lateral sclerosis, frontotemporal dementia, Alzheimer's disease, C9ORF72, hexanucleotide repeat expansions, Rab7A, autophagy.

BACKGROUND

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterised by the loss of upper motor neurons in motor cortex and lower motor neurons in the brainstem and spinal cord, that results in progressive paralysis and death due to respiratory failure (1). Familial ALS (FALS) accounts for approximately ~5 to 10% of ALS cases whilst the rest of ALS cases are sporadic (2). The C9ORF72 geneis located on chromosome 9p21.2and the gene givesrise to three transcripts which are variant 1(V1, NM_145005.6), variant 2 (V2, NM_0.18325.4) and variant 3 (V3, NM_001256054.2). Variant 2 and variant 3 encode for the long isoform (isoform a; C9ORF72-L) that consists of 481 amino acids, whereas variant 1 encodes the short isoform (isoform b; C9ORF72-S) that comprises of 222 amino acids (Figure 1). Genetic linkage analysis of a Scandinavian family with multiple family members affected with ALS and FTD had originally identified a locus on chromosome 9p21.3-p13.3 to be linked to ALS-FTD (3). Since then, the hexanucleotide repeat expansions (HRE) in C9ORF72 have been identified as the most frequent genetic cause of familial ALS and familial frontotemporal degeneration (FTD) (4, 5).

C9ORF72 HRE is important in FTD and ALS because it contributes to 25% cases of familial FTD, 39% FALS, 8% SALS cases and 7% of sporadic FTD (6) with the mutation being more frequent in Caucasian populations and more common in ALS than in FTD. C9ORF72 normal repeat size is highly polymorphic but in European populations, the typical repeat size is between 2-10 repeats (5) and at least hundreds or thousands of repeats and up to 4000 repeats are found in patients with ALS and FTD (4, 7, 8). In controls, the repeats are usually not greater than 30 repeats whereas in patients with pure ALS, pure FTD or ALS-FTD the number of repeats may be 700-1600 repeats and the expansion size may be up to 10 kb in length (4, 5).

C9ORF72 HRE gives rise toa more aggressive form of ALS than other mutation, where patients with HRE have an earlier age at onset, about ~2.5 years earlier than patients without the HRE and are more likely to have a positive family history of dementia (9). ALS patients carrying C9ORF72HRE are indistinguishable from classic ALS patients but nearly 50% of the C9-positive ALS cases develop cognitive or behavioural impairment or both, compared to those without HRE where these features are less common (10, 11). C9ORF72 protein is structurally homologous to Differentially Expressed in Normal and Neoplasia (DENN) proteins that act as Guanine nucleotide exchange factors (GEFs) to activate RAB GTPases and may therefore, regulate membrane trafficking (12, 13).

Although ALS, FTD and Alzheimer's disease (AD) have different clinical presentations, C9ORF72 repeat expansions have been reported in cases of Alzheimer's disease (6). Previous study in a large cohort of AD patients (n = 872 unrelated familial AD cases) has identified five AD families carrying abnormal C9ORF72 HRE in which three families carrying the repeat expansions in the range reported for FTD and ALS (>1000 repeats) and two families carrying 35-100 repeats (14). Previous work has also reported that C9ORF72 HRE carriers have a higher incidence of Parkinson's disease and Alzheimer's disease among relatives (15-18).

Although the precise roles of C9OR72 are still largely unknown, C9ORF72 has been demonstrated to be involved in cellular trafficking especially autophagy (13, 19).Rab proteins such as Rab1, Rab5, Rab7 and Rab11 are involved in the regulation of various autophagy stages which are important for intracellular trafficking and cellular viability (20-23). Rab7 regulates transport from early to late endosomes, biogenesis of lysosomes and autophagosome maturation into autolysosome (24-27). C9ORF72 has been demonstrated to be involved in cellular trafficking especially autophagy (13, 19). Several Rab proteins such as Rab1, Rab5, Rab7 and Rab11 have been observed to colocalize with C9ORF72 in the endolysosomal system in cortical neurons (19, 28). Furthermore, it has been reported that decreased C9ORF72 expression in neurons resulted in accumulations of p62-positive protein aggregates, suggesting dysregulation of autophagy (29, 30). It has also been observed in motor neurons of ALS patients, there is an increase of C9ORF72 colocalization with Rab1 and Rab7 but not Rab5 therefore, indicating dysregulation of endosomal trafficking in the motor neurons (19). Previous studies have also reported increases in Rab4, Rab5, Rab7 and Rab27 gene expression in cholinergic basal forebrain (CBF) neurons and CA1 pyramidal neurons in sporadic cases of Alzheimer's disease (31, 32).

