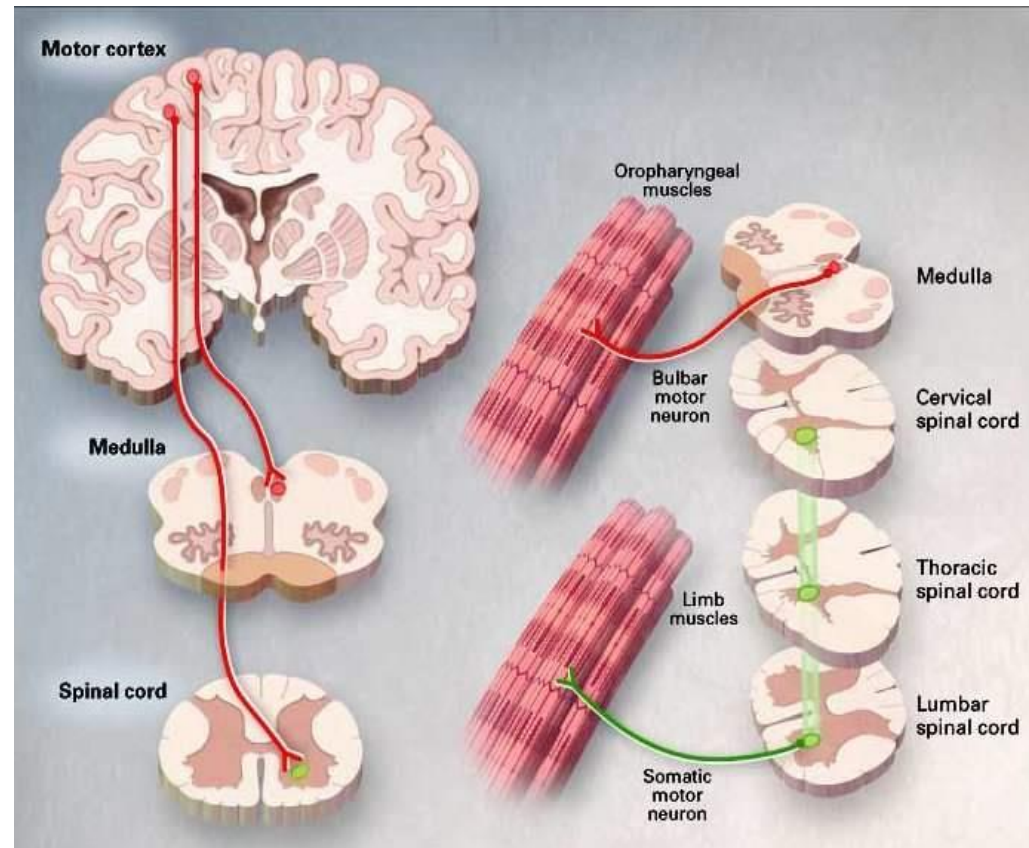
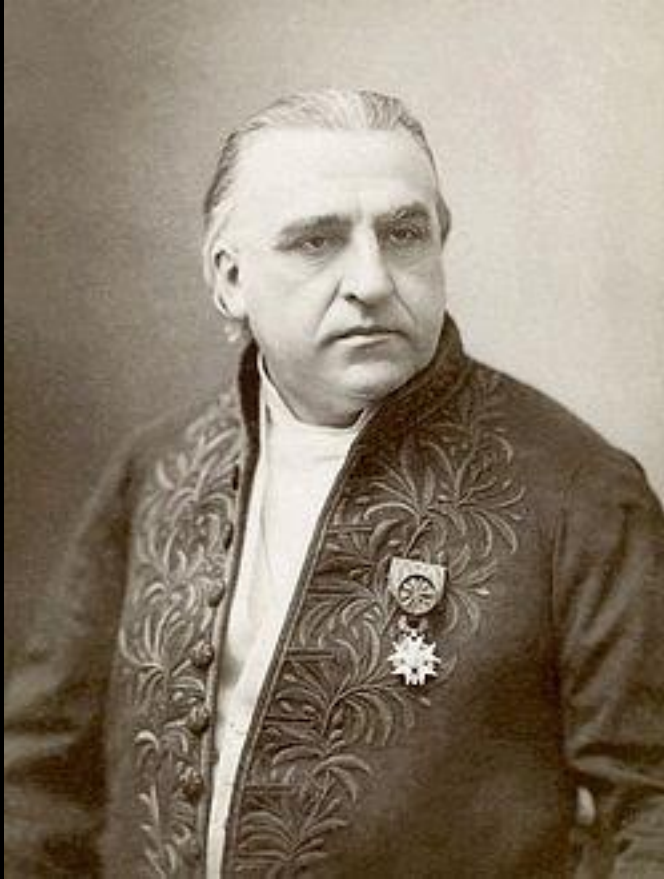


ALS = Amyotrophic Lateral Sclerosis or Motor neuron disease



Selective degeneration of motor neurons

Amyotrophic lateral sclerosis (ALS)



Jean-Martin Charcot

1825 – 1893

Paris, Hôpital de la Salpêtrière

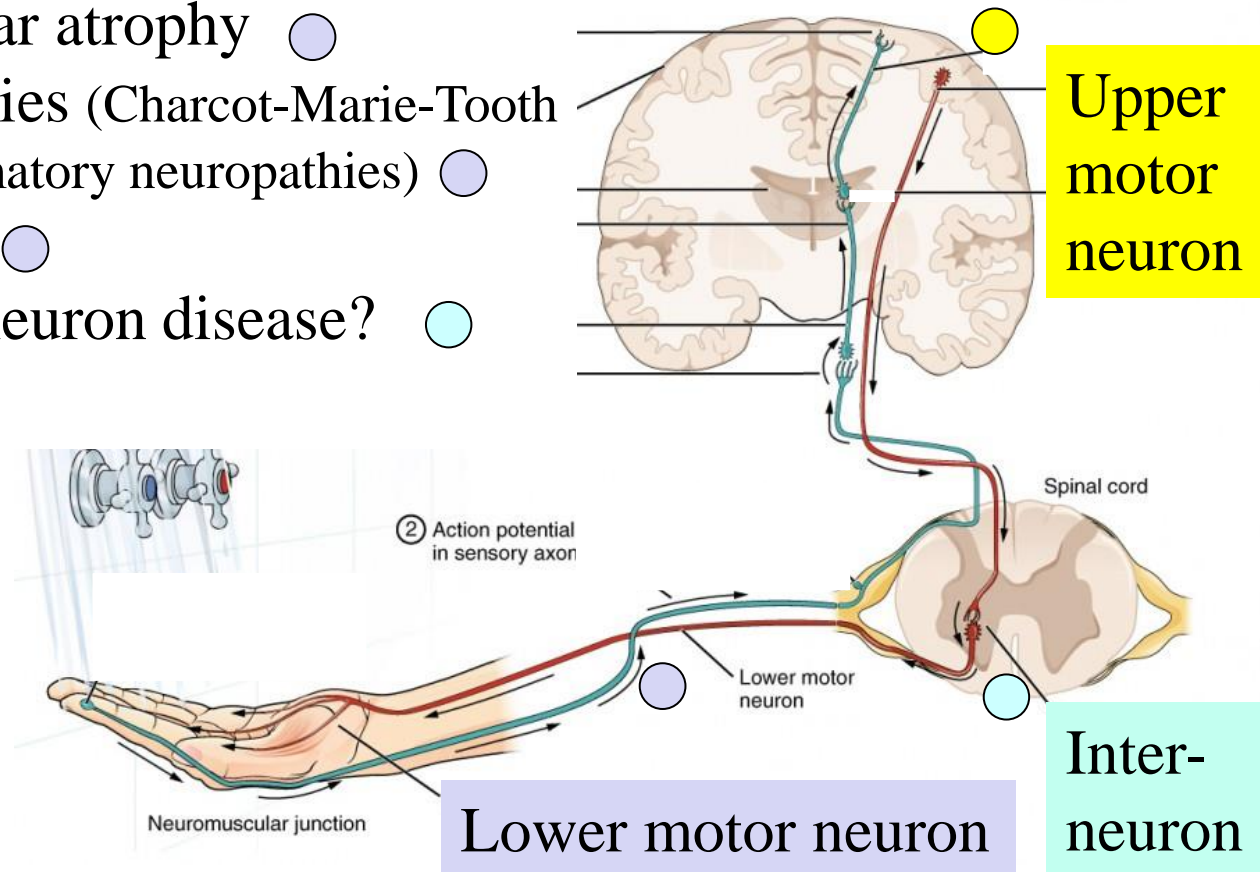
Amyotrophic = muscle atrophy

Lateral sclerosis

scarring or hardening of the
(anterior) lateral column of the
spinal cord

Motor neuron diseases (simplified)

- ALS ● ○ ○
- Primary lateral sclerosis (PLS) ●
- Spastic paraplegias ●
- Spinal muscular atrophy ○
- Polyneuropathies (Charcot-Marie-Tooth diseases, inflammatory neuropathies) ○
- Poliomyelitis ○
- Is there inter-neuron disease? ○



