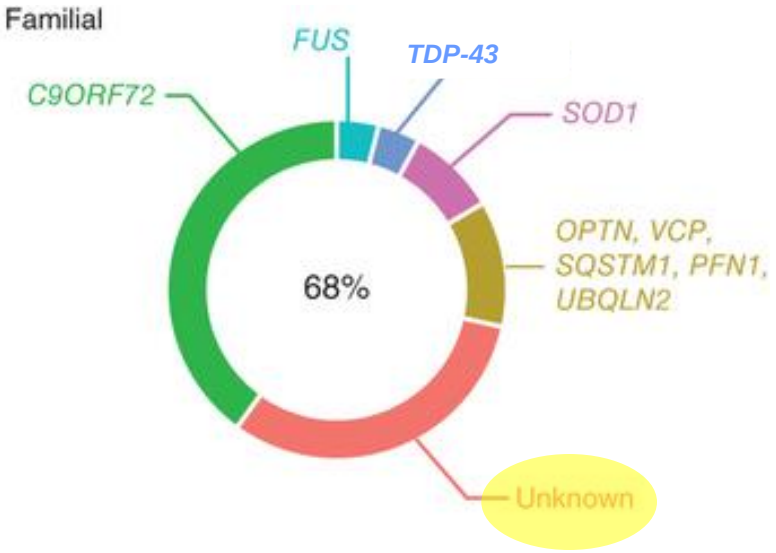
Discovery of microRNA genetic variations in human ALS patients

ENCALS Meeting
May, 2017

Chen Eitan, Weizmann Institute of Science
Supervisor Eran Hornstein

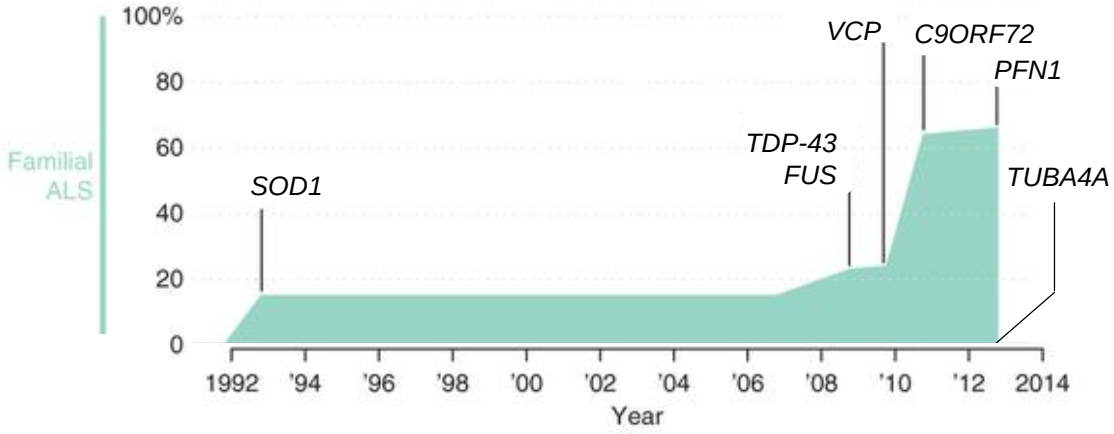
מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