Here, we examined the effect of the C9ORF72HRE on the expression of the different C9ORF72 isoforms (isoforms a and b) and Rab7A (hereafter known as Rab7) in the spinal cord from SALS cases compared to controls and in frontal and temporal cortex derived from frontotemporal dementia (FTD) individuals with C9ORF72 HRE (FTD C9-positive) or without C9ORF72 HRE (FTD C9-negative) and Alzheimer's disease (AD) cases to understand its association with ALS and proposed consequences on autophagy.

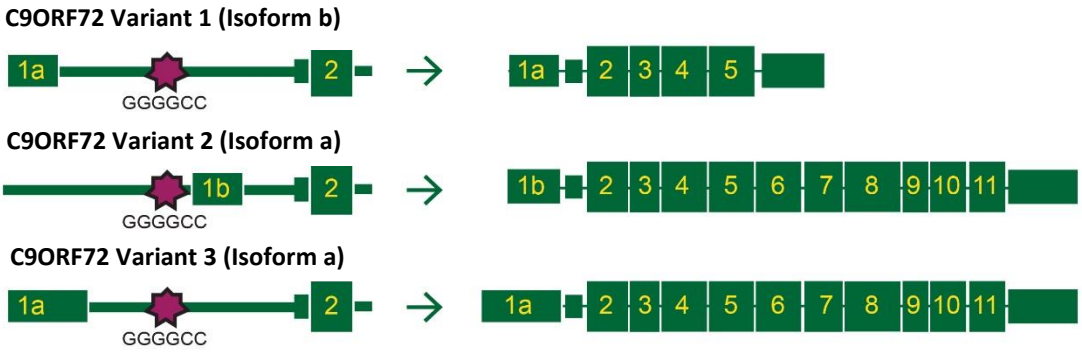


Figure 1: The C9ORF72 hexanucleotide repeat expansions (HRE). This schematic diagram shows the sites of C9ORF72 hexanucleotide repeat expansions, GGGGCC depicted as stars and the three annotated transcript variants; variant 1, variant 2 and variant 3. The pathogenic C9ORF72 HRE is found in the first intron of the variant 1 and variant 3 and is located within the predicted promoter region of variant 2.

MATERIALS AND METHODS

Spinal cord samples

Spinal cord samples were available for 41 samples: 12 controls and 29 sporadic ALS as detailed in Table 2 below. For the spinal cord cases and controls, frozen lumbar tissue sampled at levels L3 to L5 was used. These tissues have been characterised in detail with regards to motor neuron counts, Nissl staining, immunohistochemistry of neuronal markers (ChAT, VAPB and DAO) and p62 as a marker of ubiquitinated protein inclusions typical of ALS (33).

Table 2: ALS patients and healthy controls(33)

Number of cases (gender)		**Mean age at death (age range) in years (y)**	**Postmortem delay, mean ± SEM (range) in hours (h)**
Controls	12 (9 males, 3 females)	63.5 y (20-91 y)	8.88 ± 1.30 h (3-16 h)
SALS	29 (18 males, 11 females)	68.2 y (43-87 y)	14.37 ± 1.07 h (5-25 h)
Age and postmortem delay unavailable for three and two cases, respectively.			

Frontal and temporal cortex samples

Frozen prefrontal cortex (PFC) and temporal cortex (TC) tissues from frontotemporal dementia (FTD) and Alzheimer's disease (AD) subjects were obtained from the MRC London Neurodegenerative Diseases Brain Bank, a member of the Brains for Dementia Research Network. All tissue was contributed from voluntary donors in compliance with the Mental Capacity Act (2005). The Brain Bank has been approved by the National Research Ethics Service. All clinical diagnoses were confirmed neuropathologically at postmortem. Dissected brain tissue was snap frozen, then stored at −80 °C until further use. The mean age at death and the mean postmortem delay for healthy controls and disease cases are as shown in Table 3over leaf.