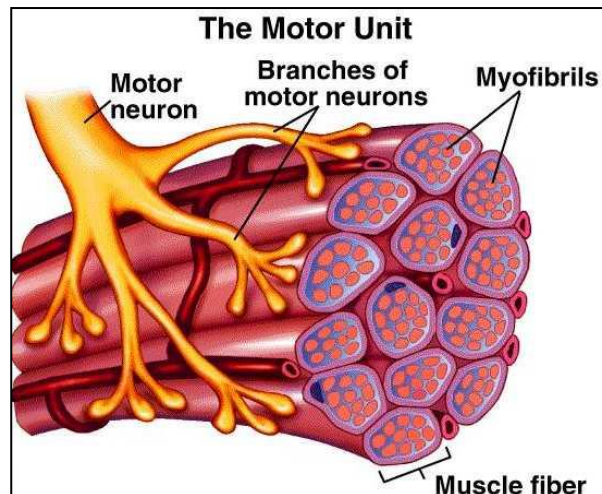
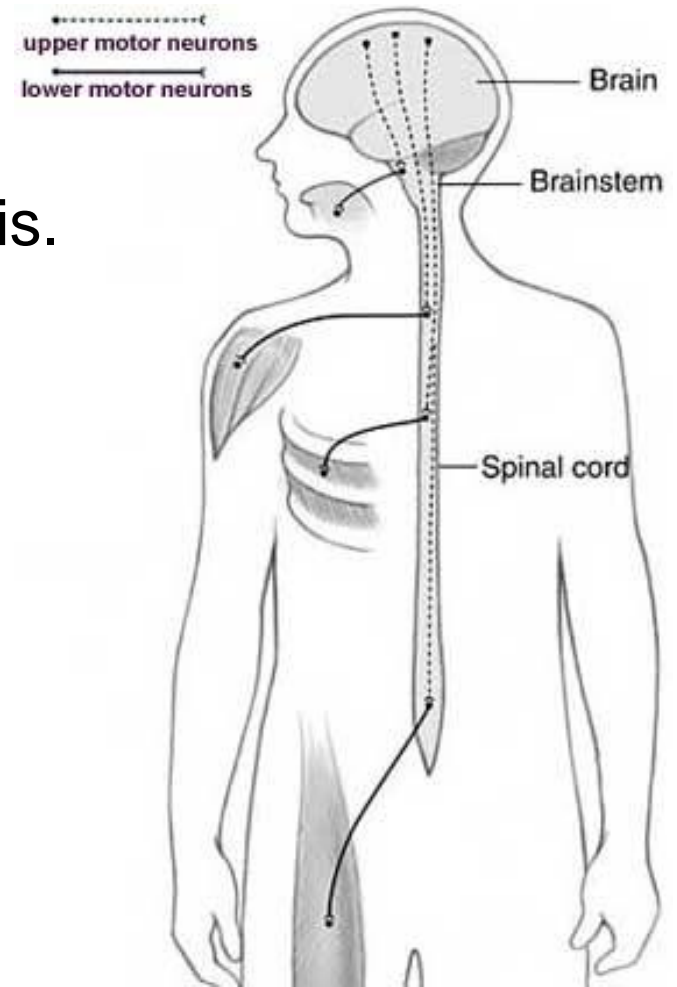
ALS in nutshell

Typical onset at age 55-75

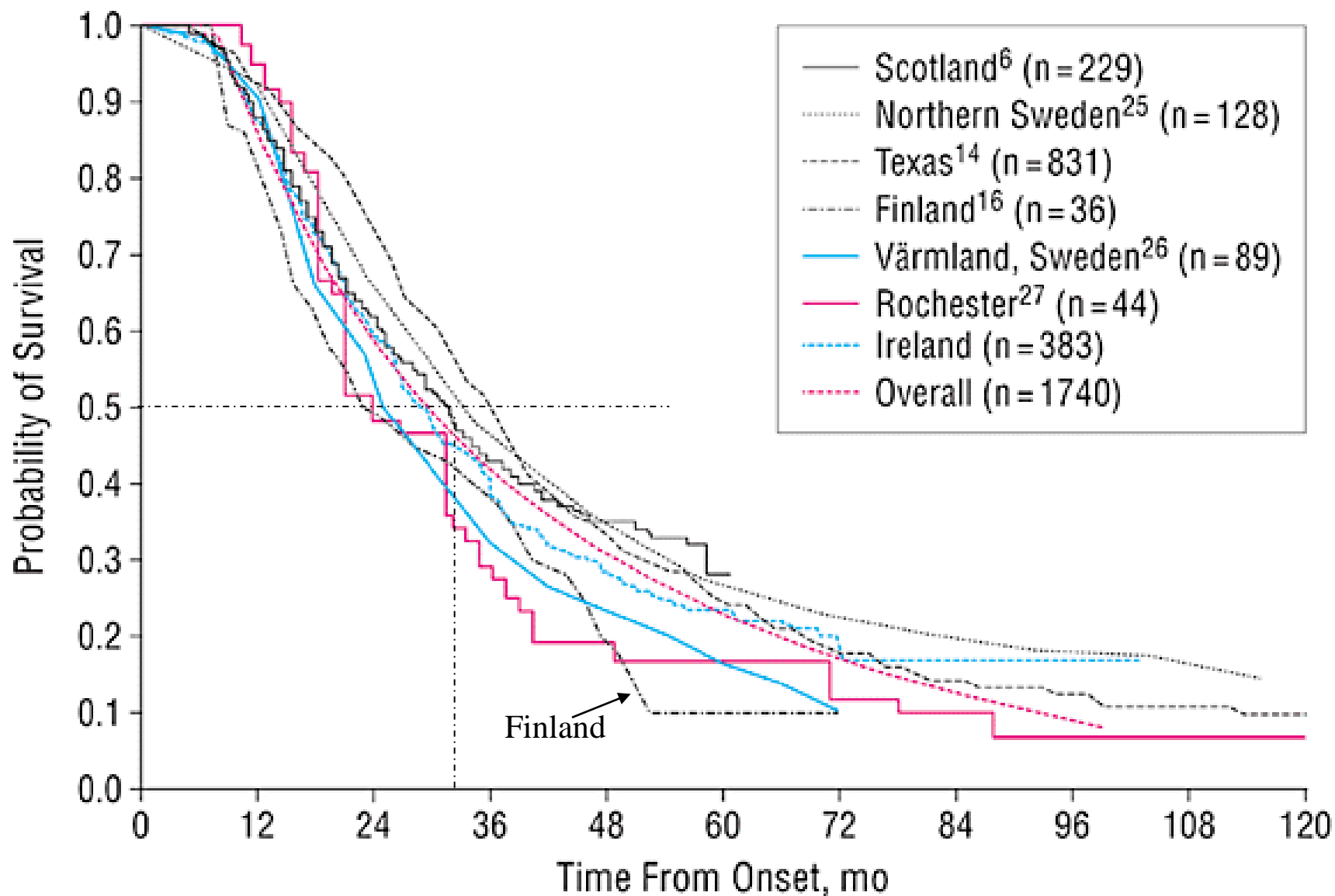
Average lifespan 3 yrs from diagnosis.

No effective treatment

5% of are familial, in Finland >10%



ALS Survival



ALS incidence and prevalence

- Finland: 3-4 new cases per week
- Finland: 500-700 living patients
- USA: 16 deaths per 24h

- ALS: $< \frac{1}{2}$ of incidence of multiple sclerosis (MS)
- ALS: $\frac{1}{20}$ of prevalence of multiple sclerosis
- Lifetime risk 1:400 (1:200 in MS)

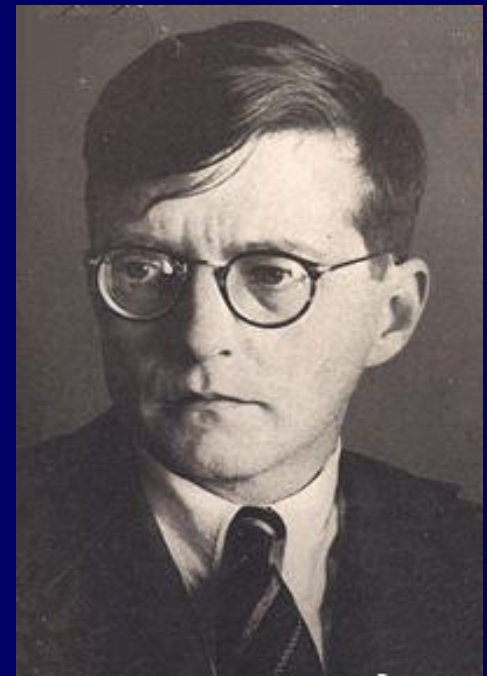
ALS with faces



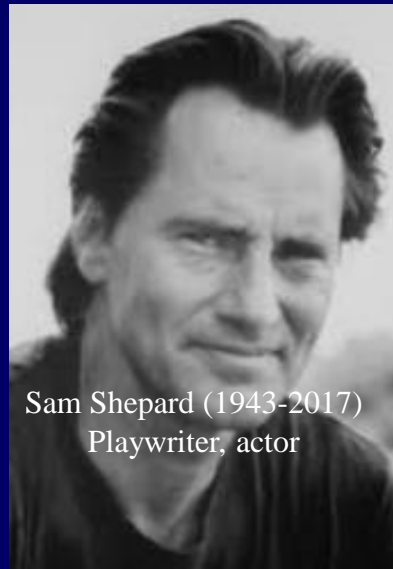
Ei "klassinen ALS"
(1942-2018)



