CLINICAL, PATHOLOGICAL AND MOLECULAR CHARACTERISATION OF *C9orf72*-ALS LEADS TO IDENTIFICATION OF NOVEL THERAPEUTIC TARGETS

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PLAN FOR TALK

1. Clinical and pathological features of C9orf72-disease
2. Southern blotting to size the C9orf72 expansion
3. RNA foci and neuropathology
4. Gene expression profiling to determine prognostic biomarkers

BRAIN
A JOURNAL OF NEUROLOGY

Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72

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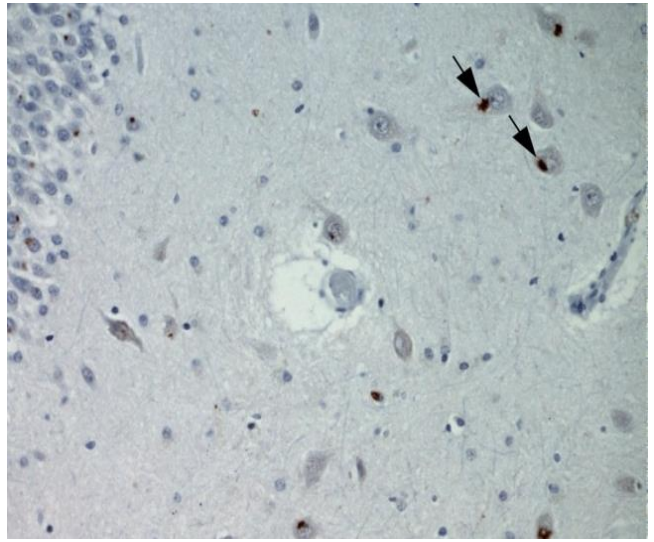
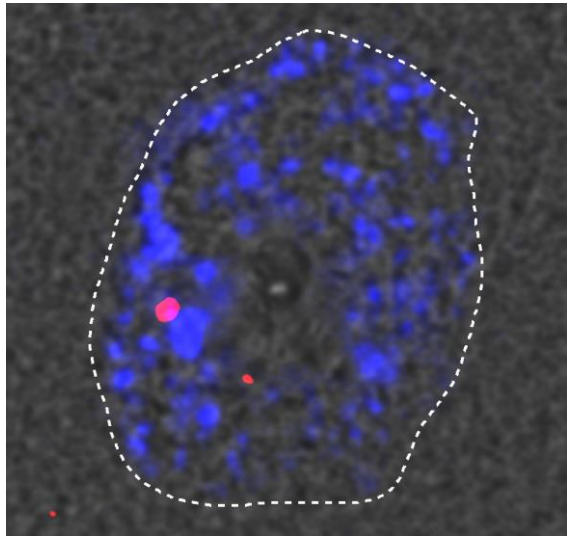
47% of familial MND cases and 7% of sporadic MND cases.

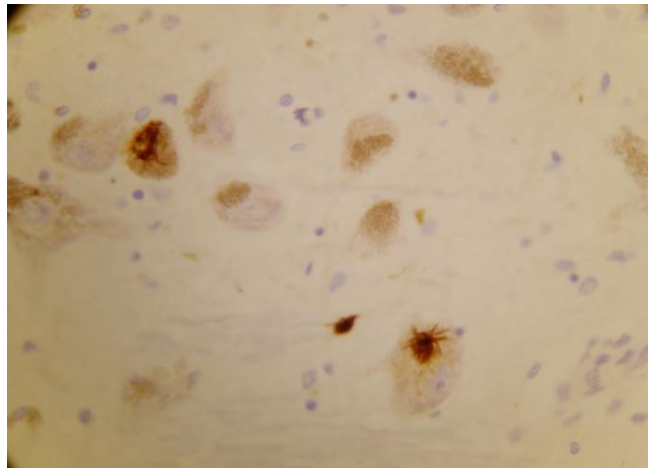
Clinically and pathologically resembles the more common sporadic disease.

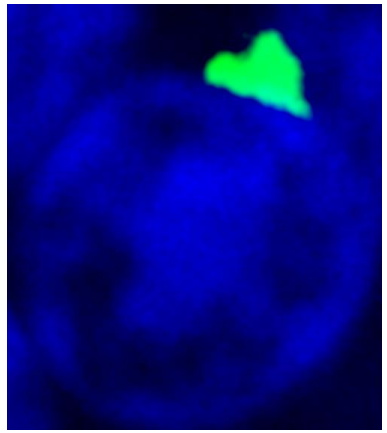
Overrepresentation of extramotor disease in patients and families.

C9orf72 NEUROPATHOLOGY









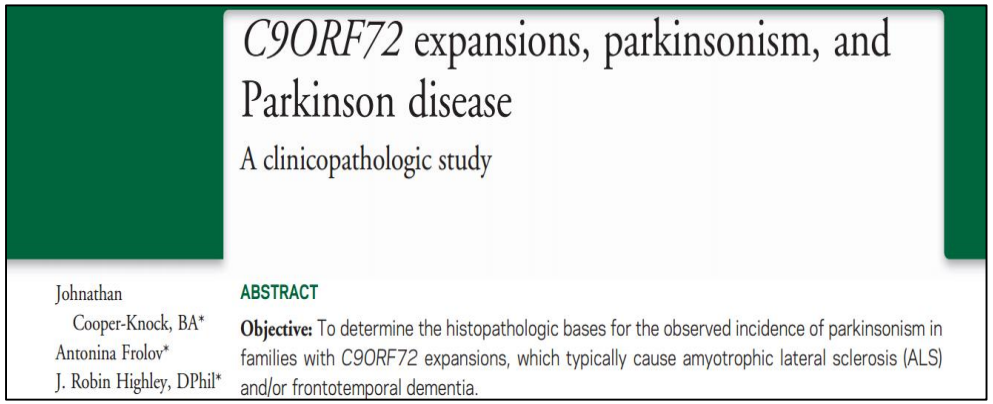
C9orf72 AND PARKINSONISM

5/61 (8.2%) patients had either a diagnosis of PD or a family history of PD.