20 yrs. of human genetics in ALS research



Genetic variations in non-coding genome

Percentage ALS explained by genetic mutation



Genetics of families harboring inherited dominant allele

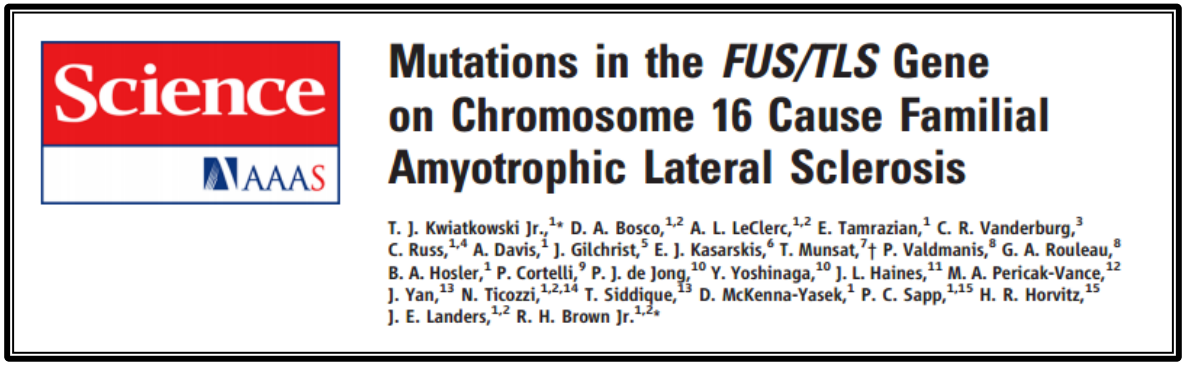
Genome wide association studies

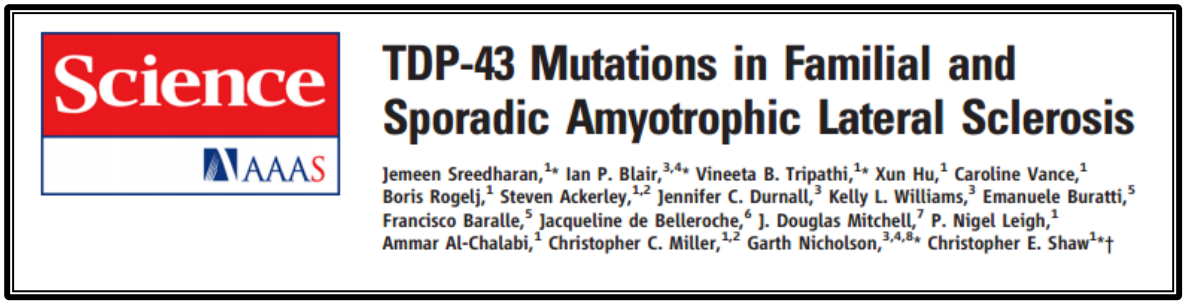
Exome Sequencing

Modified from Renton, Chiò, Traynor, Nature Neuroscience 2014

MicroRNAs in ALS

- FUS and TDP-43 are mutated in ALS patients and linked to miRNA biogenesis





MicroRNAs in ALS

- FUS and TDP-43 are mutated in ALS patients and linked to miRNA biogenesis

- Loss of miRNA activity in MNs provides a new *in vivo* model for MND

PNAS

miRNA malfunction causes spinal motor neuron disease

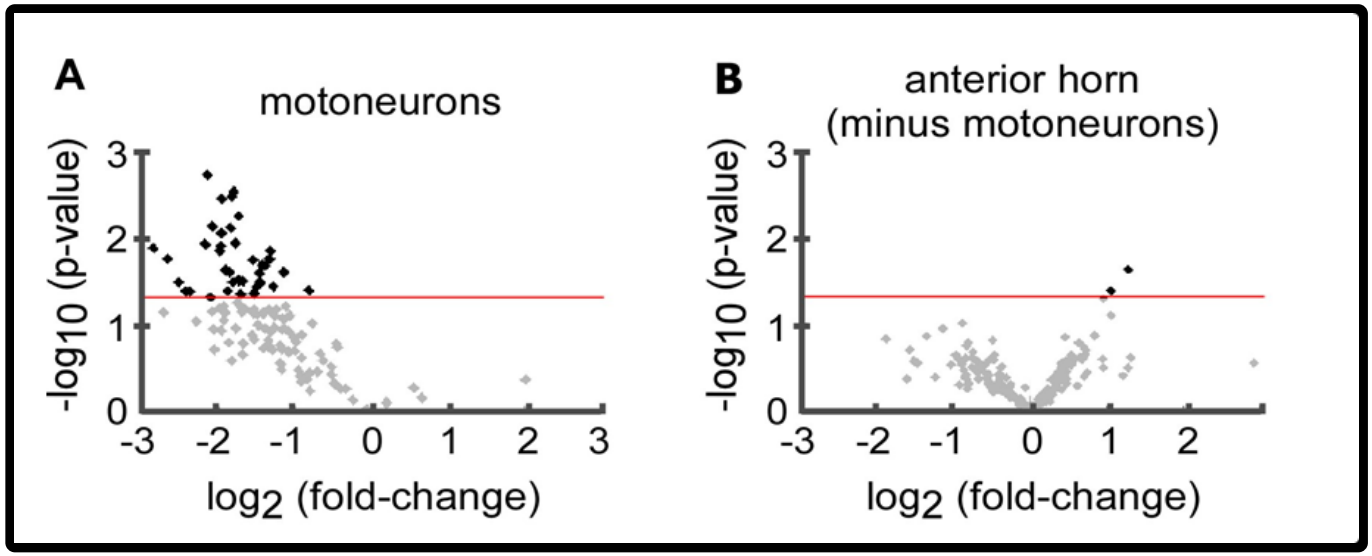
Sharon Haramati[a], Elik Chapnik[b,1], Yehezkel Sztainberg[a,c,1], Raya Eilam[d], Raaya Zwang[a], Noga Gershoni[b], Edwina McGlinn[e], Patrick W. Heiser[f], Anne-Marie Wills[g], Itzhak Wirguin[h], Lee L. Rubin[f], Hidemi Misawa[i], Clifford J. Tabin[e,2], Robert Brown, Jr.[j], Alon Chen[a,2], and Eran Hornstein[b,2]

Departments of [a]Neurobiology and [b]Molecular Genetics and [d]Veterinary Resources, Weizmann Institute of Science, Rehovot 76100, Israel; [c]The Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 52900, Israel; [e]Department of Genetics, Harvard Medical School, Boston, MA 02115; [f]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138; [g]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114; [h]Department of Neurology, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva 91352 , Israel; [i]Department of Pharmacology, Keio University Faculty of Pharmacy, Tokyo 160-8582, Japan; and [j]Department of Neurology, University of Massachusetts School of Medicine, Worcester, MA 01655

