RNA binding proteins and protein binding RNAs in ALS and FTD

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What is amyotrophic lateral sclerosis?

(AKA, motor neuron disease, Lou Gehrig's disease)

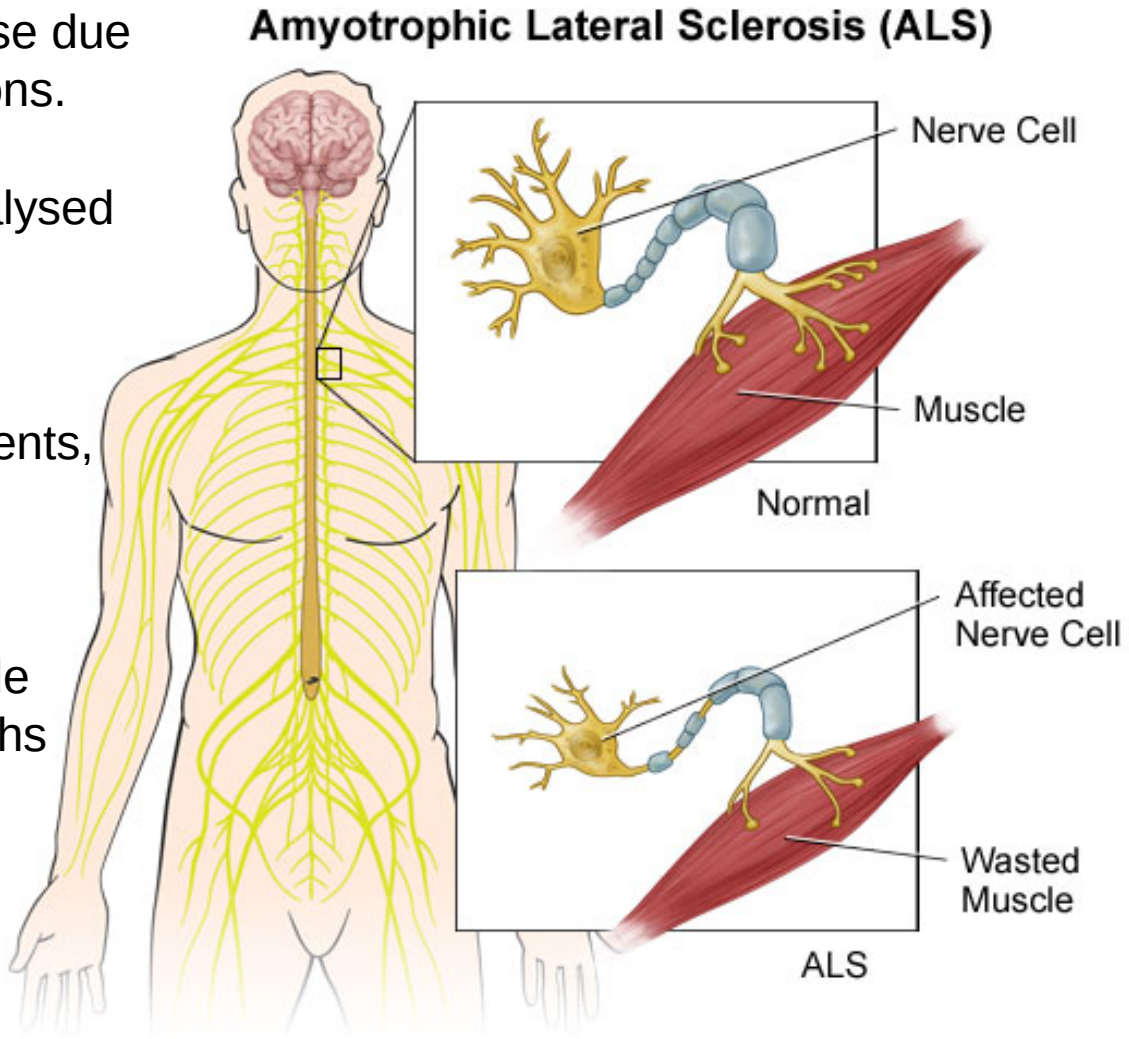
Progressive muscle wasting disease due to the degeneration of motor neurons.

People become progressively paralysed unable to walk, talk, feed or toilet themselves.

Spares sensation and eye movements, bladder and bowel function, and cognition.

People die because they are unable to breathe, death occurs ~20 months after diagnosis.

There is no effective treatment for this disease.



Epidemiology and prognosis

Average age of onset mid-50's

Mode of transmission

- Sporadic – 90-95%
- Familial – 5-10% (autosomal dominant)

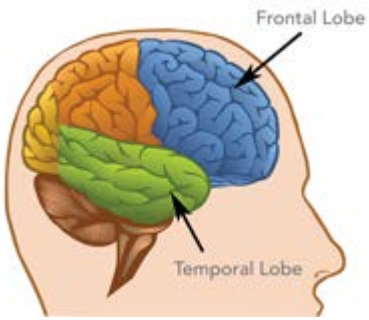
Male : Female – 3:2

Incidence 1-2.5 / 100,000

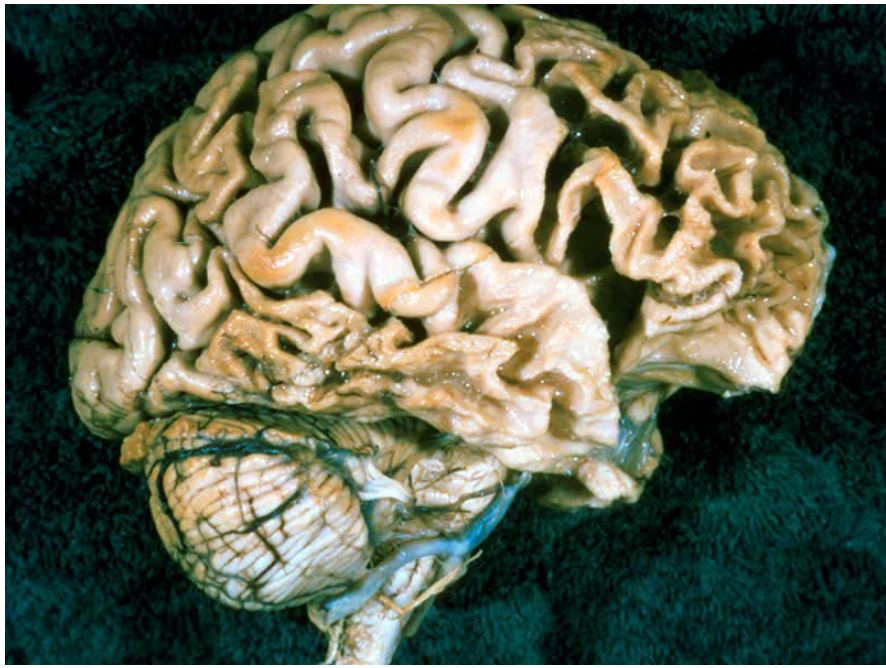
Prognosis – difficult to predict in an individual patient

- 50% live 3-4 or more years
- 20% live 5 or more years
- 10% live 10 or more years
- Occasional patients live 20 years or more.

Frontotemporal dementia



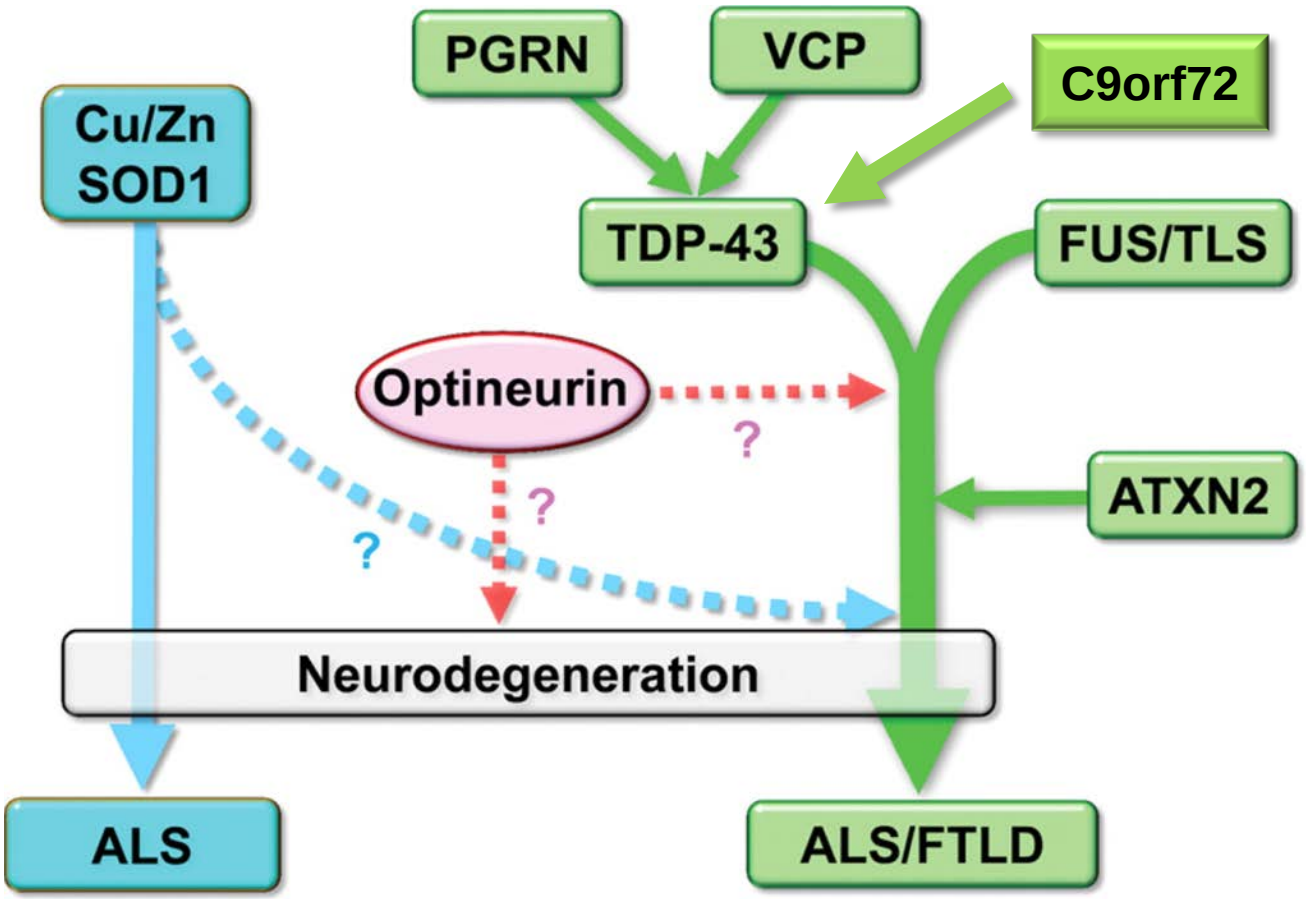
- Prominent frontal and temporal lobe atrophy.
- Deterioration of personality and cognition.



- Mirror image of AD with pronounced behaviour problems initially and memory problems later.

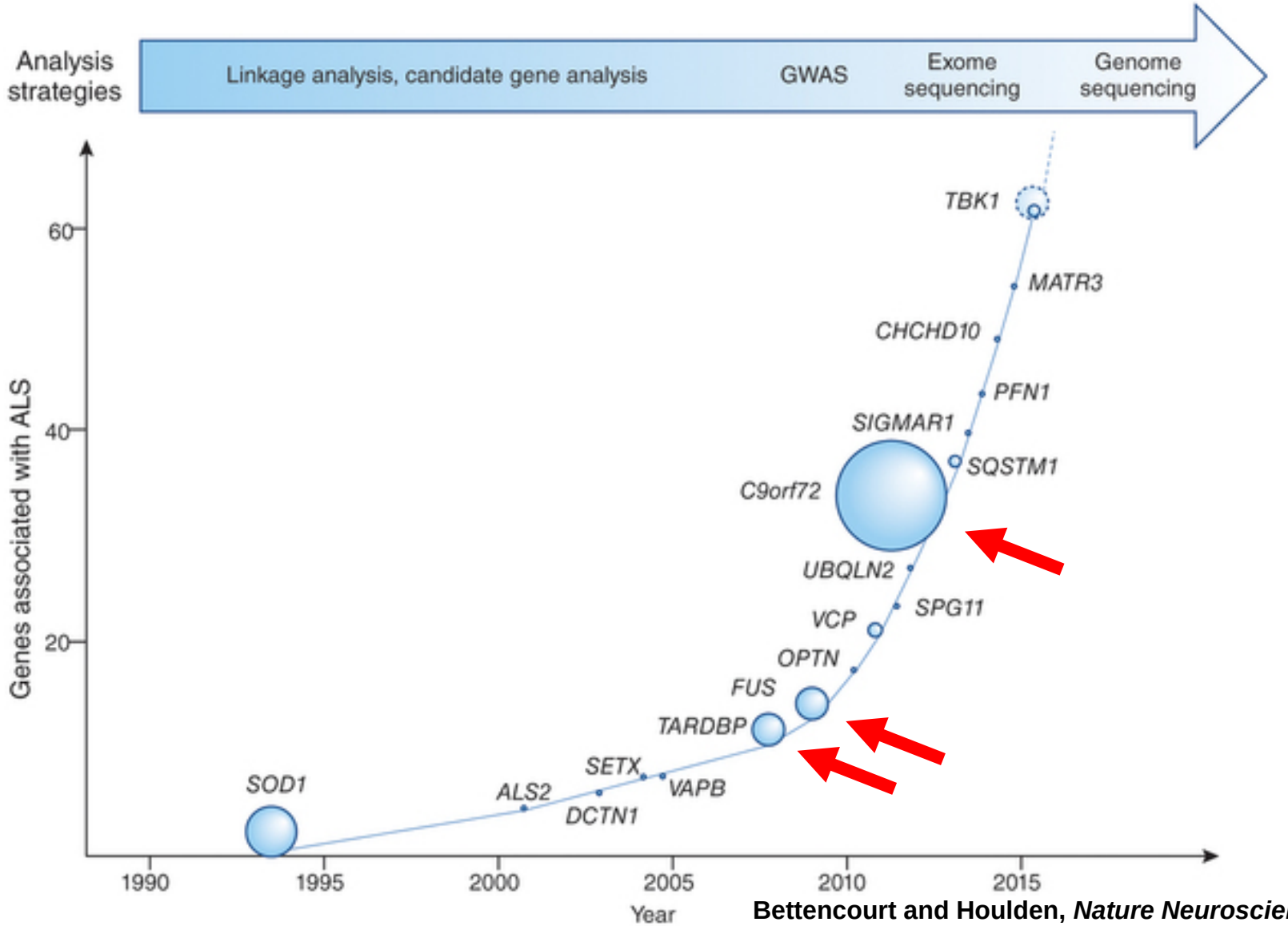
- Accounts for up to 3-20% of dementias.
- Common cause of dementia in younger population -in 45-64 age group at 15 per 100,000 (same as AD).
- Mean age of onset 52.8