Table 3: Frontotemporal dementia (FTD) and Alzheimer's disease (AD) patients and healthy controls

Number of cases (gender)		**Mean age at death (age range) in years (y)**	**Postmortem delay, mean ± SEM (range) in hours (h)**
Controls	10 (7 males, 3 females)	73.6 y (55-99 y)	36.90 ± 4.882 h (12-55 h)
FTD C9-positive	10 (4 males, 6 females)	66.1 y (53-71 y)	38.20 ± 9.690 h (3-87 h)
FTD C9-negative	10 (6 males, 4 females)	80.9 y (62-92 y)	29.85 ± 4.013 h (8.5-48.5 h)
AD	10 (4 males, 6 females)	76.5 y (53-86 y)	35.30 ± 8.249 h (3-69 h)

Extraction of mRNA and cDNA Synthesis

In brief, mRNA was extracted from tissues with the Direct-Zol RNA MiniPrep(Zymo Research) and reverse transcribed to cDNA using approximately 1ug of RNA with random hexamers 50uM (Invitrogen), AMV Reverse Transcriptase (New England Biolab), 40mM dNTP Mix (Bioline) and DEPC-treated water (Invitrogen), following which cDNA samples were stored at -20°C until further use.RNA purity and integrity for all sampleswas assessed by using multiple well-established methods.

Quantitative Polymerase Chain Reaction (qPCR)

Primers were designed using Primer 3 web software to amplify the target cDNA sequence and are listed in the Table S1 in Supplementary. Quantitative PCR (qPCR) was performed using the Power Up™ SYBR™ Green Master Mix (Thermo Fisher Scientific) and amplification was carried out using an Mx3000P Real-time PCR System (Stratagene).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software. The effect of the mutation was analysed using unpaired or paired Student's t-test (parametric) accordingly. $p<0.05$ was considered to indicate a statistically significant result. Results were given as mean ± standard error of the mean (S.E.M).

RESULTS

Gene expressions of C9ORF72 in the spinal cord insporadic ALS (SALS)

Previous studies of C9ORF72 mRNA expression levels in SALS have mainly concentrated on the frontal and temporal cortex whereas there are only two studies that have used the spinal cord for analysis of C9ORF72 mRNA expression (34, 35).Therefore, we sought to investigate the effect of the C9ORF72 mutation in spinal cord samples from our cohort of SALS and control cases. There was no difference in the C9ORF72 isoforms a and b mRNA levels in the spinal cordfrom SALS cases compared to healthy controls (Figure 4).