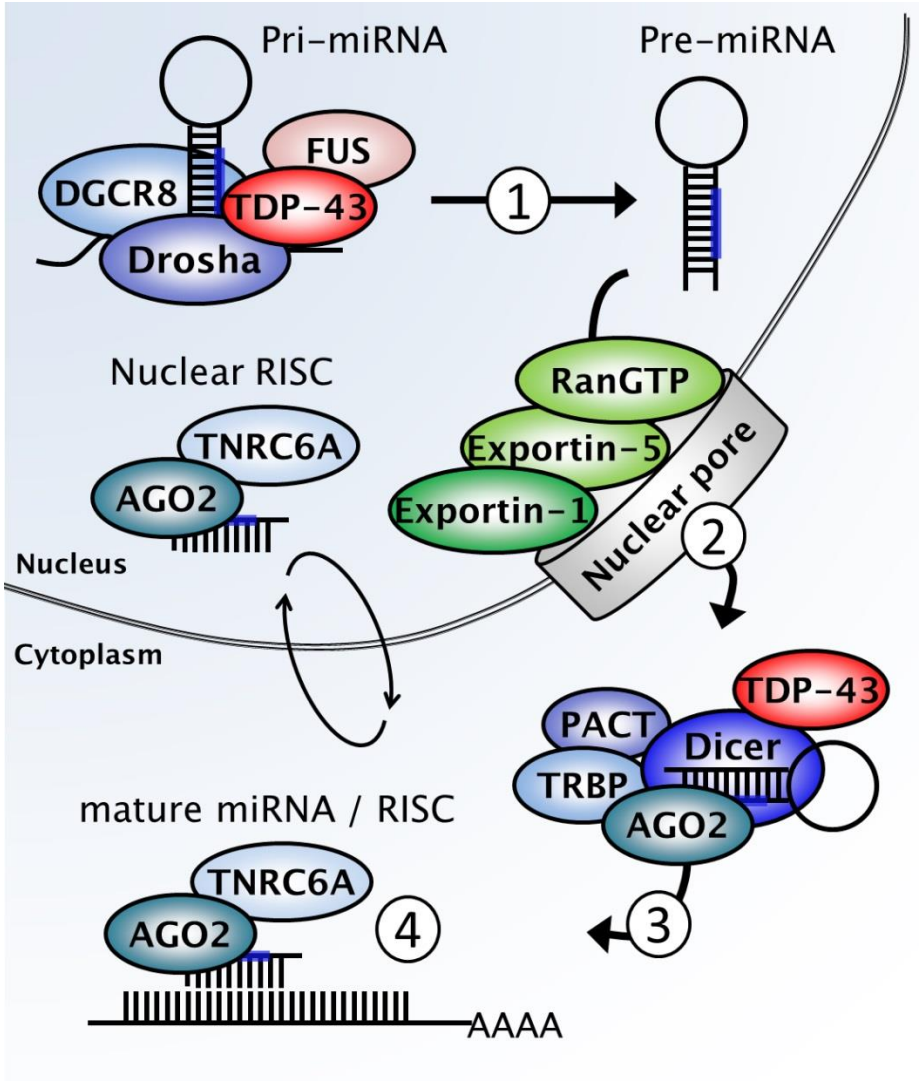
Contributed by Clifford J. Tabin, May 5, 2010 (sent for review November 30, 2009)

MicroRNAs in ALS

- FUS and TDP-43 are mutated in ALS patients and linked to miRNA biogenesis
- Loss of miRNA activity in MNs provides a new *in vivo* model for MND
- miRNAs are globally down-regulated in MNs of human ALS patients



microRNAs Biogenesis



Eitan C. & Hornstein E., Brain Res 2016

Hypothesis

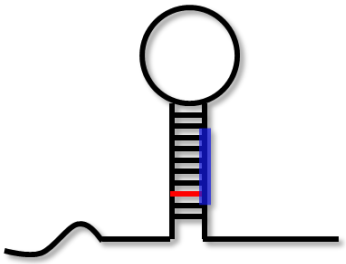
ALS-associated genetic variations can be identified in genes encoding:

(i) miRNA genes

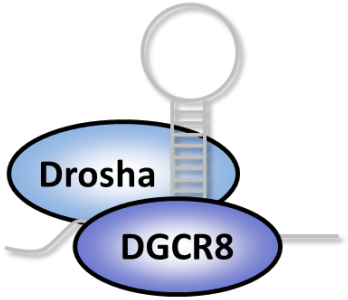
(ii) Protein co-factors required for miRNA biogenesis

(iii) 3'UTRs of disease-associated targets

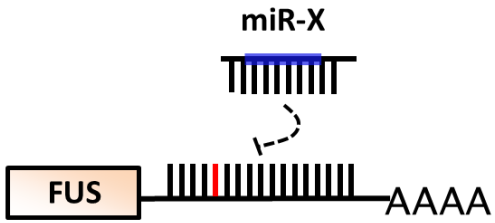
miRNA genes



miRNA biogenesis proteins



3'UTR of ALS-related genes



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nature COMMUNICATIONS

ARTICLE

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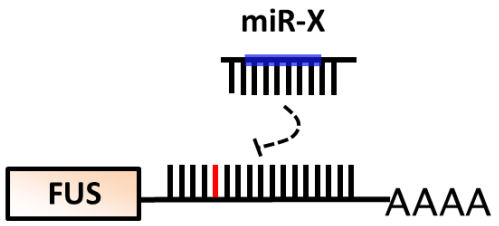
An ALS-associated mutation in the FUS 3′-UTR disrupts a microRNA–FUS regulatory circuitry

Stefano Dini Modigliani[1,*], Mariangela Morlando[2,*], Lorenzo Errichelli[1,2], Mario Sabatelli[3] & Irene Bozzoni[1,2,4]

3'UTR of ALS-related genes



Workflow for Discovery of miRNA-Related Genes Associated With ALS

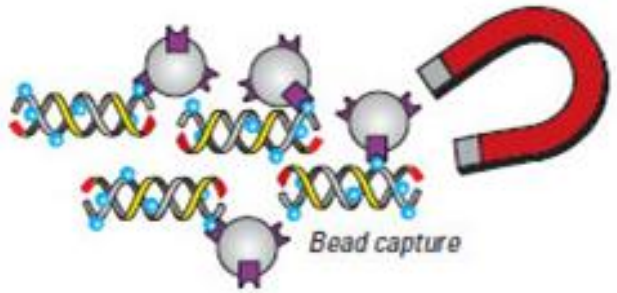
384 ALS & 96 control DNA samples (UMASS med.)