Genetic overlap of ALS and FTD



Ito *Neurology* 2011

Progress of genetic findings related to ALS etiology and pathogenesis



Bettencourt and Houlden, *Nature Neuroscience* 2015

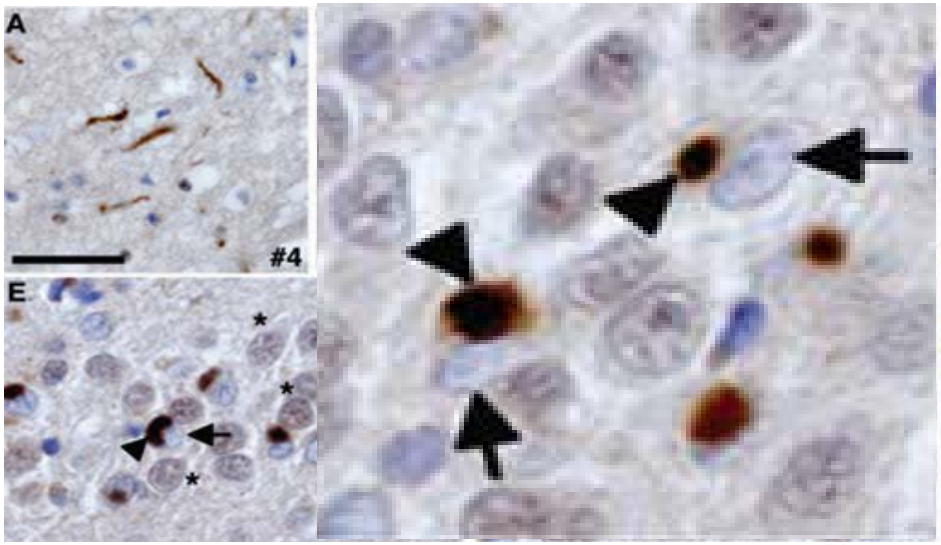
Mutations in Slovenian ALS patients

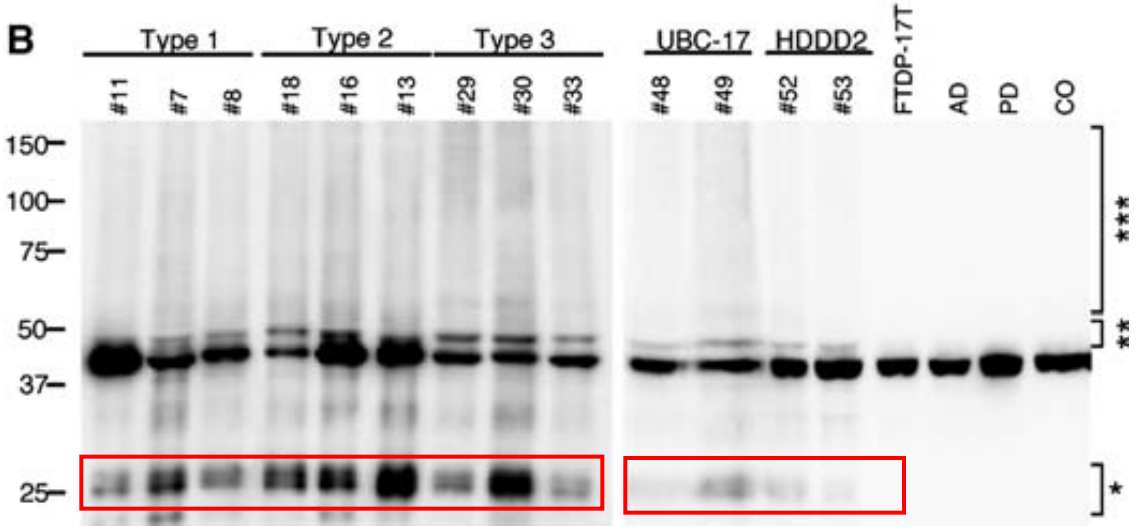
Clinical data of Slovenian ALS patients with detected genetic changes

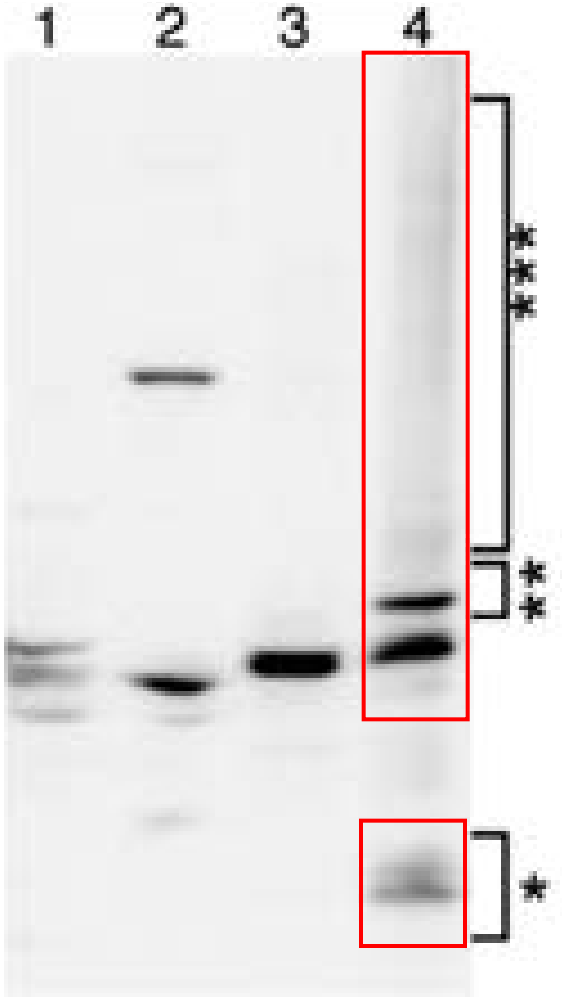
Gene	Nucleotide change	AA change	Frequency (%)	Gender	Onset; age of onset; disease duration; associated symptom	Reference
SOD1	c.43G>A	p.Val14Met	2.3	F	Spinal; 67 y; 4+ y	Deng et al., 1995
SOD1	c.280G>T	p.Gly93Cys		F	Spinal; 51 y; 5+ y	Rosen, 1993
TARDBP	c.990A>G	p.Leu330Leu	1.2			This study
FUS	c.1566G>A	p.Arg522Arg	1.2	M	Spinal; 84 y; 1 y	Ticozzi et al., 2009
C9ORF72	Expansion GGGGCC		5.9	M	Spinal; 52 y; 0.5 y	Renton et al., 2011
				F	Spinal; 60 y; 6+ yrs	
				M	Spinal; 61 y; 2+ yrs; FTD	
				F	Bulbar; 55 y; 2 y	
				M	Spinal; 70 y; 1 y	

Vrabec et al., *Neurobiol Ageing* 2014

Molecular pathology of TDP-43 in FTD



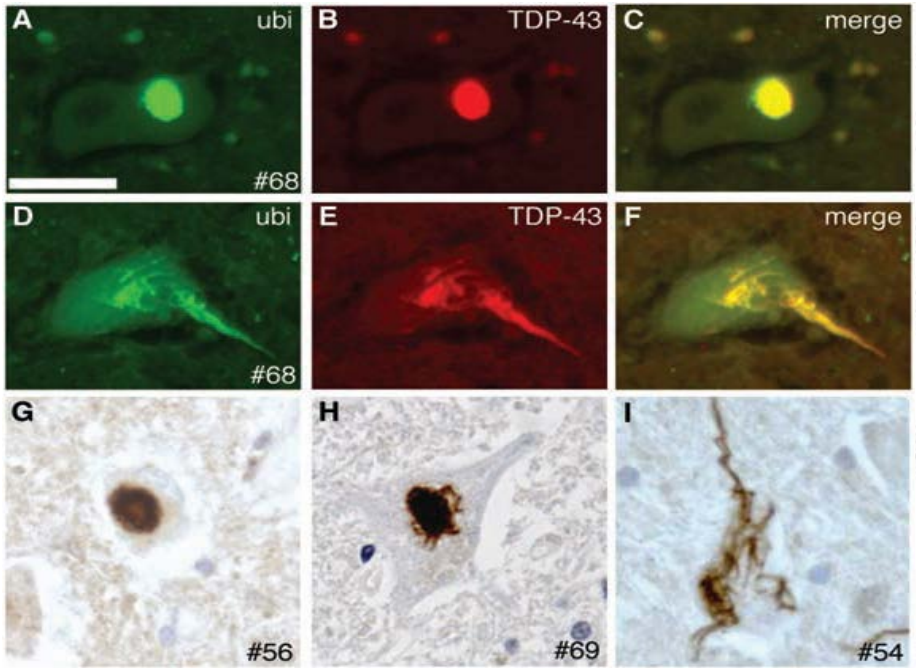


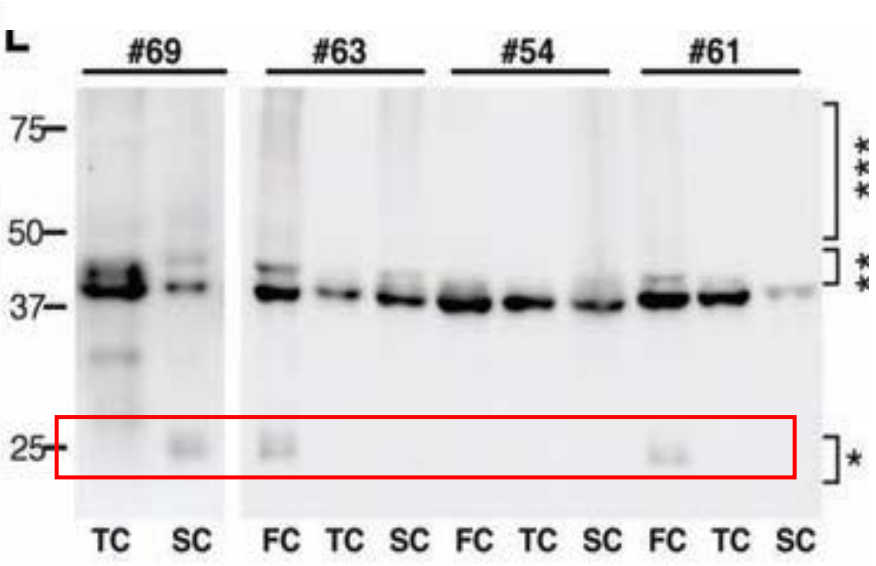


Neumann, *Science* 2006

Toxicity could be loss of nuclear function or effect of cytoplasmic aggregates.