Late-onset ALS



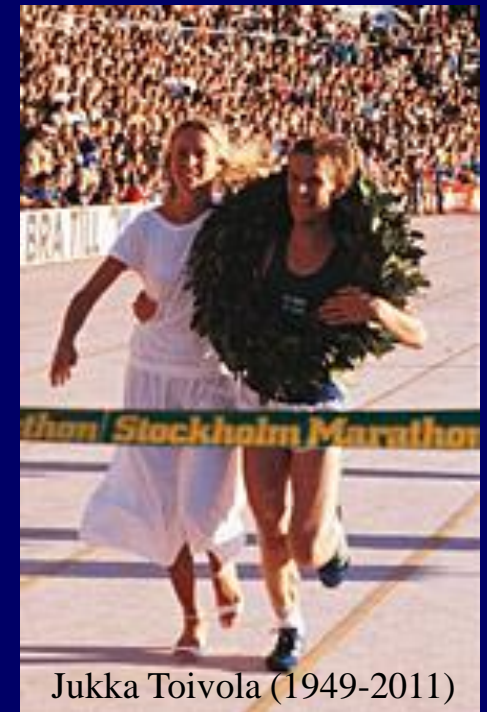
Jenifer Estess (1963-2004)
theatre producer



Sam Shepard (1943-2017)
Playwriter, actor

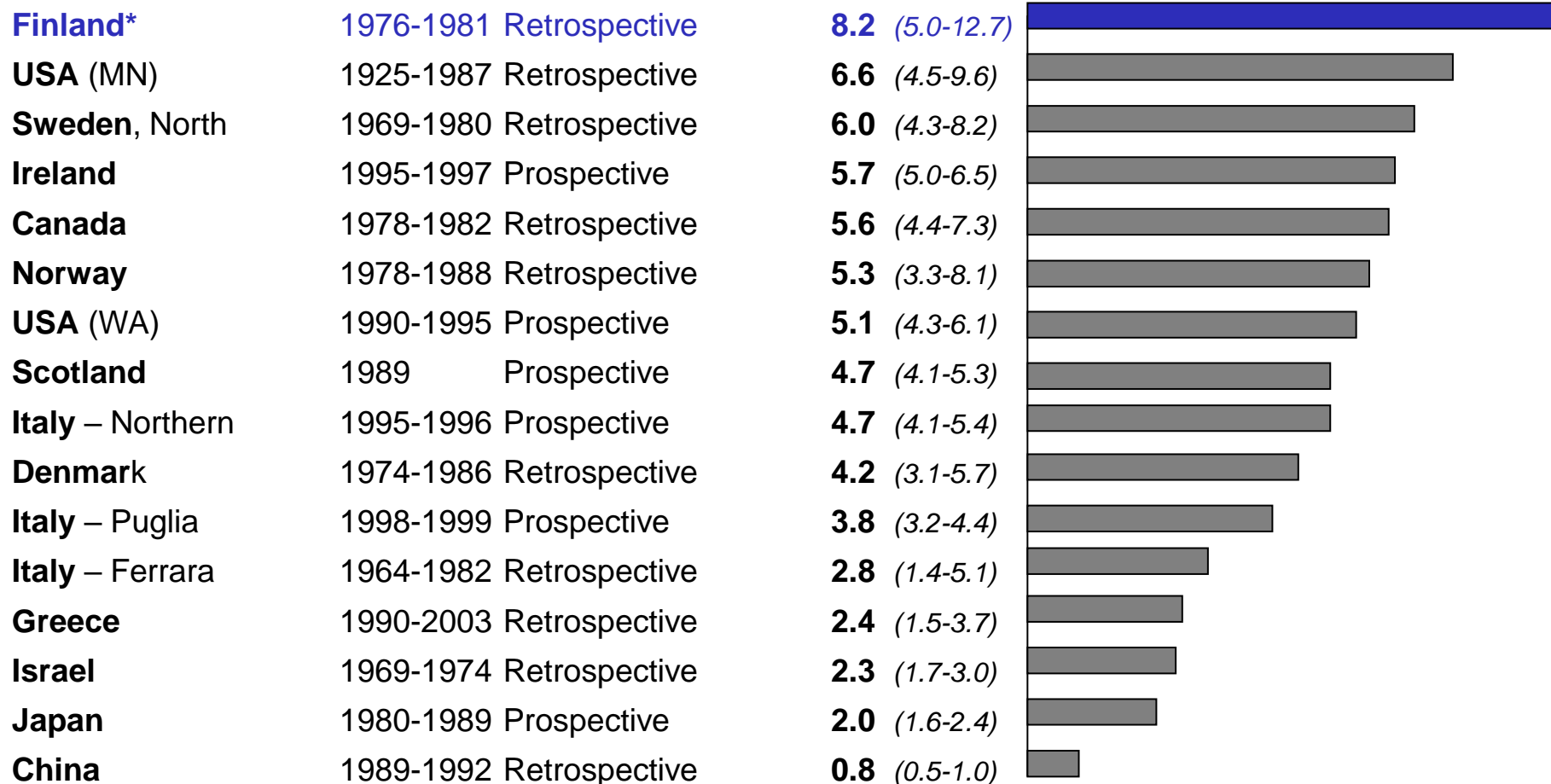


Gianluca Signorini
(1960-2002)



Jukka Toivola (1949-2011)

ALS is common in [Finland](#): Comparison of incidence studies



Incidence per year/100,000 in ageband 40-70.

***Murros K and Fogelholm R**
Acta Neurol Scand 1983 Jan;67(1):41-7

ALS - Oireet

Alussa:

- Lihasten nykinä eli faskikulaatio
- Lihaskato- ja heikkous, lihaskivut.
- Raaja-alkuinen 2/3, bulbaarialkuinen 1/3

Myöhemmin:

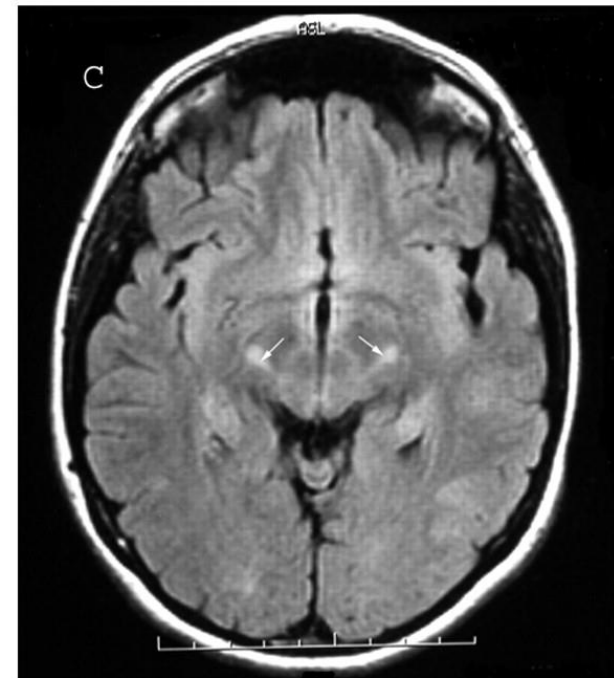
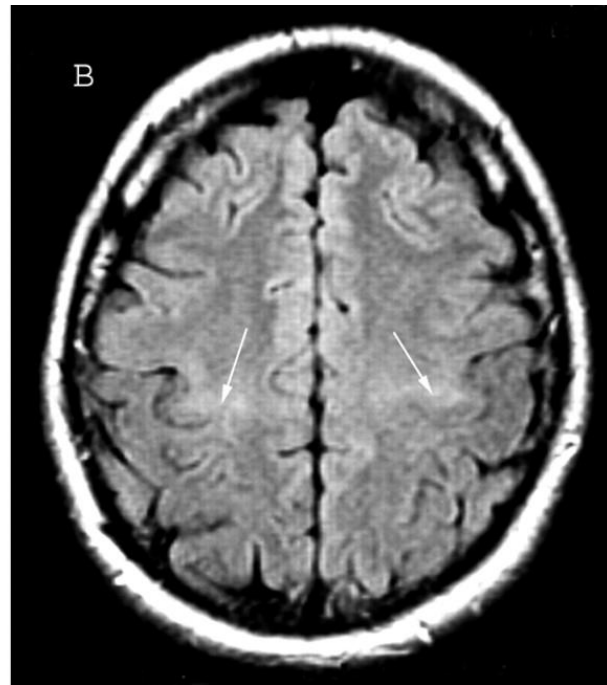
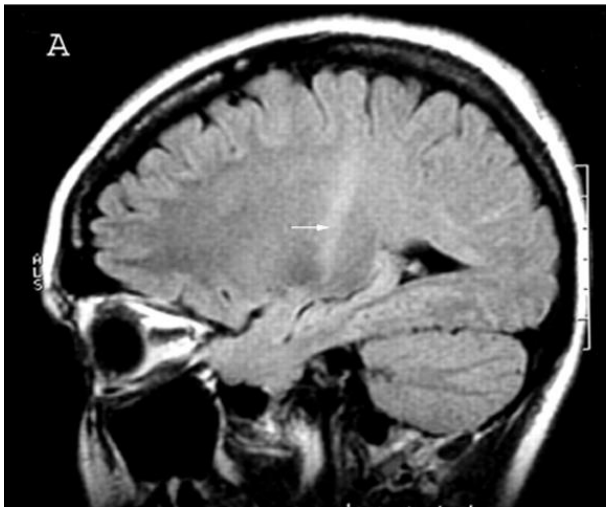
- Pään kannatus ja kävely heikkenevät
- Puhe- ja nielemisvaikeudet
- Sylki valuu hallitsemattomasti
- Hengityslihasheikkous

Tutkimuslöydökset

- KNF: EMG
 - denervaatio (mm. fibrillaatioita, faskikulaatioita ja isoja motorisia yksiköitä)
- laboratorio
 - S-CK: normaali – 4x
 - likvor: yleensä normaali
- keskushermoston MRI (aivojen ja kaularangan)
 - ei spesifejä löydöksiä
 - tarpeen yleensä muiden sairauksien poissulkemiseksi

Neurology®

MRI in ALS: Corticospinal tract hyperintensity
Yong Chern Lee, Romesh Markus and Andrew Hughes
Neurology 2003;61;1600



MRI positive in 30-40% of ALS cases

Hoito

Neuroprotektiivinen lääkehoito

-Rilutsolin (Rilutek®) 50mg 1x2 on todettu pidentävän elinaikaa n. 3kk
Rilutsolin oletetaan vaikuttavan glutamaattiprosessien estoon.