We screened 518 patients with clinical parkinsonism and α-synucleinopathy for the C9orf72 expansion.

Only one patient carried the expansion - similar to control frequency. This patient had a family history of ALS.

Two patients with clinical ALS and PD were identified, one carried the *C9ORF72* expansion, the other did not.



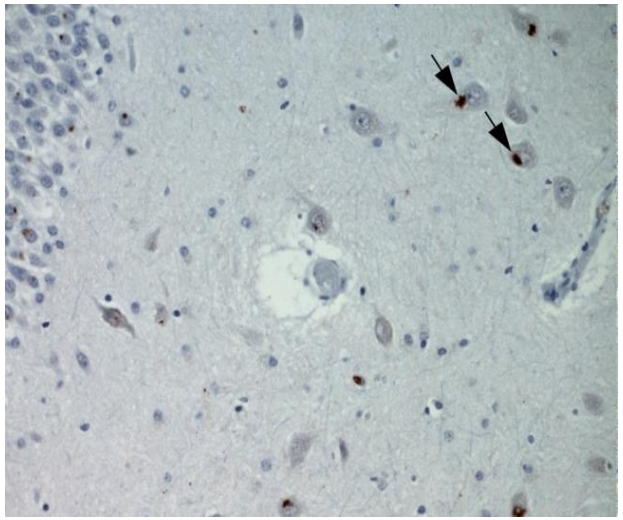
C9ORF72 expansions, parkinsonism, and Parkinson disease

A clinicopathologic study

Johnathan Cooper-Knock, BA*
Antonina Frolov*
J. Robin Highley, DPhil*

ABSTRACT

Objective: To determine the histopathologic bases for the observed incidence of parkinsonism in families with *C9ORF72* expansions, which typically cause amyotrophic lateral sclerosis (ALS) and/or frontotemporal dementia.



C9orf72 AND MULTIPLE SCLEROSIS

3/61 (5%) patients had either a diagnosis or a family history of demyelinating disease.

We prospectively identified 7 patients with MS who subsequently developed ALS

80% of MS-ALS patients carried the *C9orf72* expansion. This is a significant association (OR 3·27, p<0·001).

Zero from 215 pure MS cases carried the *C9orf2* expansion.

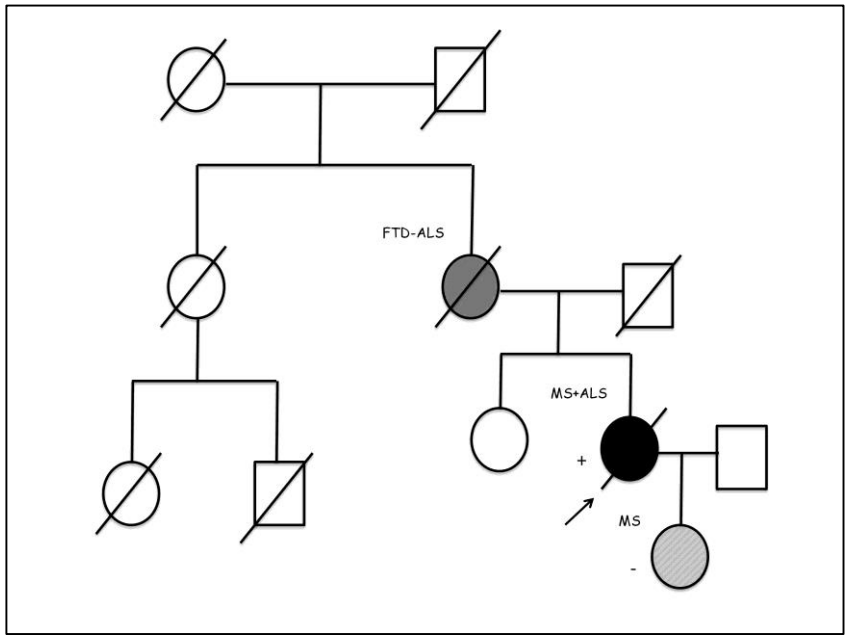
C9orf72-ALS is more rapidly progressive in the presence of MS

Neurodegeneration

RESEARCH PAPER

Concurrence of multiple sclerosis and amyotrophic lateral sclerosis in patients with hexanucleotide repeat expansions of *C9ORF72*

Azza Ismail,[1,3] Johnathan Cooper-Knock,[1,3] J Robin Highley,[1] Antonio Milano,[7] Janine Kirby,[1] Emily Goodall,[1] James Lowe,[5] Ian Scott,[5] Cris S Constantinescu,[4] Stephen J Walters,[2] Sian Price,[3] Christopher J McDermott,[1,3] Stephen Sawcer,[6] D Alastair S Compston,[6] Basil Sharrack,[1,3] Pamela J Shaw[1,3]

