Gene Expression Profiling in ALS: Past, Present & Future

Janine Kirby
University of Sheffield

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Overview

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- Introduction
- Past
  - From pooling patient samples..
  - …to profiling individual cell types
- Present
  - Profiling classes of RNA
  - Profiling cellular compartments
- Future
  - Integral to clinical trials
  - Informing diagnosis, prognosis & treatment

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Introduction

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- Gene expression profiling
- Transcriptomics
  - cDNA arrays
  - Microarrays
    - 3'IVT
    - Exon
    - Exon & splice site (HTA)
  - RNAseq
- Quantitative measure of multiple RNA transcripts at time of sampling

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Uses in ALS

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- Disease mechanisms
- Monitoring progression
- Identifying diagnostic and prognostic biomarkers

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Past: GEP in ALS

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Journal of Neurochemistry, 2001, **77**, 132–145

Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays

Andrea Malaspina,*·†·[1] Narendra Kaushik,*·[1] and Jackie de Belleroche*

**Department of Neuromuscular Diseases, Division of Neuroscience and Psychological Medicine, Imperial College School of Medicine, London, UK*

†Foundation 'Casimiro Mondino', University of Pavia, Italy

Human spinal cord homogenates; pooled RNA; antioxidant and neuroinflammation DEG

sALS and fALS spinal cord distinguished; pro-inflammatory & RNA transcription DEG

Physiol Genomics 16: 229–239, 2004.
First published November 25, 2003; 10.1152/physiolgenomics.00087.2001.

Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter

Fernando Dangond,[1] Daehee Hwang,[2] Sandra Camelo[1], Piera Pasinelli,[3] Matthew P. Frosch,[4] Gregory Stephanopoulos,[2] George Stephanopoulos,[2] Robert H. Brown, Jr.,[3] and Steven R. Gullans[5]

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Past: GEP in ALS

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First published November 25, 2003; 10.1152/physiolgenomics.00087.2001.

SOD1 mouse models: Inflammation, metal ion dysfunctions & apoptosis

Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter

Fernando Dangond,[1] Daehee Hwang,[2] Sandra Camelo[1], Piera Pasinelli,[3] Matthew P. Frosch,[4] Gregory Stephanopoulos,[2] George Stephanopoulos,[2] Robert H. Brown, Jr.,[3] and Steven R. Gullans[5]

Disease Mechanisms Revealed by Transcription Profiling in SOD1-G93A Transgenic Mouse Spinal Cord

Mary K. Olsen, BA, Steven L. Roberds, PhD, Brenda R. Ellerbrock, BA, Timothy J. Fleck, BA, Denise K. McKinley, BA, and Mark E. Gurney, PhD

Mutations of copper,zinc-superoxide dismutase (cu,zn SOD) are found in patients with a familial form of amyotrophic lateral sclerosis. When expressed in transgenic mice, mutant human cu,zn SOD causes progressive loss of motor neurons with consequent paralysis and death. Expression profiling of gene expression in SOD1-G93A transgenic mouse spinal cords indicates extensive glial activation coincident with the onset of paralysis at 3 months of age. This is followed by activation of genes involved in metal ion regulation (metallothionein-I, metallothionein-III, ferritin-H, and ferritin-L) at 4 months of age just prior to end-stage disease, perhaps as an adaptive response to the mitochondrial destruction caused by the mutant protein. Induction of ferritin–H and –L gene expression may also limit iron catalyzed hydroxyl radical formation and consequent oxidative damage to lipids, proteins, and nucleic acids. Thus, glial activation and adaptive responses to metal ion dysregulation are features of disease in this transgenic model of familial amyotrophic lateral sclerosis.

Ann Neurol 2001;50:730–740

Journal of Neurochemistry, 2002, **80**, 158–167

Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis

Tsuyoshi Yoshihara, Shinsuke Ishigaki, Masahiko Yamamoto, Yideng Liang, Jun-ichi Niwa, Hideyuki Takeuchi, Manabu Doyu and Gen Sobue

Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

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SOD1–ALS & NRF2

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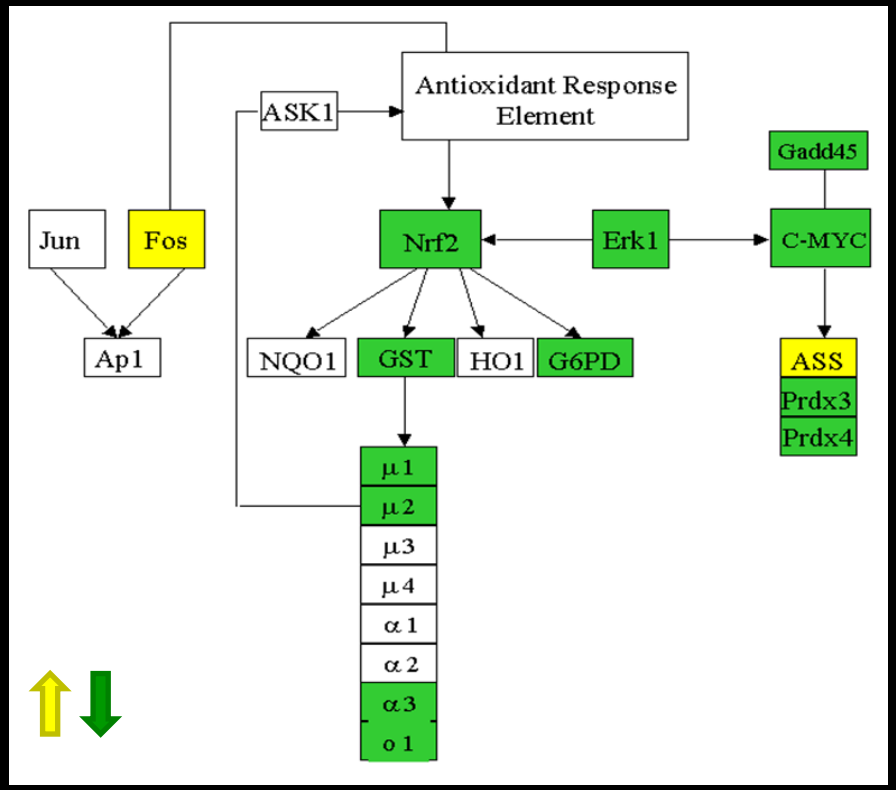
doi:10.1093/brain/awh503 *Brain* (2005), **128**, 1686–1706

Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS

Janine Kirby,[1] Eugene Halligan,[2] Melisa J. Baptista,[1] Simon Allen,[1] Paul R. Heath,[1] Hazel Holden,[1] Sian C. Barber,[1] Catherine A. Loynes,[1] Clare A. Wood-Allum,[1] Joseph Lunec[2] and Pamela J. Shaw[1]

- NSC34 cell line
- Vector only, nSOD1, G93A SOD1
- 268 genes differentially expressed
- NRF2 pathway



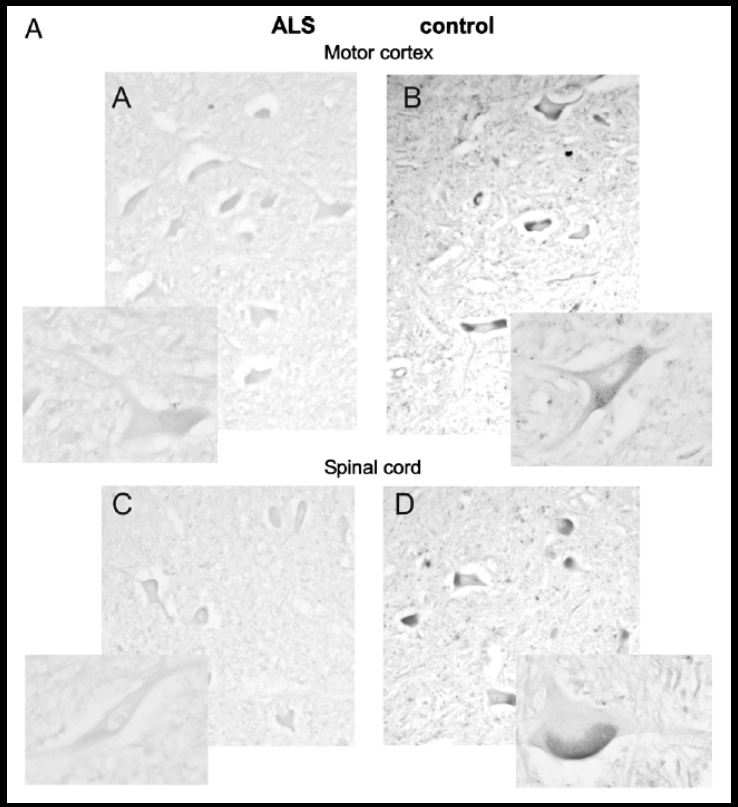
ALS & NRF2

J Neuropathol Exp Neurol. 2008 Nov;67(11):1055-62. doi: 10.1097/NEN.0b013e31818b4906.

Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis.

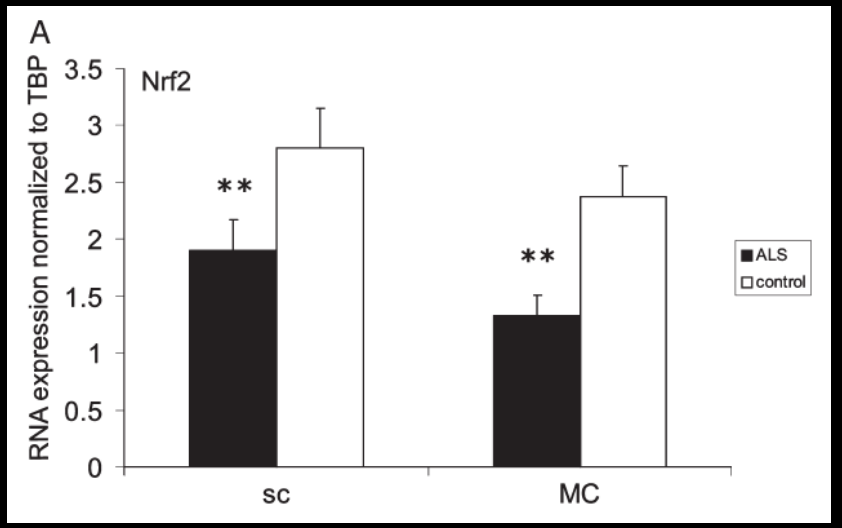
Sarlette A, Krampfl K, Grothe C, Neuhoff Nv, Dengler R, Petri S.

Department of Neurology Neuroanatomy , and Institute for Cell and Molecular Pathology , Hannover Medical School, Hannover, Germany.