-aloitettava mahdollisimman aikaisin, mikäli sitä päätetään käyttää

-ei korjaa ALS:n oireita, mikä tulee selvittää potilaalle huolellisesti

-voi paradoksaalisesti aiheuttaa lihasheikkoutta, joka korjaantuu lopetuksen jälkeen

-Loppuvaiheessa tautia (kun lihasheikkous on selkeä) ei Rilutekia ole mielekästä käyttää.

Nielemisvaikeudet, PEG

- PEG-letkusta tulee kertoa potilaalle varhain. Painotetaan elämänlaadun paranemista ravitsemustilan kohentuessa ja aspiraatoriskin vähentymistä.
- PEG:n ajoitus perustuu bulbaarioireiden vaikeuteen, ravitsemustilaan ja hengityskapasiteettiin.
- **Käytännössä PEG on tarpeen silloin, kun kohtuuton osa potilaan ajasta menee nielemisen kanssa taisteluun.**
- PEG-ravitseminen aloitetaan osastolla ja käyttö opetetaan potilaalle ja omaiselle, seurataan mahdollisia infektiioireita.

Hengitysvaikeudet

- **Non-invasiivinen nasaalinen ventilaatiohoito (NINV)** parantaa ja ylläpitää elämänlaatua. Se tulisi aloittaa kun VC laskee 50%:iin lähtötasosta. VC mitataan istuen ja maaten spirometrialla.

Vaihtoehdot hoitolinjoille

I konservatiivinen hoito = hengitystä ei tueta mekaanisesti

– hoitomuodot: vuoteen päätypuolen kohottaminen, lääkehoito

II maskilla varustettu noninvasiivinen nasaalinen ventilaattori
(NINV)

III trakeostomia ja pysyvä respiraattorihoito (TR)

Hoitopäätökset hengitysvajauksen suhteen

- Sairauden edetessä NINV ei tule riittämään hengitysvajauksen hoidoksi: valittavana on **palliatiivinen lääkehoito NINV:n lisäksi** tai **trakeostooma ja pysyvä respiraattorihoito**. NINV ei näytä lisäävän potilaiden halukkuutta trakeostoomaan ja pysyvään respiraattoriin.
- **pysyvä respiraattorihoito** pidentää elinaikaa, mutta elämänlaatu yleensä laskee, infektiokerkyys lisääntyy, hoitoisuus lisääntyy ja hoito on raskasta omaisille. Käytännössä tehdään hengityshalvauspäätös, ja potilas tarvitsee hoitoringin (5 hoitajaa).

Hyvänlaatuiset myokymiat ja faskikulaatiot

- elohiiri silmässä
- faskikulaatioita raajoissa, vartalolla
- kahvi, tupakka, liikunta, stressi
- eivät ole yhteydessä ALS-sairauteen

Geenitestaus

- 5-10%:lla ALS liittyy SOD1*D91A mutaatioon (peittyvästi periytyvä)
- Tautimuoto alkaa yleensä alaraajakrampeilla ja -jäykkyydellä, mitä seuraa toisesta alaraajasta alkava hitaasti etenevä heikkous. Taudin kesto on hidas, ennuste yli 15 vuotta. Potilaan sisarusten riski olla homotsygootteja on 25 %, ja potilaan lapset ovat kantajia.
- SOD-D91A-mutaatiota harkitaan tutkittavaksi, jos taudinkuva on tähän viittaava. Merkittävä ennusteellinen informaatio.
- Vallitsevasti periytyviä mutaatioita ei toistaiseksi suositeta tutkittavaksi diagnostisen selvittelyn osana. Tämä voi muuttua mikäli mutaatiospesifejä kliinisiä lääketutkimuksia alkaa.

- *SOD1***D91A*-mutation cluster in Tornio river valley

- Andersen PM et al. *Brain*, 1996;119(4) 1153-72

- First Finnish family described in Kainuu

- Myllylä VV et al, *Acta Neurol Scand*, 1979; 60: 170-7

rs80265967 (A/C) (p.Asp91Ala) , SOD1

Genotypes of the rs80265967 in the FINNGEN data

rs80265967	
Major HOM (A/A)	51 404
HET (A/C)	1 013
Minor HOM (C/C)	1
Total	52 418

→ **2% suomalaisista on *SOD1***D91A* kantajia
Riski: 1 case per 10,000**



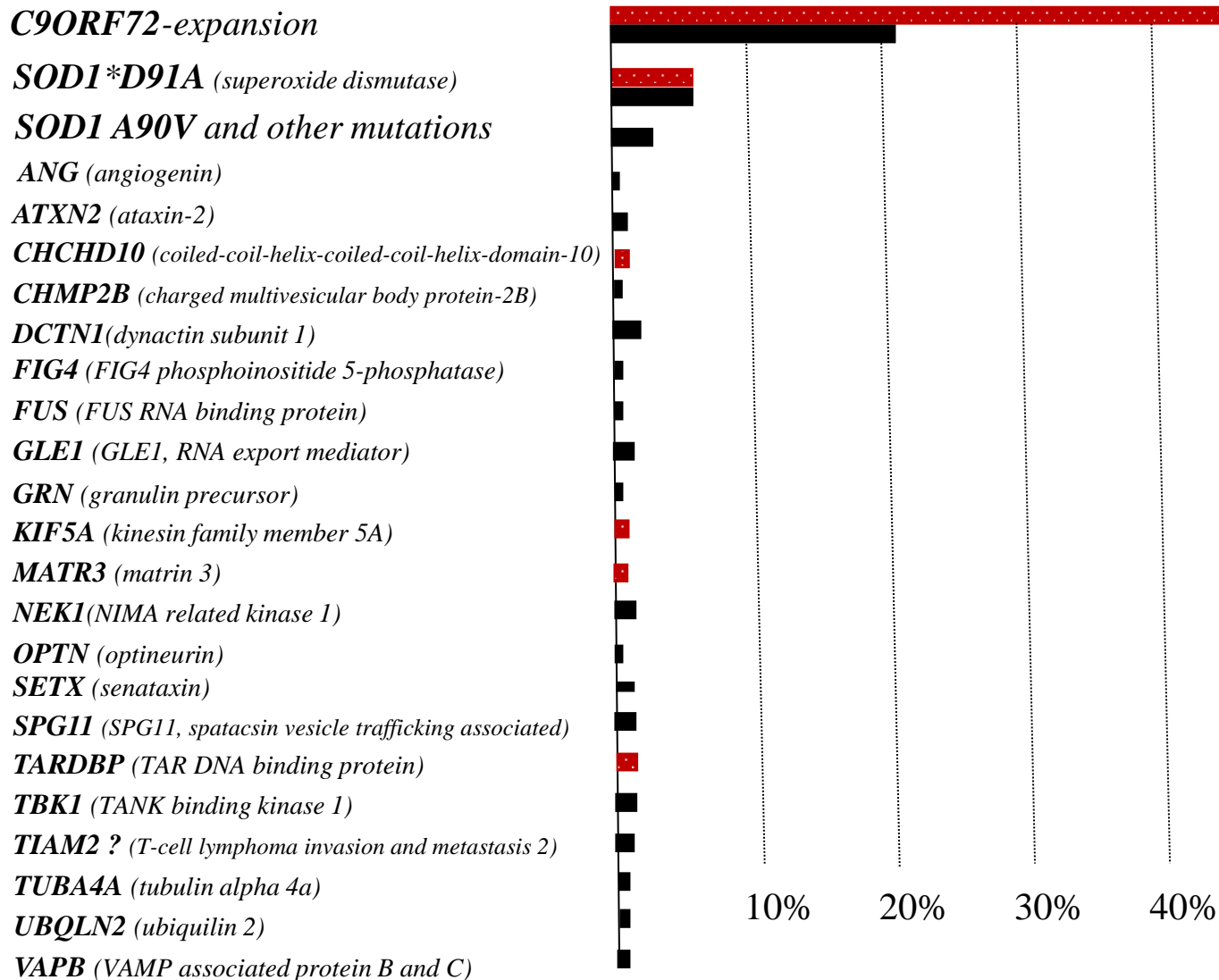
ALS genetics in Finland: The two towers



Two mutation hotspots (*C9ORF72* and *SOD1*) - other mutations heterogeneous in Finnish ALS.