Molecular pathology of TDP-43 in ALS

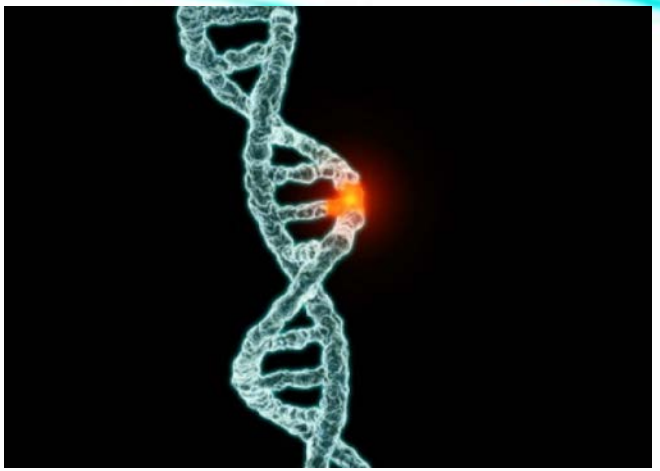




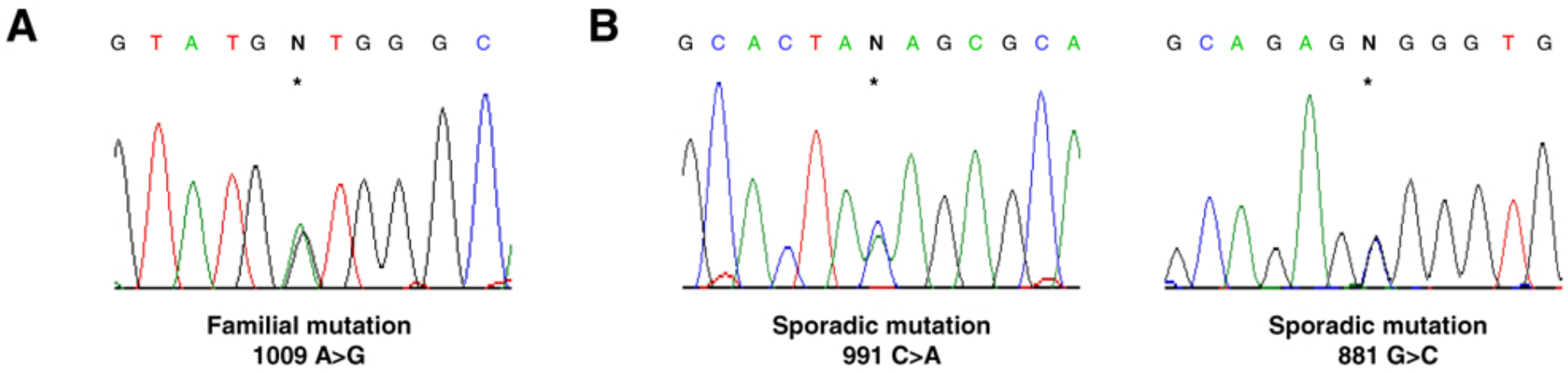
Neumann, *Science* 2006

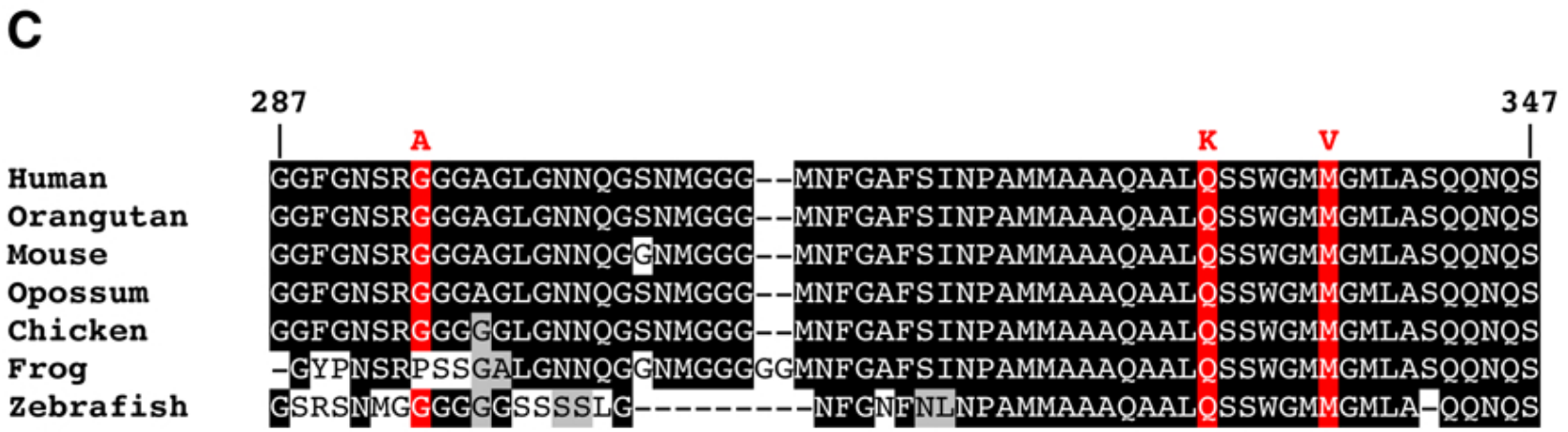
TDP-43 +ve inclusions, lost from nucleus, insoluble phos+ 25kDa fragments.

TDP-43 mutations in ALS



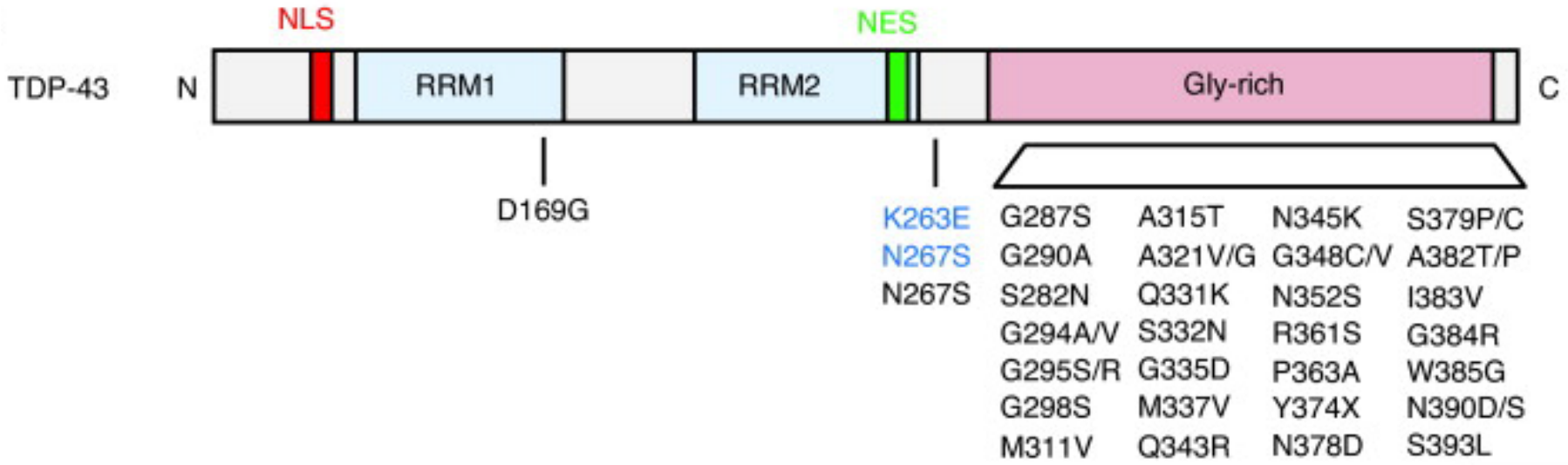
TARDBP mutations





Sreedharan et al. *Science* 2008

TDP-43 mutations in familial and sporadic ALS

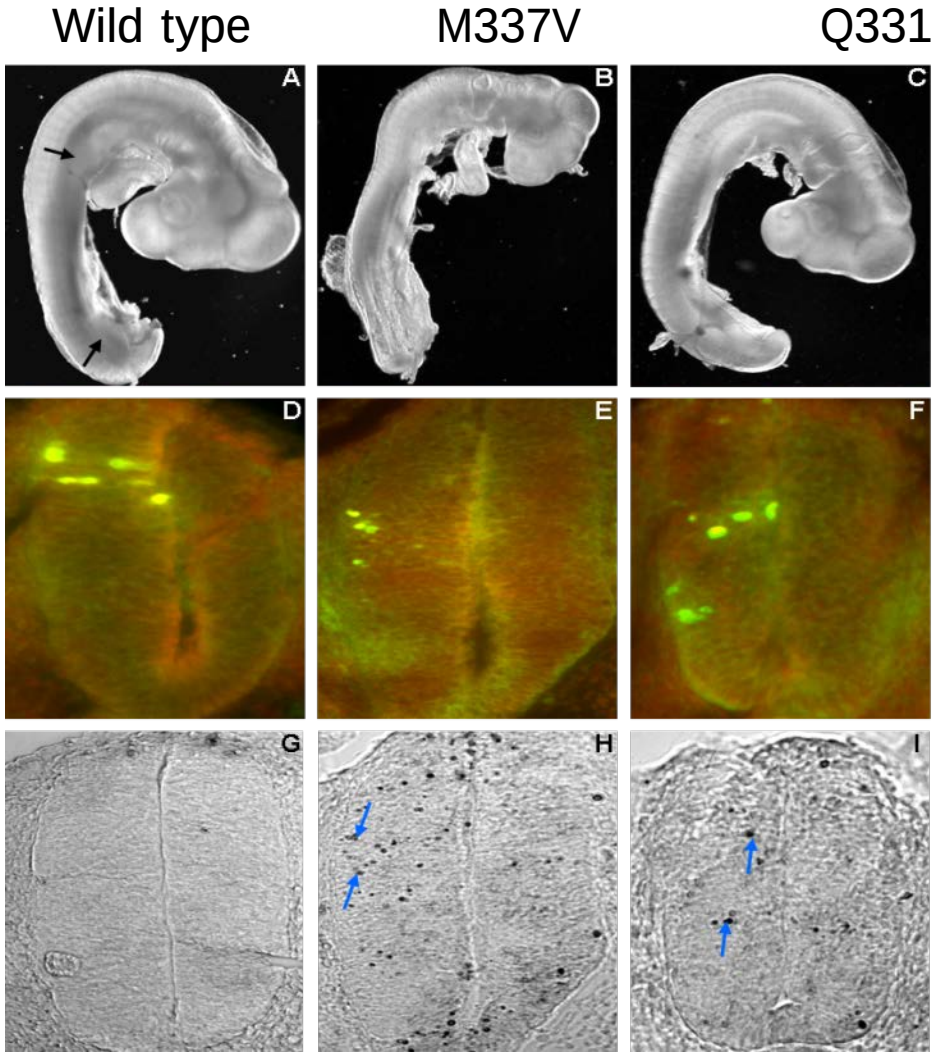


Dormann, *TiNS* 2011

Mutations present in 1-3% of familial and sporadic ALS,

- ~40 mutations in ALS all but one are in the G-rich C-terminal domain
- ~40% affect a potential phosphorylation site

TDP-43 is toxic to neural tissues



Chick embryo

Developmental delay

Transverse Section IHC

Transgene expression

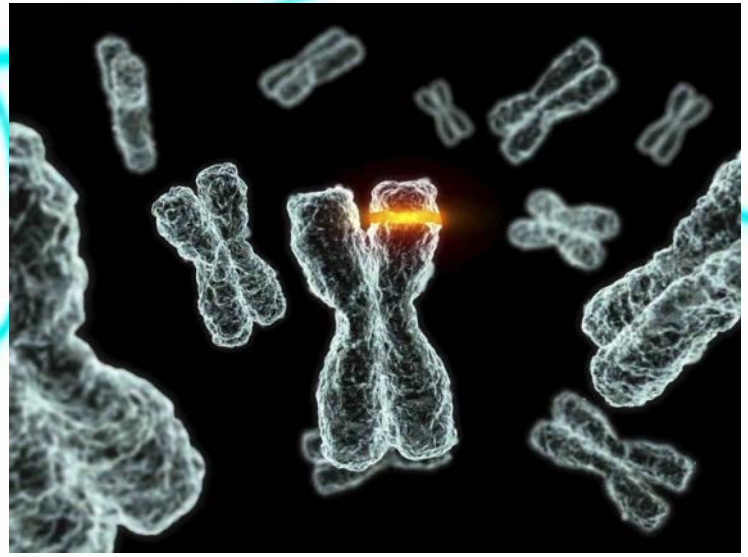
Tunel Staining

Apoptotic Cells

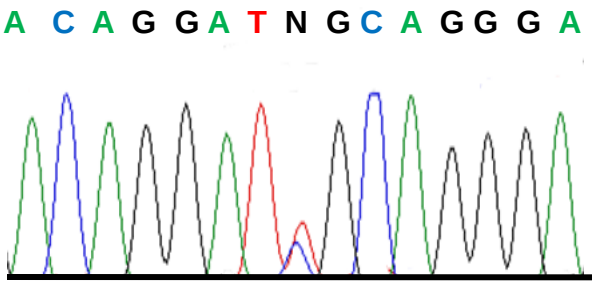
Electroporation with mt TDP-43 but not wild-type causes death of motor neurons.