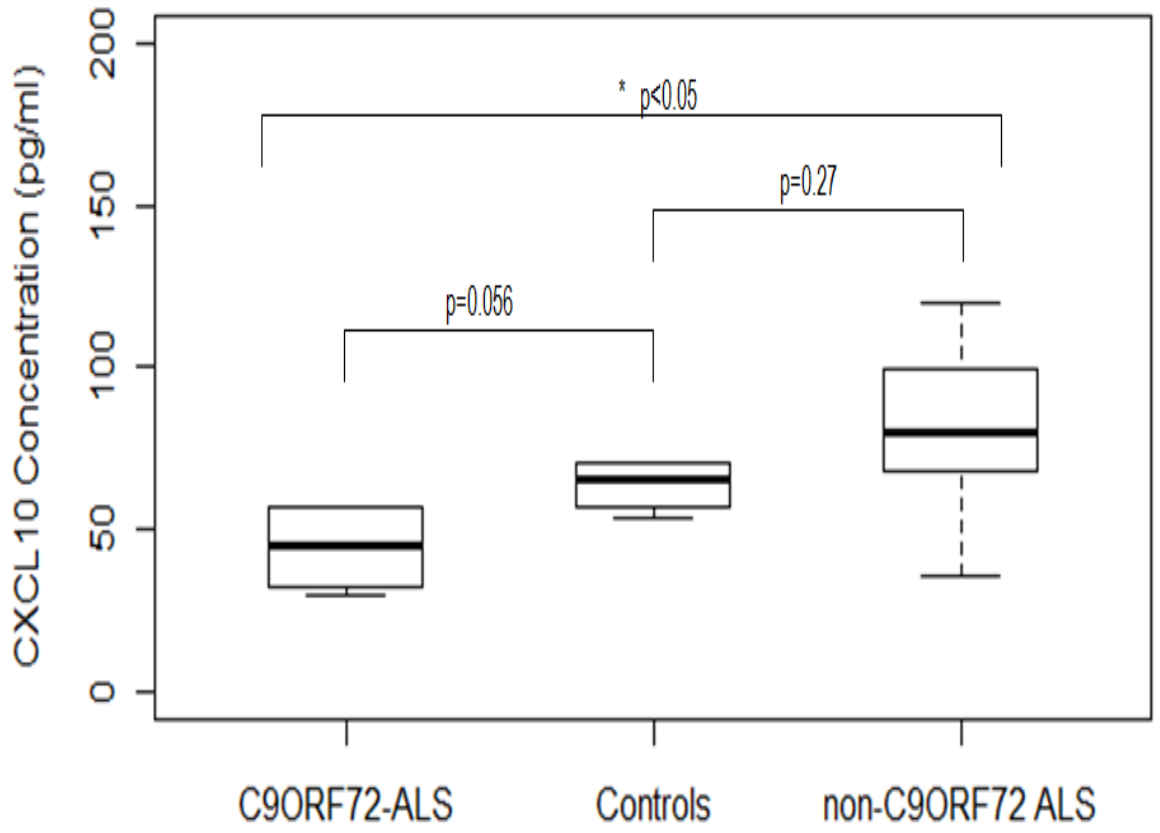


C9orf72 AND MULTIPLE SCLEROSIS

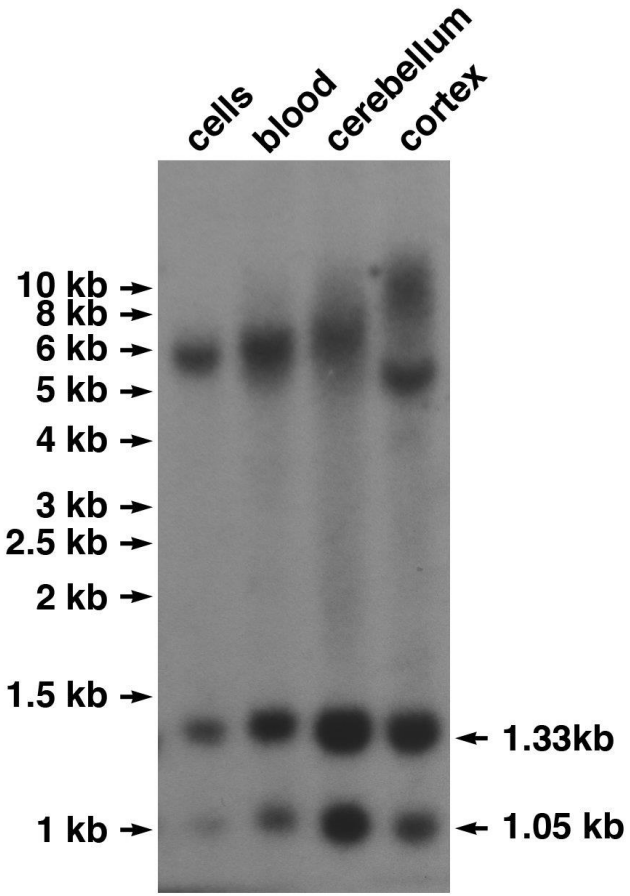
CXCL10 is a cytokine thought to be neuroprotective in ALS.

CXCL10 is normally elevated in MND but in *C9orf72*-ALS it is reduced.

This is a potential therapeutic target.