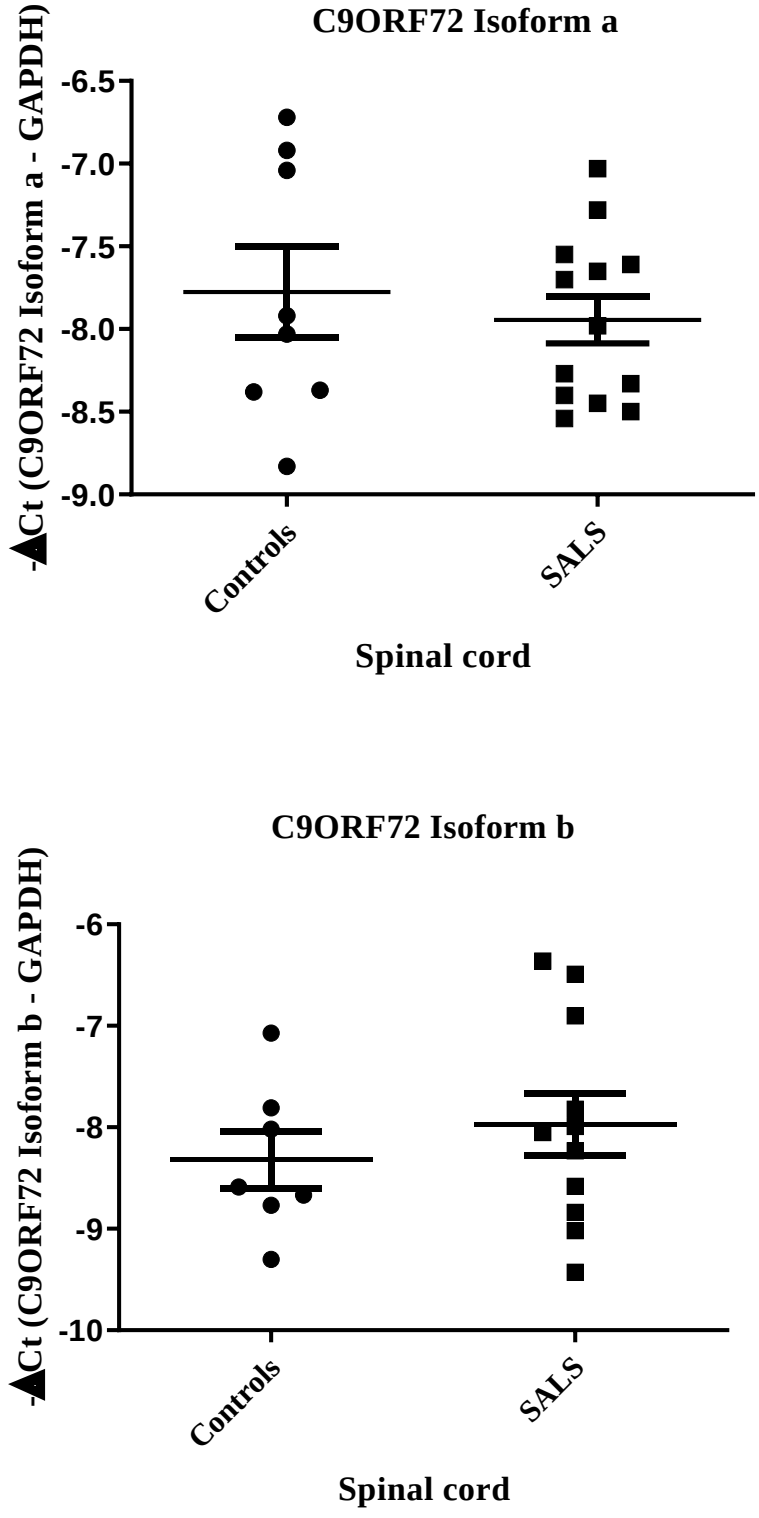


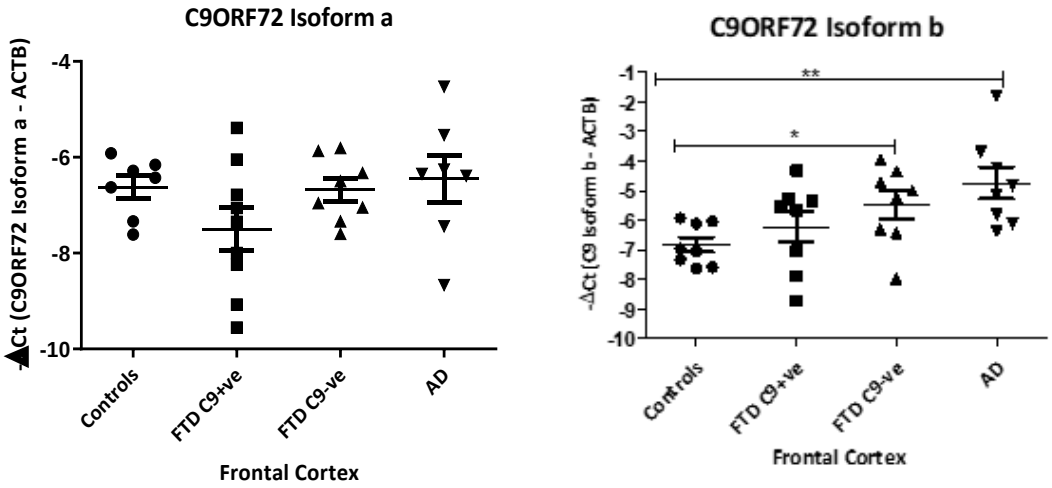
Figure 4: Quantitative PCR analysis of C9ORF72 isoforms a and b mRNA in the spinal cord normalised against GAPDH showed no change between SALS and control individuals.

Gene expressions of C9ORF72 in the motor cortexin frontotemporal dementia (FTD) and Alzheimer's disease (AD)

C9ORF72 isoforma mRNA expression has been reported to be reduced in the frontal and temporal cortex in familial ALS and frontotemporal degeneration (FTD) patients carrying the GGGGCC repeat expansions(4, 34, 36-40). However, we observedno change in the C9ORF72 isoform a mRNA expression levels between our cohort of FTD C9-positive, FTD C9-negative and Alzheimer's disease (AD) subjects and control individuals in frontal and temporal cortex samples (Figure 5).

In the frontal cortex, there was a significant upregulation in C9ORF72 isoform b mRNA expression in FTD C9-negative and AD cases when compared to healthy controls. These findings suggest that C9ORF72 isoform b may be associated with FTD lacking the C9ORF72 HRE (C9-negative) and Alzheimer's disease (AD) pathogenesis.

In the temporal cortex, we found that C9ORF72 isoform b mRNA expression was significantly downregulated in FTD C9-positive individuals compared to healthy subjects whereas no significant changes were found in C9ORF72 isoform b mRNA expression in FTD C9-negative and AD cases compared to healthy controls (Figure 5).