Sreedharan et al. *Science* 2008

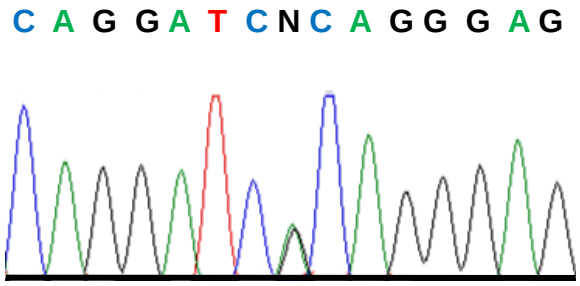
FUS mutations in ALS



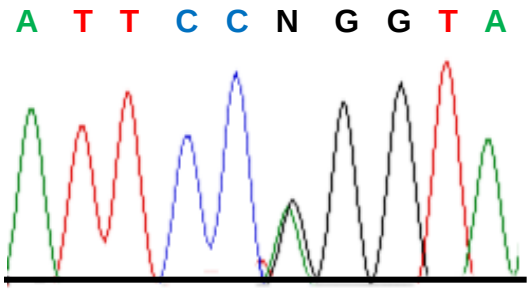
Three Mutations in FUS in 8 kindreds



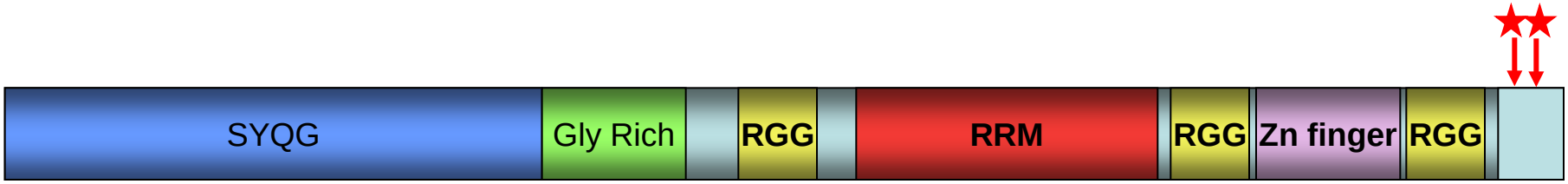
1561 C>T R521C



1562 G>A R521H



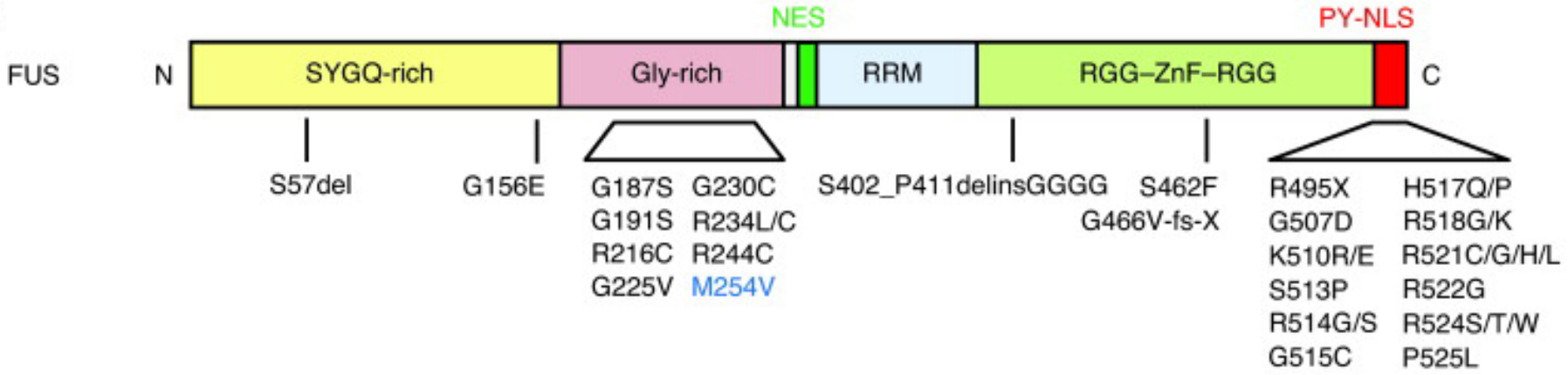
1540 A>G R514G



Human	RGGDRGGFRGGRGGGDRGGFGPGKMDSRGEHRQDRRERPY
Mouse	RGGDRGGFRGGRGGGDRGGFGPGKMDSRGEHRQDRRERPY
Chick	RGGDRGNFRGGRGG-ERGGFGPGKMDSRGDHRQDRRERPY
Xenopus	RGGDRGGFRGGRGG-DRGGFGPGKMDSRGDHRQDRRDRPY
Zebrafish	RGGDRGGFRGGRGG-DRGGFGPGKMDSRGDHRHDRRDRPY

Vance/Rogelj, *Science* 2009

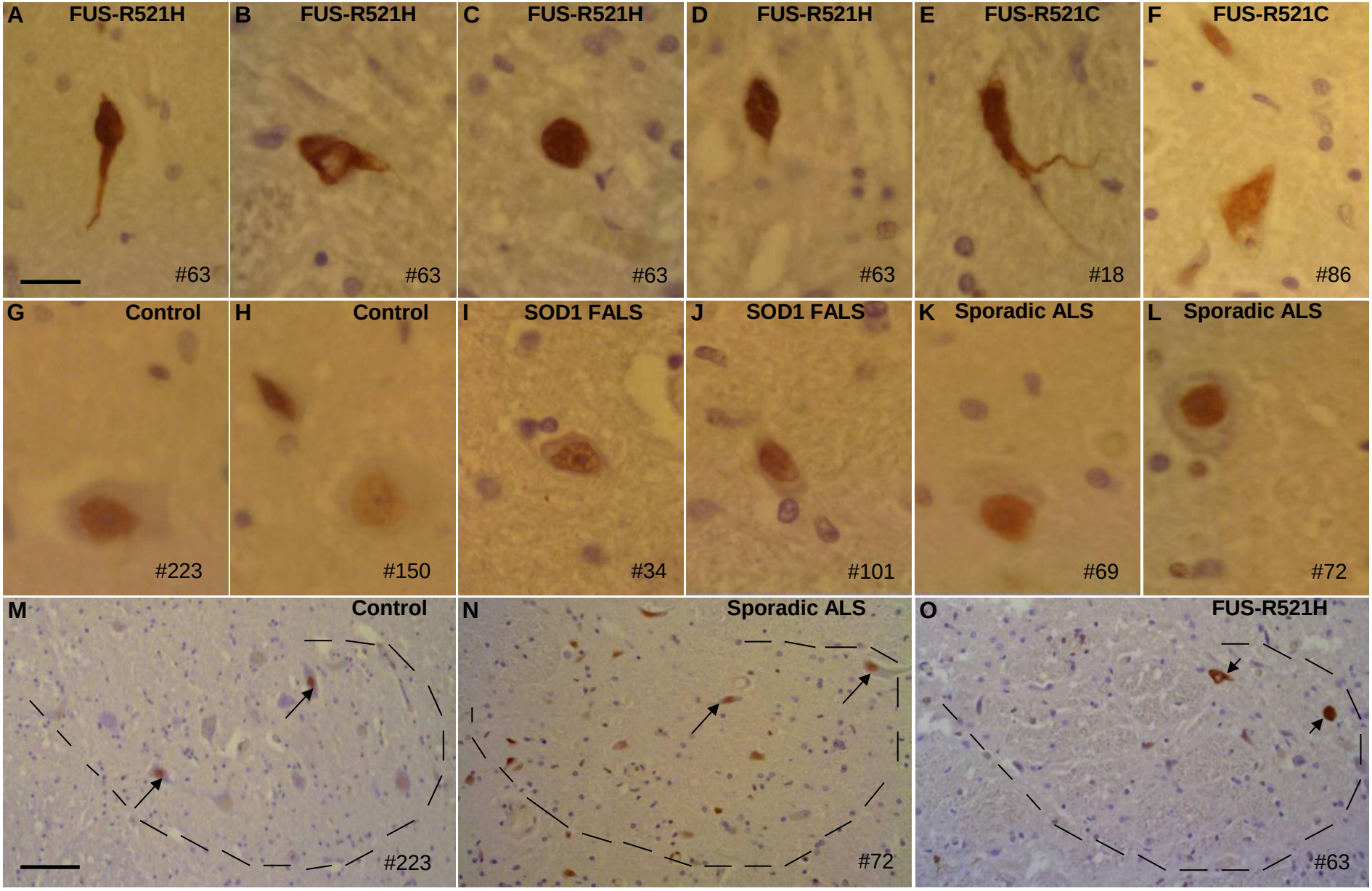
FUS mutations in familial ALS



Dormann, *TiNS* 2011

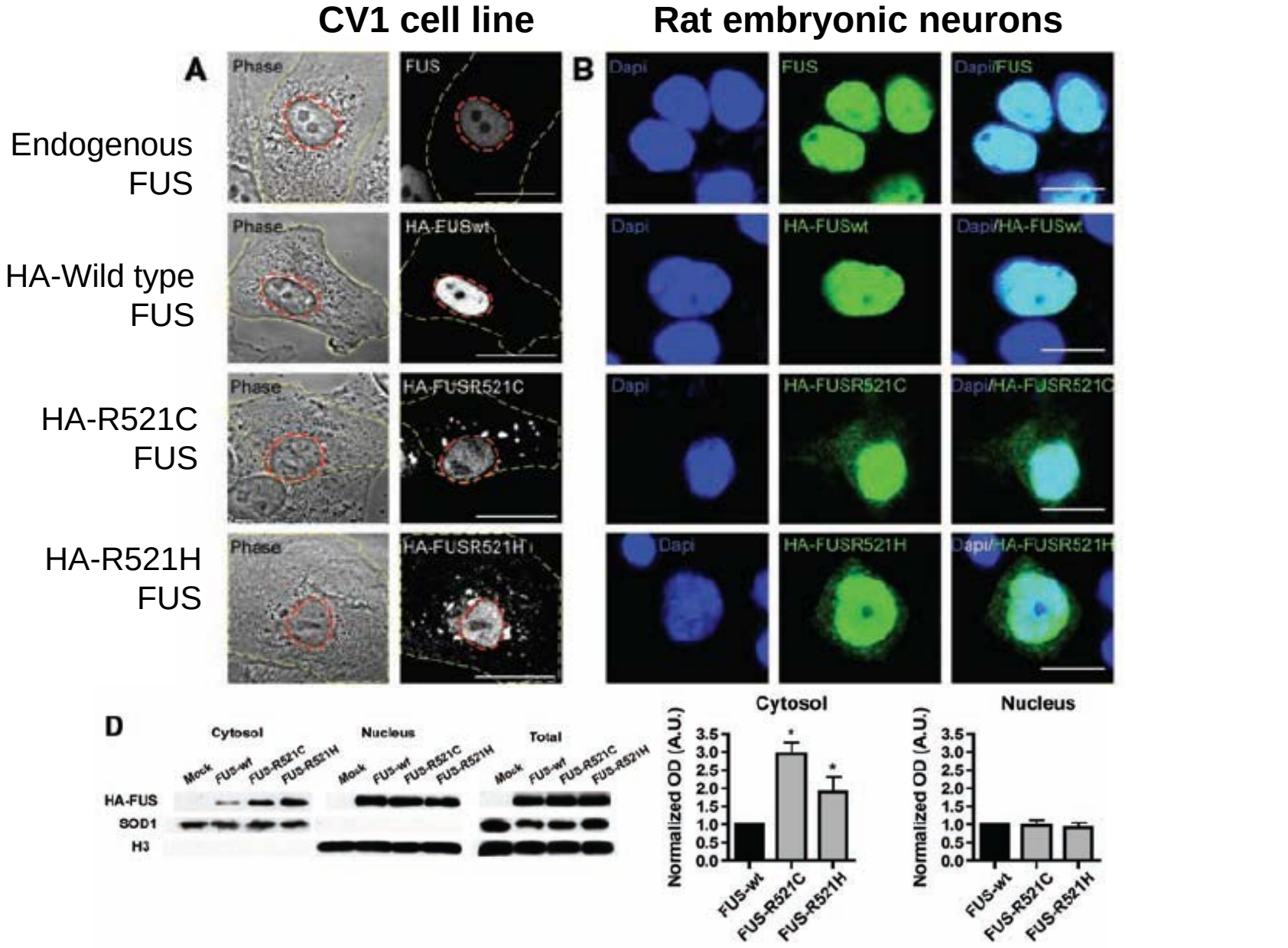
- ~ 40 mutations to date.
- 2/3 of the mutations are at the C terminus of the protein.
- 1/3 of the mutations in the Glycine-rich region.