SOUTHERN BLOTTING TO SIZE THE *C9orf72* EXPANSION



Buchman *et al. Molecular Neurodegeneration* 2013, **8**:12
http://www.molecularneurodegeneration.com/content/8/1/12

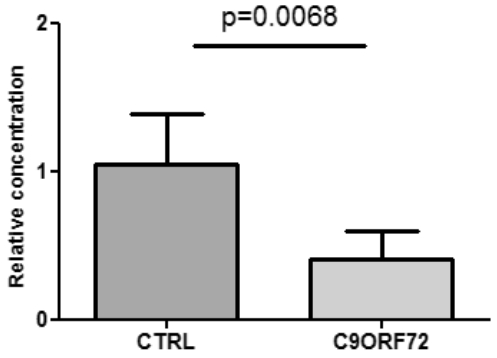
MOLECULAR NEURODEGENERATION

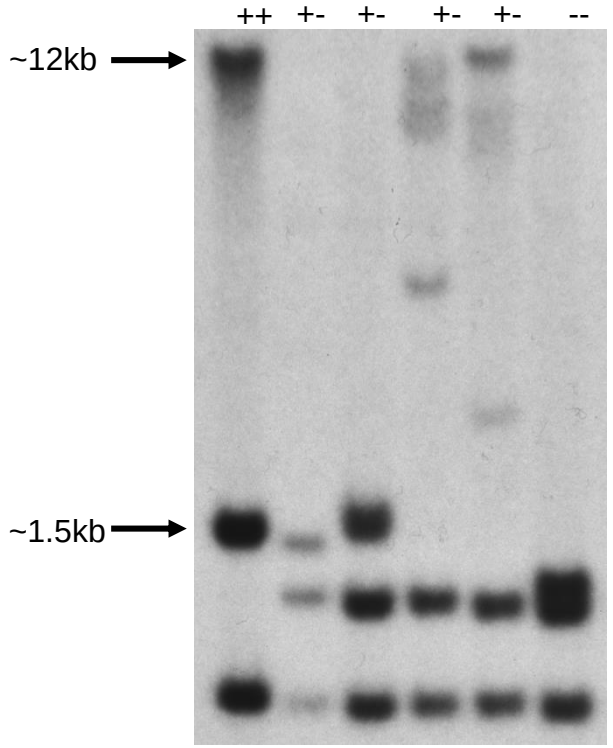
METHODOLOGY Open Access

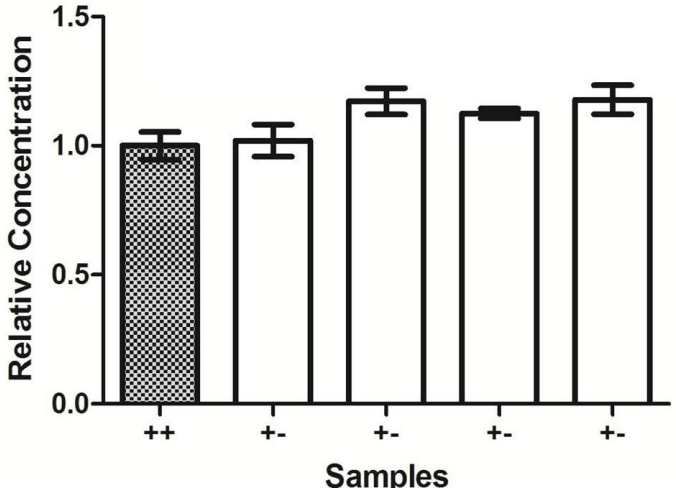
Simultaneous and independent detection of C9ORF72 alleles with low and high number of GGGGCC repeats using an optimised protocol of Southern blot hybridisation

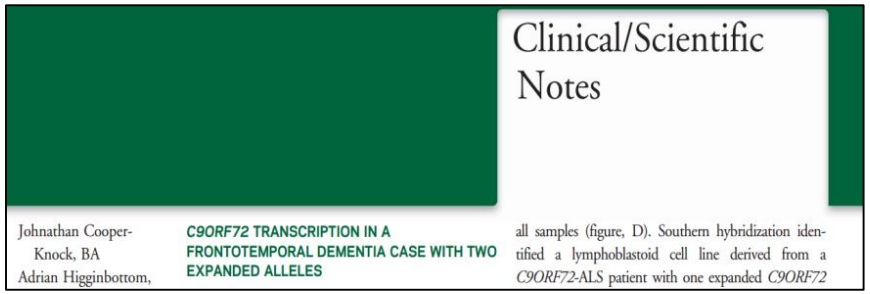
Vladimir L Buchman[1,4*], Johnathan Cooper-Knock[2], Natalie Connor-Robson[1], Adrian Higginbottom[2], Janine Kirby[2], Olga D Razinskaya[3], Natalia Ninkina[1,4] and Pamela J Shaw[2]

EFFECT OF REPEAT LENGTH ON *C9orf72* TRANSCRIPTION








Clinical/Scientific Notes

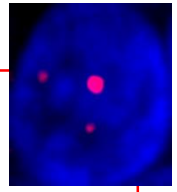
Johnathan Cooper-Knock, BA
Adrian Higginbottom,

C9ORF72 TRANSCRIPTION IN A FRONTOTEMPORAL DEMENTIA CASE WITH TWO EXPANDED ALLELES

all samples (figure, D). Southern hybridization identified a lymphoblastoid cell line derived from a *C9ORF72*-ALS patient with one expanded *C9ORF72*

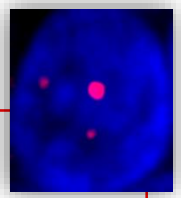
C9orf72 AND RNA FOCI

Sense Transcripts:



$[GGGGCC]_n$

Antisense Transcripts:



$[GGCCCCC]_n$

Acta Neuropathol (2015) 130:63–75
DOI 10.1007/s00401-015-1429-9

ORIGINAL PAPER

Antisense RNA foci in the motor neurons of *C9ORF72*-ALS patients are associated with TDP-43 proteinopathy