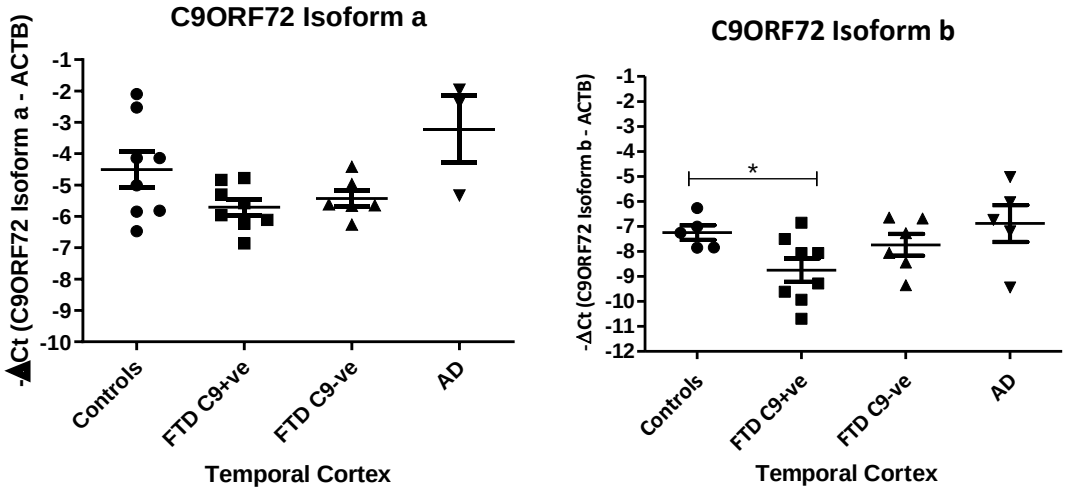


Figure 5: Quantitative PCR analysis of C9ORF72 isoforms a and b mRNA levels in the motor cortex normalised against ACTB in FTD C9-positive, FTD C9-negative, AD and control individuals.See also Table S2 (Supplementary).*,p<0.05, **,p<0.01

Gene expression of Rab7 in the motorcortexin frontotemporal dementia (FTD) and Alzheimer's disease (AD)

Previous study has reported that C9ORF72 colocalized and coprecipitated with Rab7 in neuronal cell lines and primary cortical neurons (19), therefore, we investigated the Rab7 mRNA expression in the brain tissues. In the frontal cortex we observed no change in the Rab7 mRNA expression level in FTD C9-positive and FTD C9-negative compared to healthy controls (Figure 6). However, there was a significant downregulation of Rab7 mRNA in Alzheimer's disease subjects compared to healthy subjects (Figure 6).

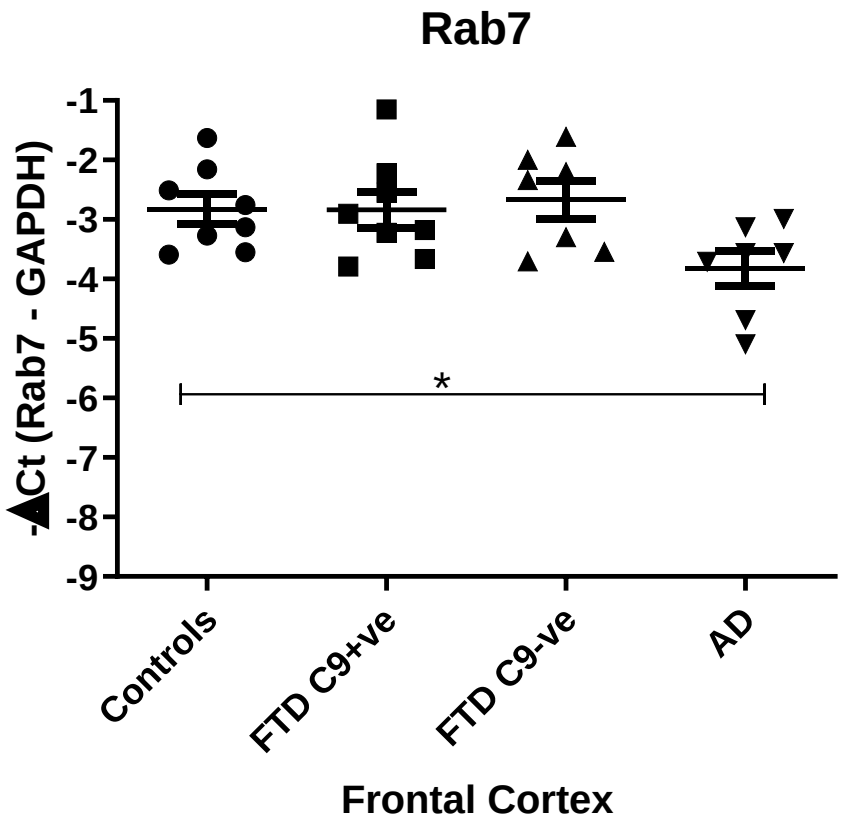


Figure 6: Quantitative PCR analysis of Rab7 mRNA in the frontal cortex normalised against GAPDH showed the Rab7 expression was downregulated in Alzheimer's disease (AD) subjects compared to controls.*,$p<0.05$