FUS inclusions in patients carrying mutations



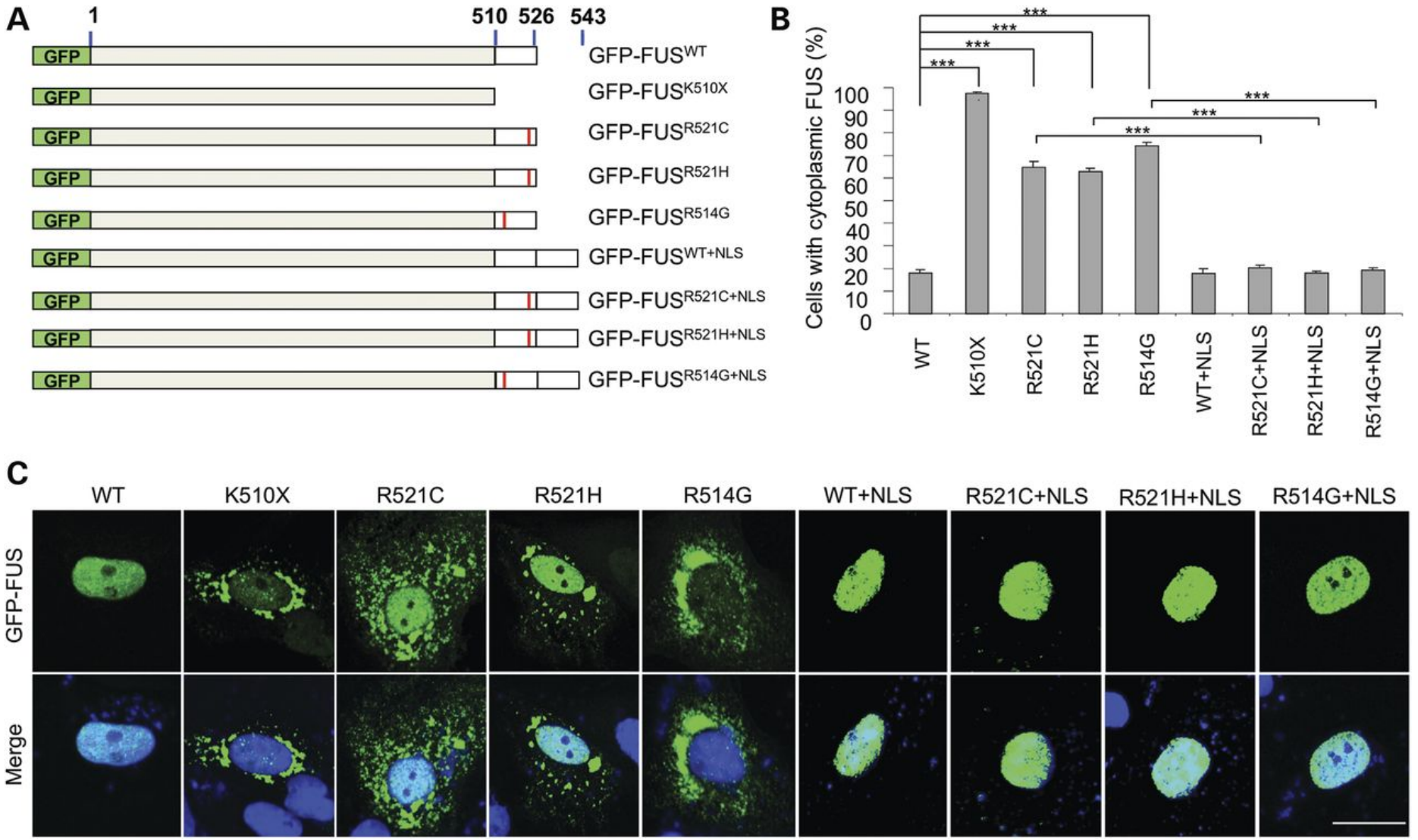
Vance/Rogelj, *Science* 2009

FUS mutations affect subcellular localisation



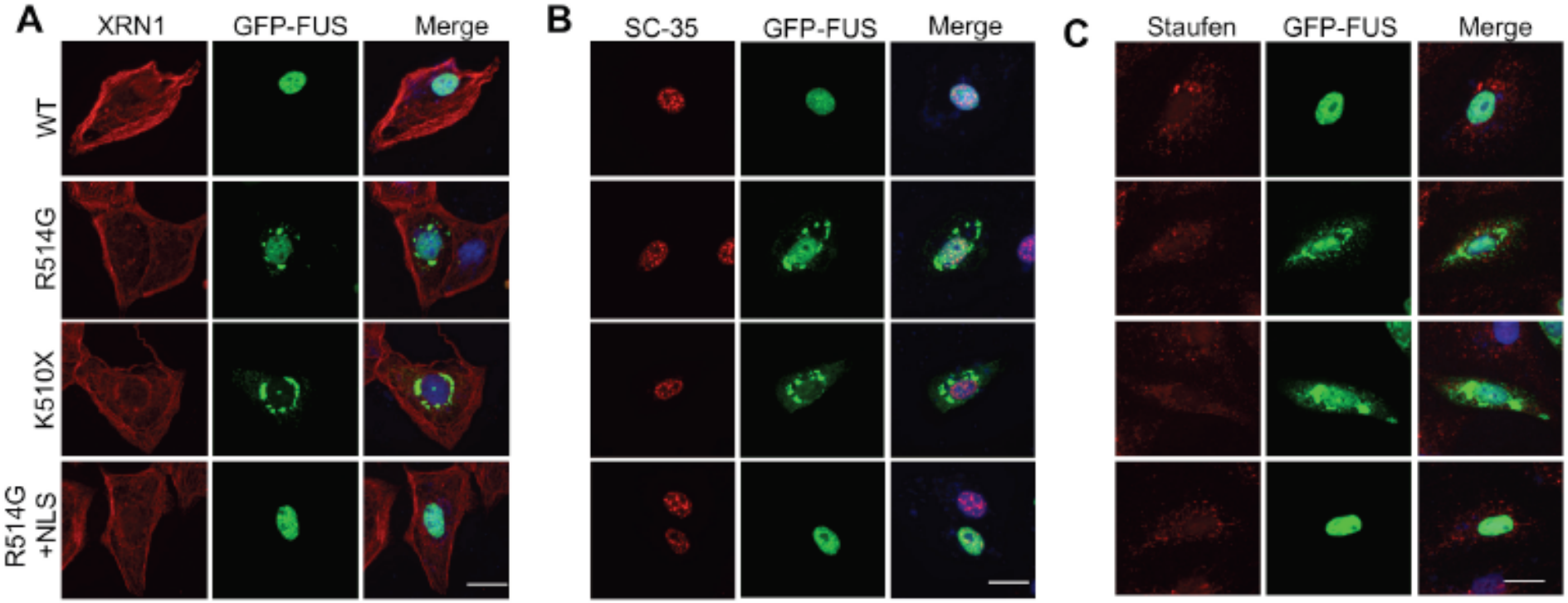
Vance/Rogelj, *Science* 2009

C terminus of FUS contains an NLS



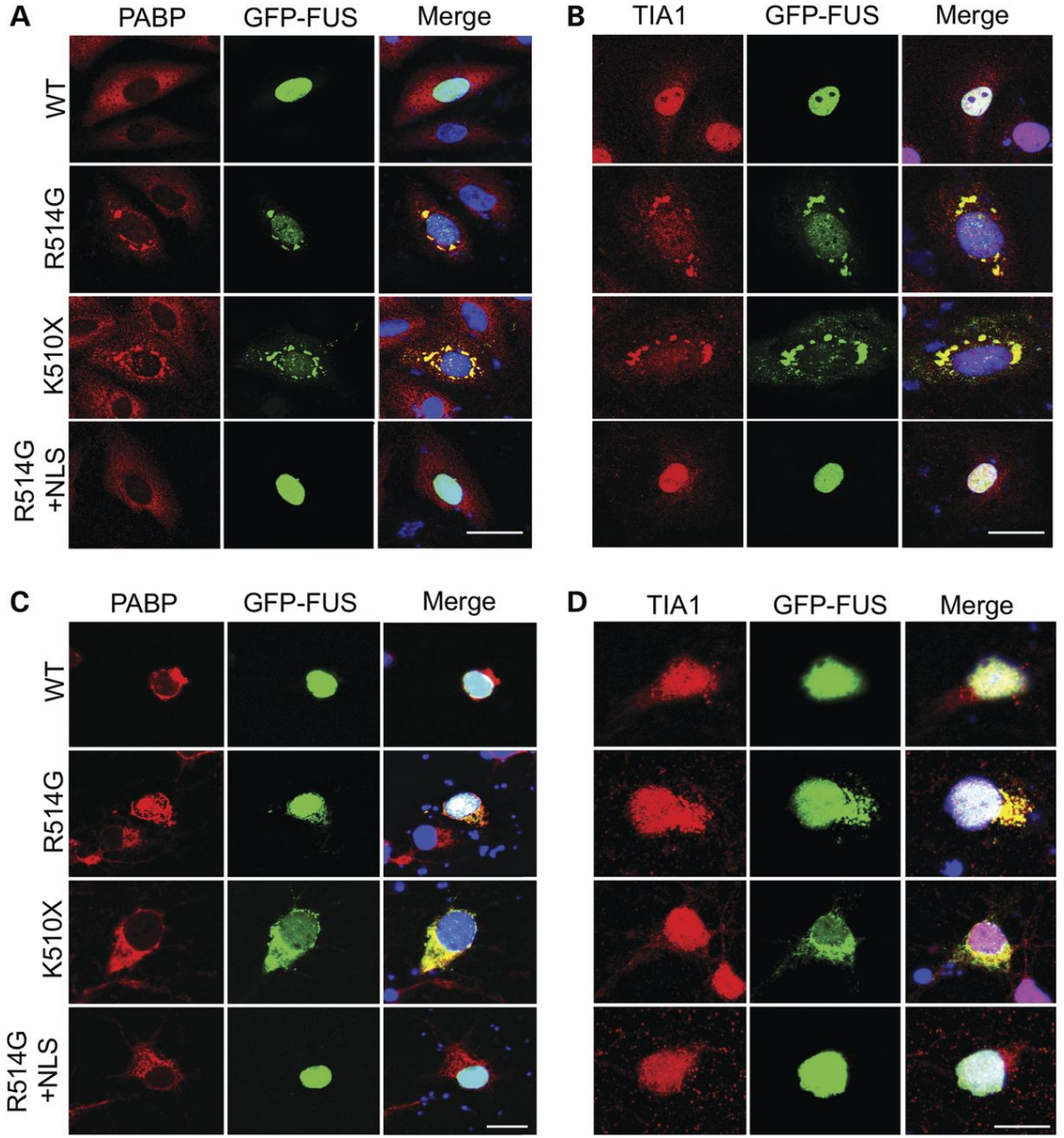
Vance C et al. Hum. Mol. Genet. 2013

Mutant FUS does not colocalize with P bodies, nuclear speckles, RNA transport granules



Vance C et al. Hum. Mol. Genet. 2013

Mutant FUS colocalizes with stress granules

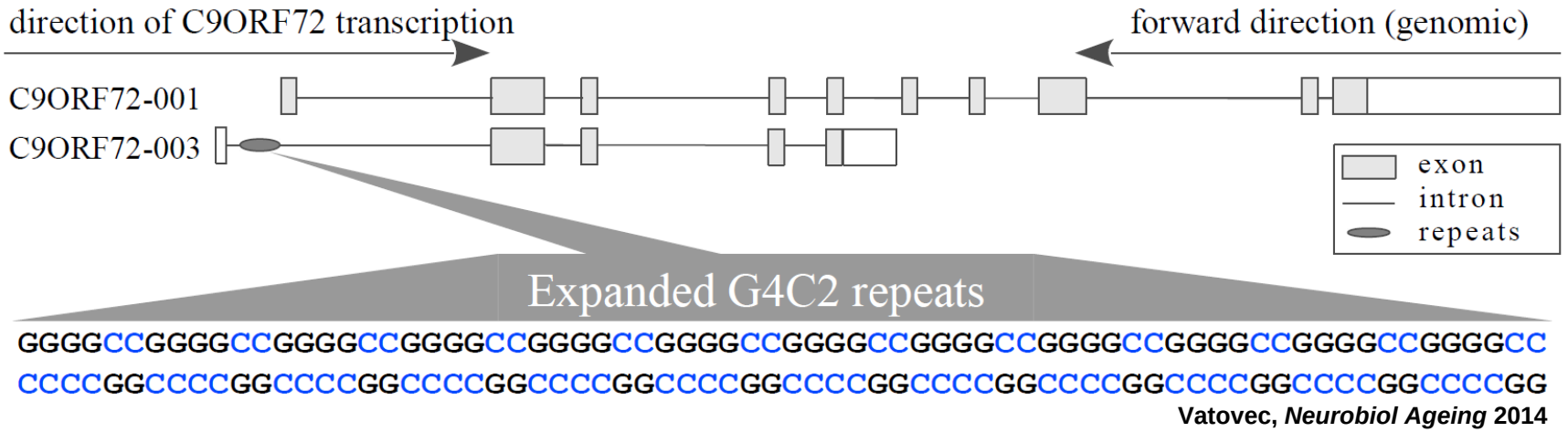


Vance C et al. Hum. Mol. Genet. 2013

GGGGCC

C9orf72

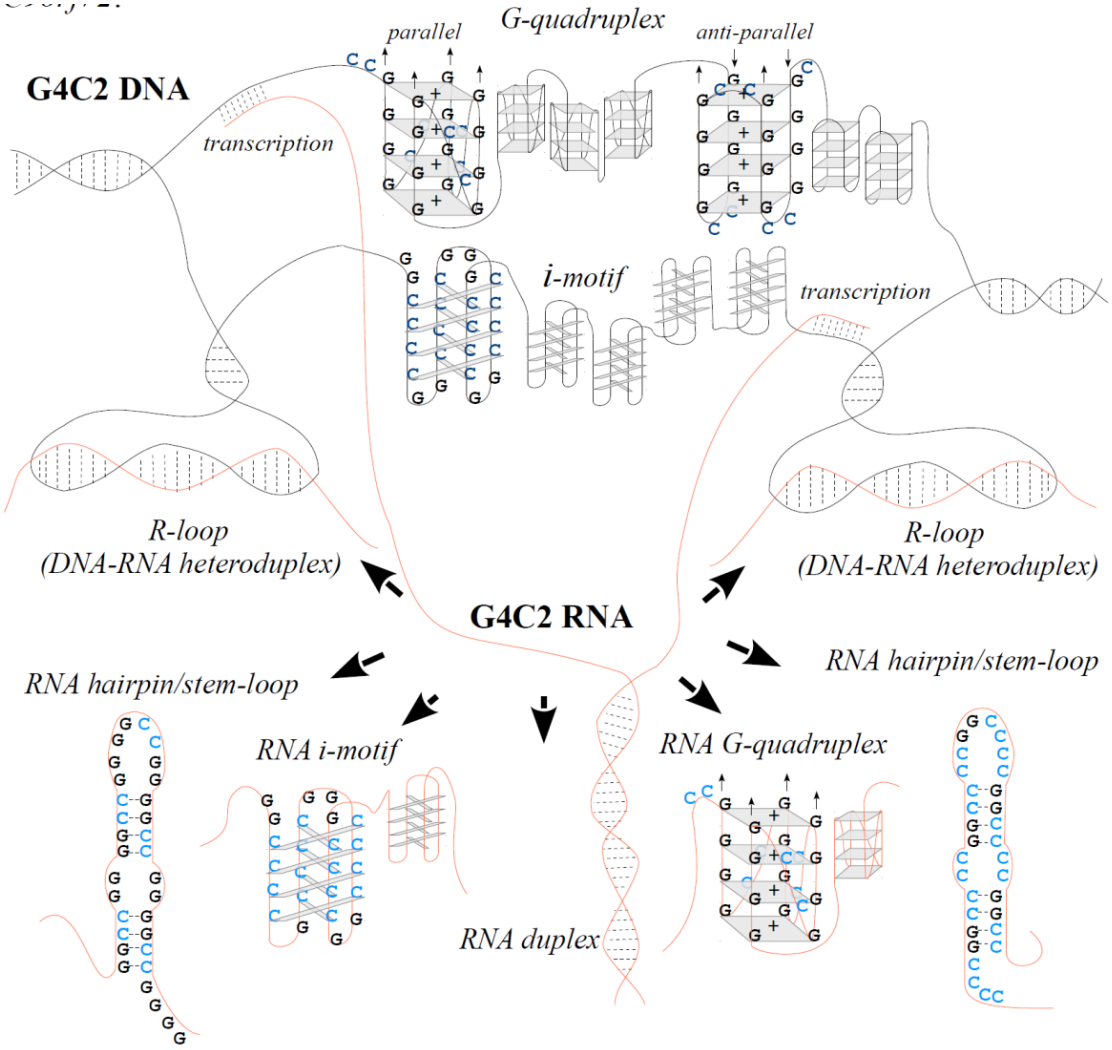
C9orf72 mutation



Vatovec, *Neurobiol Ageing* 2014

- Expansion recently associated with approximately 40% of familial ALS, 25% of familial FTLD, and 6%–7% of sporadic cases.
- 500 to >5000 repeats in patients with ALS or FTLD. Individuals without disease possess 2–19 repeats (average ~2).
- The intronic location of G4C2 repeat strongly suggests a disease mechanism directly involving RNA.
- TDP-43 proteinopathy.

