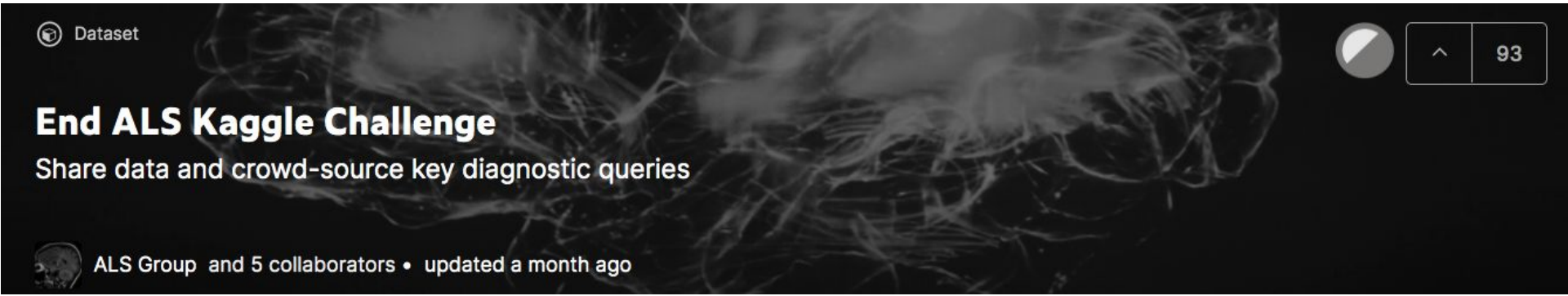
When the outlier is the signal



Searching new genetic causes of ALS
by aberrant expression analysis

15th May 2021

TUM - UCI team

Task 1

Does ALS have one mechanism of action?

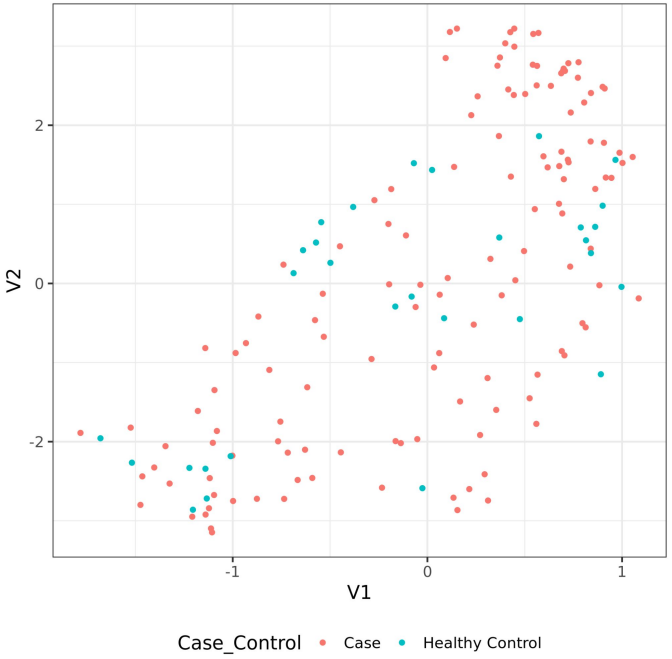
→ one pathway

Or is it caused by multiple independent or different mechanisms of action?