Preliminary data (analyses in progress)

 Familial ALS
 Sporadic ALS



C9orf72 taudin patogeneesi

Finnish ALS-GWAS was important in finding the *C9ORF72* mutation



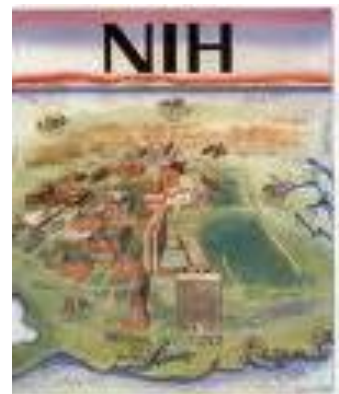
Hannu Laaksovirta, MD neurologist

- Has personally examined almost all ALS patients in Southern Finland since 1990s.
- Samples of >500 Finnish ALS-patients



Terhi Peuralinna, MSc

- Carried out the GWAS.



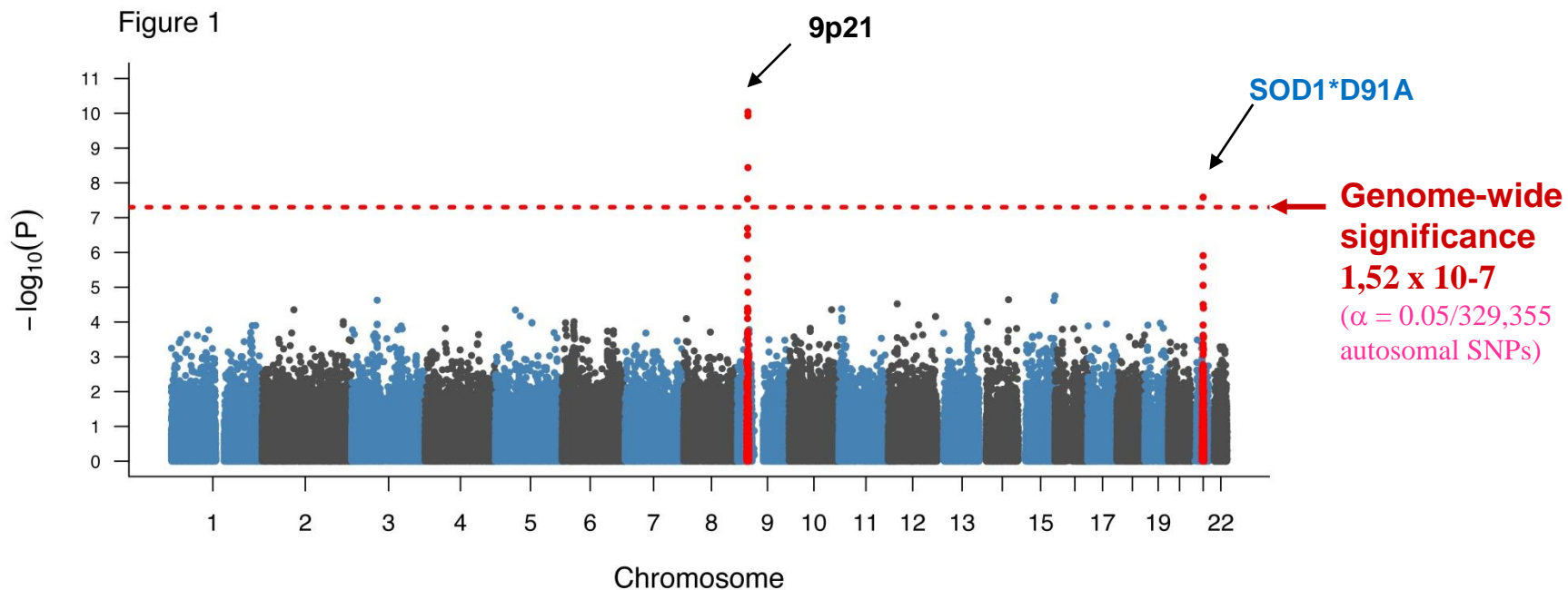
Illumina HumanCNV370 chip,

Finnish ALS-GWAS

Laaksovirta et al. *Lancet Neurol* 2010 Oct;9(10):978-85.

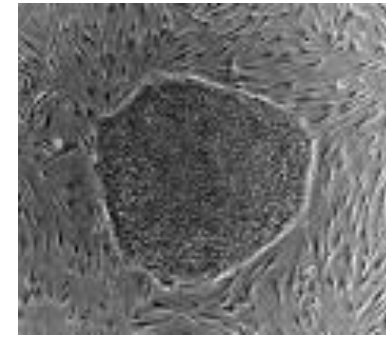
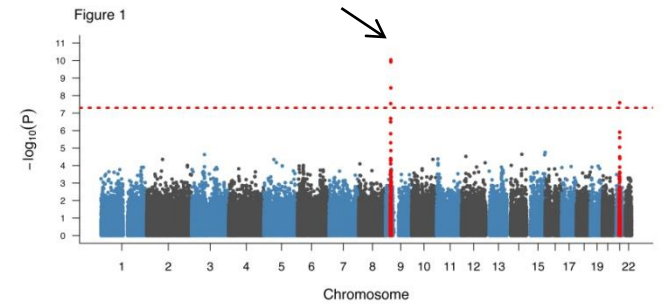
Chromosome 9p21 in amyotrophic lateral sclerosis: A genome-wide association study

Manhattan plot: 405 ALS patients and 497 controls



SOD1*D90A is a previously known recessive mutation, common in Scandinavia
(Andersen PM et al. *Nat Genet*;10:61,1995)

Two patients alive with the 9p21 risk haplotype



Skin biopsy



Fibroblast culture



Induced pluripotent stem cell (iPS) clones

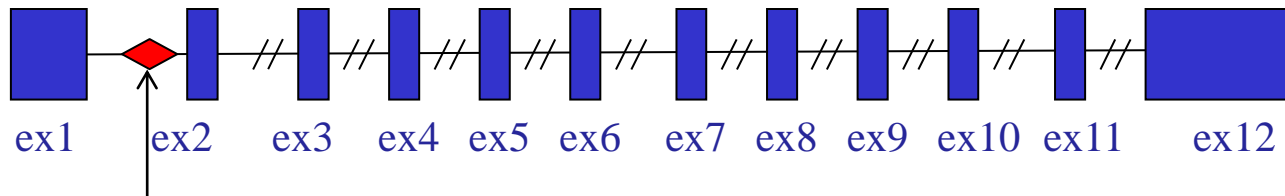
Biomedicum stem cell center

$(GGGGCC)_n$ hexanucleotide expansion in c9orf72 intron-1

Normal: 2-45 repeats

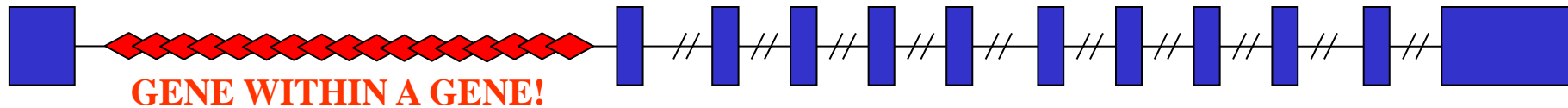
Expansion: 100 - 4000 repeats

C9ORF72 gene (28 kb)



$(GGGGCC)_n$

C9orf72 mRNAs



Renton et al Neuron 2011; DeJesus-Hernandez et al Neuron 2011

Major role of the **C9ORF72** expansion in Finnish ALS

	Family history+ (n=93)	Family history- (n=312)	All ALS (n=402)	Controls* (n=3300)
ALS	46%	21%	28%	0.15%*

Reduced penetrance?
Blood mosaicism?

* RP-PCR assay on blood DNA

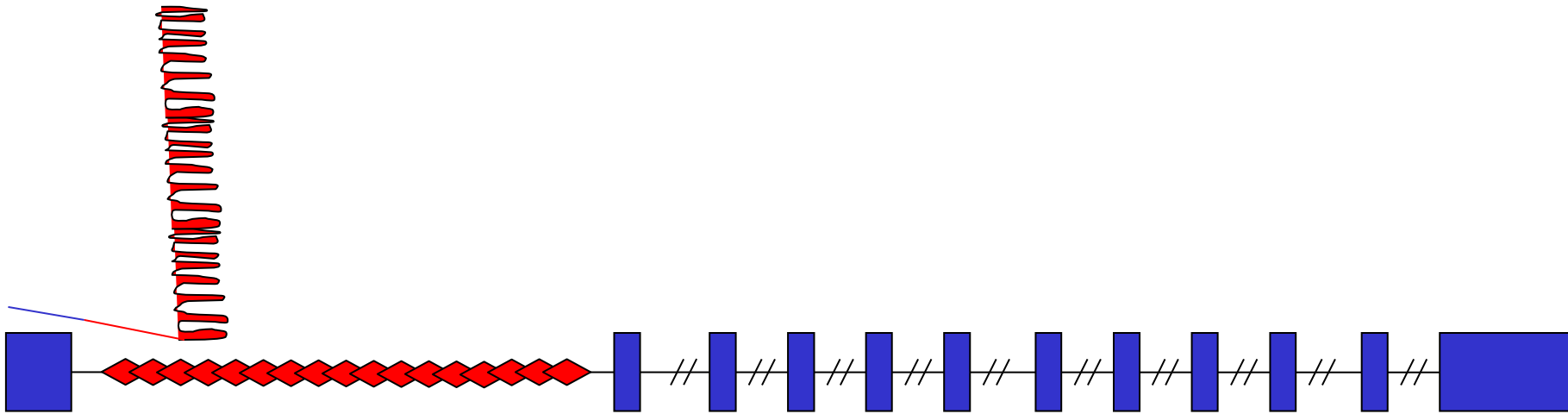
C9ORF72 expansion is also common
in Finnish frontotemporal dementia (FTD)

	Family history+ (n=27)	Family history- (n=48)	All FTD (n=75)
FTD	48%	19%	29%
(ALS	46%	21%	28%)

- RP-PCR assay on blood DNA.
- FTD patients from Dr. Anne Remes Univ. Oulu,

GAIN-OF-FUNCTION mutation

RNA G-quadruplexes



Fratta P et al *Sci Rep.* 2012;2:1016; Haeusler AR et al *Nature* 2014;507:195

C9orf72 expansion-encoded protein!