Immunohistochemistry revealed decreased protein Nrf2 immunoreactivity in MNs ALS v Controls

q-PCR revealed decreased Nrf2 mRNA in MNs ALS v Controls



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ALS & NRF2

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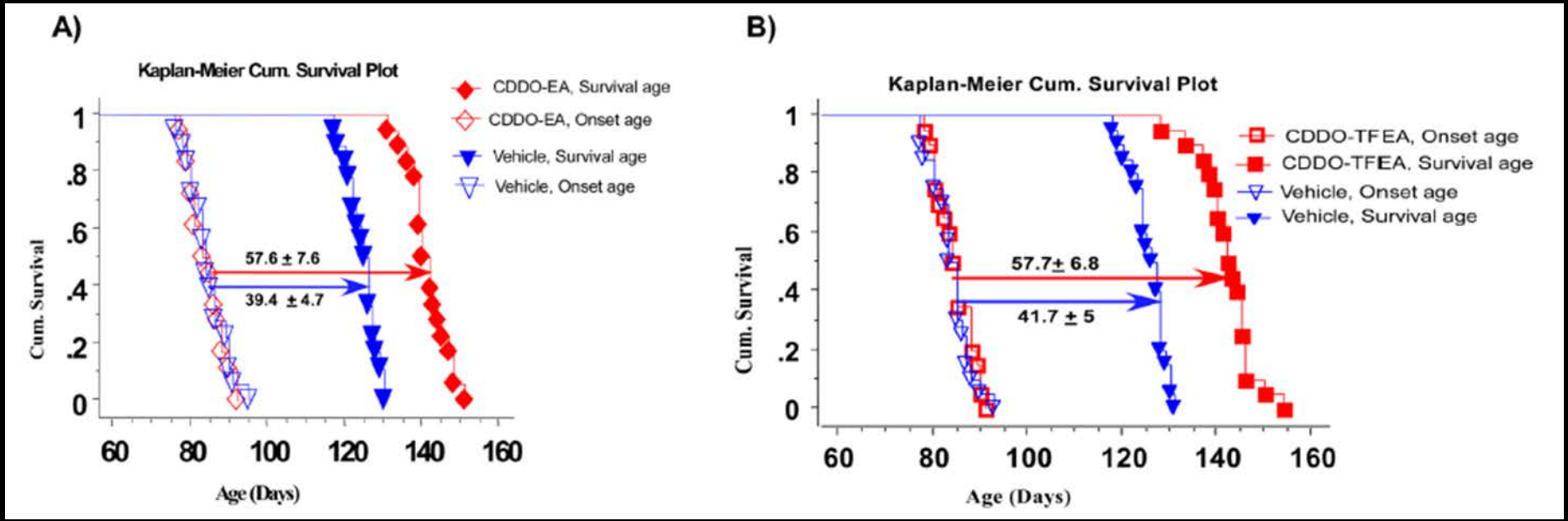
Free Radic Biol Med. 2011 Jul 1;51(1):88-96. doi: 10.1016/j.freeradbiomed.2011.03.027. Epub 2011 Mar 30.

Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis.

Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M, Liby KT, Risingsong R, Sporn M, Beal MF, Kiaei M.

Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York-Presbyterian Hospital, New York, NY 10065, USA.



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ALS & NRF2

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Free Radical Biology and Medicine 61 (2013) 438–452

Contents lists available at SciVerse ScienceDirect

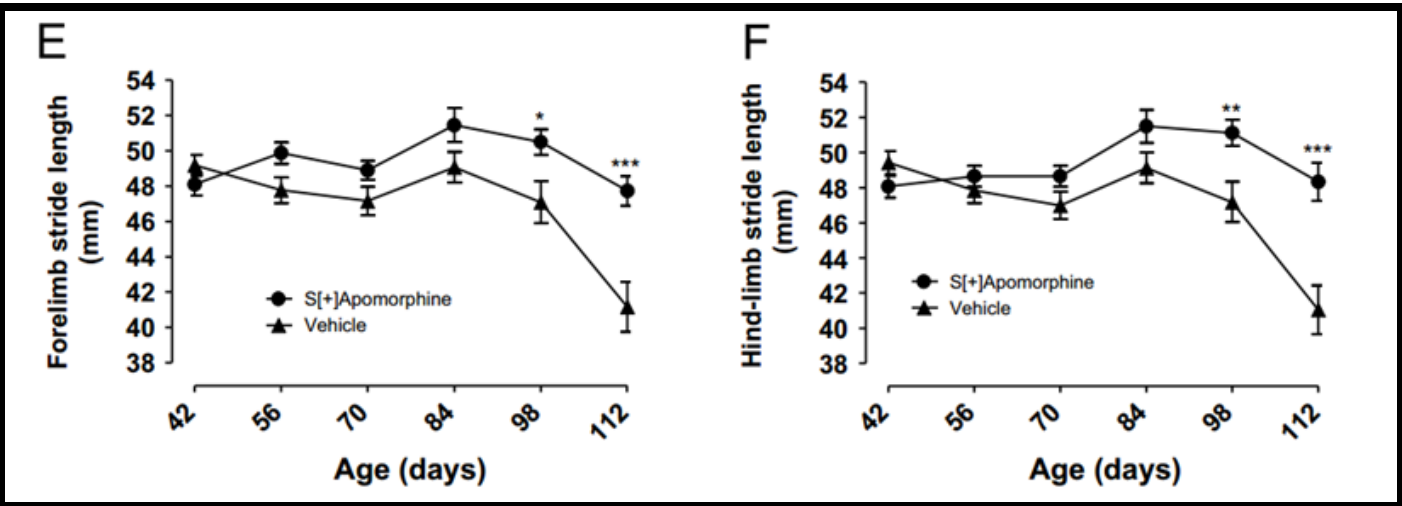
Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

S[+] Apomorphine is a CNS penetrating activator of the Nrf2-ARE pathway with activity in mouse and patient fibroblast models of amyotrophic lateral sclerosis ☆

Richard J. Mead [a,1], Adrian Higginbottom [a,1], Scott P. Allen [a], Janine Kirby [a], Ellen Bennett [a], Siân C. Barber [a], Paul R. Heath [a], Antonio Coluccia [b], Neelam Patel [a], Iain Gardner [c], Andrea Brancale [b], Andrew J. Grierson [a], Pamela J. Shaw [a,*]



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ALS & PTEN/PI3K

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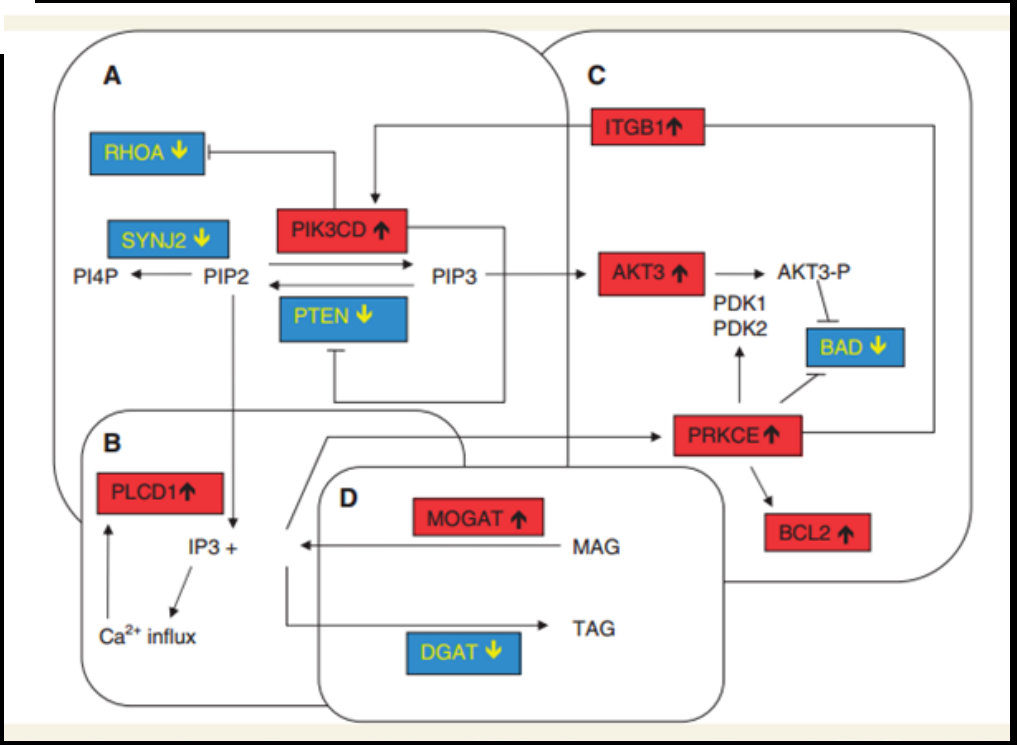
doi:10.1093/brain/awq345 Brain 2011: Page 1 of 12

BRAIN
A JOURNAL OF NEUROLOGY

Phosphatase and tensin homologue/protein kinase B pathway linked to motor neuron survival in human superoxide dismutase 1-related amyotrophic lateral sclerosis

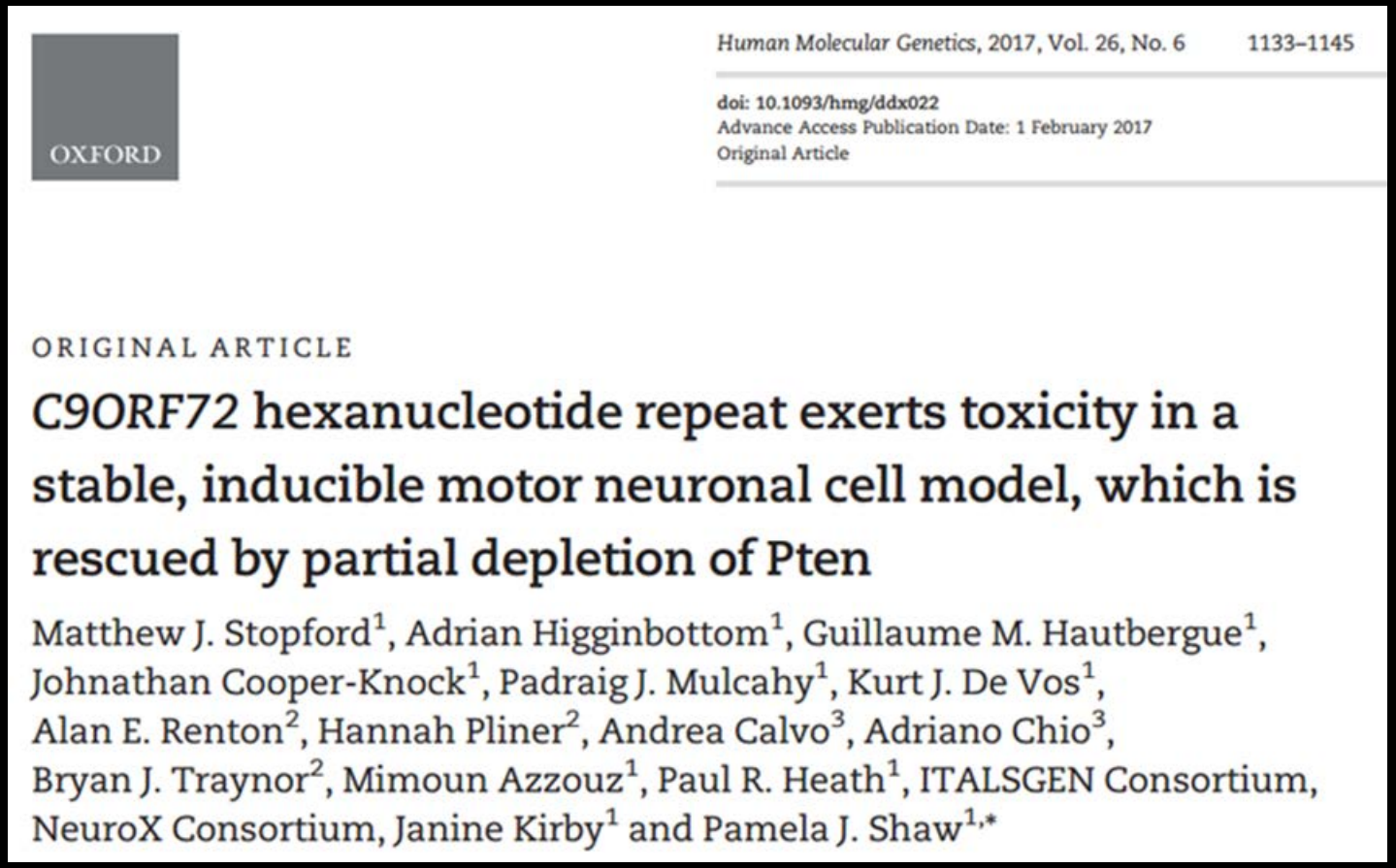
Janine Kirby,[1,*] Ke Ning,[1,*] Laura Ferraiuolo,[1] Paul R. Heath,[1] Azza Ismail,[1] Su-Wei Kuo,[1] Chiara F. Valori,[1] Laura Cox,[1] Basil Sharrack,[2] Stephen B. Wharton,[3] Paul G. Ince,[3] Pamela J. Shaw[1,†] and Mimoun Azzouz[1,†]