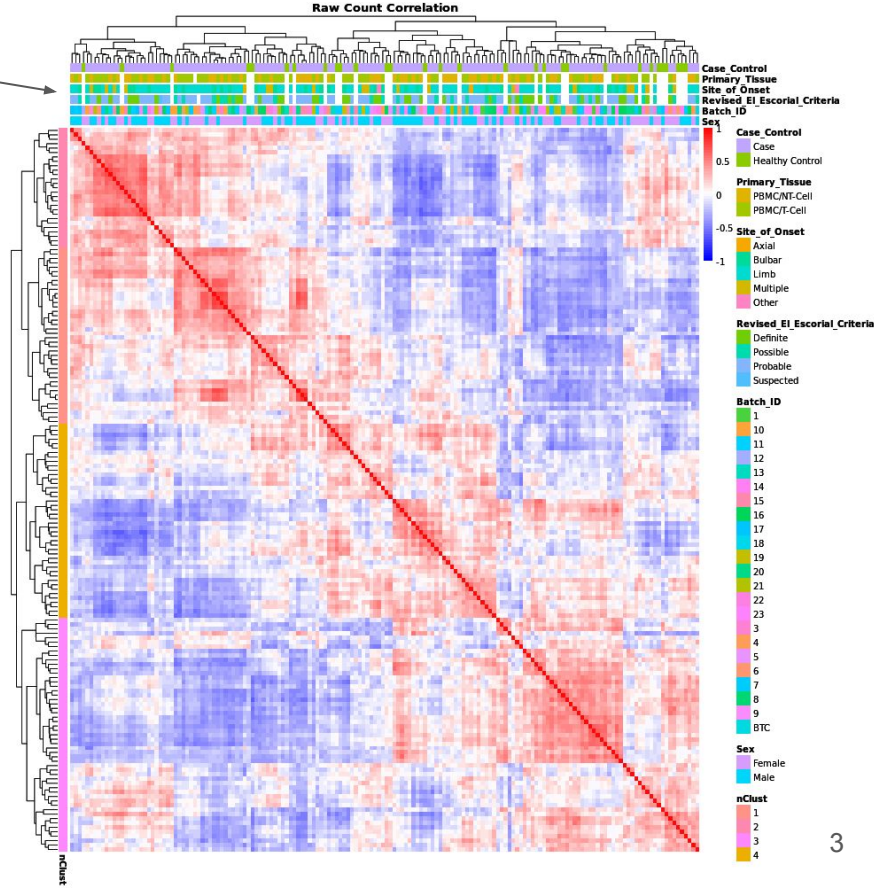
→ multiple pathways

Gene expression does not naturally cluster donor groups

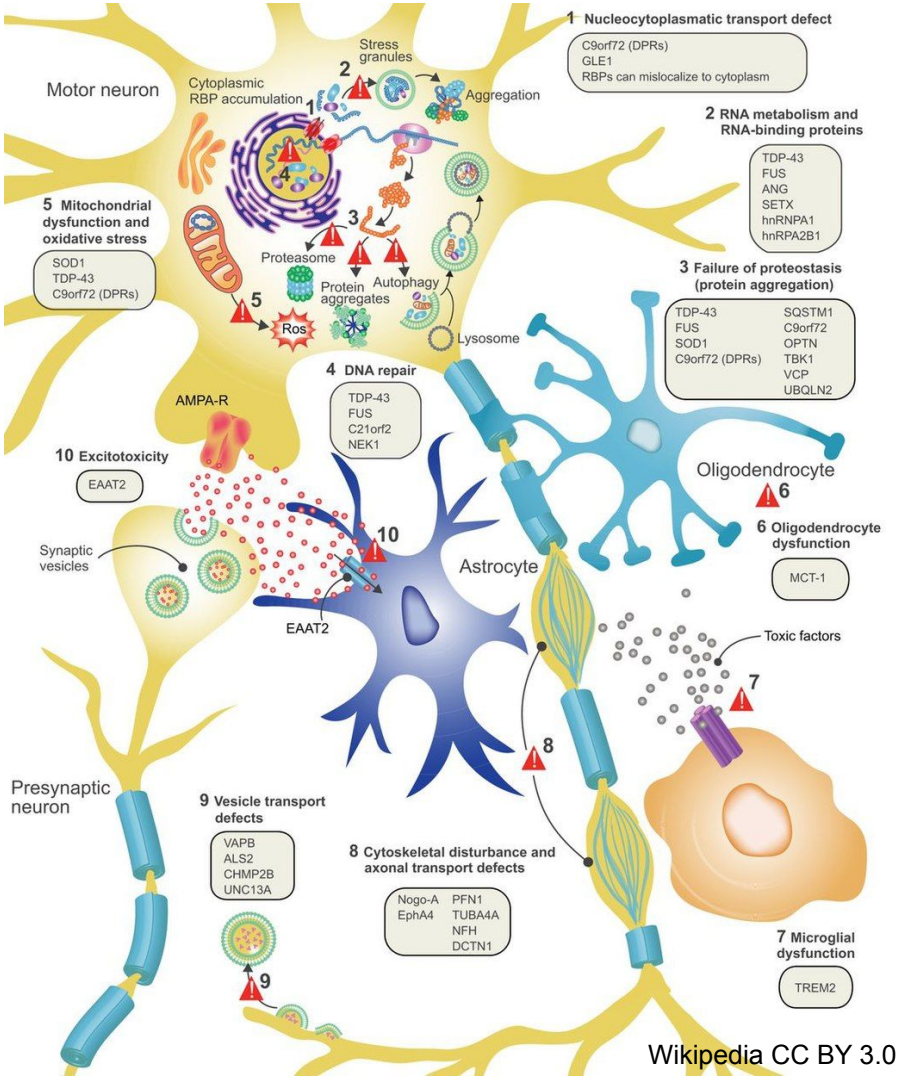
Cases and controls
visualized in first two principal components (V1 and V2)