As shown in Figure7,in the temporal cortex samples, there was a significant upregulation of Rab7 expression in the AD patients whereas there was no change in the FTD C9-positive when compared with healthy controls. No statistical analysis could be performed for FTD C9-negative subjects due to the very few numbers of samples (n = 2).

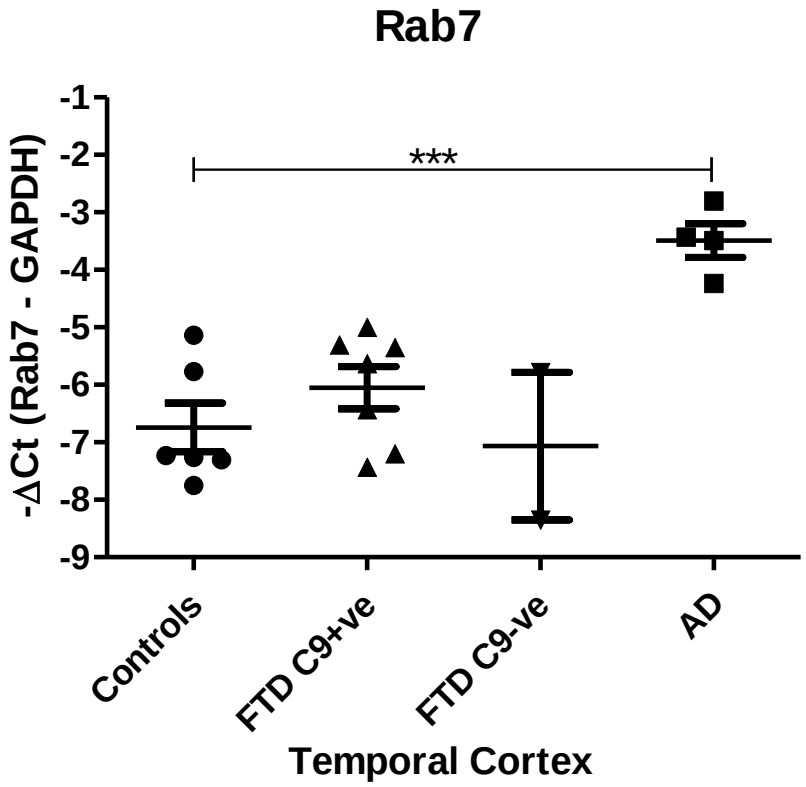


Figure 7: Quantitative PCR analysis of Rab7 mRNA in the frontal cortex normalised against GAPDH showed the Rab7 expression was upregulated in Alzheimer's disease (AD) subjects compared to controls.***,$p<0.001$

DISCUSSION

C9ORF72 hexanucleotide repeat expansions (HRE) have been reported as a common mutation of ALS and FTD (4, 5, 36). Initially, we aimed to determine the effects of C9ORF72 repeat expansions on C9ORF72 isoforms mRNA levels in the spinal cord samples of SALS cases. Several studies have reported reduced mRNA levels of C9ORF72 in brain tissues for examples frontal cortex, motor cortex and cerebellum from ALS and FTD subjects (4, 36, 37, 39-42) and a reduction in the C9ORF72 variant 2 (isoform a) in spinal cord from SALS subjects(35).

With regards to C9ORF72 isoform b, there were only two studies been reported to investigate the C9ORF72 isoform b mRNA levels in SALS cases and these studies have been focusing on brain tissues and not the spinal cord. This is probably due to C9ORF72 isoform b is not the major transcript and might be of lower abundance in tissues compared to C9 isoform a, therefore are more difficult to detect. C9ORF72 isoform b RNA levels in C9-negative FTD cases were observed to be decreased significantly in frontal cortex samples from twoC9-negative FTD individuals compared to nine healthy controls (36). In a study of four controls and six C9-positive ALS subjects, it has been reported that

the C9ORF72 isoform b RNA levels were reduced significantly in cerebellum, motor cortex and cervical spinal cord (34) whereas another study in frontal cortex samples that compared the C9ORF72 isoform b RNA levels between five controls and ten C9-positive ALS/FTD subjects found that there was no change in the RNA levels (39). To date, our study is the first study that utilizes the lumbar spinal cord in a large number of samples to examine the relative expressions of C9ORF72 isoforms aand b in SALS and we did not observe any significant changes in both of C9ORF72 isoformsmRNA levels between SALS cases and healthy subjects.