↓

Targeted DNA capture & NGS

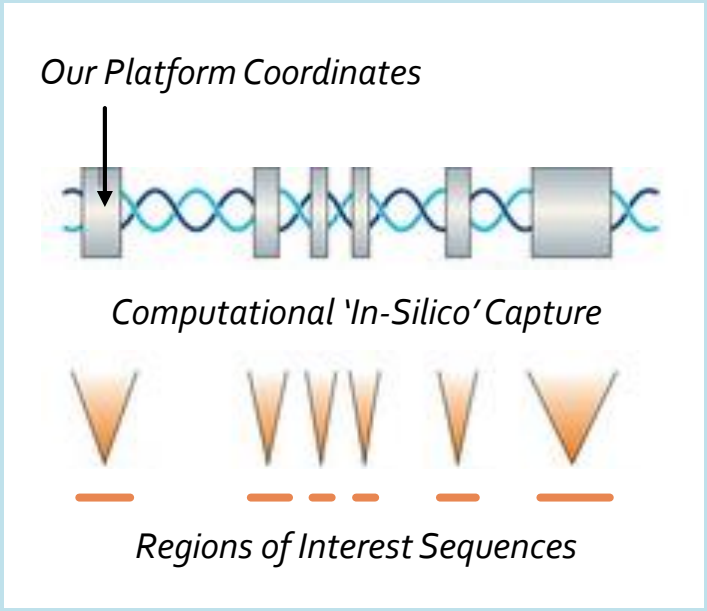
↓

Variant detection & Computational analysis



Targeted capture & enrichment platform (SureSelect, Agilent)