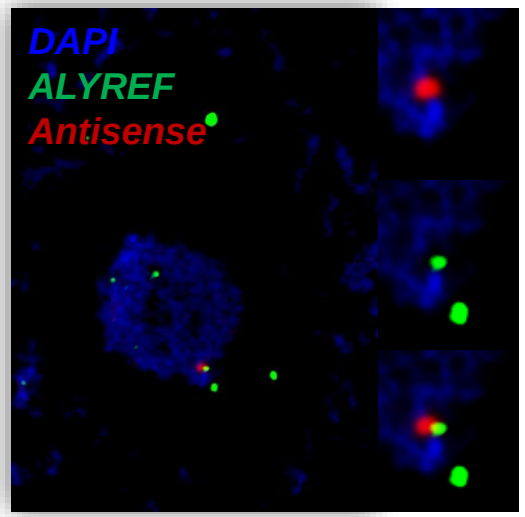
Johnathan Cooper-Knock[1] · Adrian Higginbottom[1] · Matthew J. Stopford[1] · J. Robin Highley[1] · Paul G. Ince[1] · Stephen B. Wharton[1] · Stuart Pickering-Brown[2] · Janine Kirby[1] · Guillaume M. Hautbergue[1] · Pamela J. Shaw[1]

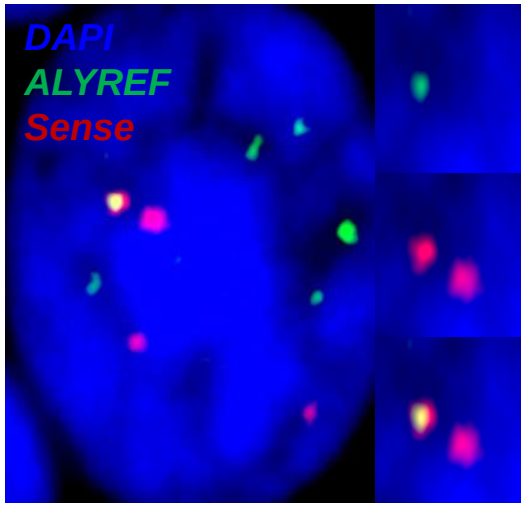
BRAIN
A JOURNAL OF NEUROLOGY

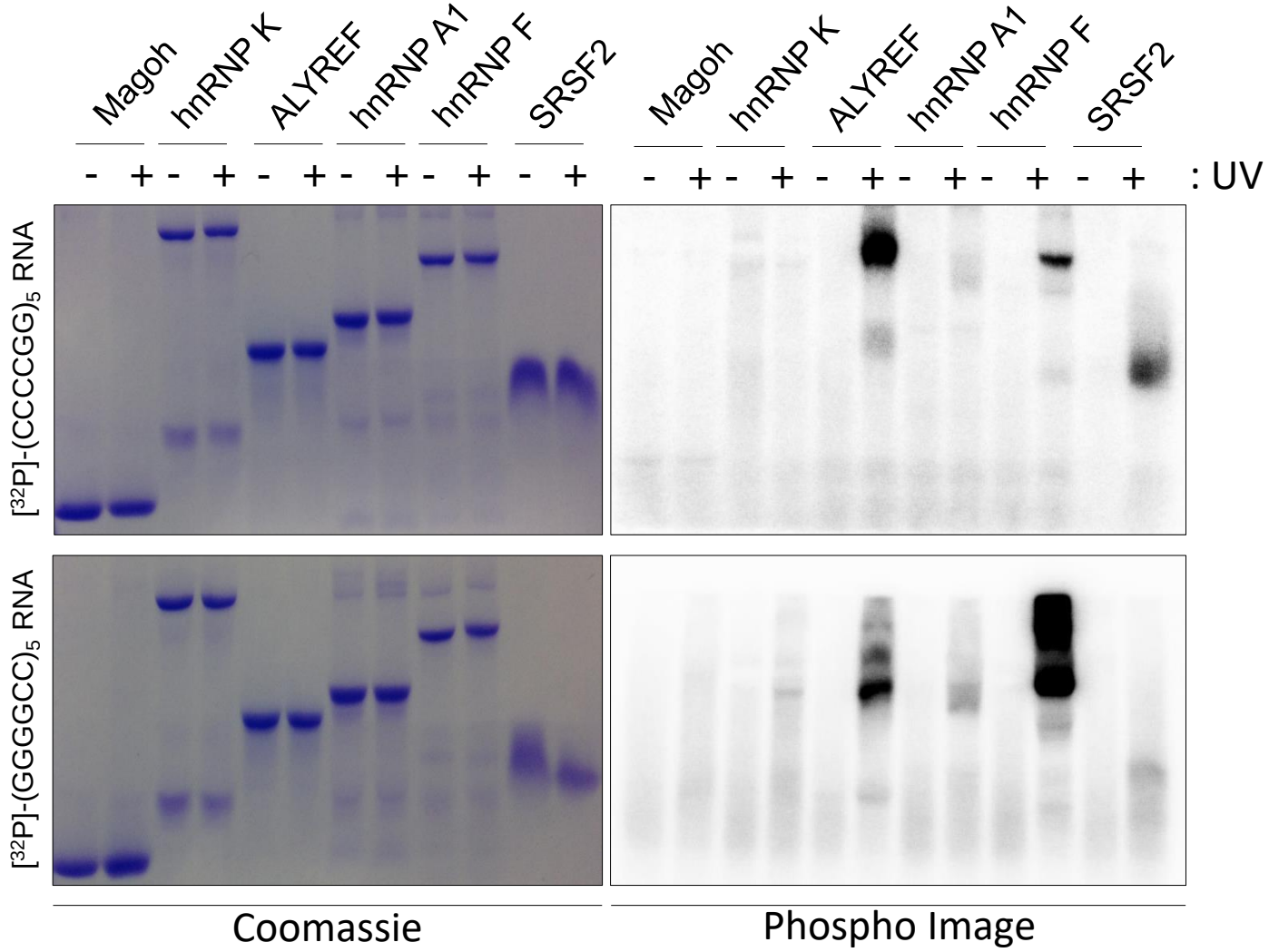
Sequestration of multiple RNA recognition motif-containing proteins by *C9orf72* repeat expansions