The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science*. 2013 Mar 15;339:1335-8.

Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretschmar HA, Cruts M, Van Broeckhoven C, Haass C, Edbauer D.

Unconventional translation of C9ORF72 ggggcc expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron*. 2013 Feb 20;77(4):639-46.

Ash PE, Bieniek KF, Gendron TF, Caulfield T, Lin WL, DeJesus-Hernandez M, van Blitterswijk MM, Jansen-West K, Paul JW 3rd, Rademakers R, Boylan KB, Dickson DW, Petrucelli L.

C9orf72 expansion-encoded protein!

Formation of dipeptide repeat proteins from ggggcc-repeat expansion
by
Repeat-Associated Non-atg (RAN) translation*

Sense

GGG **GCC** GGG **GCC** (**GA**)

GGG **CCG** GGG **CCG** (**GP**)

GG**C** **CGG** GG**C** **CGG** (**GR**)

Antisense

GGC **CCC** GGC **CCC** (**GP**)

GCC **CCG** **GCC** **CCG** (**GP**)

CCC **CGG** **CCC** **CGG** (**PR**)

GA, GP, GR, or PR dipeptides depending on the reading frame.

* Zu et al. Non-ATG–initiated translation directed by microsatellite expansions.
PNAS; 108:260, 2011. (SCA8 and DM1 expansion)

Study highlights the role of toxic RNA

RNA Toxicity from the ALS/FTD C9ORF72 Expansion Is Mitigated by Antisense Intervention

Donnelly CJ, Zhang PW, Pham JT, Heusler AR, Mistry NA, Vidensky S, Daley E, Poth E, Hoover B, Fines D, Maragakis N, Tienari PJ, Petrucelli L, Traynor BJ, Wang J, Rigo F, Bennett CF, Blackshaw S, **Sattler R**, **Rothstein JD**.

Neuron. 2013 Oct 16;80(2):415-28.

Based on iPSC-neurons and ALS autopsy samples



Jeffrey Rothstein
Johns Hopkins Univ.

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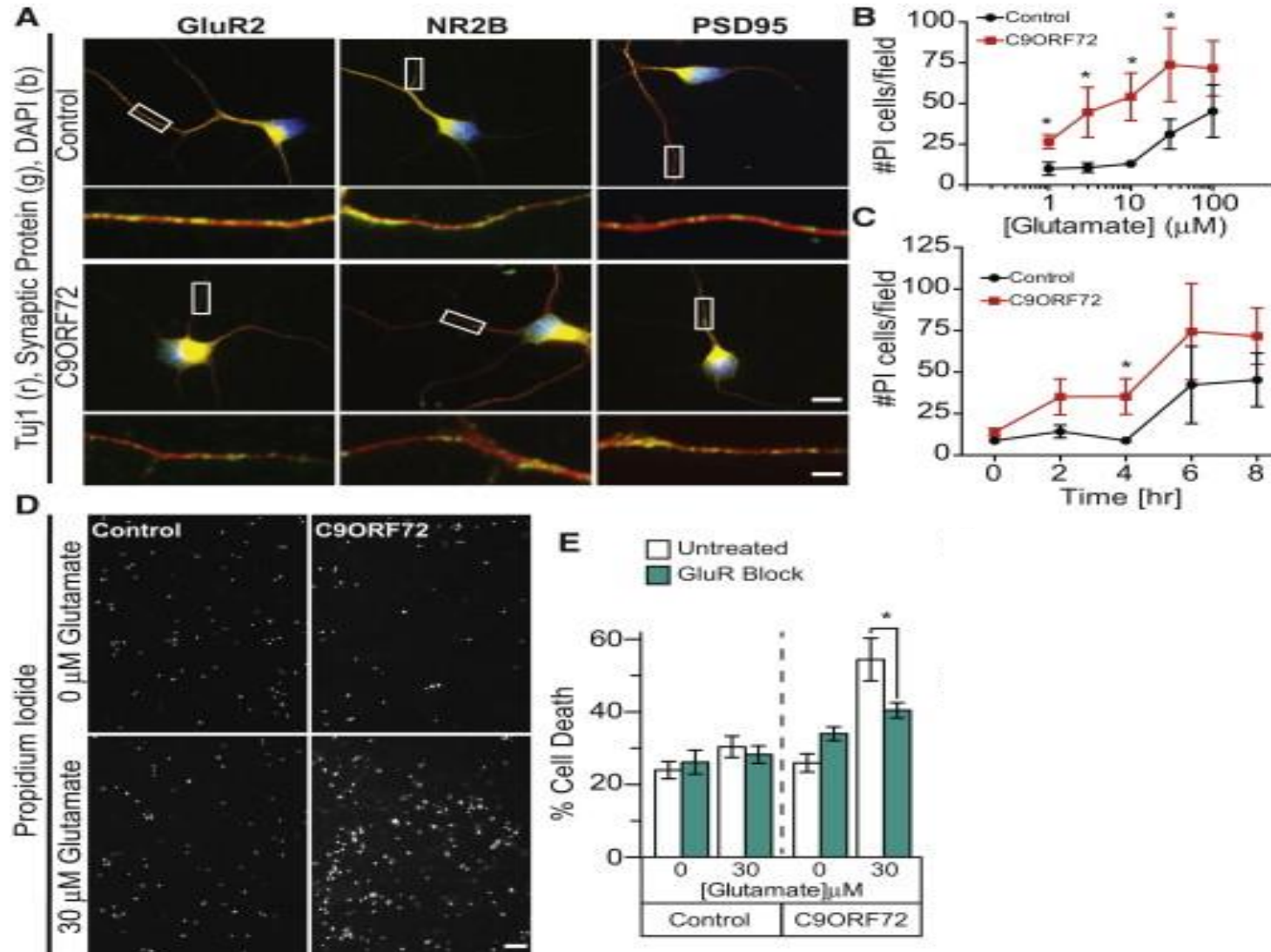
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Based on iPSC-neurons and ALS autopsy samples

- 1. Glutamate sensitivity as the consequence of the c9orf72 expansion.**

Glutamate sensitivity as the consequence of the *c9orf72* expansion



iPS neurons express glutamate receptor (GluR2), NMDA receptor (NR2B), and postsynaptic density protein-95 (PSD95),

New study highlights the role of toxic RNA

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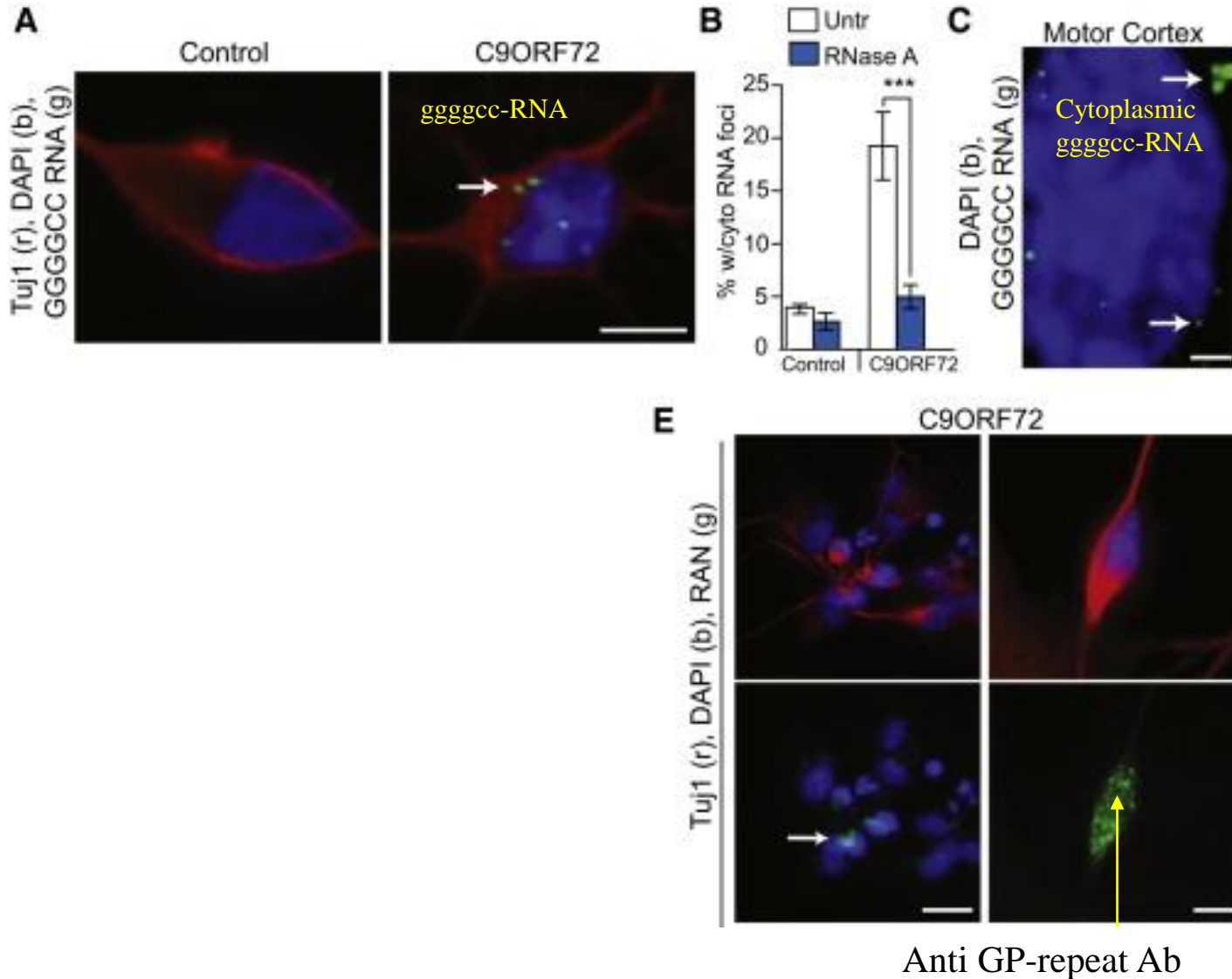
1. Glutamate sensitivity as the consequence of the c9orf72 expansion.
2. Demonstration of nuclear c9orf72 RNA ggggcc-repeat foci and dipeptide repeat proteins in ALS iPSC-neurons and brains.