No covariate drives the sample clusters



In fact, mutations in ca. 40 genes have been implicated in ALS over various molecular pathways[1]



1. Gregory et al. Curr Genet Med Rep (2020)
2. Hardiman O et al. Nat. Rev. Dis. Primers (2017)
3. van Damme et al. Disease Models and Mechanisms (2017)

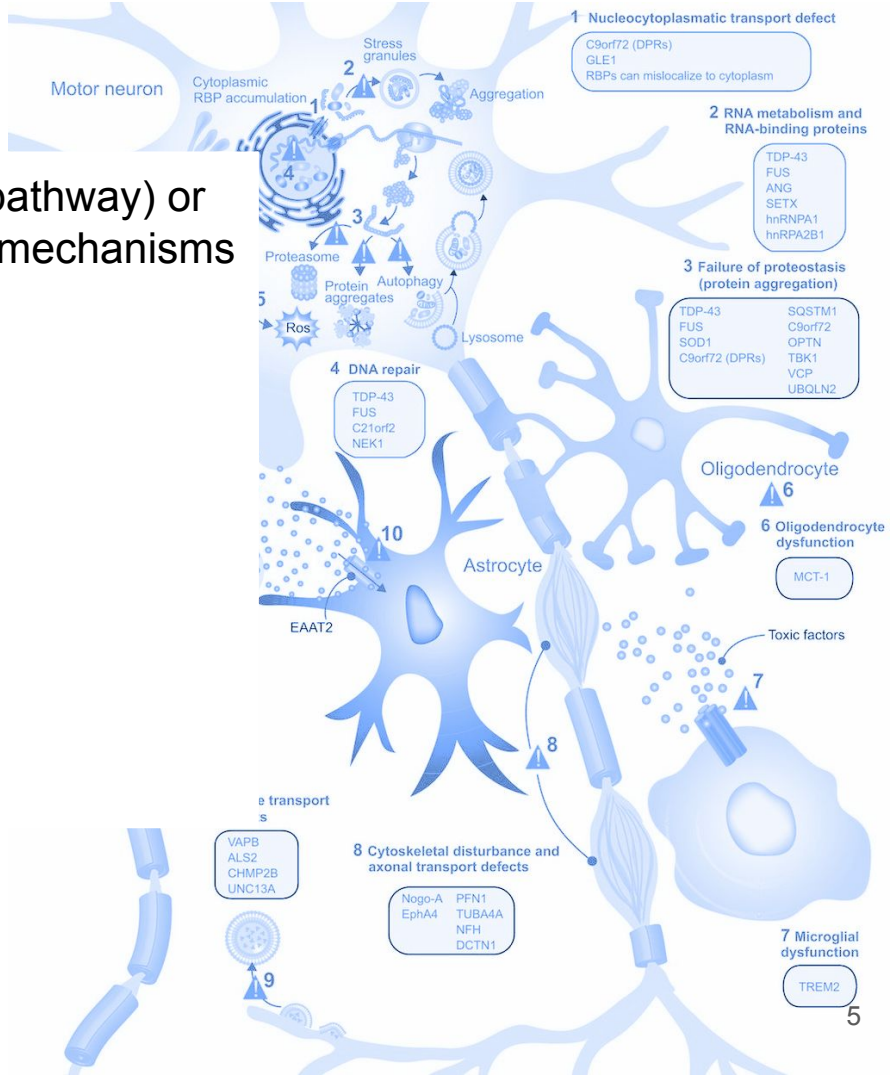
Does ALS have one mechanism of action (one pathway) or is it caused by multiple independent or different mechanisms of action (multiple pathways)?