"Surviving" motor neurones at post-mortem express cell survival genes



ALS & PTEN/PI3K

PTEN/PI3K also implicated in C9ORF72-ALS through transcriptomic analysis



OXFORD

Human Molecular Genetics, 2017, Vol. 26, No. 6 1133–1145

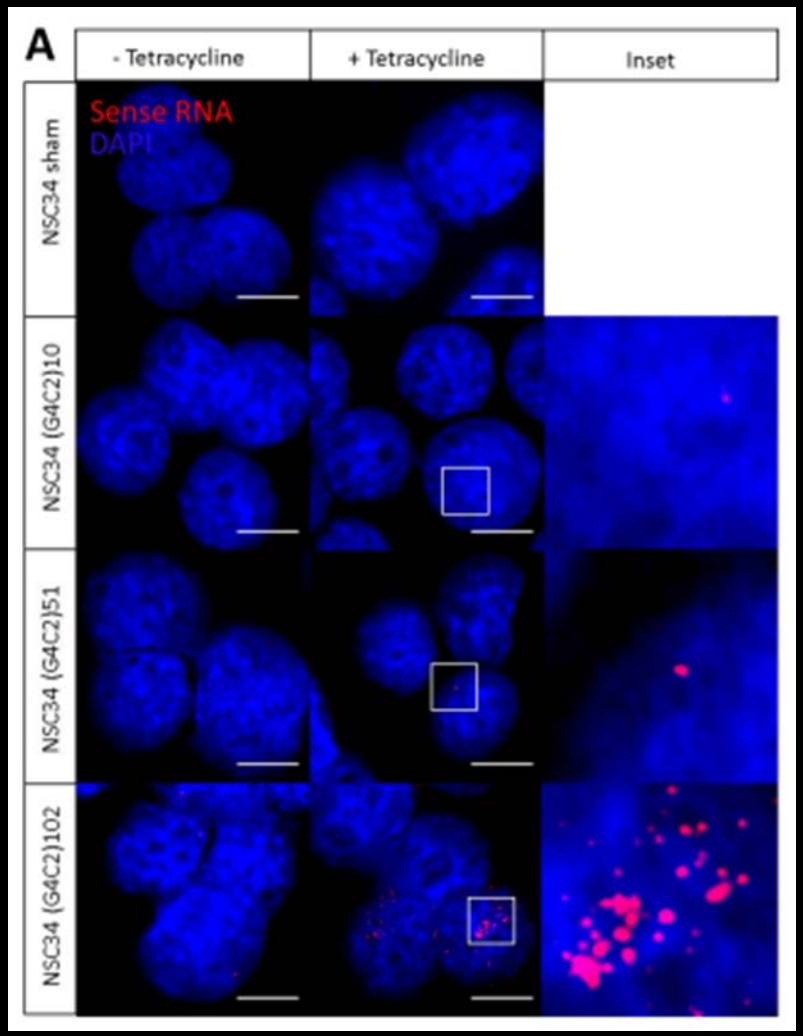
doi: 10.1093/hmg/ddx022
Advance Access Publication Date: 1 February 2017
Original Article

ORIGINAL ARTICLE

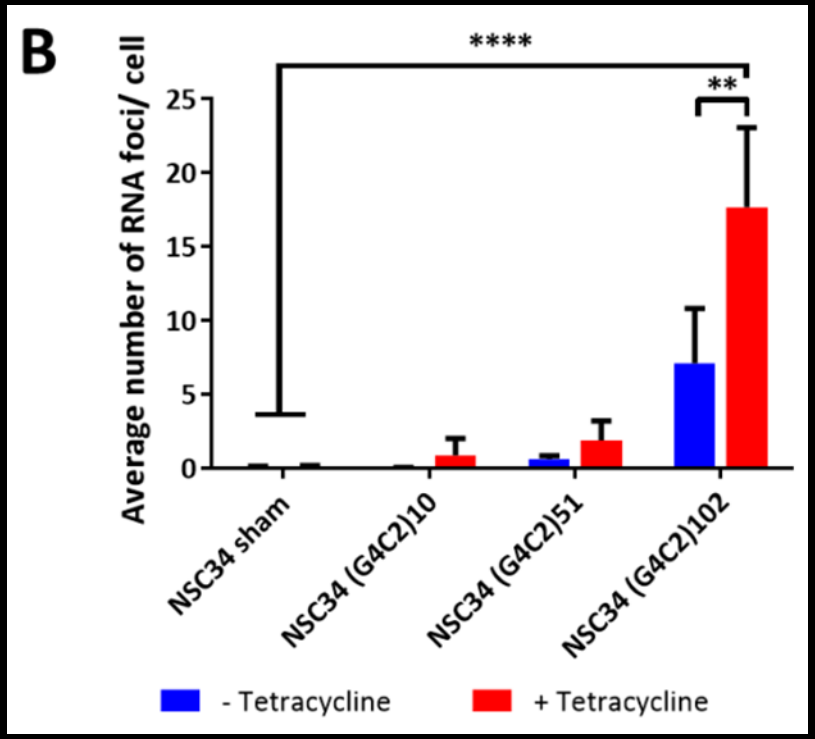
C9ORF72 hexanucleotide repeat exerts toxicity in a stable, inducible motor neuronal cell model, which is rescued by partial depletion of Pten

Matthew J. Stopford[1], Adrian Higginbottom[1], Guillaume M. Hautbergue[1], Johnathan Cooper-Knock[1], Padraig J. Mulcahy[1], Kurt J. De Vos[1], Alan E. Renton[2], Hannah Pliner[2], Andrea Calvo[3], Adriano Chio[3], Bryan J. Traynor[2], Mimoun Azzouz[1], Paul R. Heath[1], ITALSGEN Consortium, NeuroX Consortium, Janine Kirby[1] and Pamela J. Shaw[1,*]

(G4C2)n Cell Model



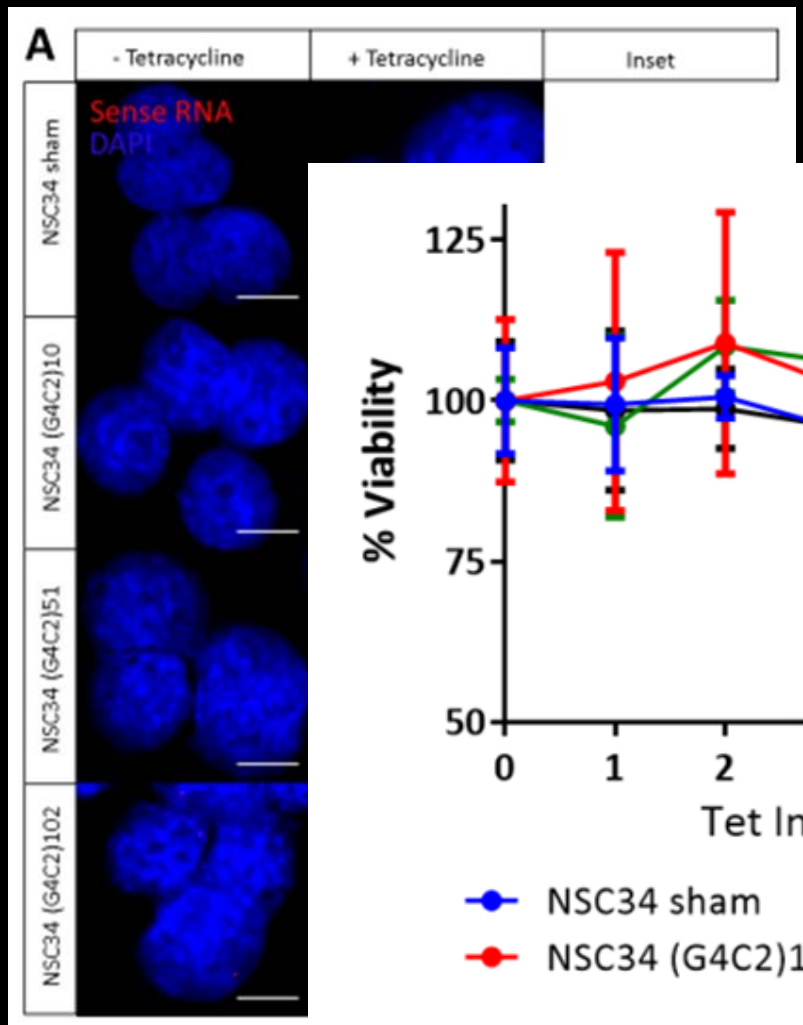
Isogenic inducible NSC34 model with 10, 51 & 102 (G4C2) interrupted repeats

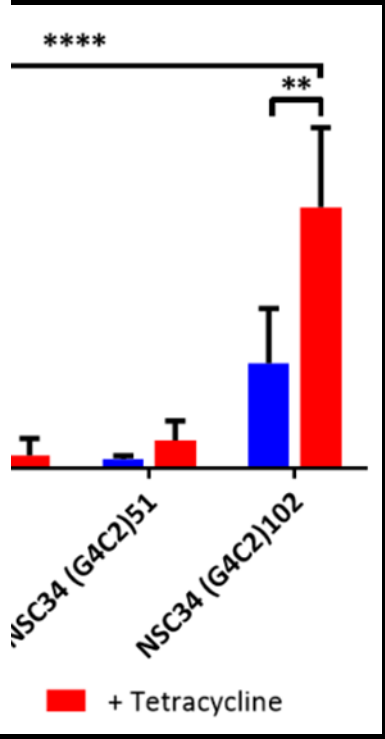


Matt Stopford

(G4C2)n Cell Model

Isogenic inducible NSC34 model with 10, 51 & 102 ...bted repeats





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(G4C2)n Cell Model

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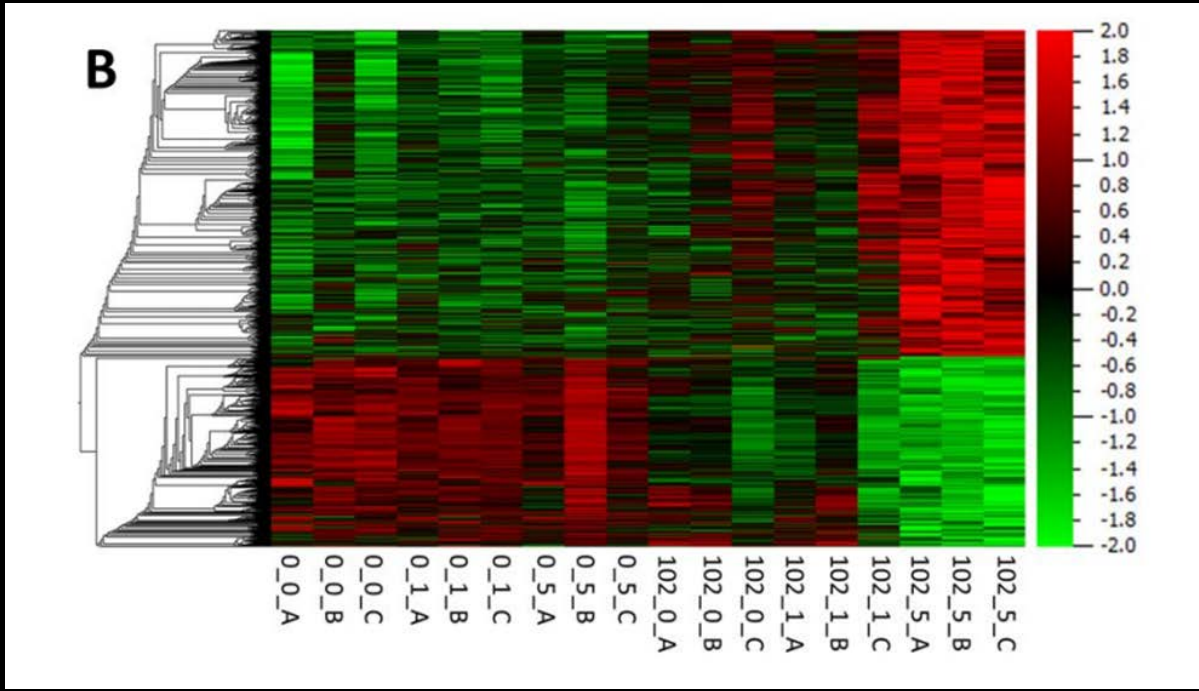
NSC34 Sham (G4C2) 102 rpts