The reasons for the discrepancy are unclear however, the different tissues used in the studies and limitations in the PCR-based mRNA quantification method that rely on various primers and correct transcript splicing are possible explanations. Our findings may reflect the very low abundance of C9ORF72 proteins in the spinal cord as shown in previous work by another group(43). Our data also showed that C9ORF72 isoform b is very low in abundance compared with isoform a which is the main transcript of C9ORF72. This is probably due to C9ORF72 isoform b contains the hexanucleotide repeat expansions in the first intron of the gene and may produce pre-mRNA that produces RNA foci therefore, decreasing the C9ORF72 isoform b mRNA abundance (44).

In addition, our data report no change in the C9ORF72 isoforms a and b expression in the motor cortex from FTD subjects with or without C9ORF72 HRE compared to healthy subjects therefore suggesting loss-of-function of C9ORF72alone is not sufficient to cause toxicity in the neurons because evidence have shown that no missense or truncation C9ORF72 mutations have been reported in patients and neural-specific ablation of C9ORF72 or knockdown using antisense oligonucleotides (ASOs) in mice has also failed to recapitulate pathology (45-49).

Several studies have studied the C9ORF72 HRE in Alzheimer's disease, however these studies are focused on the number of C9ORF72 repeats in the Alzheimer's disease patients and not the level of C9 gene expression in the cases. To date, our study is the first that investigates the C9ORF72 transcripts level in Alzheimer's disease individuals. Previous works on the effects of C9ORF72 HRE in Alzheimer's disease have reported that C9ORF72 HRE pathogenic repeats (>30 repeats) are rare in Alzheimer's disease cases(50-53).

Rab GTPases have been linked with various neurodegenerative disorders either directly for example, in Charcott-Marie-Tooth type 2B disease (CMT2B) (54) or indirectly as in Alzheimer's disease (AD) (55-58). Rab GTPases alternate between GTP-bound active state and GDP-bound inactive state and are essential in endosomal intracellular trafficking. Accumulations of autophagosomes are commonly observed in neurons of AD individuals but the mechanisms driving autophagic stress in AD are still unknown. Autophagosomes are reported to aggregate at axons in AD individuals and are

associated with Amyloid-ß aggregates to disrupt the interaction of dynein that results in impaired autophagy process in AD patients (59).

C9ORF72 colocalized with Rab1, Rab5, Rab7 and Rab11, which are involved in endolysosomal trafficking (13, 19). A previous study has also reported that Rab7 mRNA is upregulated in basal forebrain from patients and this upregulation correlates with the cognitive decline in AD patients (32). Rab7 was also observed to be elevated in the hippocampus and frontal cortex of AD individuals (56). In agreement with another study in neurons and hippocampus (56), we observeda significant upregulation of Rab7 mRNA level in temporal cortex from our AD cases compared with healthy subjects. This suggests that the maturation of late endosome is disrupted therefore causing impaired autophagy in the neurons of AD individuals.

It has been reported that C9ORF72 forms a complex with SMCR8 to promote GDP/GTP exchange for the Rab8a and Rab39b which are involved in macroautophagy(60, 61). C9ORF72 has been recognised as a Rab1a effector by regulating the Unc-51-like kinase 1 (ULK1) autophagy initiation complex to the phagophore (30). To further confirm the role of C9ORF72 in autophagy, we investigated the gene expression level of Rab7 in in temporal and frontal cortex samples in healthy subjects and subjects with FTD with and without C9ORF72HRE and AD subjects. A previous study has shown that Rab7 mRNA level was upregulated in the CA1 pyramidal neurons of AD subjects and in the hippocampus of end-stage AD subjects suggesting of alteration of Rab7 expression that is involved in late endosome may contribute to CA1 neurodegeneration by impairing neurotrophin receptor signalling (56). In keeping with this, our data show that in subjects with AD, Rab7 mRNA levels are significantly upregulated in the temporal cortex but are significantly downregulated in the frontal cortex. There was no difference in the Rab7 mRNA level in the frontal cortex or temporal cortex samples from FTD individuals with or without C9ORF72 HRE when compared to healthy controls.