GA
GP GR

C9-ALS iPS neurons and brains exhibit RNA foci and cytoplasmic RAN translation peptides

Chris Donnelly
Ping-Wu Zhang
Rita Sattler



New study highlights the role of toxic RNA

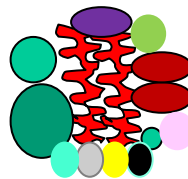
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3. 19 proteins bind to the RNA foci, one is glutamate receptor editor ADARB2.



New study highlights the role of toxic RNA

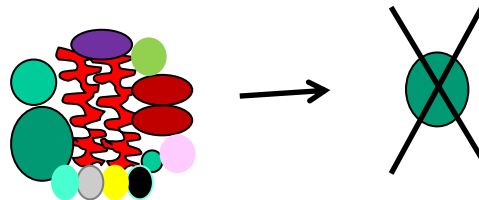
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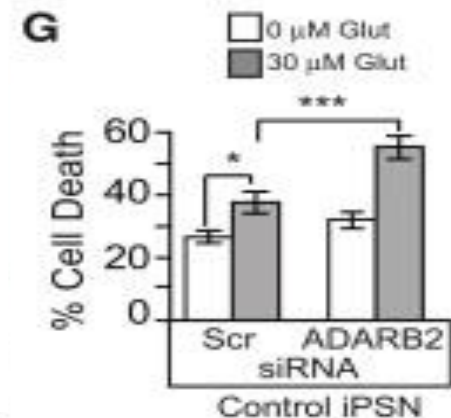
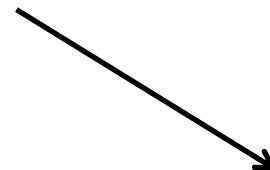
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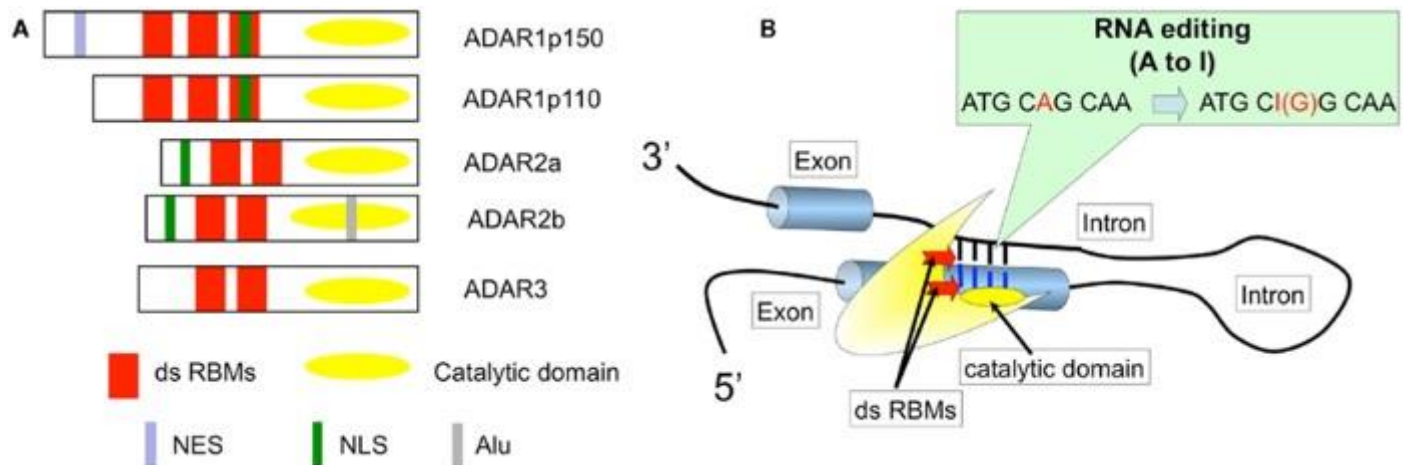
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4. ADARB2-knockdown identified as a major factor in the glutamate sensitivity



Knock-down of ADARB2 via siRNA renders **control iPS neurons** sensitive to glutamate-mediated excitotoxicity.

Central role of ADARB2 depletion in glutamate sensitivity.





ADARB2

Adenosine to inosine conversion (A-to-I RNA editing)

New study highlights the role of toxic RNA

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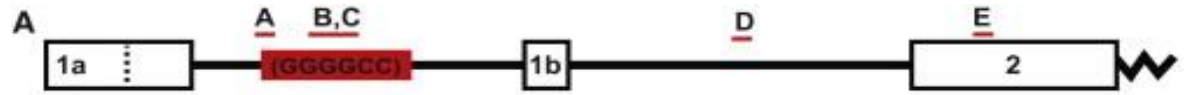
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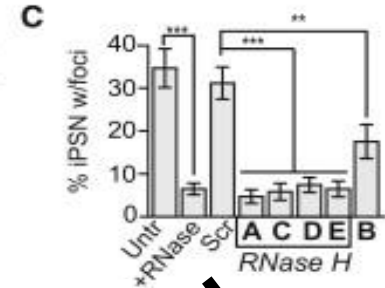
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4. ADARB2-knockdown identified as major factor in the glutamate sensitivity
5. Therapy in iPS neurons with antisense oligonucleotides.

Antisense oligonucleotide (ASO) therapy in C9-ALS iPS neurons

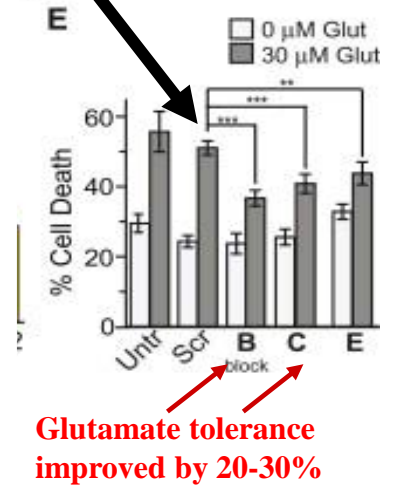
A. Position of the ASOs
(B without RNase H)



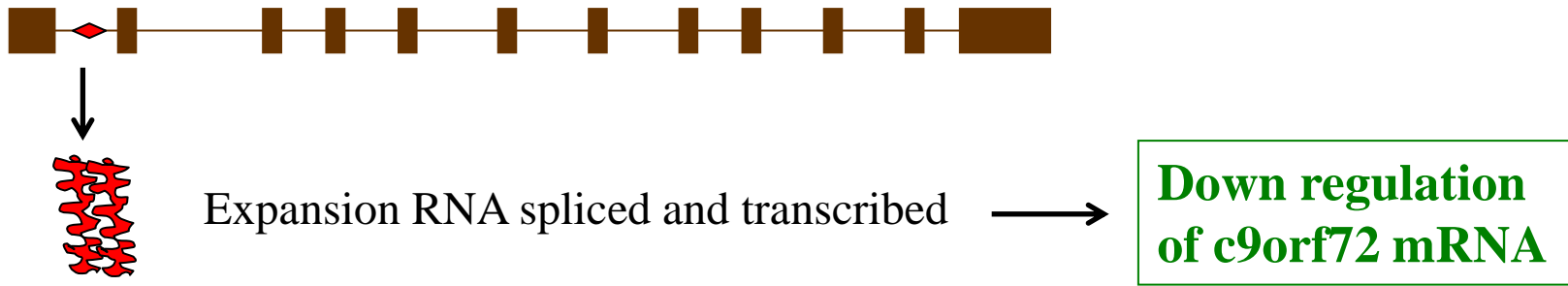
C. All ASOs inhibit the formation of RNA foci