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- (G4C2) 102 rpts v NSC34 sham (+5d Tet)
  - Protein transport, vesicle-mediated transport
  - RNA metabolism
  - PI3K/AKT pathway

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PTEN/PI3K/AKT

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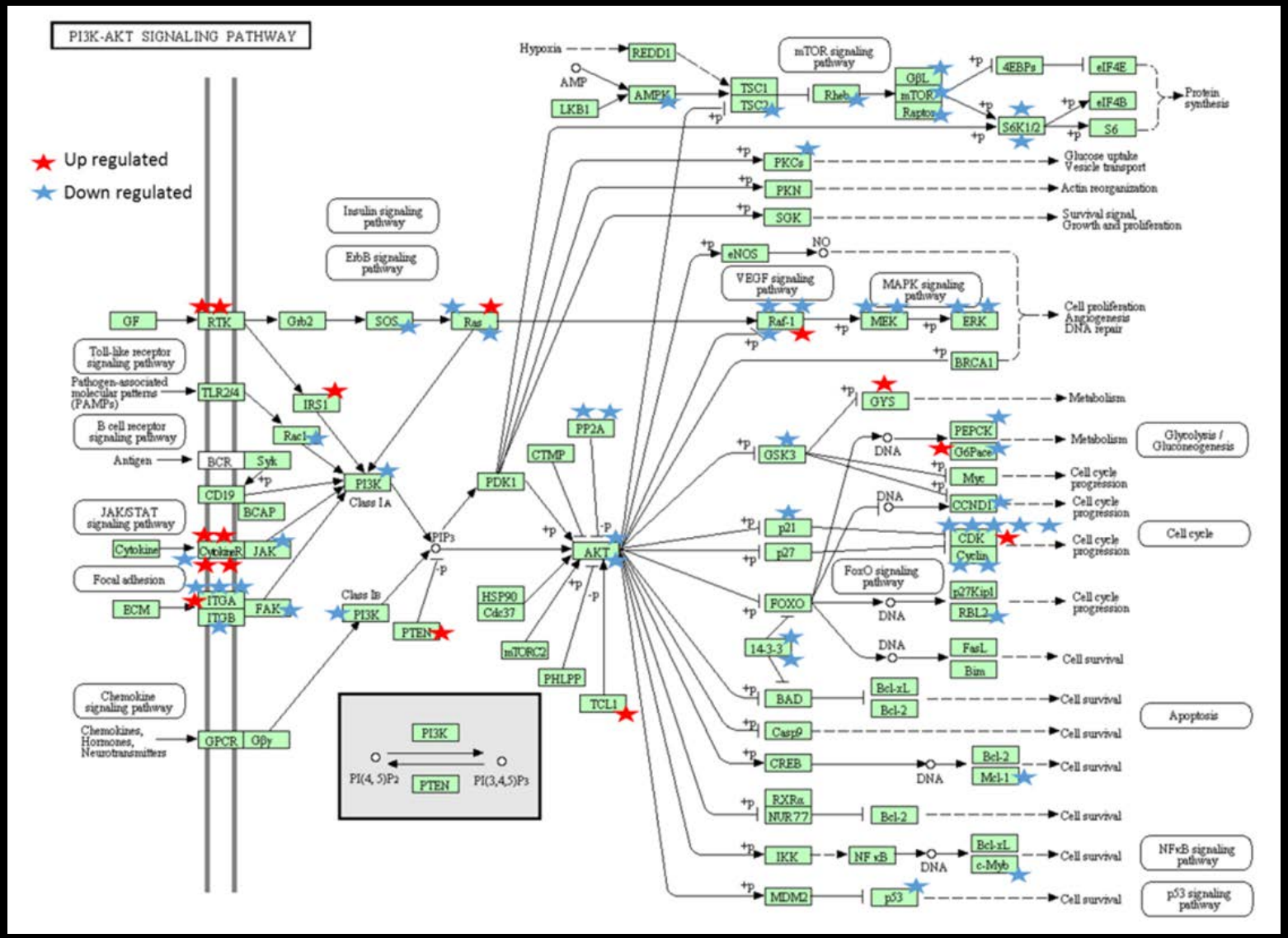

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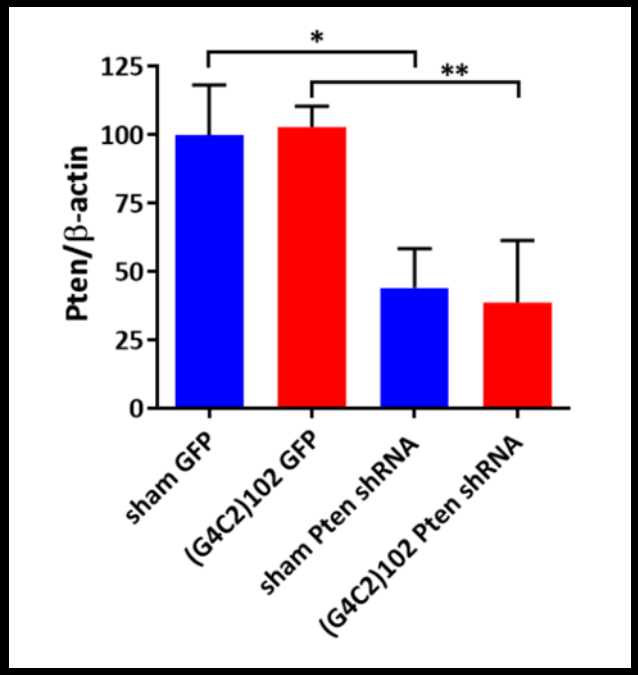
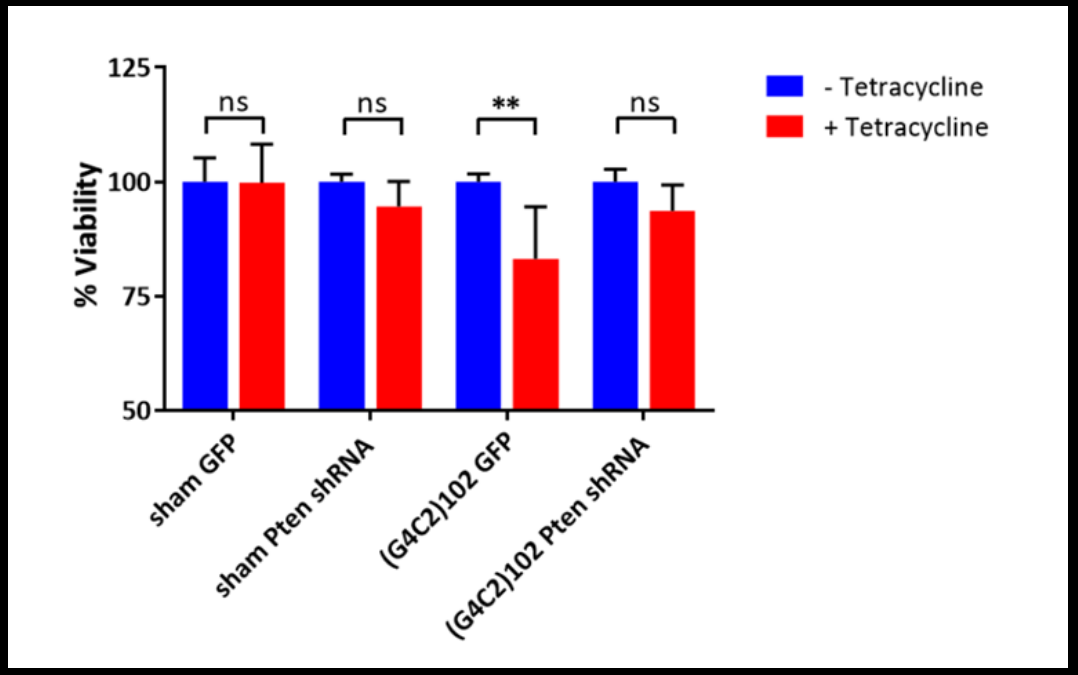
PTEN Knockdown Rescues Toxicity

Q-PCR: PTEN knockdown in sham & (G4C2)102



Cell Viability Assay: Knockdown of PTEN in induced (G4C2)102 cells rescues cell viability



Matt Stopford

RNA Splicing

Neuropathology and Applied Neurobiology (2014), **40**, 670–685 doi: 10.1111/nan.12148

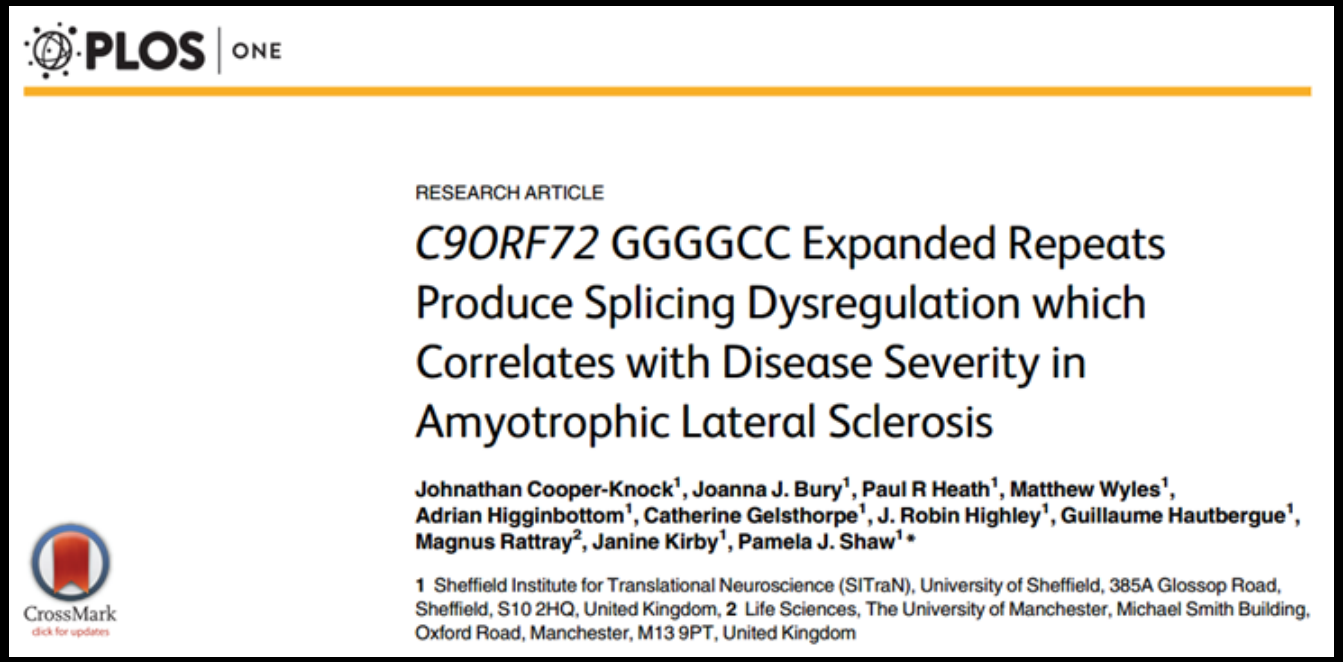
Loss of nuclear TDP-43 in amyotrophic lateral sclerosis (ALS) causes altered expression of splicing machinery and widespread dysregulation of RNA splicing in motor neurones