Workflow for Discovery of miRNA-Related Genes Associated With ALS



4327 ALS & 1855 control WGS data (Project MinE)

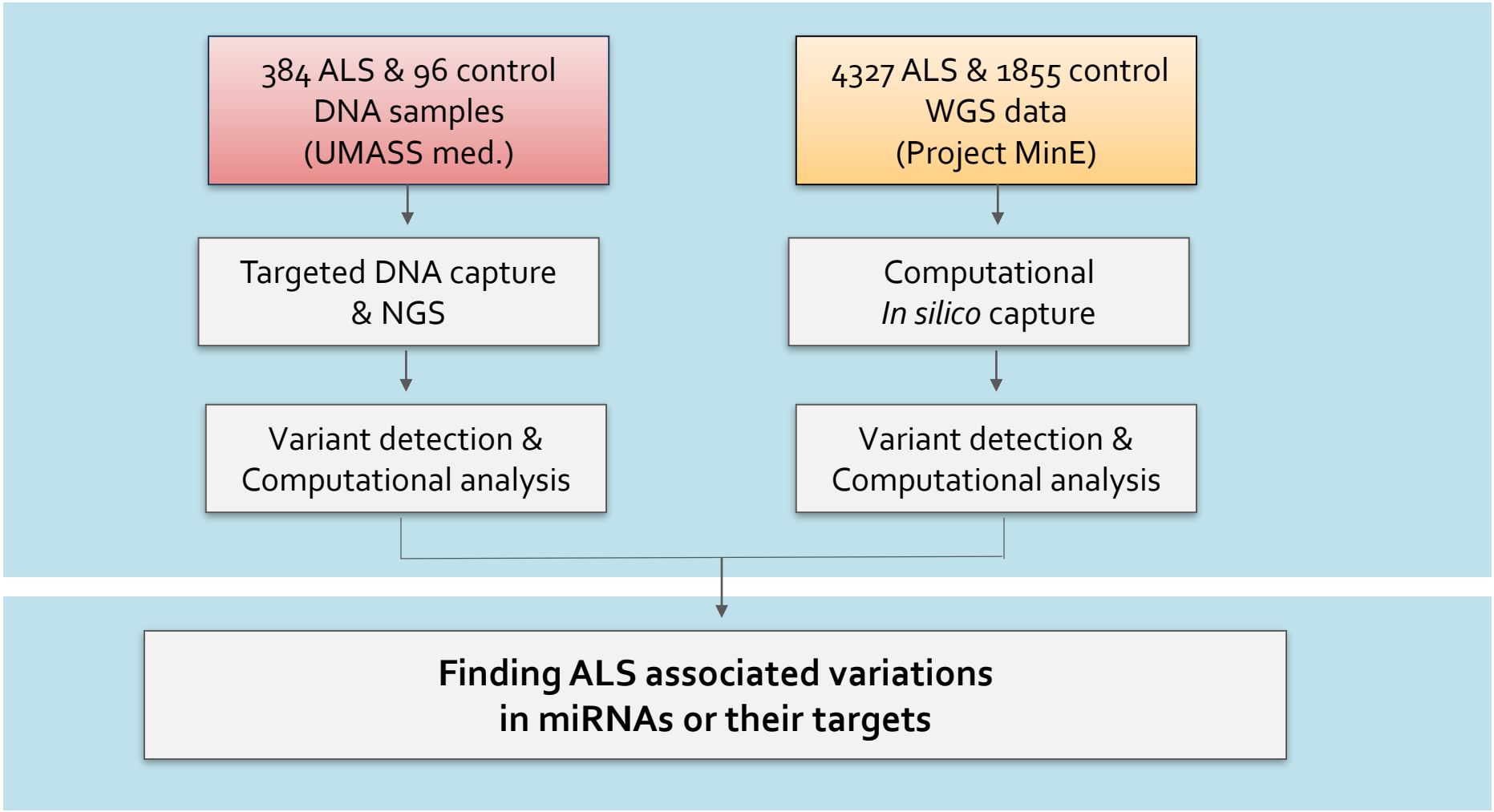
↓

Computational *In silico* capture

↓

Variant detection & Computational analysis

Workflow for Discovery of miRNA-Related Genes Associated With ALS



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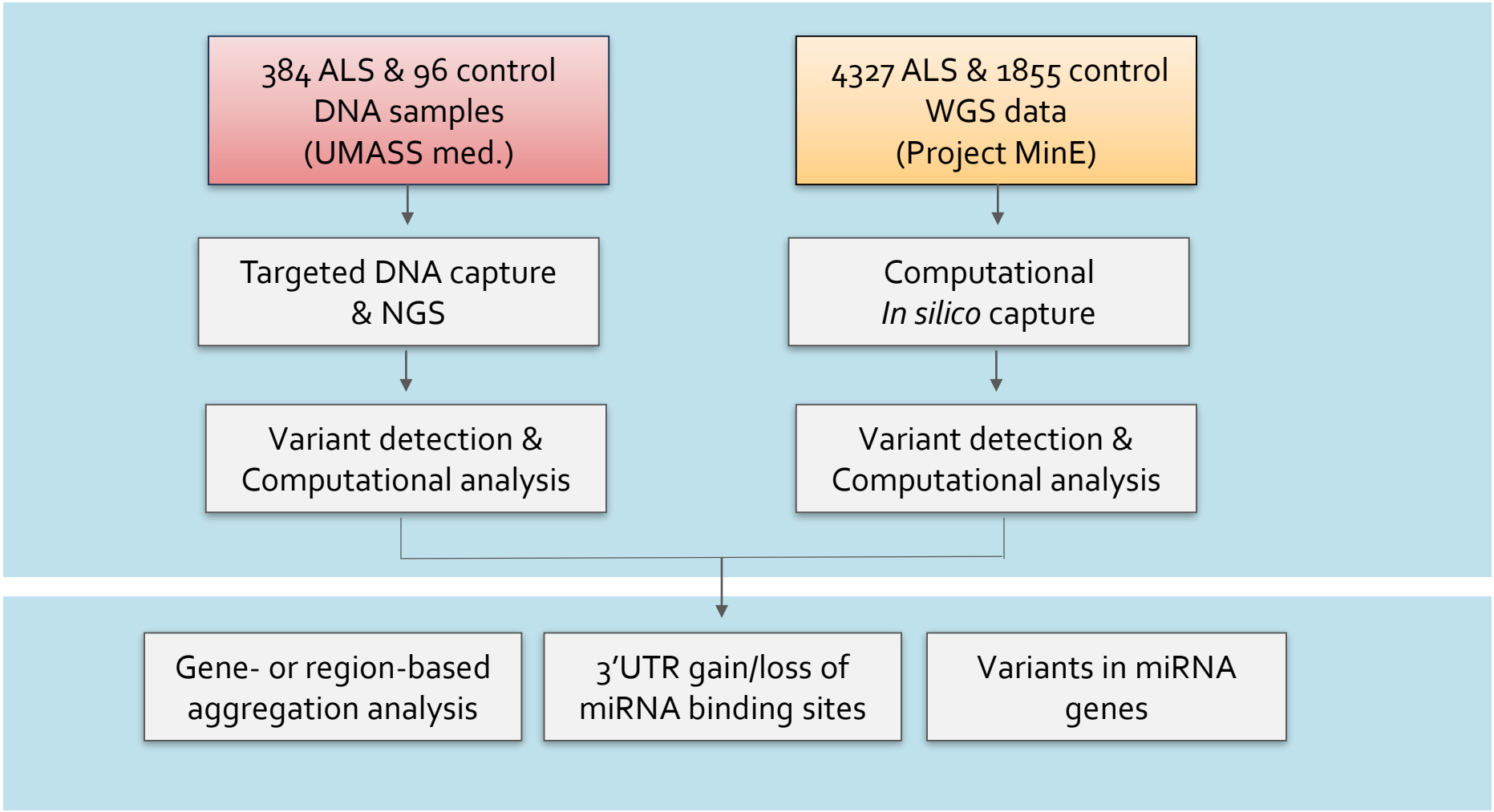


Bioinformatics Assay, Functional Annotation & Statistical test

Gene- or region-based aggregation analysis	3'UTR gain/loss of miRNA binding sites	Variants in miRNA genes

DNA CGGATGTAGCGCTACATCAGT

DNA With Mutations

CGGATGTAGTGCTACATCAGT

CGGATGTAGCGCTACATCACT

CGAATGTAGCGCTACATCAGT

CGGATGTAGCGCTACATGAGT

Gene based Aggregation

CGAATATAGTGCTACATGACT

Bioinformatics Assay, Functional Annotation & Statistical test

Gene- or region-based aggregation analysis

3'UTR gain/loss of miRNA binding sites

Variants in miRNA genes

CGGATGTAGCGCTACATCAGT

Bioinformatics Assay, Functional Annotation & Statistical test

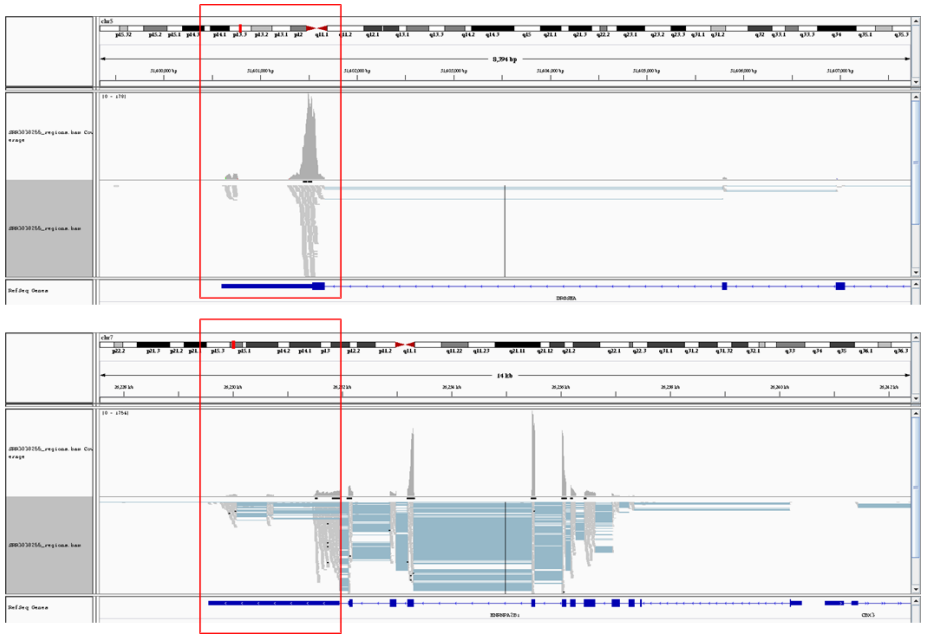
Gene- or region-based aggregation analysis