Johnathan Cooper-Knock,[1,*] Matthew J. Walsh,[1,*] Adrian Higginbottom,[1] J. Robin Highley,[1] Mark J. Dickman,[2] Dieter Edbauer,[3] Paul G. Ince,[1] Stephen B. Wharton,[1] Stuart A. Wilson,[4] Janine Kirby,[1] Guillaume M. Hautbergue[1] and Pamela J. Shaw[1]

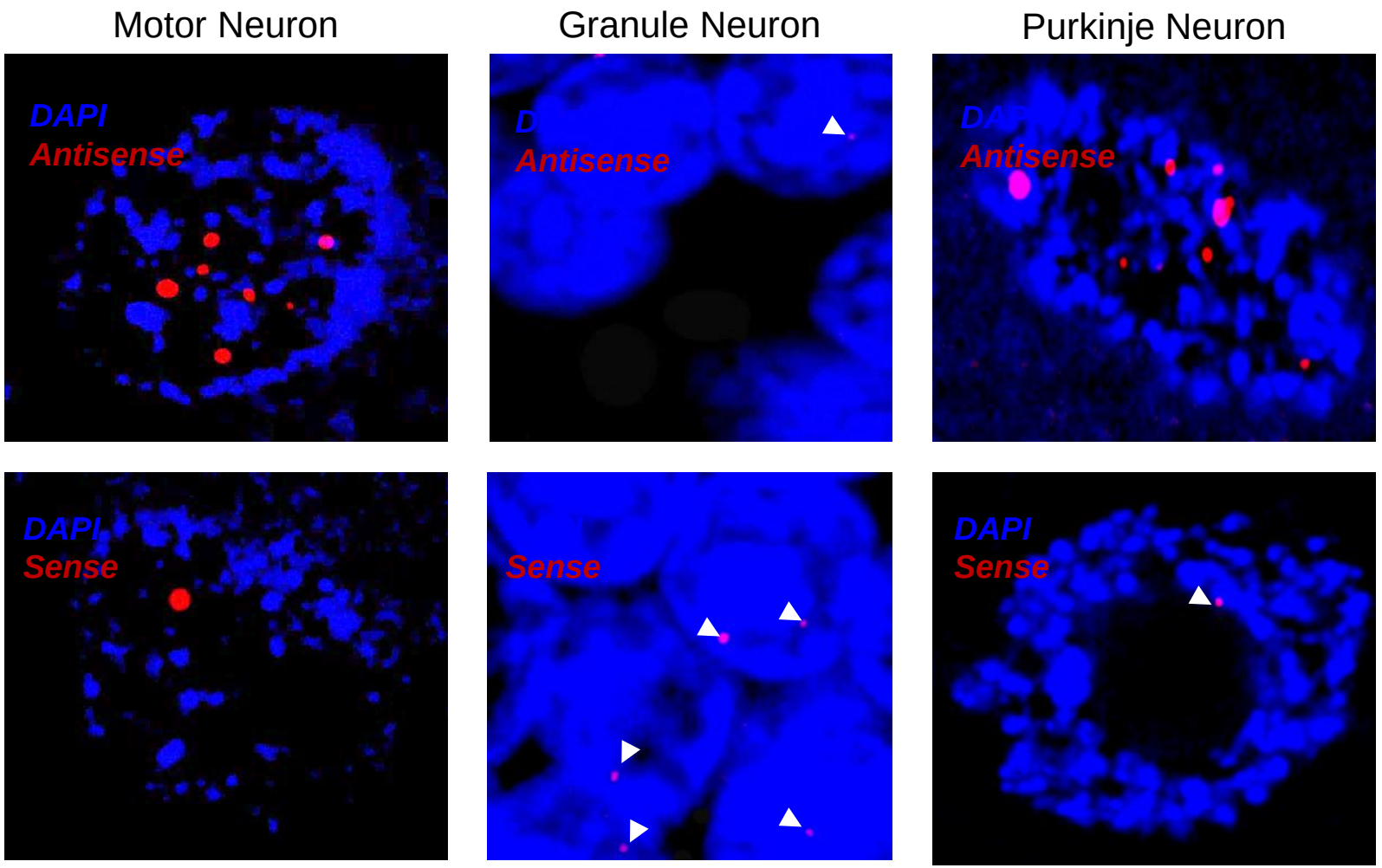
RNA FOCI AND RRM-CONTAINING PROTEINS





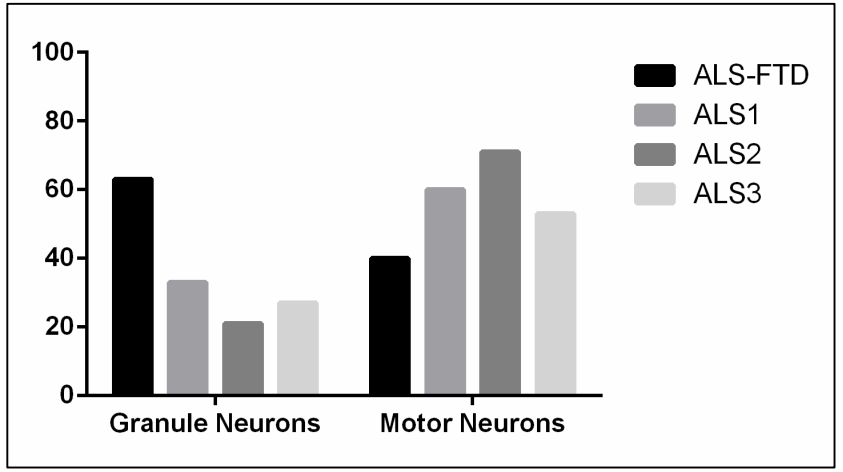


RELATIVE DISTRIBUTION OF SENSE AND ANTISENSE RNA FOCI

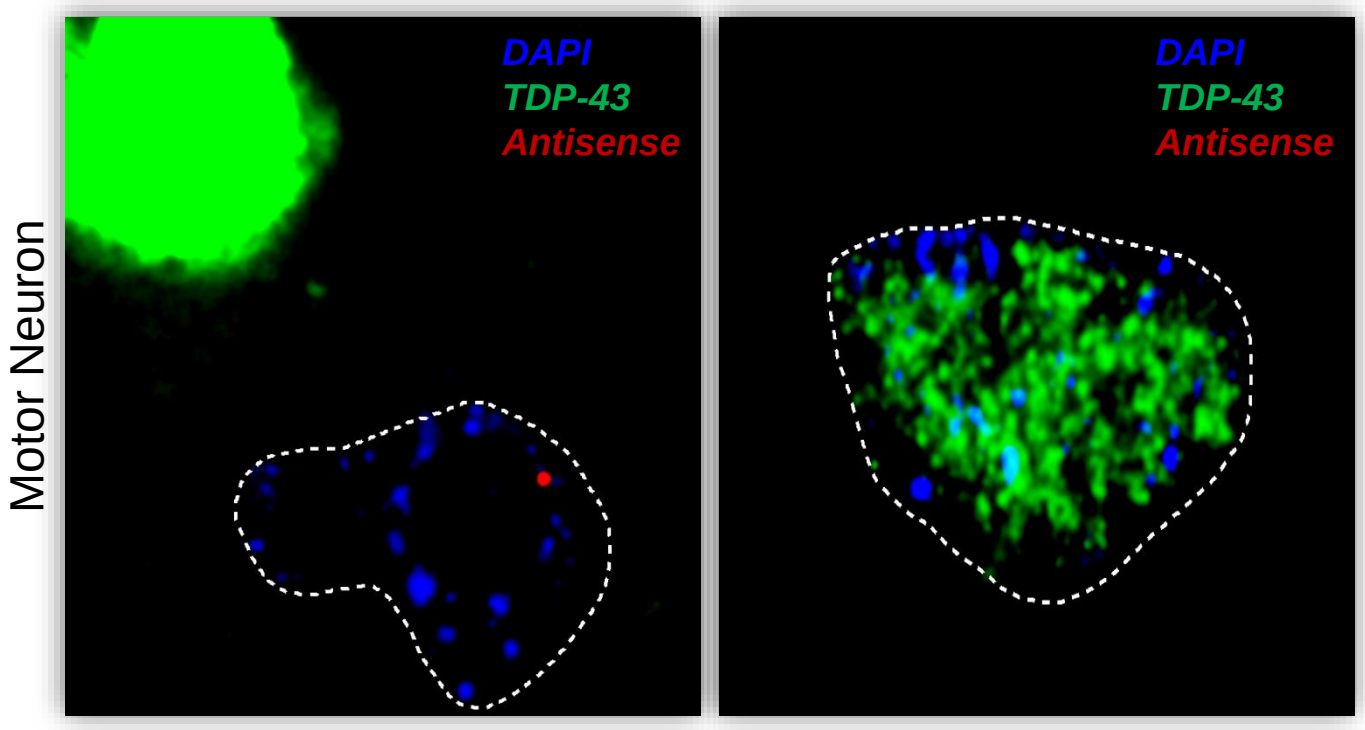


RELATIVE DISTRIBUTION OF SENSE AND ANTISENSE RNA FOCI

	Purkinje Neurons			Granule Neurons			Motor Neurons		
Case	Antisense	Sense	P-value	Antisense	Sense	P-value	Antisense	Sense	P-value
1	26.40	6.40	2.37E-14	0.00	0.56	1.37E-12	3.33	1.00	0.02
2	4.30	1.10	0.002	0.03	1.10	9.63E-18	3.00	1.00	0.02
3	4.60	1.30	0.002	0.01	0.34	1.17E-07	5.40	2.44	0.02
4	6.30	1.40	6.88E-05	0.02	0.40	3.1E-07	14.90	1.50	5.65E-14



CELLULAR DISTRIBUTION OF RNA FOCI AND TDP-43



CONCLUSIONS FROM RNA FOCI WORK

The presence of antisense foci correlates with TDP-43 mislocalisation in motor neurons.

Sense and antisense transcripts share similar binding partners.

The relative frequency of sense and antisense foci/dipeptide-repeat proteins varies between neuronal populations.

A key event determining toxicity might be a propensity to produce antisense transcripts.

Brief Communication

Cell Chemical Biology

c9orf72 Disease-Related Foci Are Each Composed of One Mutant Expanded Repeat RNA

Highlights

- Quantitative biochemical link between RNA numbers and disease
- Less than four mutant *c9orf72* molecules per cell on average
- ~1:1 correspondence between *c9orf72* foci and mutant intronic RNA
- Small numbers of disease RNA molecules can have major consequences

Authors

Jing Liu, Jiaxin Hu, Andrew T. Ludlow, Jacqueline T. Pham, Jerry W. Shay, Jeffrey D. Rothstein, David R. Corey

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In Brief

Knowing absolute numbers of cellular RNAs is critical for understanding molecular mechanism. The *c9orf72* gene is a suspected cause of ALS. Liu et al. find that a handful of mutant *c9orf72* transcripts are present per cell. Small numbers of RNA molecules may have a big impact on disease.