RNA/DNA structures

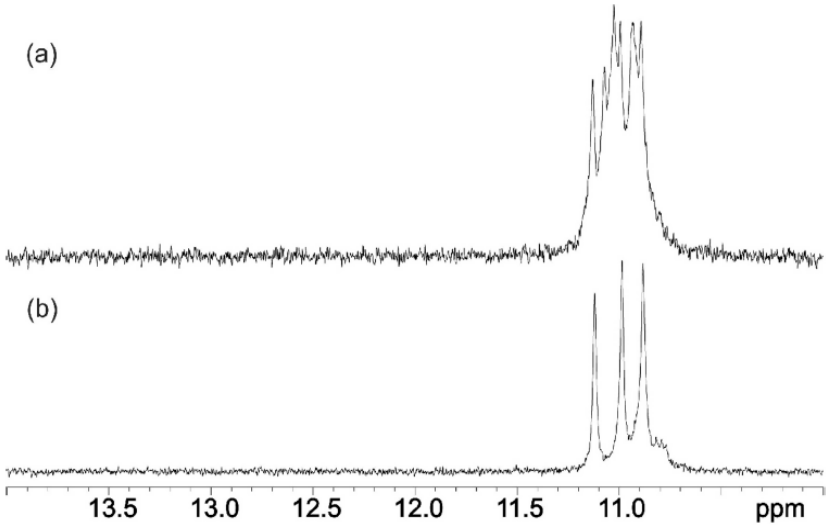


- Repeat may cause highly complex DNA and/or RNA structures.
- Sense and antisense transcripts.

Vatovec, *Neurobiol Ageing* 2014

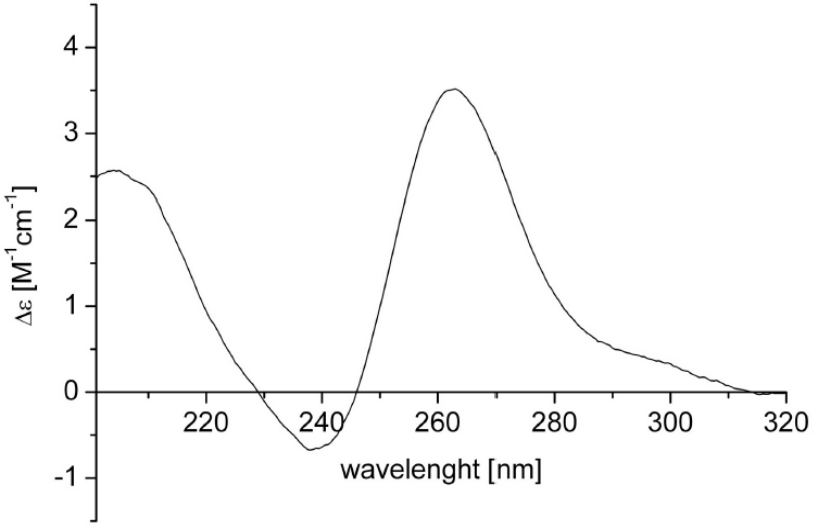
dG4C2 forms parallel GQs

Figure 1: NMR and CD spectra of d(G_4C_2)



Imino regions of 1H NMR 600 MHz spectra of d(G_4C_2) immediately after addition of 100 mM KCl (a) and one day latter (b).

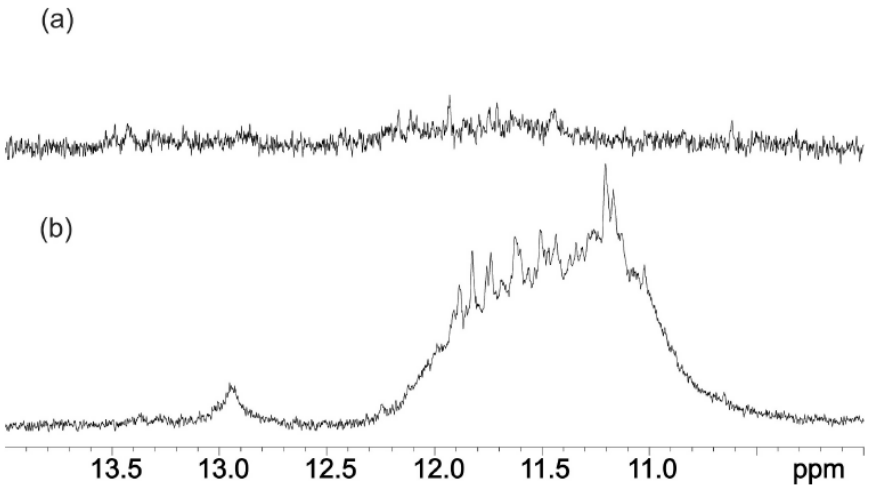
(c) CD specter of d(G_4C_2) in 100 mM KCl.



Šket, *Neurobiology of Aging* 2014

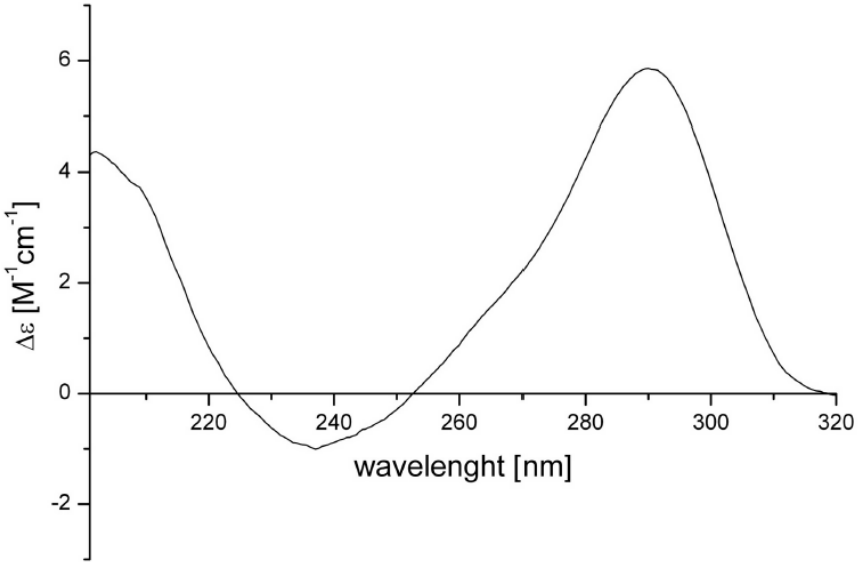
Uniform structure breaks down with higer repeat number d(G4C2)4

Figure 4: NMR and CD spectra of d(G_4C_2)4

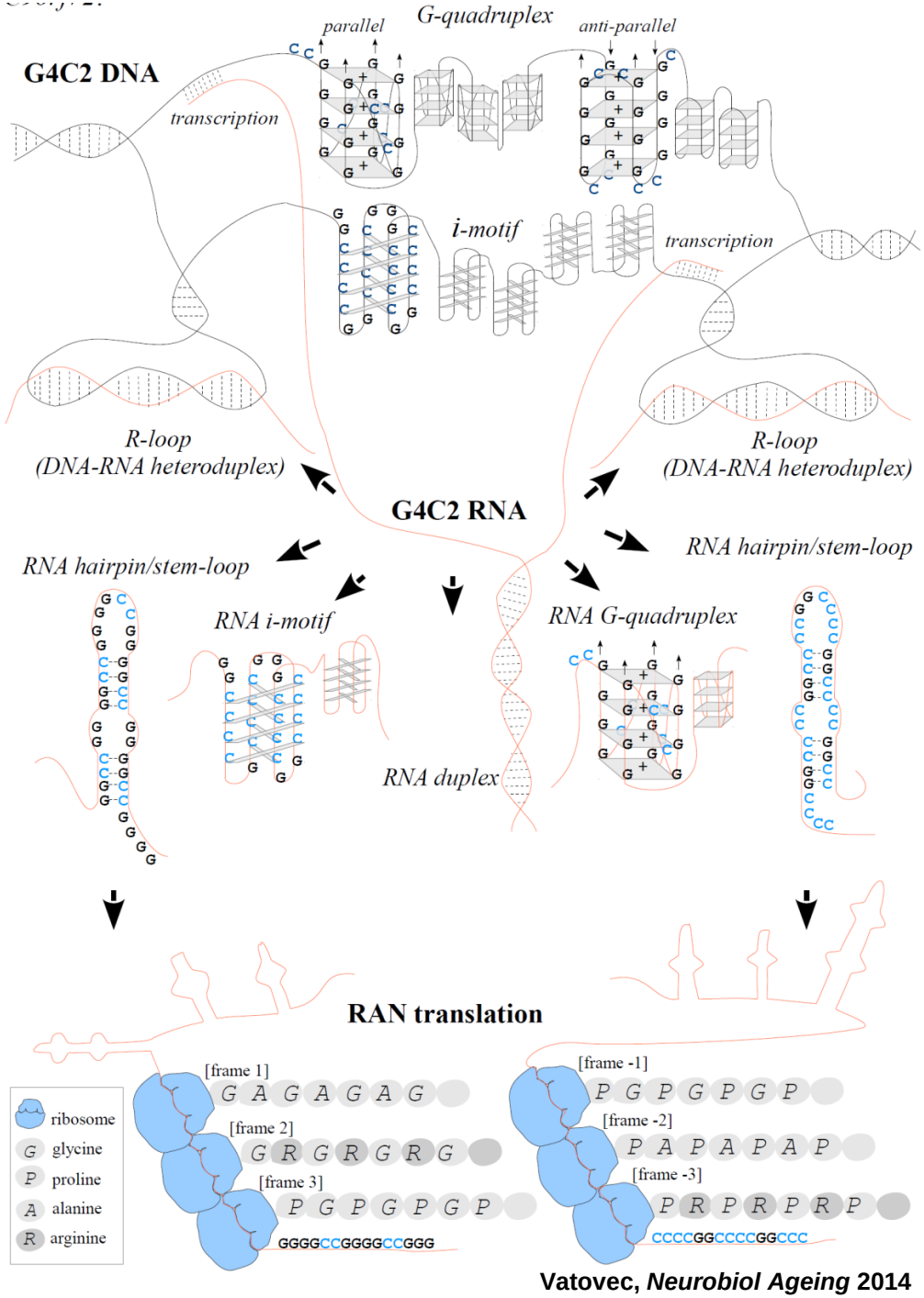


Imino regions of 1H NMR spectra of d(G4C2)4 (a) without KCl and (b) after two weeks after addition of 100 mM KCl.

(c) CD specter of d(G_4C_2)4 in 100 mM KCl.



Šket, *Neurobiology of Aging* 2014



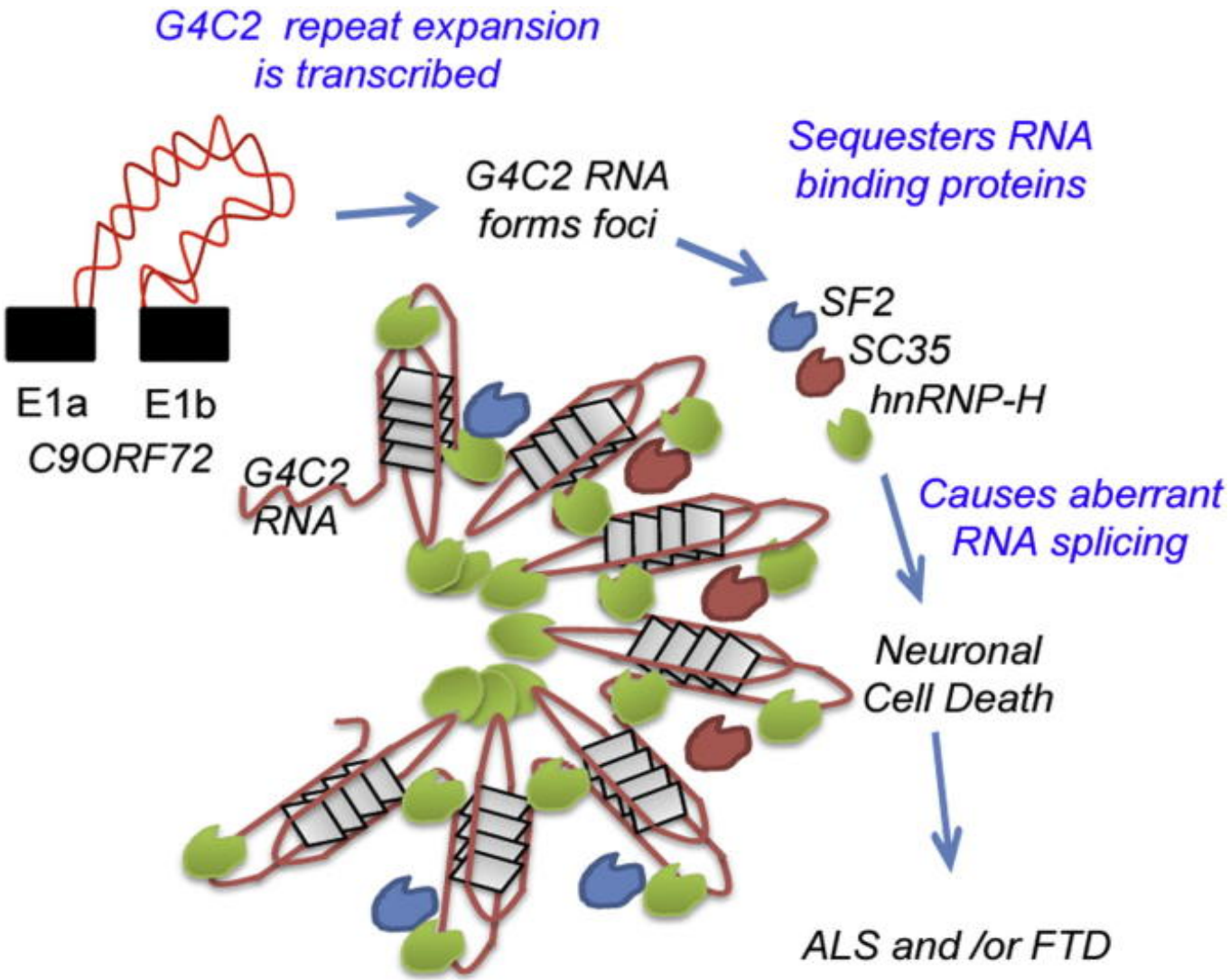
Vatovec, *Neurobiol Ageing* 2014

Mechanism?