3'UTR gain/loss of miRNA binding sites

Variants in miRNA genes

DROSHA

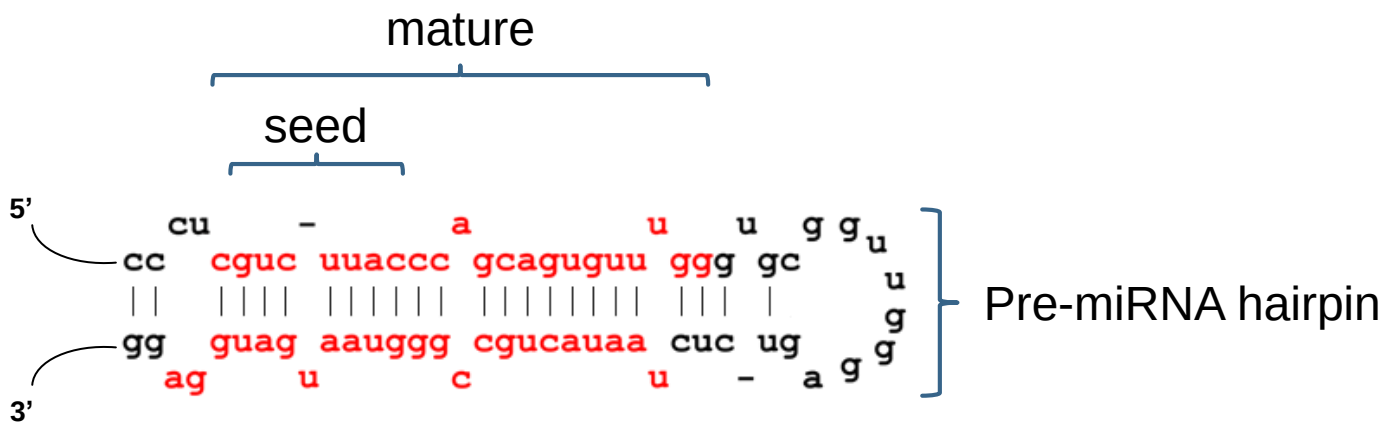
HNRNPA2B1



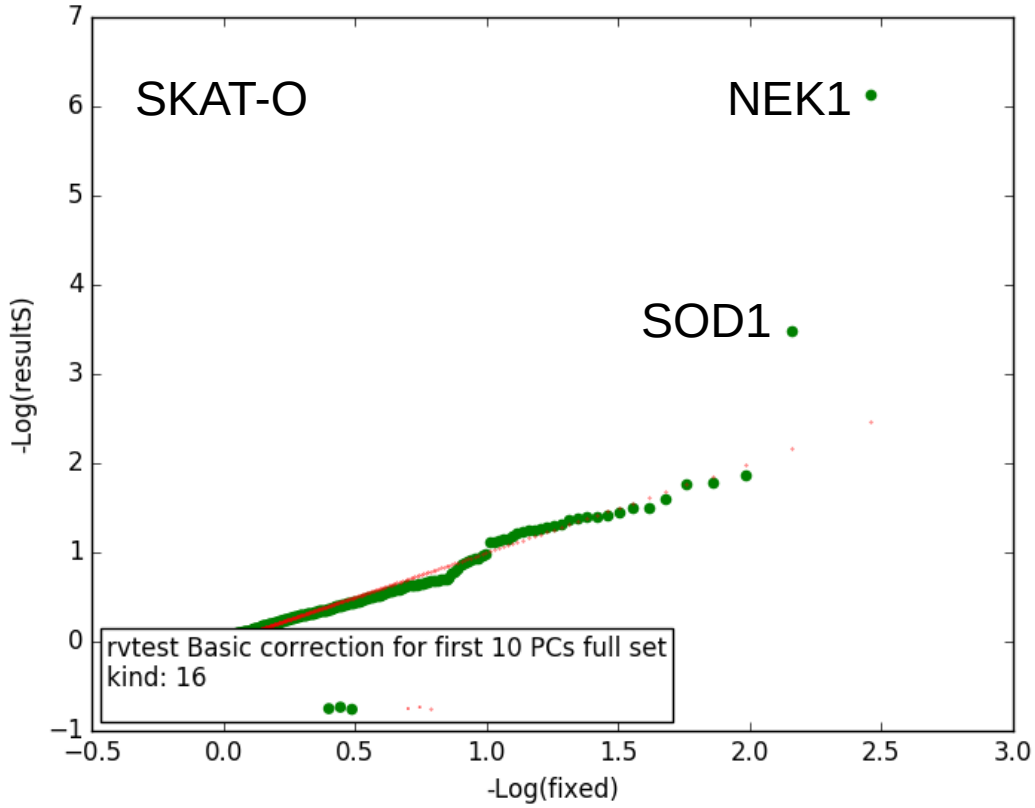
Ravits & Yeo labs, 2016

Bioinformatics Assay, Functional Annotation & Statistical test

Gene- or region-based aggregation analysis	3'UTR gain/loss of miRNA binding sites	Variants in miRNA genes