➔ **Multiple pathways**

However:

Do we find evidence for new implicated genes?

…. and new pathways involved?

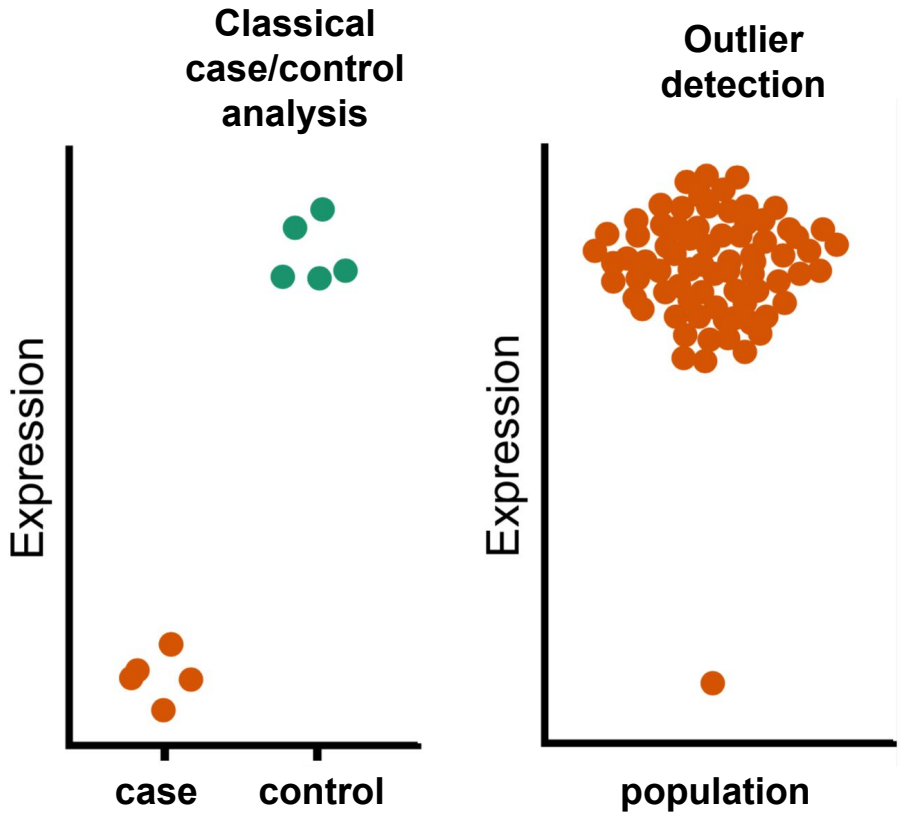


Outlier detection for gene discovery

- No common pattern of gene expression among patients
- Many pathways involved

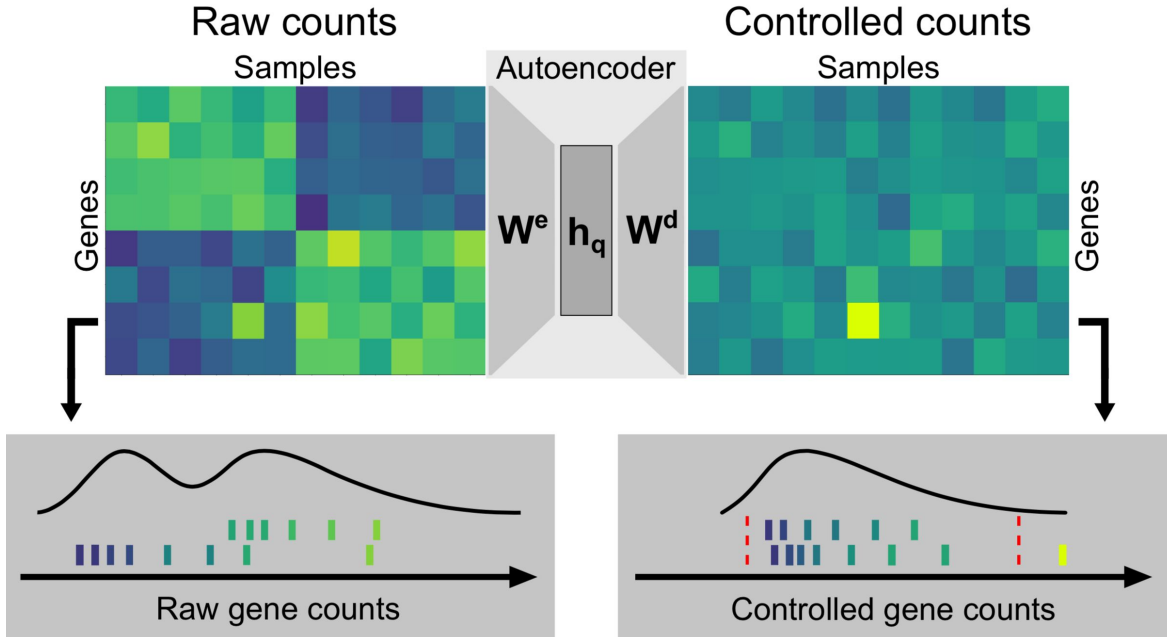
➔ To search for new genes we instead ask: What makes every patient unique?

➔ Expression outlier detection



A denoising autoencoder with a negative binomial loss to control for latent factors in RNA-Seq data

- Negative Binomial loss
- Number of latent factors set to maximise precision-recall of artificial outliers



OUTRIDER, Brechtmann et al. AJHG (2018)