Three possible mechanisms:

1. Haploinsufficiency.
2. Repeat-associated RNA toxicity.
3. RAN translation resulting in DPRs.

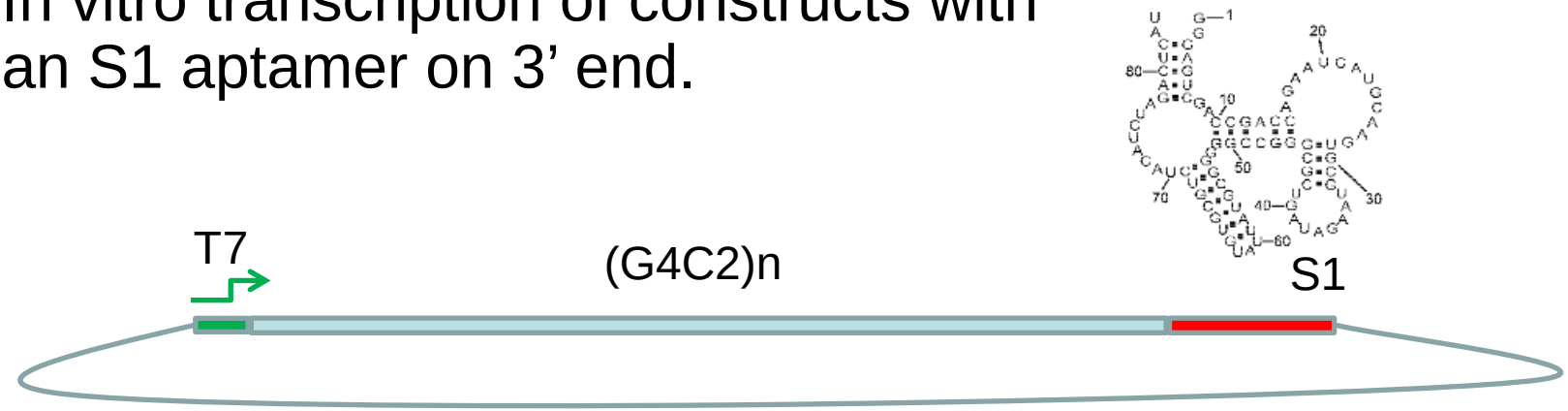
RNA toxicity hypothesis



Lee, *Cell Reports* 2013

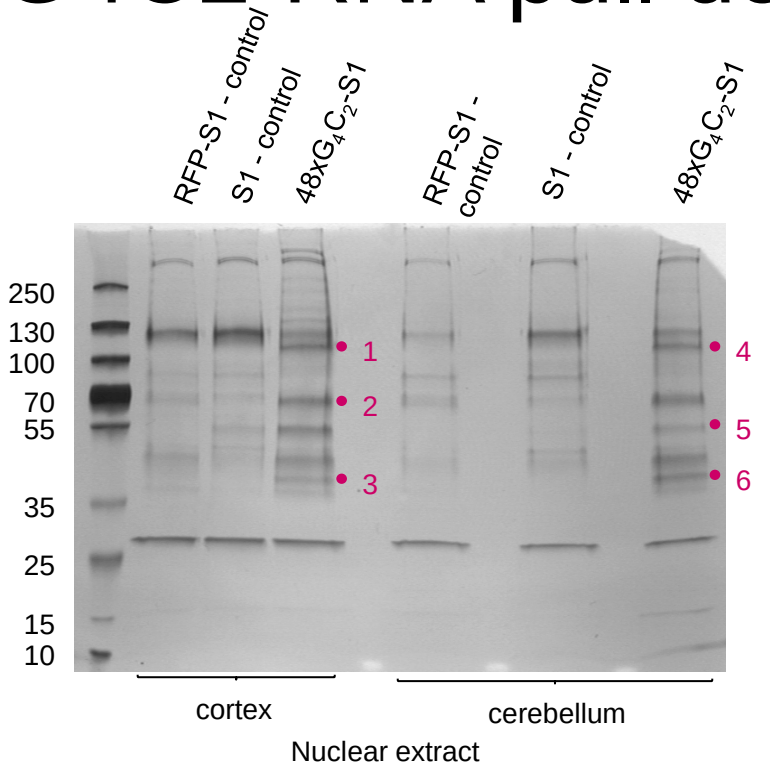
RNA pulldown

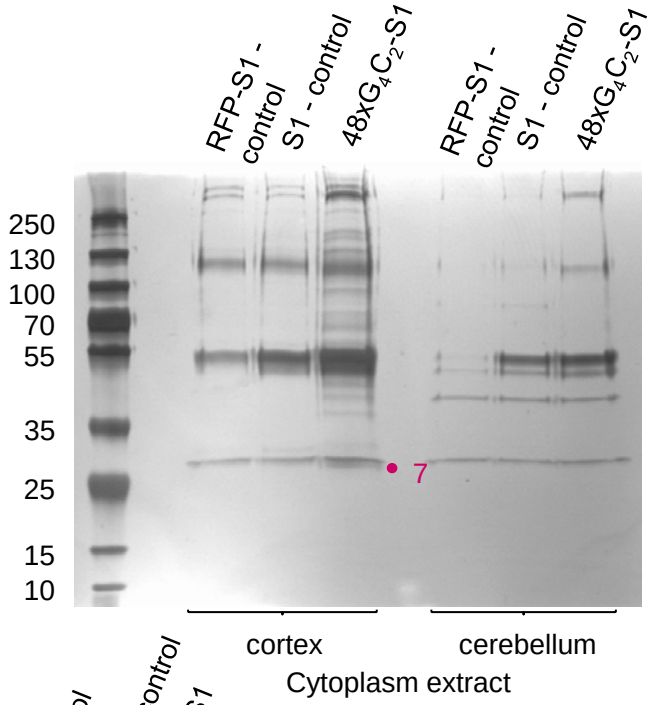
- In vitro transcription of constructs with an S1 aptamer on 3’ end.



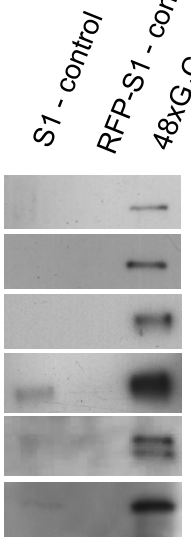
- 48x(G4C2)-S1; Controls: RFP(1-300)-S1 and S1 only
- Incubate in fresh nuclear or cytoplasmic brain extract from rats.

G4C2-RNA pull down from rat brain





Sample	Protein code*
1, 4	SFPQ
2	NONO
3,6	nucleophosmin
5	EEF1A2
5	hnRNP H
7	PRDX1



Targeted screeing of hnRNPs and other RBPs

A

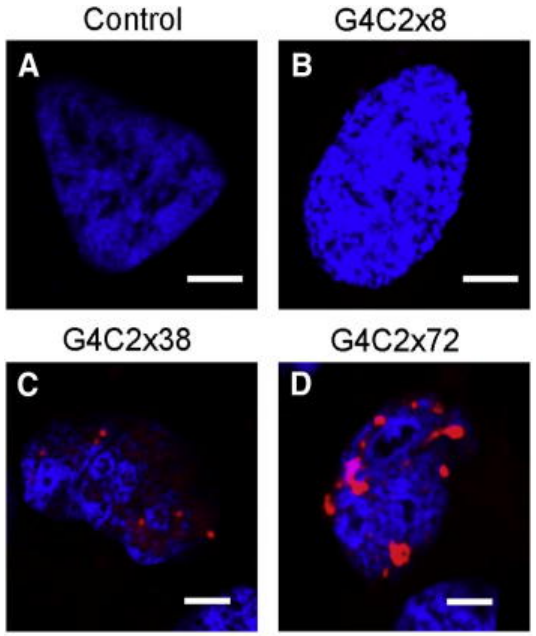
RNA binding proteins	
GENE	G4C2-Foci
TDP-43	-
EWS	-
PABP	-
PABPN1	-
9GB	-
TAFIIp68	-
TAP	-
PUR-a	-
MBNL	-
CUGBP	-
CUGBP2	-
RBM4	-
RBMX	-
U2AF65	-
SF2	+
SC35	+

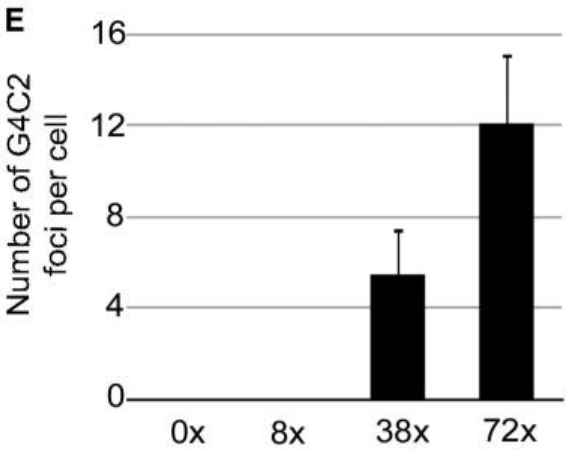
B

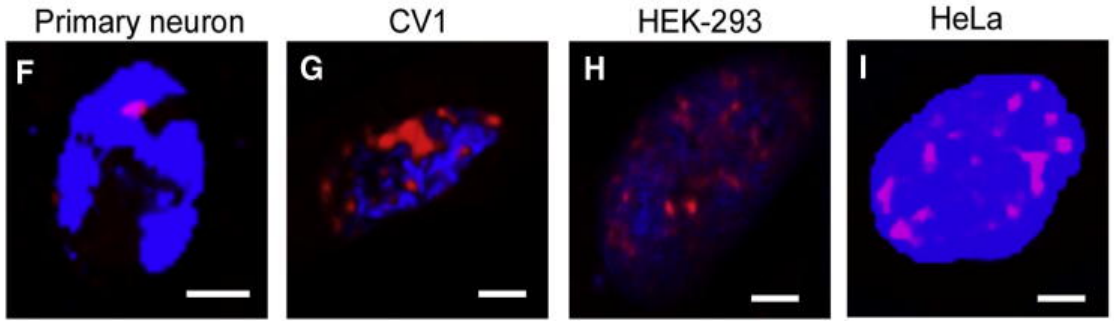
hnRNPs	
GENE	G4C2-Foci
hnRNP-pan	-
hnRNP-A1	-
hnRNP-A2B1	-
hnRNP-A3	-
hnRNP-C1/C2	-
hnRNP-E1	-
hnRNP-F	-
hnRNP-H	+
hnRNP-K	-
hnRNP-L	-
hnRNP-M	-
hnRNP-Q	-
hnRNP-R	-
hnRNP-U	-