Article

Neuron

C9orf72 BAC Mouse Model with Motor Deficits and Neurodegenerative Features of ALS/FTD

Highlights

- *C9orf72* BAC mice with behavioral, neurodegenerative, and molecular features of ALS/FTD
- These mice express *C9orf72* sense and upregulated antisense transcripts
- Antisense RNA foci accumulate preferentially in ALS/FTD-vulnerable cell populations
- RAN aggregates increase with age and disease with TDP-43 aggregates at end stage

Authors

Yuanjing Liu, Amrutha Pattamatta, Tao Zu, ..., David R. Borchelt, Anthony T. Yachnis, Laura P.W. Ranum

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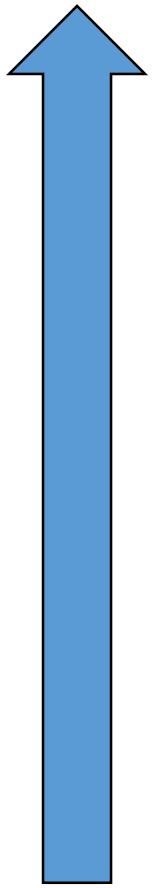
In Brief

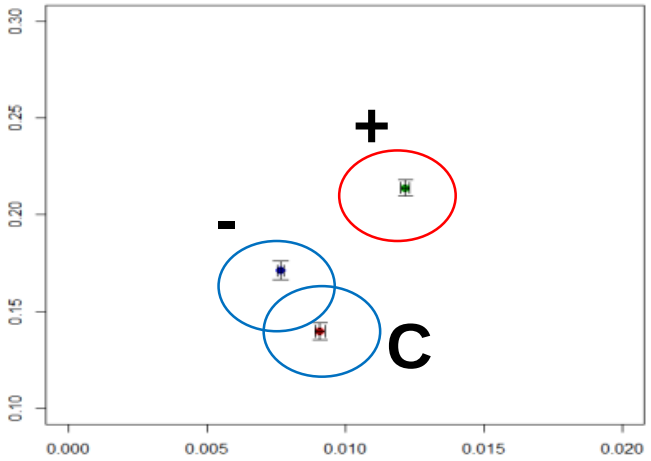
Liu et al. report the generation of the first *C9orf72* BAC mouse model that recapitulates the molecular, behavioral, and neurodegenerative features of ALS/FTD. Antisense RNA foci accumulate in vulnerable regions, and RAN protein accumulation increases with age and disease.

GENE EXPRESSION PROFILING IN MOTOR NEURONS

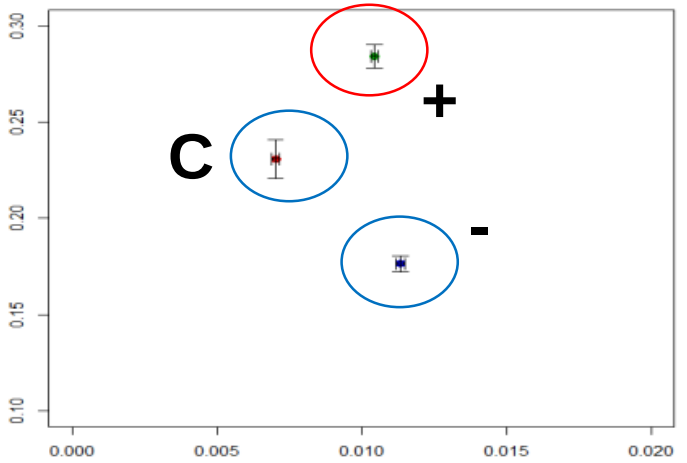
MORE ERRORS

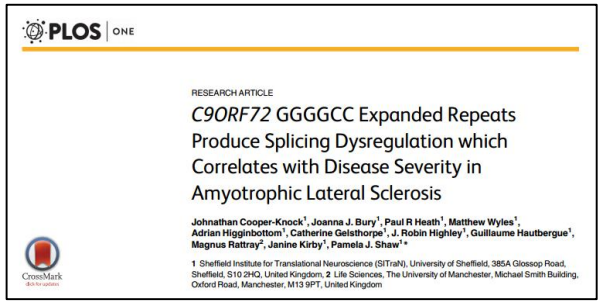


Exon Inclusion :



Exon Exclusion :





PLOS ONE

RESEARCH ARTICLE

C9ORF72 GGGGCC Expanded Repeats Produce Splicing Dysregulation which Correlates with Disease Severity in Amyotrophic Lateral Sclerosis

Johnathan Cooper-Knock[1], Joanna J. Bury[1], Paul R Heath[1], Matthew Wyles[1], Adrian Higginbottom[1], Catherine Gelsthorpe[1], J. Robin Highley[1], Guillaume Hautbergue[1], Magnus Rattray[2], Janine Kirby[1], Pamela J. Shaw[1]*

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NEXT STEPS...

In *C9orf72*-disease we look forward to the forthcoming ASO trial

Building *C9orf72*-disease models with particular focus on antisense transcription

Fascinating insight into genetic architecture of ALS from early project MinE data - mt*C9orf72* probably never works alone.

THANKS

Many, many people at SITraN but especially:

- Adrian Higginbottom
- Guillaume Hautbergue
- Winston Hide
- Paul Heath
- Robin Highley
- **Janine Kirby**
- **Pamela Shaw**

THANKS

Collaborators:

- Vladimir Buchman
- Magnus Rattray
- John Hardy
- Bryan Traynor
- Dieter Edbauer
- Michael Baughn
- MNDA LCL Bank