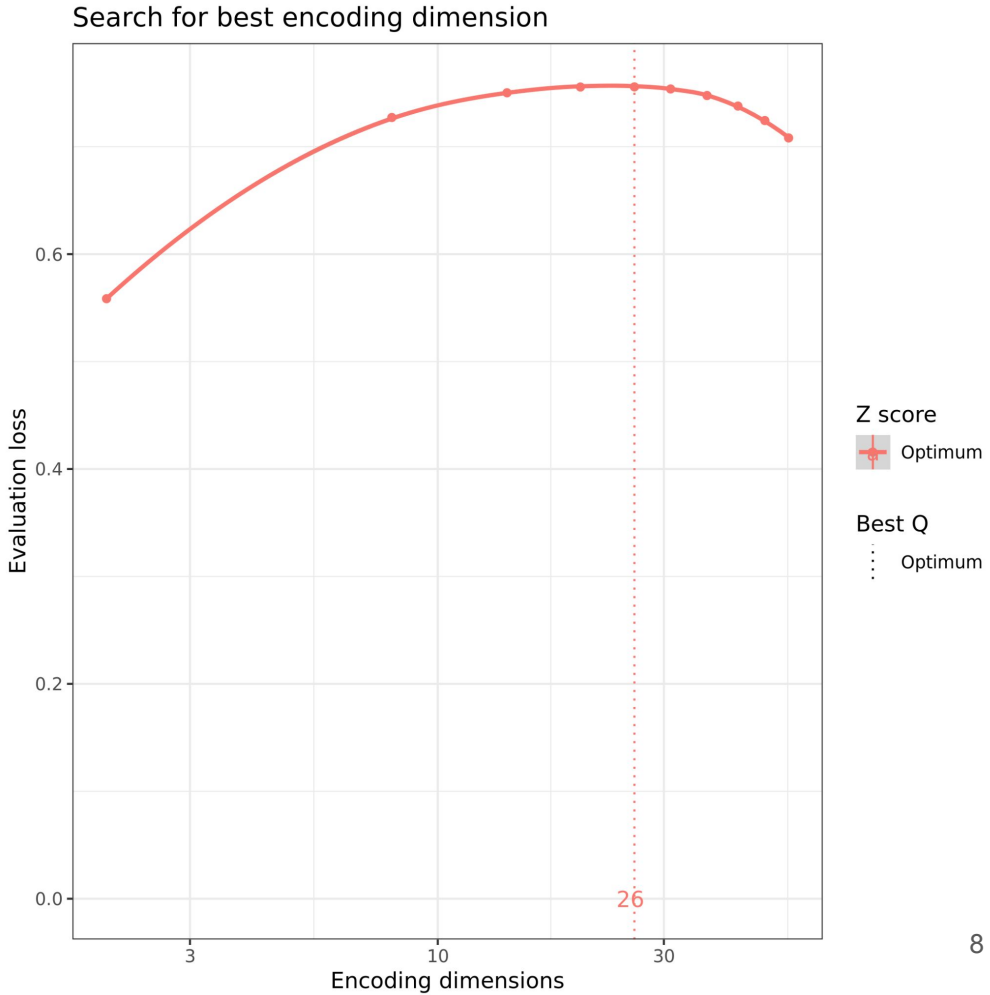
Hyperparameter optimization

As with image processing denoising autoencoders (AEs), OUTRIDER is optimized to remove artificially injected noise

$\mathbf{X}$ 

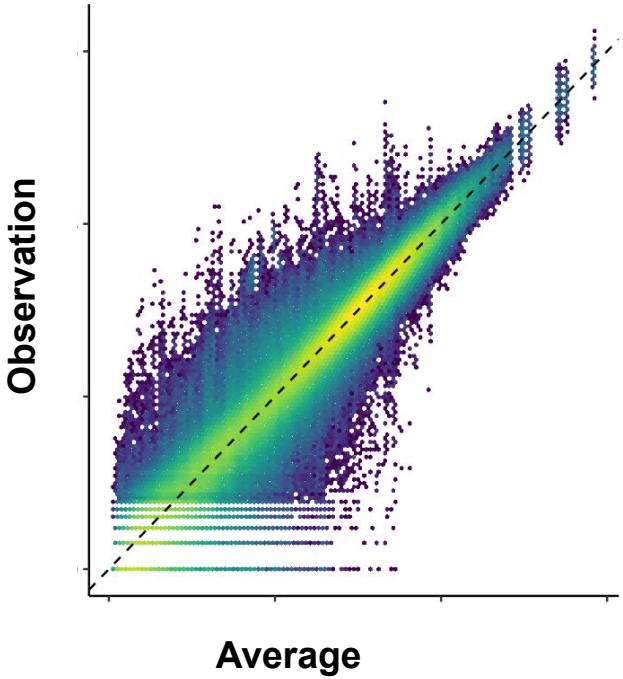