Rare allele association on genes

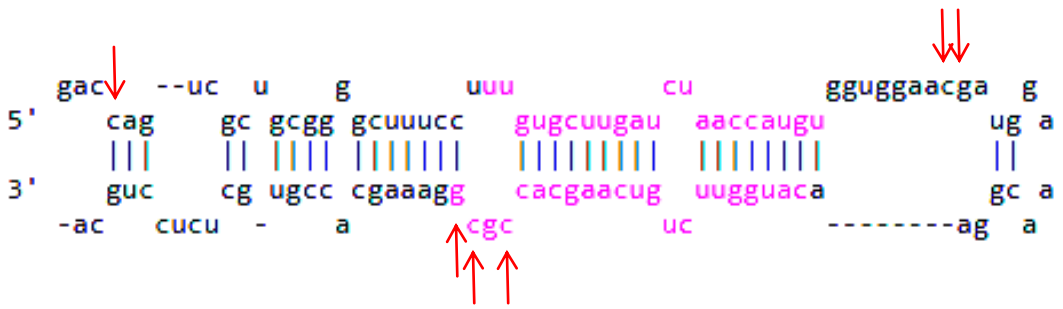


SKAT-O - combine burden and variance-component tests (combined tests)

Preliminary Results
Variants in miRNA genes

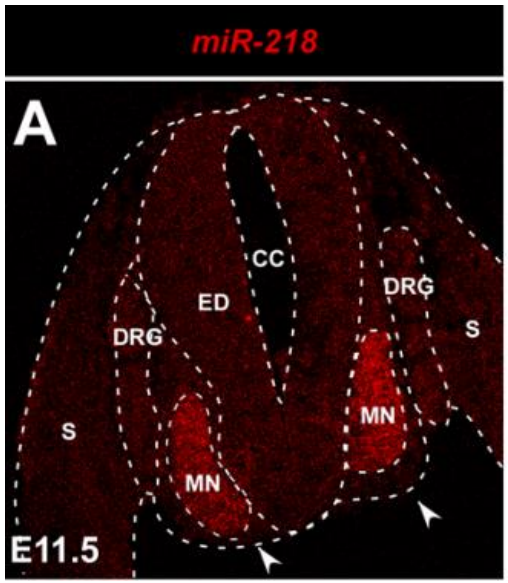
Variant Info				Count											
				UMASS		UK		Netherlands		USA		Ireland		Total	
Variant in gene	Genomic Position	Nuc Alt	Location	cases (341)	ctrl (96)	cases (1111)	ctrl (277)	cases (1794)	ctrl (1022)	cases (433)	ctrl (70)	cases (269)	ctrl (136)	cases (3948)	ctrl (1601)
miR-218	5:168195174	G>A	pre-miR	1				2	1	1		1		5	1
	5:168195173	C>T	pre-miR			2								2	
	5:168195176	G>A	pre-miR			1								1	
	5:168195207	C>T	pre-miR					1						1	
	5:168195257	G>A	pre-miR					1						1	
	5:168195208	G>T	pre-miR							1				1	
	Total			**1**	**0**	**3**	**0**	**4**	**1**	**2**	**0**	**1**	**0**	**11**	**1**

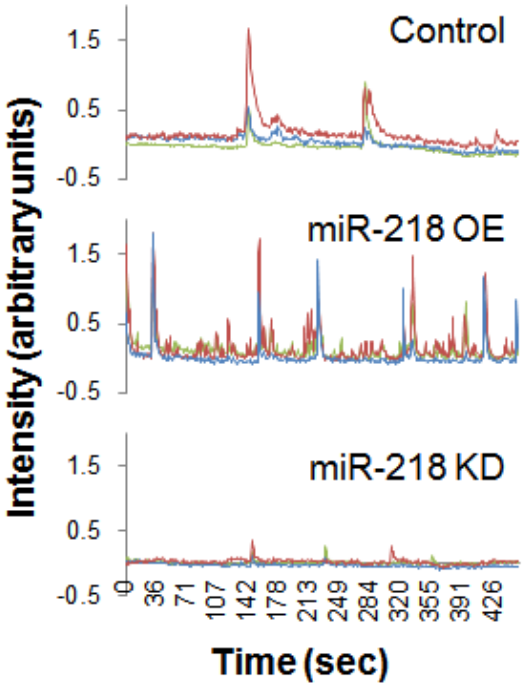
miR-218



Motor Neuron Specific miR-218

microRNA	Biological Relevance	Target	Ref.
miR-218	Motor neuron specific miRNA, essential for establishing MN fate. miR-218 loss causes systemic neuromuscular failure.	SLC1A2 SLC6A1 PTEN	Thiebes et al. 2015 Amin et al. 2015