Consequently, we proposed that different C9ORF72 isoforms may play different roles in the pathogenesis of ALS and Alzheimer's disease (AD) and it may be possible that C9ORF72 isoform a contributes to the C9ORF72 haploinsufficiency whereas C9ORF72 isoform b may enhance the aggregation of other proteins than dipeptide repeat (DPR) proteins. It has been reported that mislocalized TDP-43 inclusions are more commonly found in neurons with decreased expression of the short form of C9ORF72, encoded by C9ORF72 isoform b(62). Furthermore,misregulated RNA processing events may represent a common pathway connecting ALS and FTD, although the mechanism by which C9ORF72 repeat expansions results in different clinical manifestations remains under investigation. Our results have demonstrated strongly in humanpostmortem tissue that C9ORF72 isoform b and Rab7 expressions areaffected in the motor cortex of FTD and AD cases. The

identification of these possible interactions may provide a valuable source of informationto elucidate the pathogenesis of the diseases.

DECLARATIONS

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Contributions

IB and JB designed research; IB performed research;JB contributedreagents/analytical tools;

IB and JB analysed data; and IB wrote the manuscript. All authors read and approved the final

manuscript.

Ethics declarations

Ethics approval and consent to participate

This study was approved by the Riverside Research Ethics Committee and was carried out according to their guidelines. All clinical diagnoses were confirmed neuropathologically at postmortem.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Data availability statement

Anonymized data supporting the findings of this study will be shared by reasonable request from any qualified investigator during three years after the publication of the study. The data are not publicly available due to privacy or ethical restrictions.

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SUPPLEMENTARY MATERIALS

Gene	Primer Name	Primer Sequence (5'-3')	cDNA Size (bp)
C9ORF72	Isoform aF	CCTTCCTGGATCAGGTCTTTCA	76
	Isoform aR	ACAAGTAGAAACTGTGCAAGGAAAGTAC	
	Isoform bF	AAATCATGGCCCCTTGCTT	69
	Isoform bR	TGAAAGGATTCTGTTAGCTTTAATGAGA	
Rab7	Rab7F	CAGACAAGTGGCCACAAAGC	130
	Rab7R	AAGTGCATTCCGTGCAATCG	
Gene	**Primer Name**	**Primer Sequence (5'-3')**	**cDNA Size (bp)**
GAPDH	GAPDH	Ready-made primer pair ordered from Primer Design (UK)	118
ACTB	ACTBF	GACAACGGCTCCGGCATGTG	121
	ACTBR	CCTTCTGACCCATGCCCAC	

Table S1: Primers pairs used for quantitative PCR (qPCR).(Legends: F = forward primer, R = reverse primer).

CNS Tissues	C9ORF72 Isoform	No. of Controls	Mean ± S.E.M	No. of Diseased States	Mean ± S.E.M	p-value
Spinal cord	a	8	-7.776± 0.2772	SALS, n = 13	-7.945± 0.1404	0.533(ns)

	b	7	-8.319 ± 0.2791	SALS, n = 11	-7.974 ± 0.3073	0.451(ns)
Frontal cortex	a	7	-6.624 ± 0.2366	FTD C9-positive n = 9	-6.301 ± 0.2144	0.1387(ns)
				FTD C9-negative, n = 8	-5.807 ± 0.2176	0.8644(ns)
				Alzheimer's disease (AD) n = 7	-5.711 ± 0.3272	0.7636(ns)
	b	8	-6.826 ± 0.2476	FTD C9-positive n = 8	-6.228 ± 0.5298	0.3233(ns)
				FTD C9-negative, n = 8	-5.494 ± 0.4679	0.0246(*)↑
				Alzheimer's disease (AD) n = 8	4.745 ± 0.5336	0.0033 (**)↑
Temporal cortex	a	8	-4.508 ± 0.5607	FTD C9-positive n = 8	-5.708 ± 0.2543	0.0716(ns)↓
				FTD C9-negative, n = 6	-5.430 ± 0.2619	0.2068(ns)
				Alzheimer'sdisease (AD) n = 7	-3.220 ± 1.062	0.2775(ns)
	b	5	-7.246 ± 0.2938	FTD C9-positive n = 8	-8.748 ± 0.4687	0.0397(*)↓

				FTD C9-negative, n = 6	-7.738 ± 0.4371	0.3950(ns)
				Alzheimer'sdisease (AD) n = 7	-6.882 ± 0.7367	0.6585(ns)

Table S2: C9ORF72 gene expression in the spinal cord and motor cortex in healthy subjects and diseased states