$\mathbf{X}^{\mathbf{corrupt.}}$ 

$f(\mathbf{X}^{\mathbf{corrupt.}}, \boldsymbol{\theta})$ 

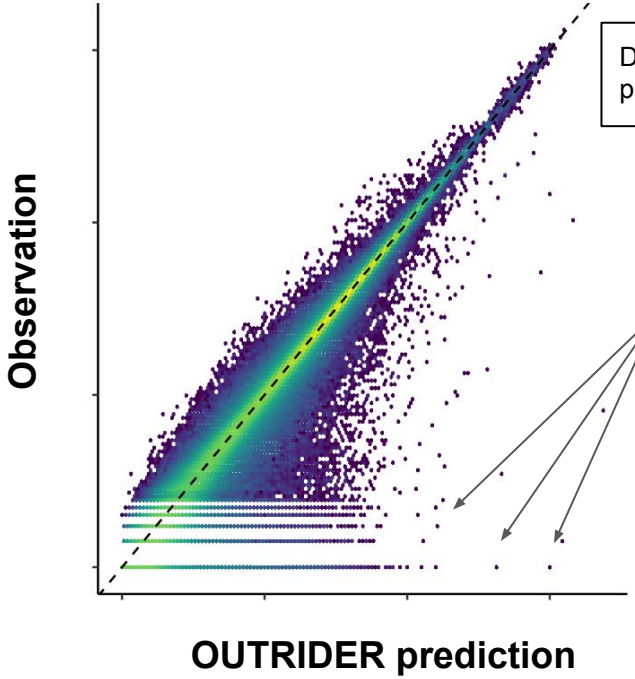


OUTRIDER accurately predicts expression of each gene per sample and reveals outliers