Lee, *Cell Reports* 2013

RNA foci are dependent on repeat length

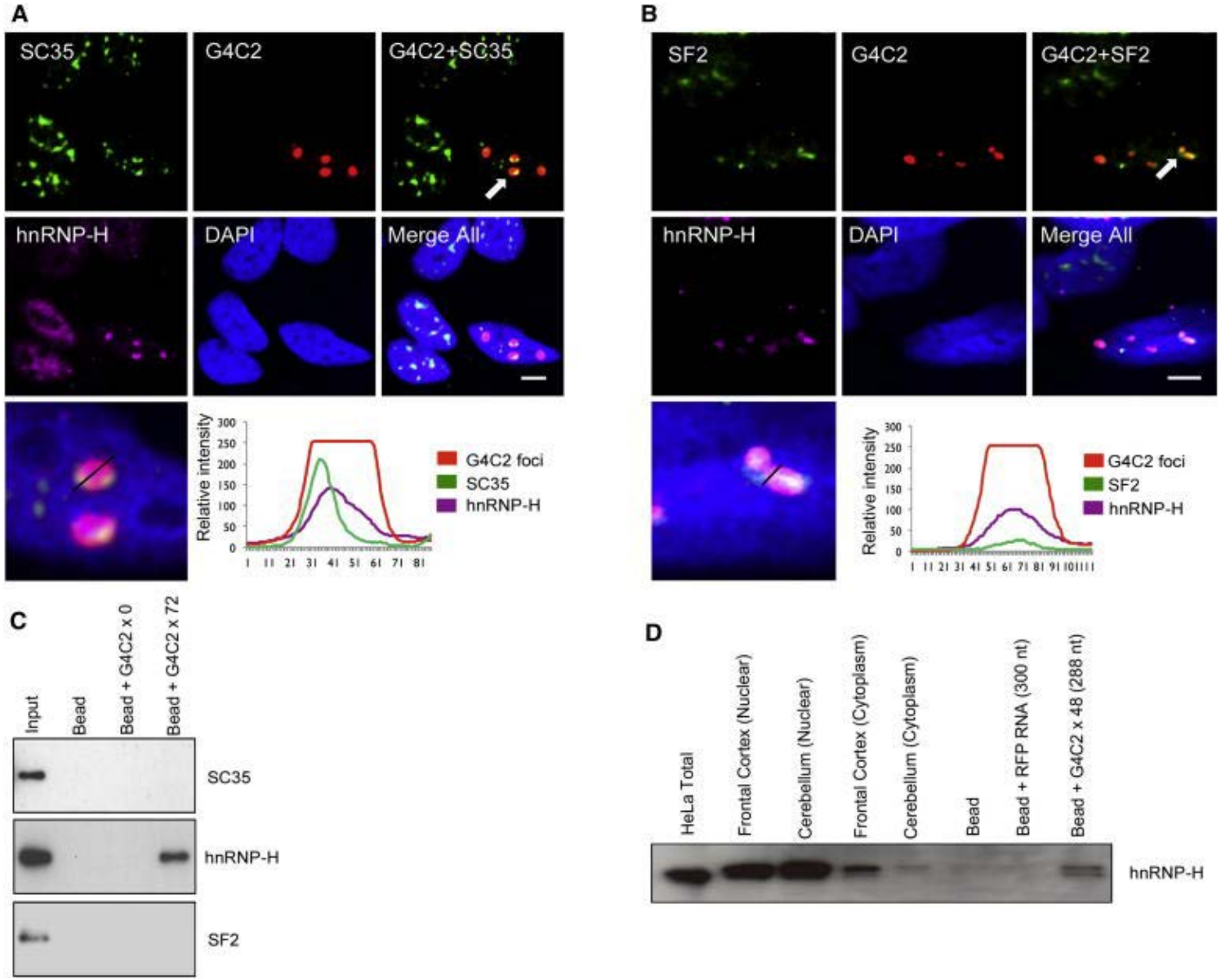






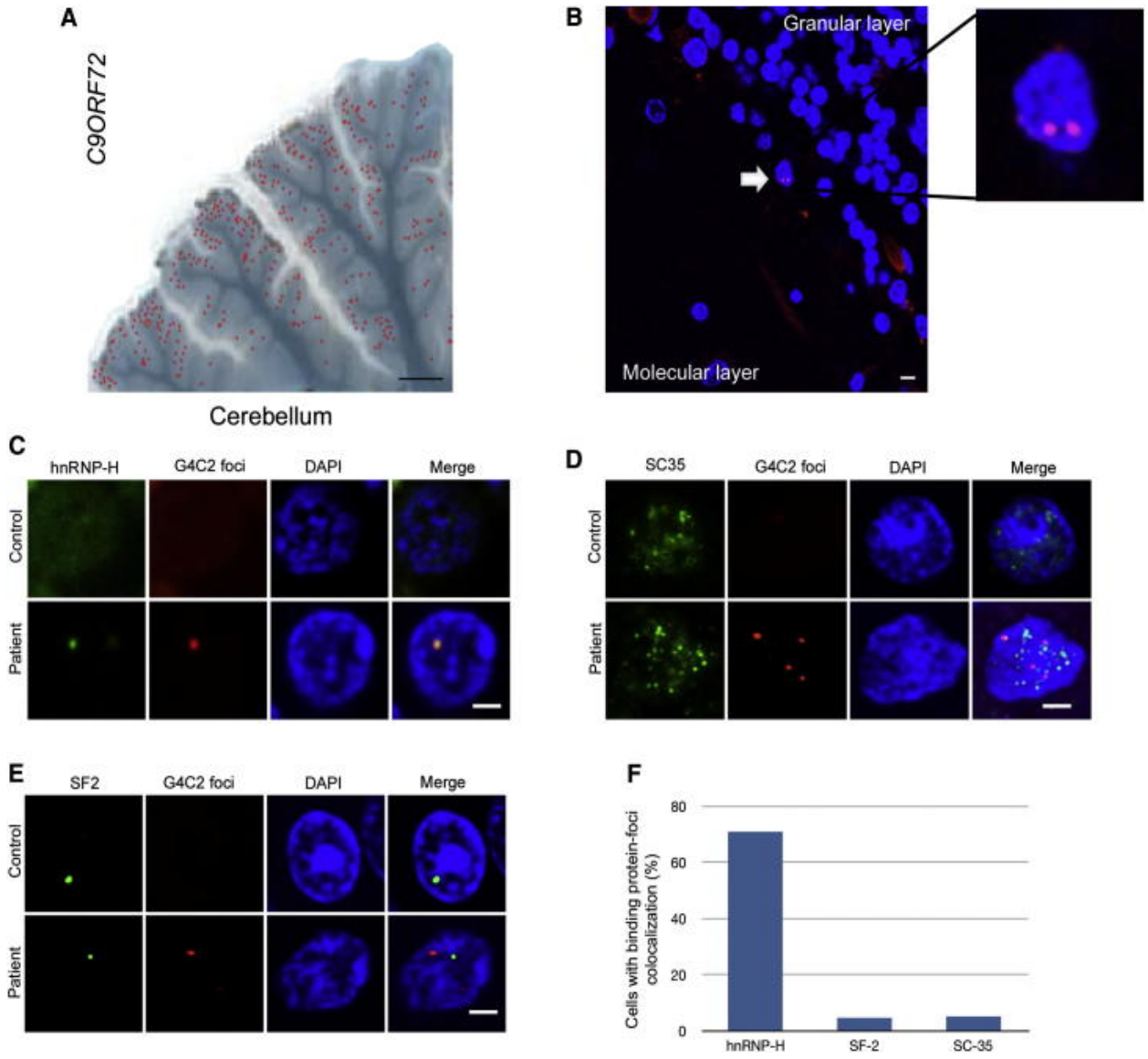
Lee, *Cell Reports* 2013

SC35, SF2, and hnRNP-H colocalize with G4C2 nuclear foci



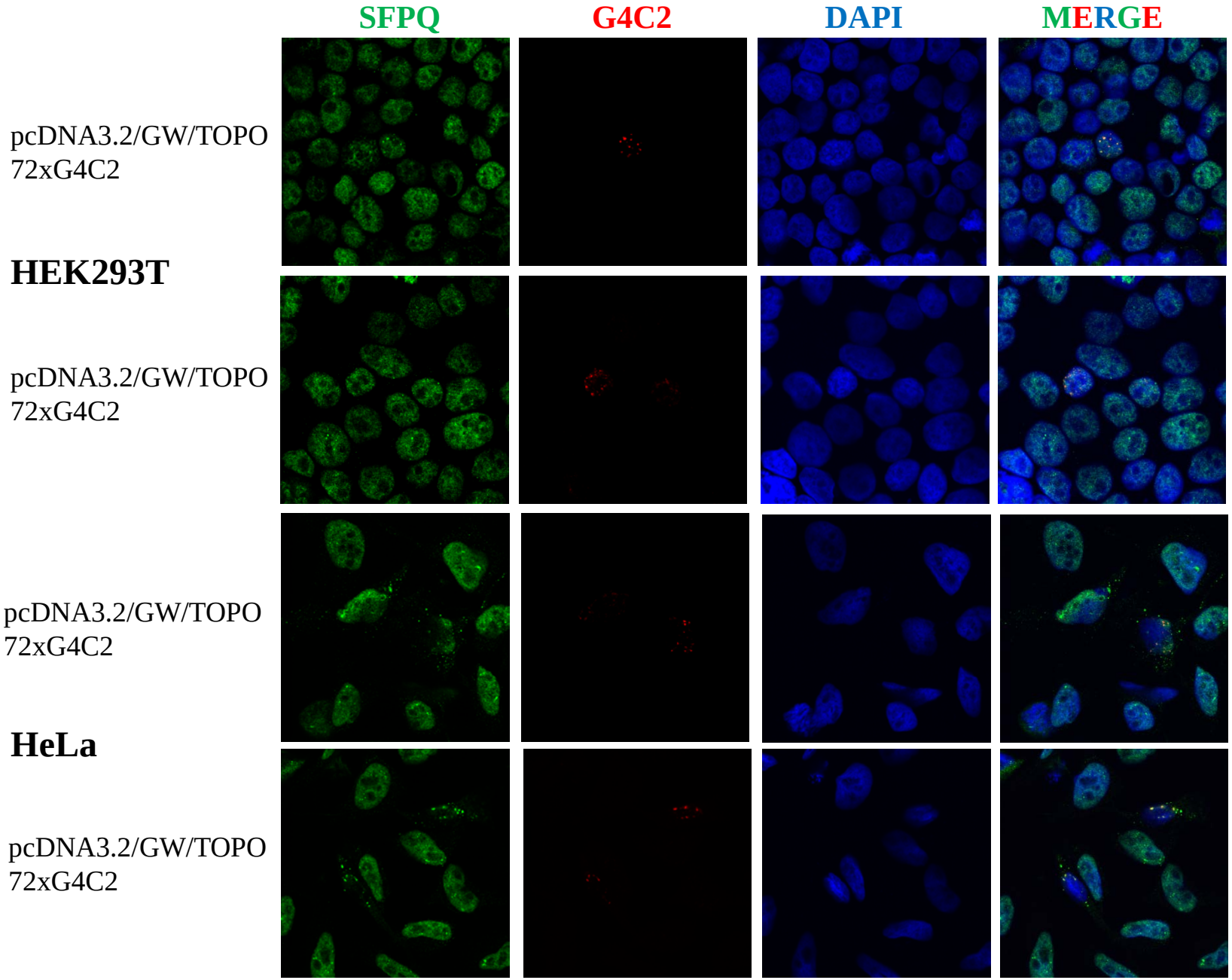
Lee, *Cell Reports* 2013

RNA foci in C9ORF72 brain tissues colocalize with hnRNP-H

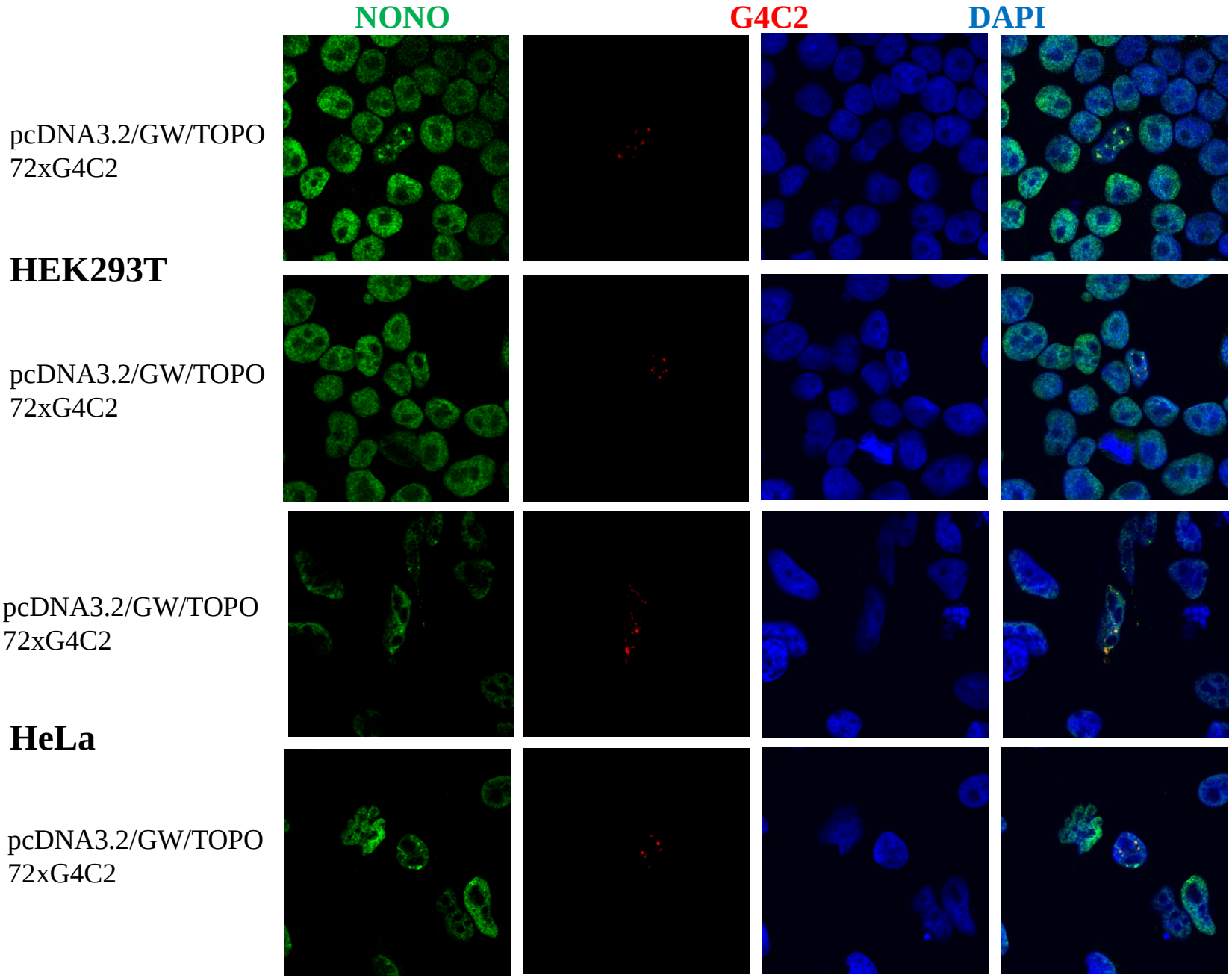


Lee, *Cell Reports* 2013

SFPQ *Splicing factor, proline- and glutamine-rich*



NONO *Non-POU domain-containing octamer-binding protein*



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