J. R. Highley*[1], J. Kirby*[1], J. A. Jansweijert, P. S. Webb*, C. A. Hewamadduma*‡, P. R. Heath*, A. Higginbottom*, R. Raman*, L. Ferraiuolo*, J. Cooper-Knock*, C. J. McDermott*, S. B. Wharton*, P. J. Shaw*[1] and P. G. Ince*[1]

*Sheffield Institute for Translational Neuroscience (SITraN) and ‡MRC Centre for Developmental and Biomedical Genetics, Firth Court, University of Sheffield, Sheffield, UK, and †Academisch Medisch Centrum, Amsterdam, The Netherlands

- Spinal cord MNs with TDP-43 pathology revealed significant dysregulation of splicing
  - Differentially spliced genes enriched for ribonucleotide binding
  - Also enriched for ALS genetic loci (15/24 loci diff. spliced)
- Mutant TARDBP fibroblasts also showed splicing dysregulation
  - Differentially spliced genes enriched for ribonucleotide binding
  - More diff. spliced genes in TARDBP-ALS v SOD1-ALS & SALS

RNA Splicing



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]*

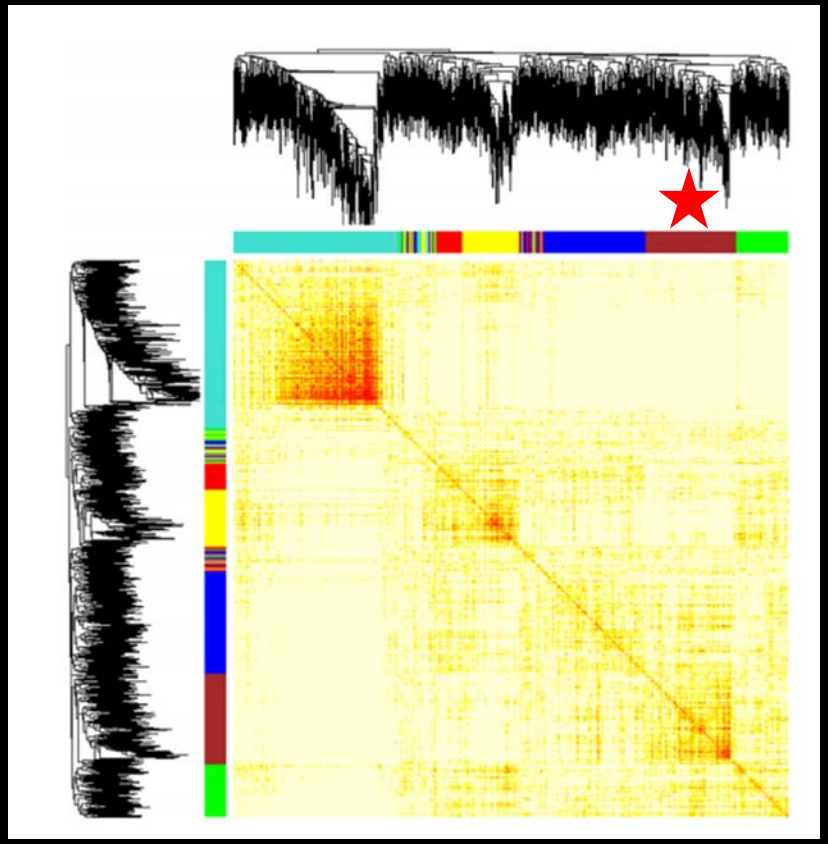
1 Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, 385A Glossop Road, Sheffield, S10 2HQ, United Kingdom, **2** Life Sciences, The University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, United Kingdom

CrossMark click for updates

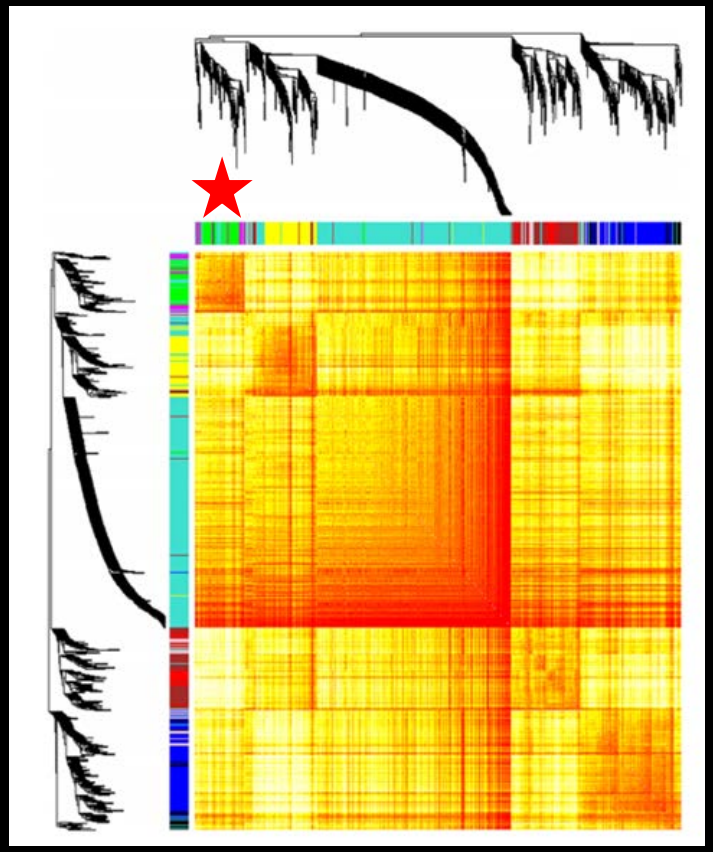
- C9ORF72-ALS spinal cord MNs – 3'IVT arrays
- Lymphoblastoid cell lines – exon arrays
- Weighted gene co-expression network analysis (WCGNA) performed

RNA Splicing

Isolated C9ORF72-ALS spinal cord MNs



C9ORF72-ALS LCLs



54% “RNA processing” gene DE in MNs also DE in LCLs

Past: Disease Mechanisms

- Investigating disease mechanisms
  - NRF2 pathway
  - PTEN/PI3K/AKT pathway

  These pathways are viable targets for therapeutic invention

  - Further evidence for dysregulated RNA splicing

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Uses in ALS

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- Disease mechanisms
  - NRF2 pathway
  - PTEN/AKT pathway
  - RNA splicing
- Monitoring progression
- Identifying diagnostic and prognostic biomarkers

Monitoring Progression

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LCM of MNs G93A SOD1 mouse; very few changes in presymptomatic; increase in cell growth / maintenance genes

Human Molecular Genetics, 2005, Vol. 14, No. 21 **3309–3320**
doi:10.1093/hmg/ddi357
Advance Access published on September 28, 2005

No widespread induction of cell death genes occurs in pure motoneurons in an amyotrophic lateral sclerosis mouse model

Florence E. Perrin[1], Gaelle Boisset[1], Mylene Docquier[2], Olivier Schaad[2], Patrick Descombes[2] and Ann C. Kato[1,*]

Neurobiology of Disease

Microarray Analysis of the Cellular Pathways Involved in the Adaptation to and Progression of Motor Neuron Injury in the SOD1 G93A Mouse Model of Familial ALS

Laura Ferraiuolo, Paul R. Heath, Hazel Holden, Paul Kasher, Janine Kirby, and Pamela J. Shaw
Academic Neurology Unit, Section of Neuroscience, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield S10 2RX, United Kingdom

Changes in presymptomatic SOD1 G93A mice due to homogenous background; late-stage transcriptional repression

Monitoring Progression

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LCM of MNs G93A SOD1 mouse; very few changes in presymptomatic; increase in cell growth / maintenance genes

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Florence E. Perrin[1], Gaelle Boisset[1], Mylene Docquier[2], Olivier Schaad[2], Patrick Descombes[2] and Ann C. Kato[1,*]

Changes in presymptomatic SOD1 G93A mice due to homogenous background; late-stage transcriptional repression

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Laura Ferraiuolo, Paul R. Heath, Hazel Holden, Paul Kasher, Janine Kirby, and Pamela J. Shaw
Academic Neurology Unit, Section of Neuroscience, School of Medicine and Biomedical Sciences, University of Sheffiel
United Kingdom

Evidence of reduced metabolic support in astrocytes, with reduced lactate levels and p75 activation.

doi:10.1093/brain/awr193 Brain 2011: 134; 2627–2641 | 2627

BRAIN
A JOURNAL OF NEUROLOGY

Dysregulation of astrocyte–motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis

Laura Ferraiuolo,[1] Adrian Higginbottom,[1] Paul R. Heath,[1] Sian Barber,[1] David Greenald,[2] Janine Kirby[1] and Pamela J. Shaw[1]

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ALS Biomarkers

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Published Ahead of Print on February 22, 2017 as 10.1212/WNL.0000000000003741

Urinary p75^{ECD}

A prognostic, disease progression, and pharmacodynamic biomarker in ALS

OPEN

Stephanie R. Shepheard, PhD
Joanne Wuu, ScM
Michell Cardoso, MSc
Luke Wiklendt, PhD
Phil G. Dinning, PhD
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ABSTRACT

Objective: To evaluate urinary neurotrophin receptor p75 extracellular domain (p75^{ECD}) levels as disease progression and prognostic biomarkers in amyotrophic lateral sclerosis (ALS).

Methods: The population in this study comprised 45 healthy controls and 54 people with ALS, 31 of whom were sampled longitudinally. Urinary p75^{ECD} was measured using an enzyme-linked immunoassay and validation included intra-assay and inter-assay coefficients of variation, effect of circadian rhythm, and stability over time at room temperature, 4°C, and repeated freeze-thaw cycles. Longitudinal changes in urinary p75^{ECD} were examined by mixed model analysis, and the prognostic value of baseline p75^{ECD} was explored by survival analysis.

Results: Confirming our previous findings, p75^{ECD} was higher in patients with ALS (5.6 ± 2.2 ng/mg creatinine) compared to controls (3.6 ± 1.4 ng/mg creatinine, $p < 0.0001$). Assay reproducibility was high, with p75^{ECD} showing stability across repeated freeze-thaw cycles, at room temperature and 4°C for 2 days, and no diurnal variation. Urinary p75^{ECD} correlated with the revised ALS Functional Rating Scale at first evaluation ($r = -0.44$, $p = 0.008$) and across all study visits ($r = -0.36$, $p < 0.0001$). p75^{ECD} also increased as disease progressed at an average rate of 0.19 ng/mg creatinine per month ($p < 0.0001$). In multivariate prognostic analysis, bulbar onset (hazard ratio [HR] 3.0, $p = 0.0035$), rate of disease progression from onset to baseline (HR 4.4, $p < 0.0001$), and baseline p75^{ECD} (HR 1.3, $p = 0.0004$) were predictors of survival.