E. ASOs B, C and E decrease the glutamate sensitivity of the C9-ALS iPS neurons.



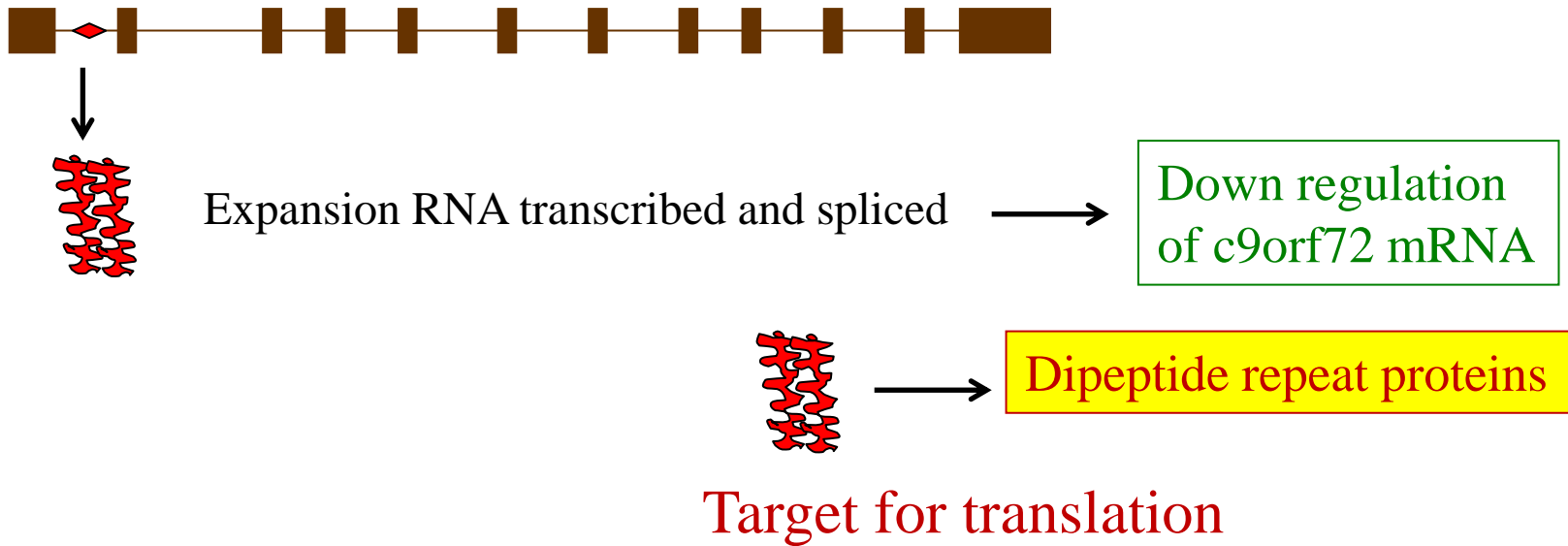
C9orf72 pathogenetic model



Probably not pathogenic:

C9ORF72 knock-out mice do not show motor neuron degeneration

C9orf72 pathogenetic model

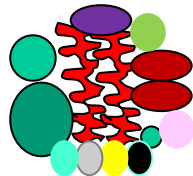


C9orf72 pathogenetic model – **toxic RNA**



Expansion RNA transcribed and spliced

Down regulation
of c9orf72 mRNA



Protein trap



Target for translation

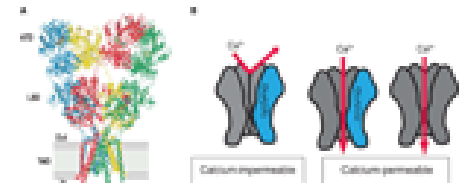
Dipeptide repeat proteins



ADARB2 knock-down

Lack of GluR2 Q/R607 site editing

Glutamate toxicity



C9orf72 pathogenetic model – 3 consequences of the toxic RNA



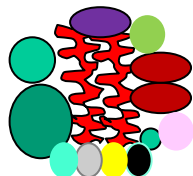
Expansion RNA transcribed and spliced



Dipeptide repeat proteins

Target for translation

Nuclear stress



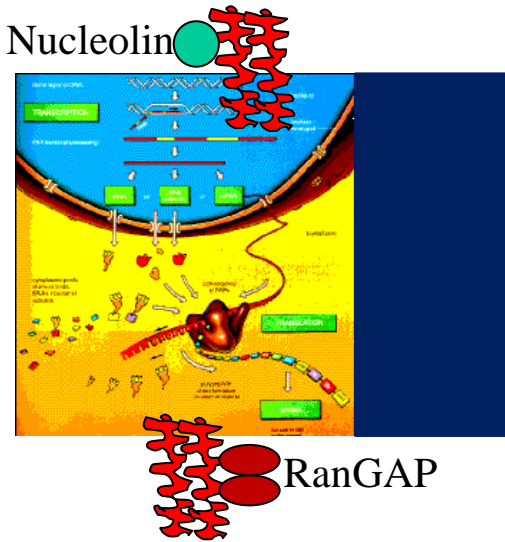
Protein trap

ADARB2 knock-down

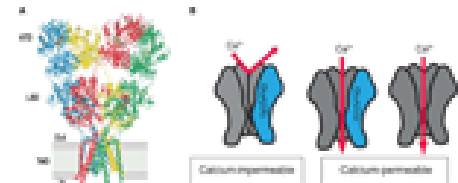


Lack of GluR2 Q/R607 site editing

Glutamate toxicity



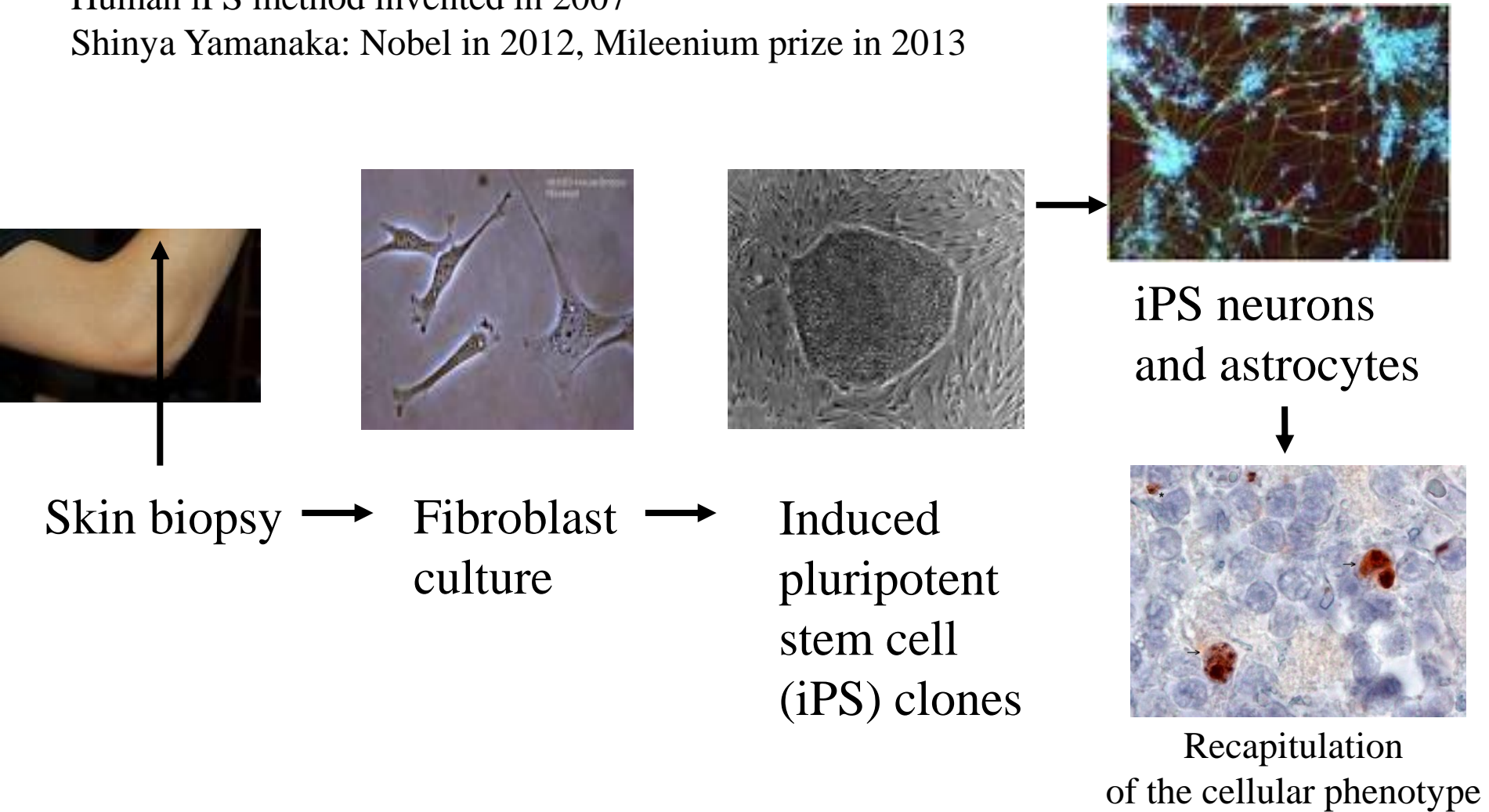
Impaired nuclear transport



Revolution in research of neurodegenerative diseases

Human iPS method invented in 2007

Shinya Yamanaka: Nobel in 2012, Mileenium prize in 2013



preALS approach to facilitate early diagnosis