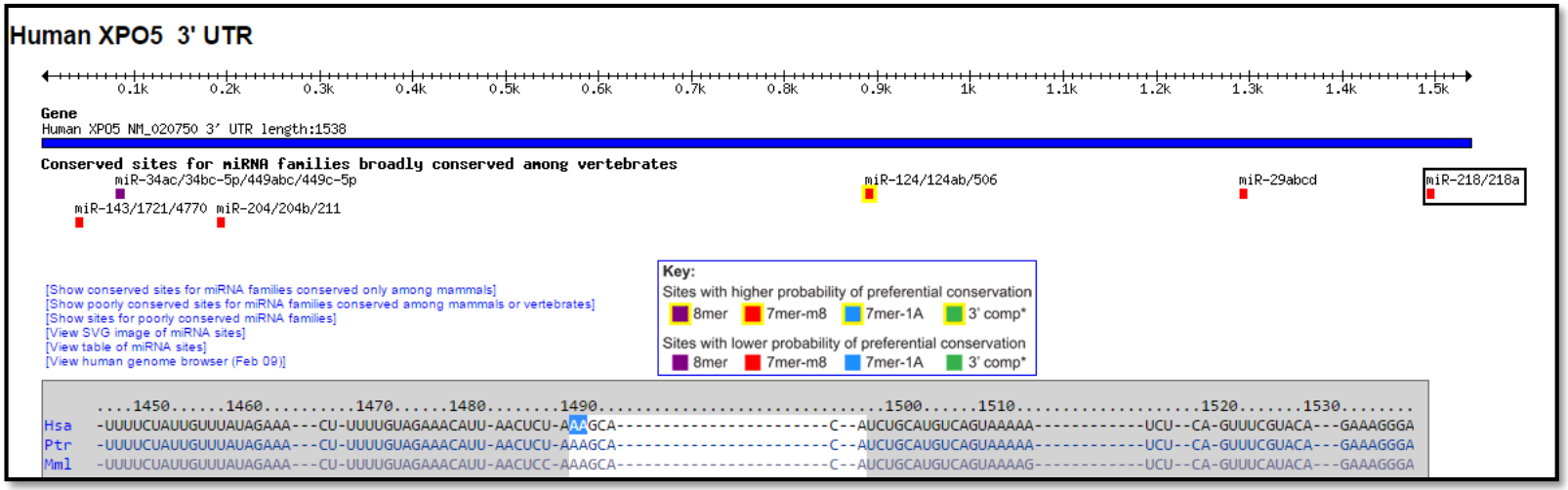




Hornstein lab, unpublished

Preliminary Results
Loss of miRNA binding site

Loss of binding sites for miRNAs					
Variant Info					
Variant in 3'UTR	Genomic Position	Nuc Alt	Lossed miRNA Regulation	Total	
				cases (3948)	ctrl (1601)
XPO5	6:43490112	G>T	miR-218-5p	1	
	6:43490115-43490120	TTAGAG>-	miR-218-5p	3	
	6:43490114	T>C	miR-218-5p	1	
	Total			**5**	**0**



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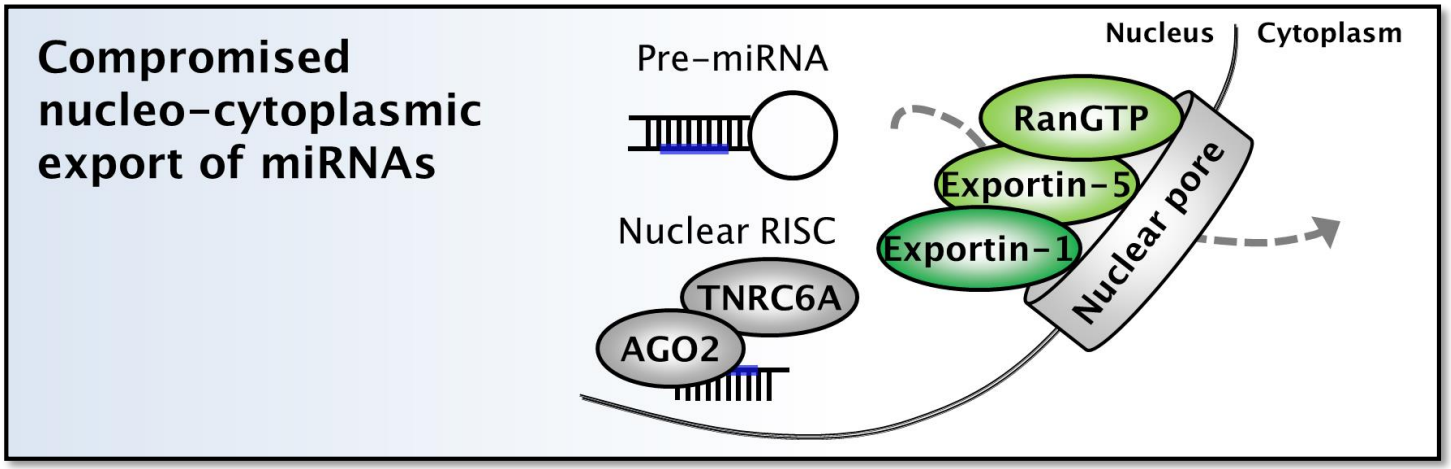
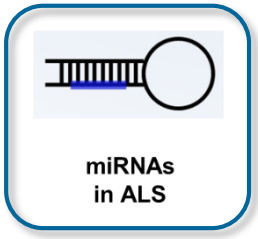
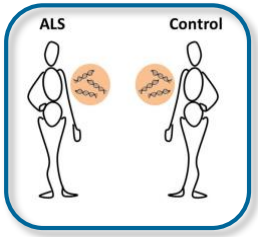


Summary



miRNAs & their targets are known to play a role in neurodegeneration, including ALS.



We explore variations in miRNA genes & target genes, in collaboration with Project MinE.

Endpoints:

I. **Discover new candidate ALS genes**
II. **Decipher miRNA roles in pathogenesis**

Thank you for listening!!

Project MinE Consortium

UMC Utrecht | Leonard van den Berg, Jan Veldink & lab
Sara Pulit, Kristel Kool-Van Eijk, Lindy Kool, Joke van Vugt

King's College | Ammar Al-Chalabi & lab
William Sproviero

UMASS med. | Robert Brown, John Landers

WIS

Tsviya Olender, Elik Chapnik, Eran Segal & lab

Elad Barkan, Daphna Rotschild

Eran Hornstein & lab

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