Conclusions: The assay for urinary p75^{ECD} is analytically robust and shows promise as an ALS biomarker with prognostic, disease progression, and potential pharmacodynamic application. Baseline urinary p75^{ECD} provides prognostic information and is currently the only biological fluid-based biomarker of disease progression. *Neurology*® 2017;88:1-7

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Overview

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  - …to profiling individual cell types
- Present
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  - Profiling cellular compartments
- Future
  - Integral to clinical trials
  - Informing diagnosis, prognosis & treatment

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Present: miRNA Biomarkers

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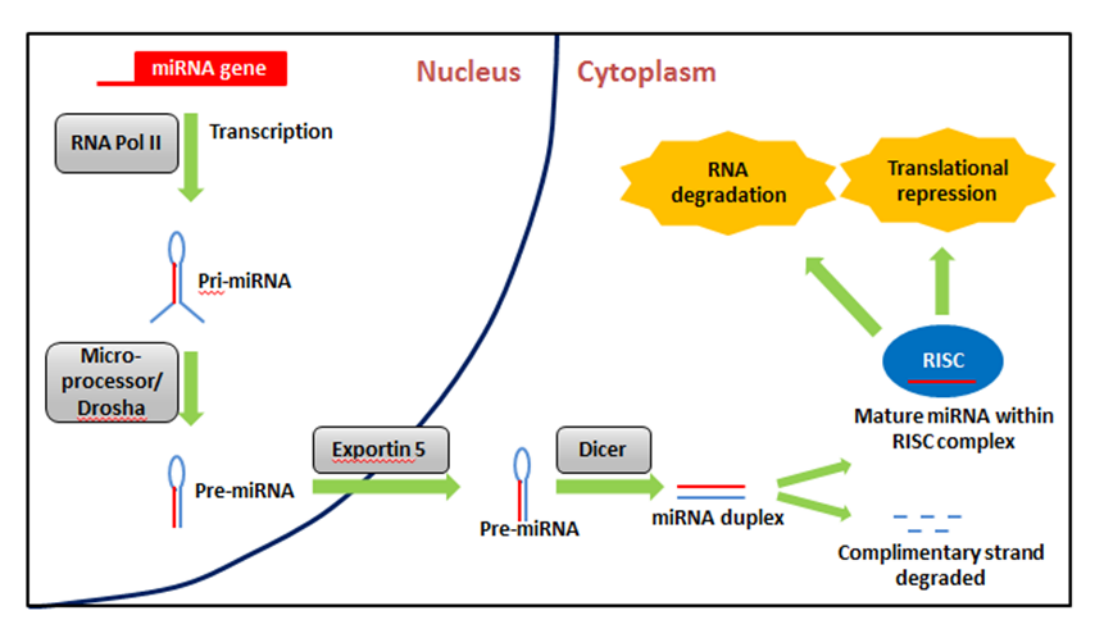
- Non-coding single stranded 22-mer RNA
  - Regulate gene expression; multiple targets
  - Stable and detectable in blood
  - Reflect physiological conditions



Emily Goodall

The University Of Sheffield.

miRNA Biomarkers

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Neurobiology of Aging xxx (2017) 1–9

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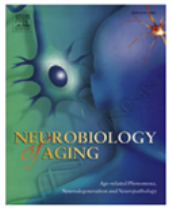
Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging



Serum miRNAs miR-206, 143-3p and 374b-5p as potential biomarkers for amyotrophic lateral sclerosis (ALS)

Rachel Waller[a,1], Emily F. Goodall[a,1], Marta Milo[b], Jonathan Cooper-Knock[a], Marc Da Costa[a], Esther Hobson[a], Mbombe Kazoka[a], Helen Wollff[a], Paul R. Heath[a], Pamela J. Shaw[a,1], Janine Kirby[a,*,1]

[a] Department of Neuroscience, Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK
[b] Department of Biomedical Science, University of Sheffield, Sheffield, UK

- 27 SALS patients, 25 controls & 36 ALS mimics
- Taqman Low Density Arrays (TLDA cards) 750 miRNAs
- Validated miRNA changes in 2nd cohort using mirScript
- Investigated miRNA changes over disease progression

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miRNA Biomarkers

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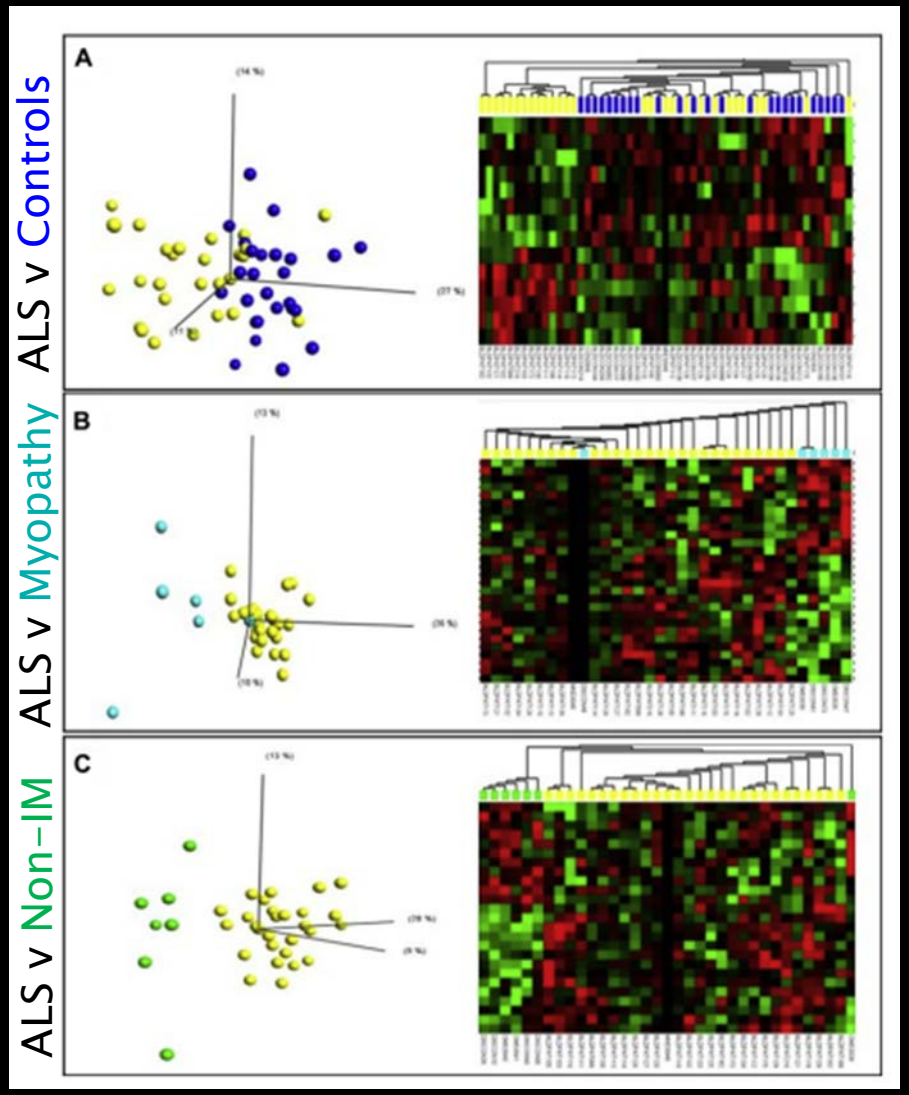
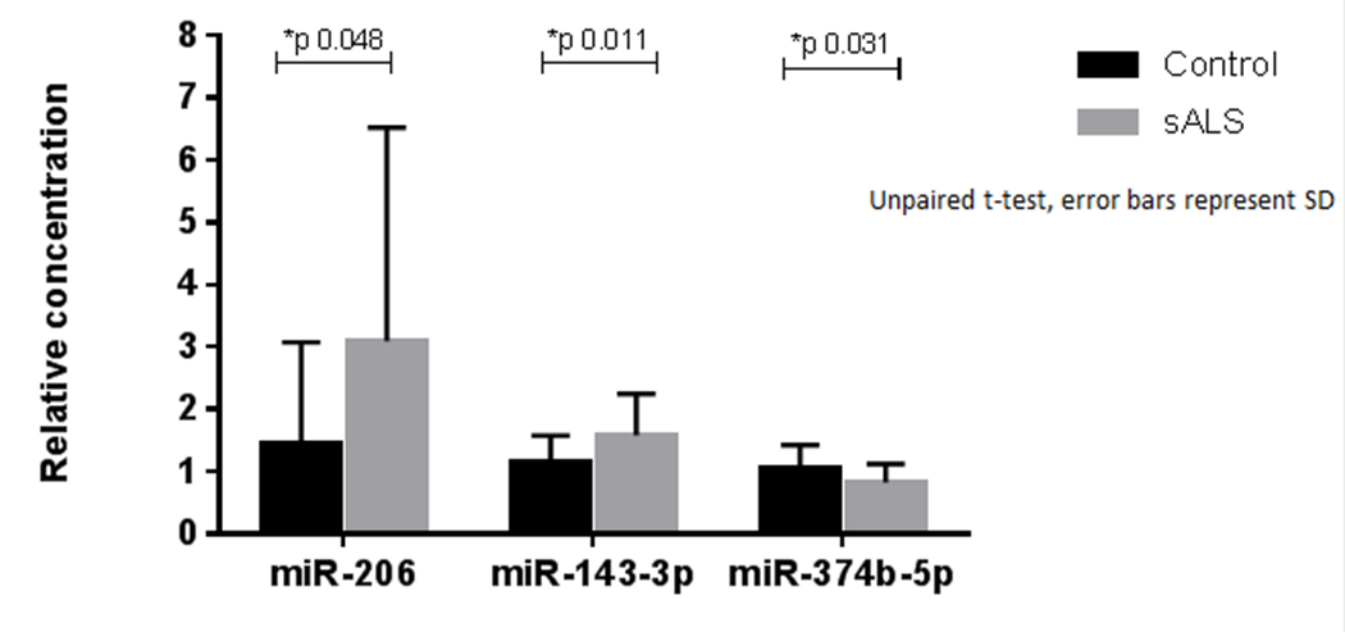
- Diff Exp miRNA ($p<0.05$)
  - sALS v Con 12
  - sALS v Myopathy 28
  - sALS v Non-IM 25
- Validation cohort
  - 23 sALS v 22 Controls
  - 12 miRNAs tested
  - 3 validated



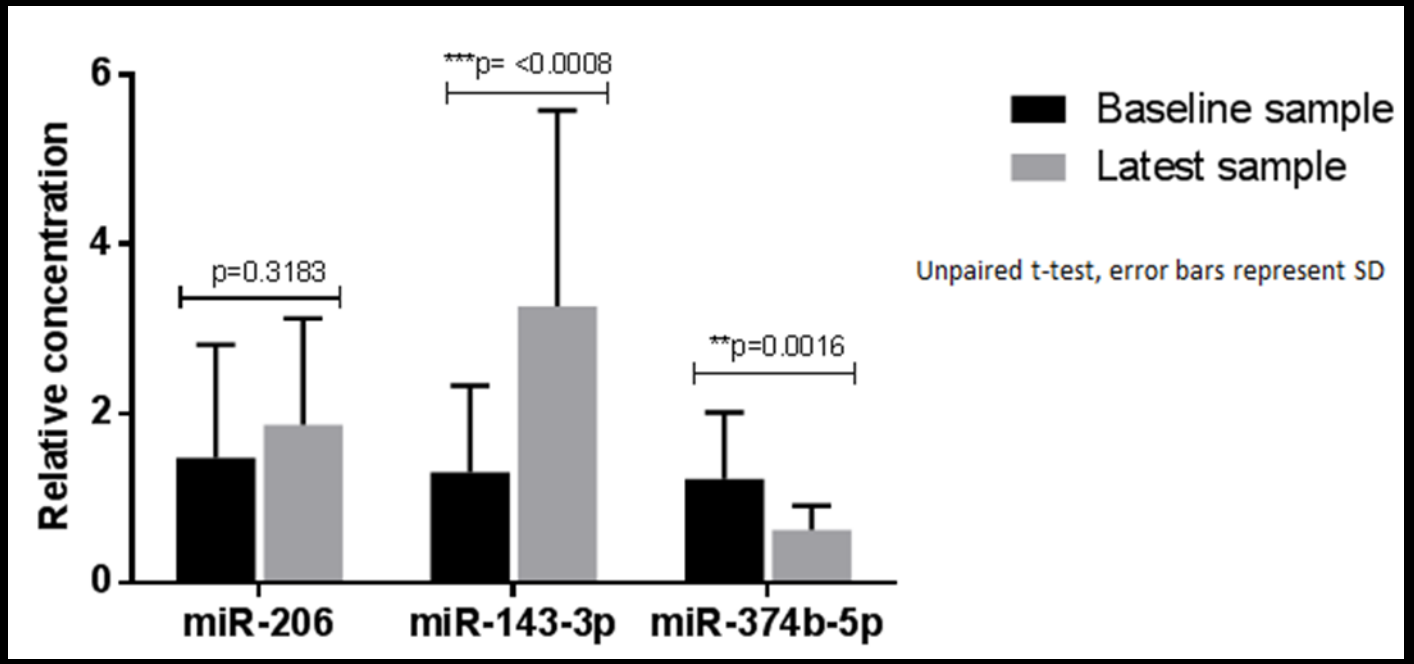
miRNA Biomarkers



- miR-206 ⇧
- miR-143-3p ⇧
- miR-374b-5p ⇩
- No change in response due to riluzole or site of disease onset (B v UL v LL)

miRNA Biomarkers

- Longitudinal Study
  - Diagnosis v >3mths later (n=21 ALS)
  - miR-143-3p increased with progression
  - miR-374b-5p decreased with progression



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Profiling Cellular Compartments

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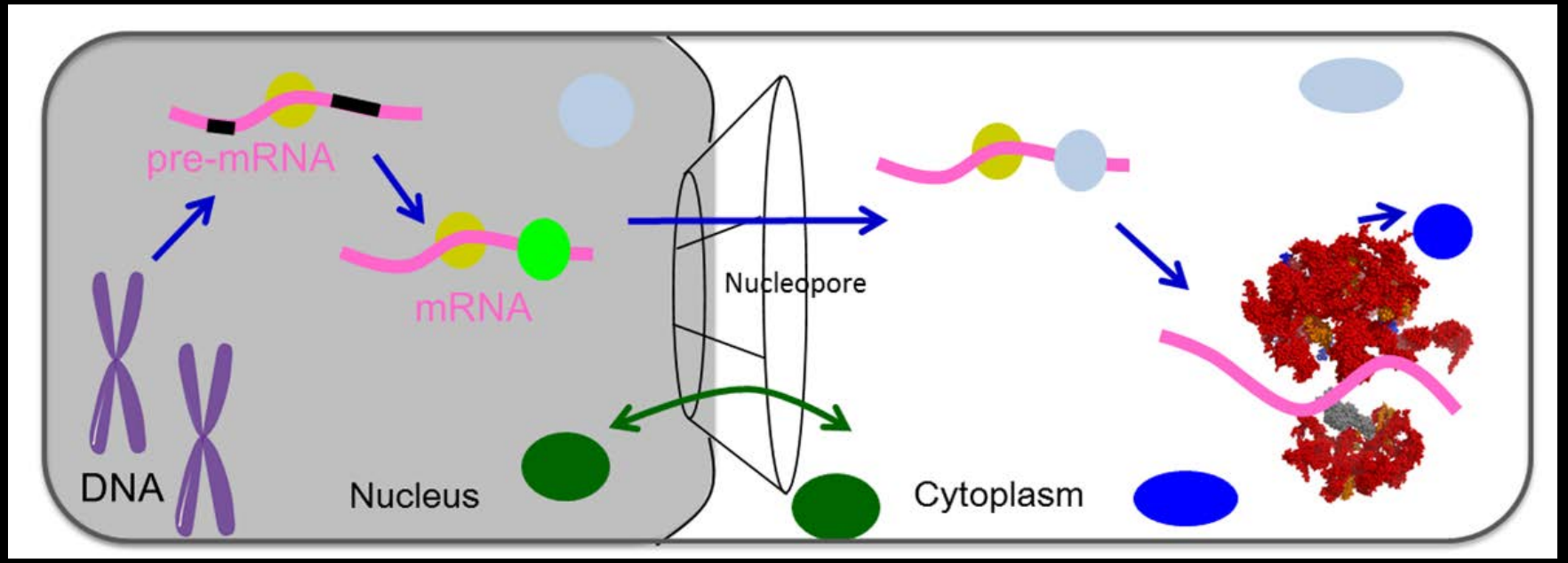
- Total RNA
- Nuclear v Cytoplasmic



Guillaume Hautbergue

Cyto v Nuclear

- Fibroblast samples
  - 4 controls
  - 3 missense TARDBP (p.A321V, p.M337V, p.G287S)
  - Exon arrays
    - Gene level
    - Exon level
  - Nuclear v cytoplasmic RNA

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Cyto v Nuclear

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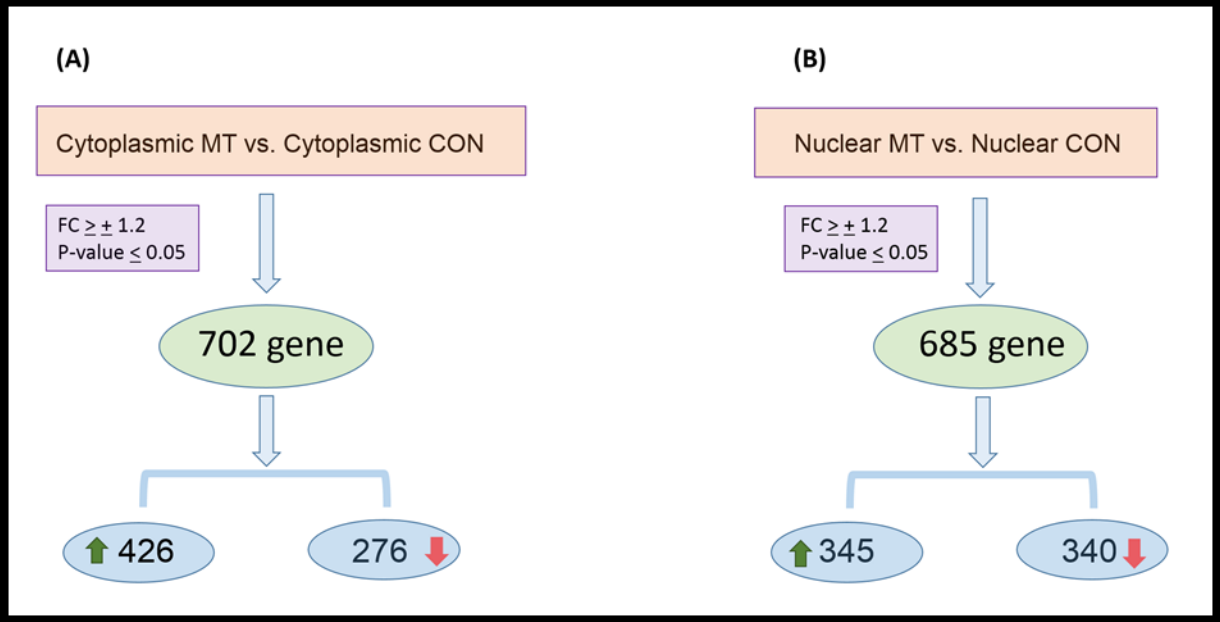

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Cyto v Nuclear

SITraN
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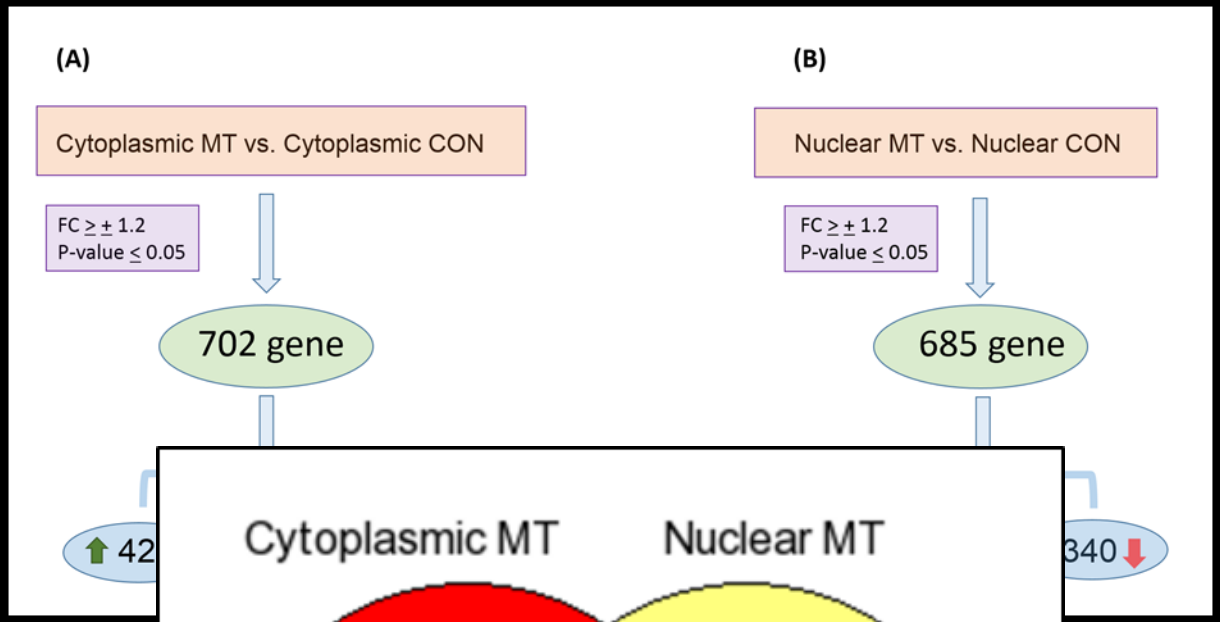

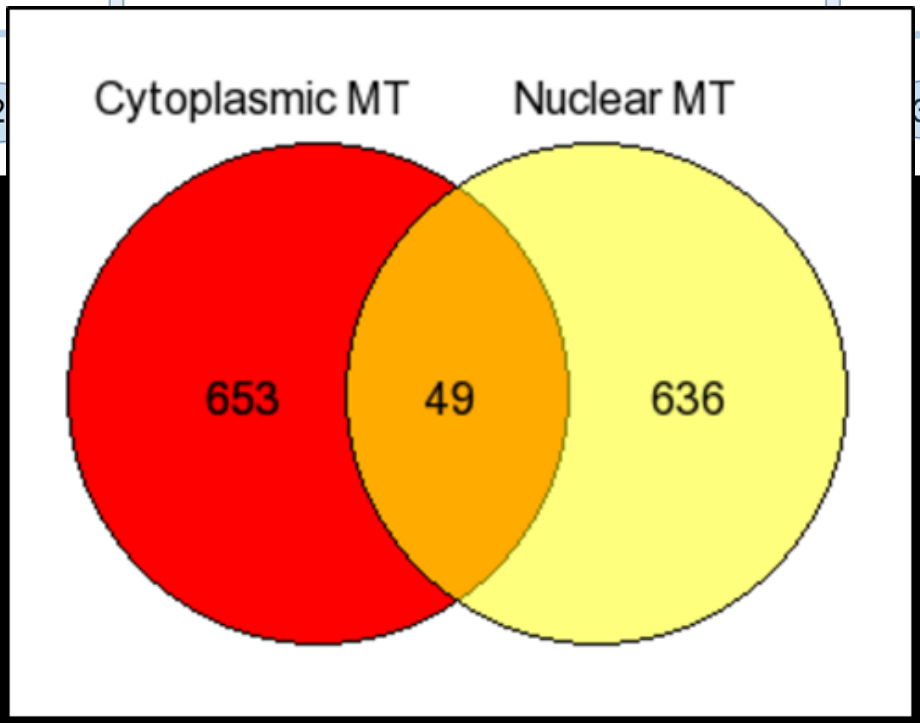

DEG:

- mRNA splicing
- Transcription

DEG:

- Mitotic nuclear division
- DNA replication
- mRNA splicing

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Missense v Truncation

- Fibroblast samples
  - 4 controls
  - 3 missense TARDBP (p.A321V, p.M337V, p.G287S)
  - 3 truncating TARDBP (p.Y374X)

  - Cytoplasmic RNA

  - RNA sequencing

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Present: RNA-Seq

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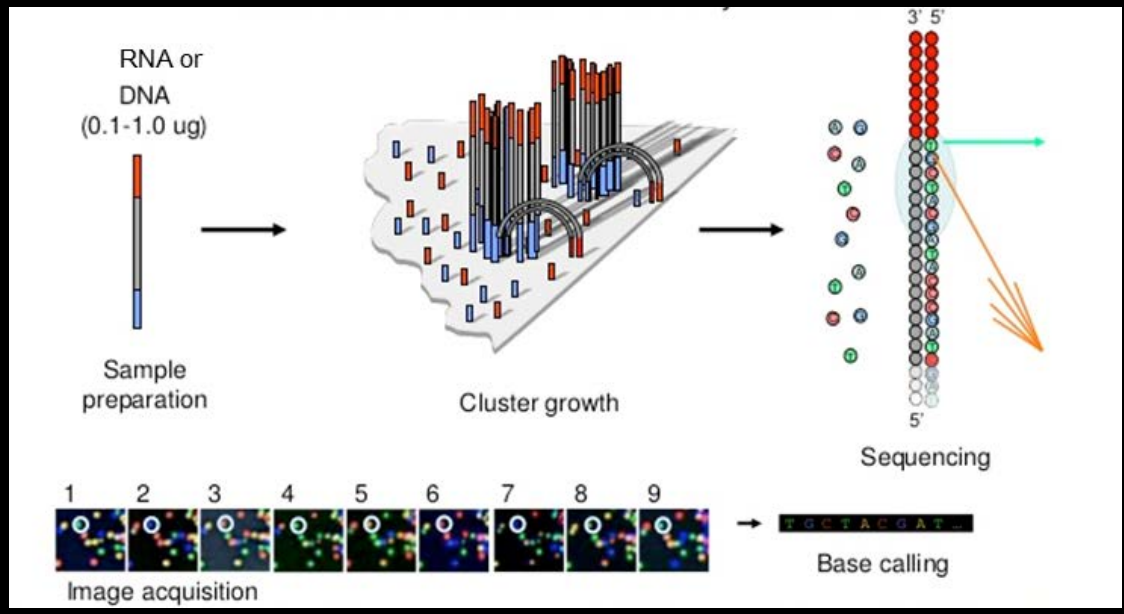
- Not limited by predefined probes
- Identification of novel transcripts & RNA species
- Wider dynamic range
- Sequence specific information

- Challenges:
  - Volumes of data
  - Analysis pipelines



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Missense/Truncation

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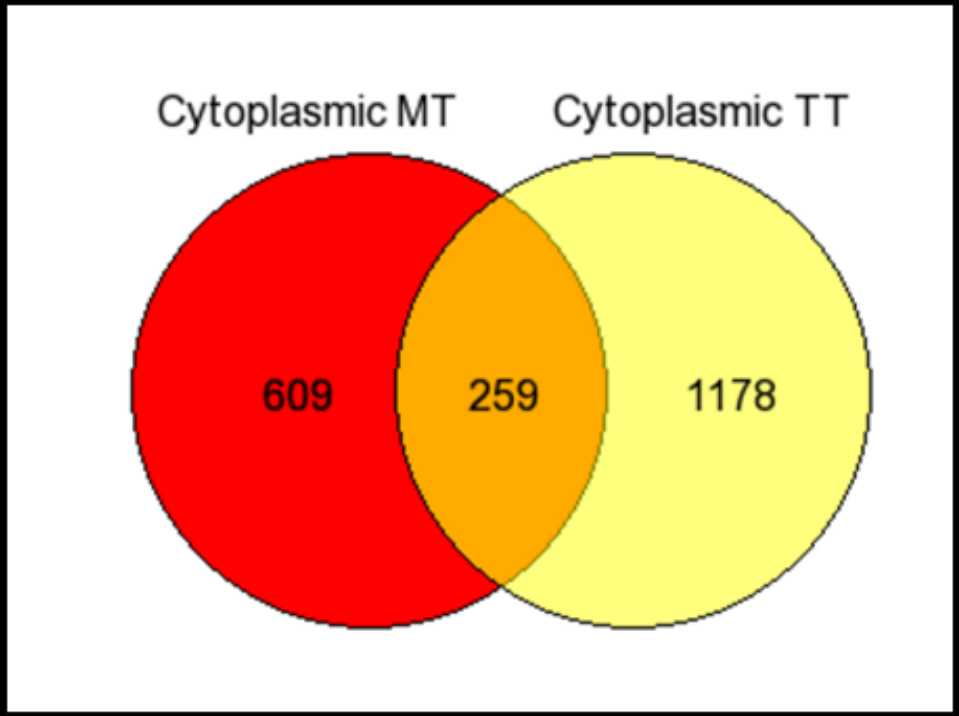

- Missense DEG
  - Regulation of RNA metabolic process
  - Cell adhesion
- Truncation DEG
  - Vesicle mediated transport
  - Response to organic substance

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Profiling Cellular Compartments

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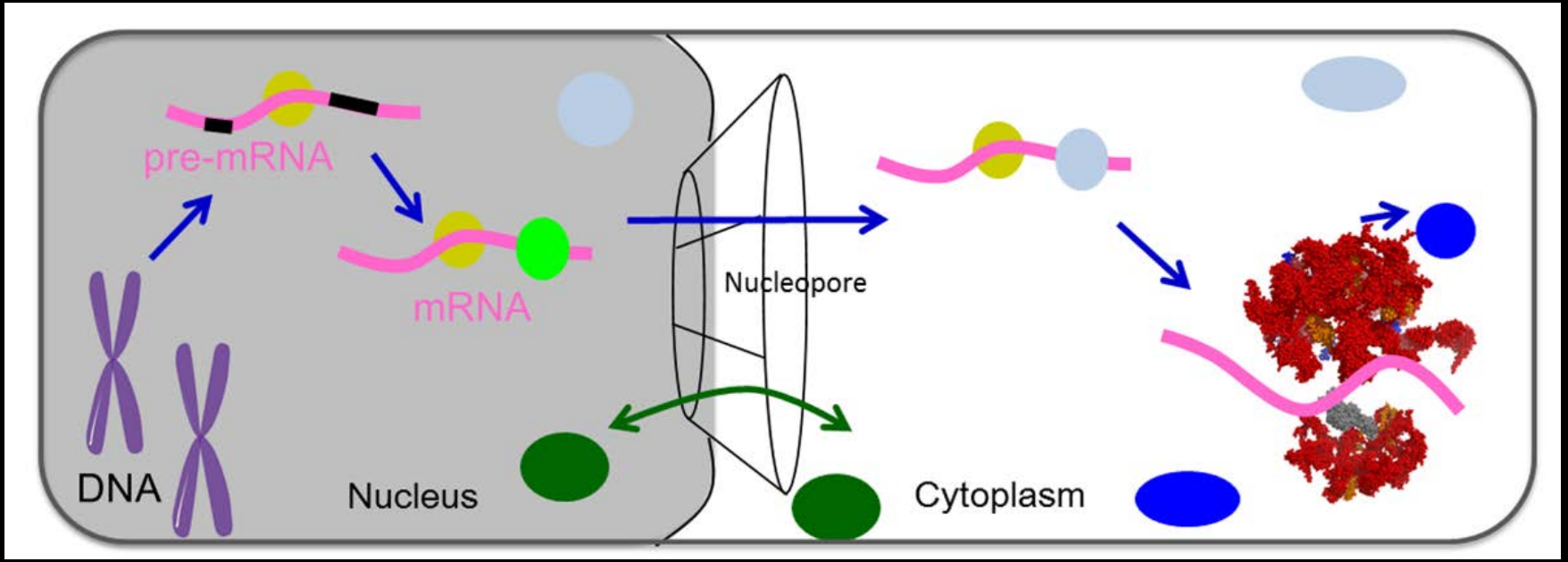
- Total RNA
- Nuclear v Cytoplasmic
- Translatome



Guillaume Hautbergue

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Profiling Cellular Compartments

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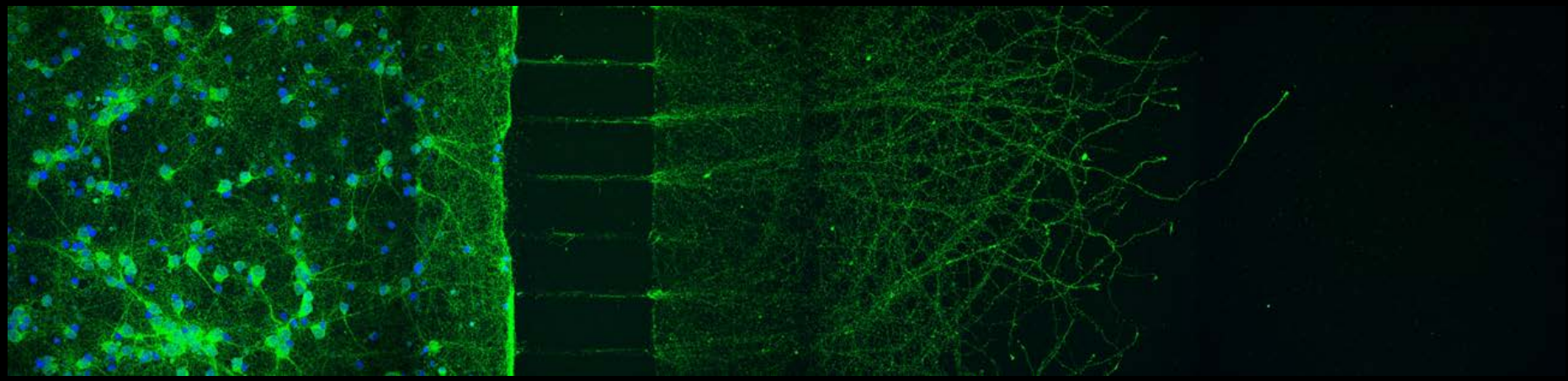
- Total RNA
- Nuclear v Cytoplasmic
- Translatome
- Axon v Cell Body



Wen-Yo Tu & Paul Heath

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Overview

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- Introduction
- Past
  - From pooling patient samples..
  - …to profiling individual cell types
- Present
  - Profiling classes of RNA
  - Profiling cellular compartments
- Future
  - Integral to clinical trials
  - Informing diagnosis, prognosis & treatment

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Future: Clinical Trials

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- Collect DNA & RNA
- Clinical data – MUNE/MUNIX/Imaging/Cognitive ability
- Systems biology approach

- Sub-classify patients
  - Responders v non-responders
  - Correlations with clinical, biochemical & genetic data

Future: Clinical Care

- Diagnostic & Prognostic Biomarkers
  - Skyline Diagnostics AMLprofiler
    - Replaced 7 different tests, 3-4 weeks
    - Single Affymetrix based assay
      - Assesses prognostic markers t(16;16)/inv(16), t(8;21) and t(15;17)
      - CEBPA double mutations & NPM1 A/B/D mutations
      - BAALC and EVI1 expression
    - Can be completed in 3 days

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Future…

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Considerations…

RNA-seq

Microarrays

Total RNA

Cytoplasmic

Nuclear

Translatome

Axonal

Cell body

mRNA

miRNA

xRNA

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Future GEP?

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Modifying Immune Response & Outcomes in Amyotrophic Lateral Sclerosis
MRC Medical Research Council
wellcome trust

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Any Questions?

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