Before OUTRIDER



After OUTRIDER



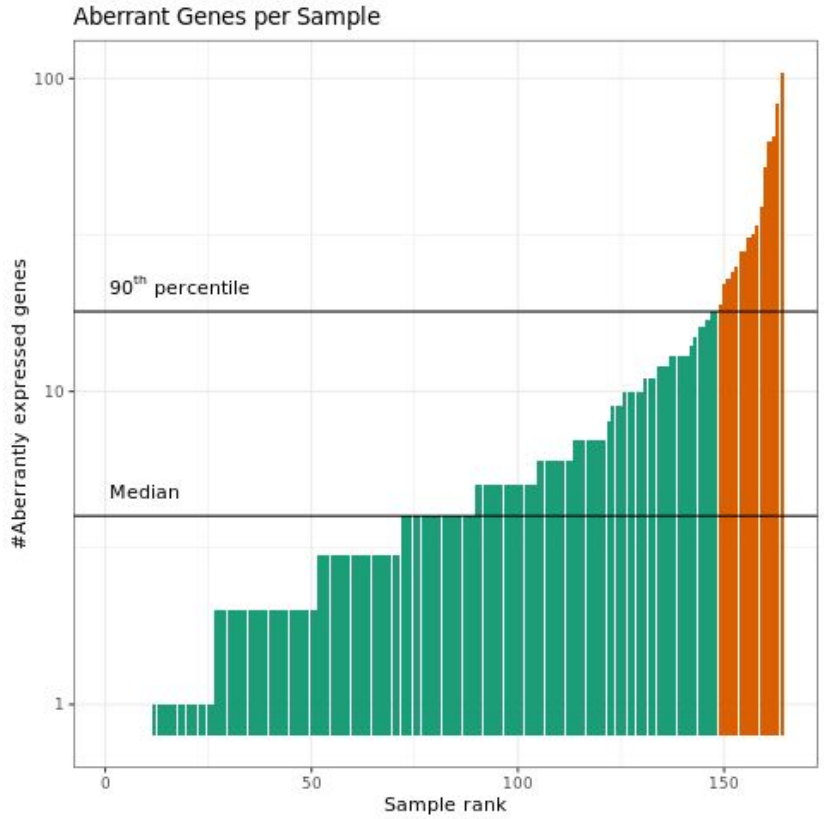
Deviations from model predictions are outliers.

In these individuals, those genes are at levels far below what one would expect given the expression of all other genes….

Are rare genetic variants disrupting those genes in these individuals?

Scale: $\log_{10}$(count +1)

Individuals typically have 4 outliers



Genetically supported outliers

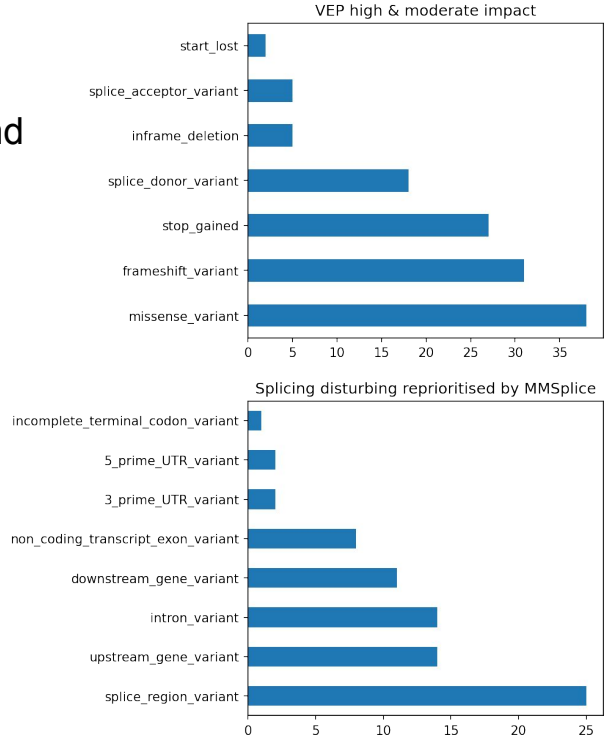
Filtering outliers for having **rare** and **deleterious** genetic variants

Frequency in general population < 0.1% (gnomAD[5])

At most 6 samples in the cohort

Standard VEP high and moderate impact annotations

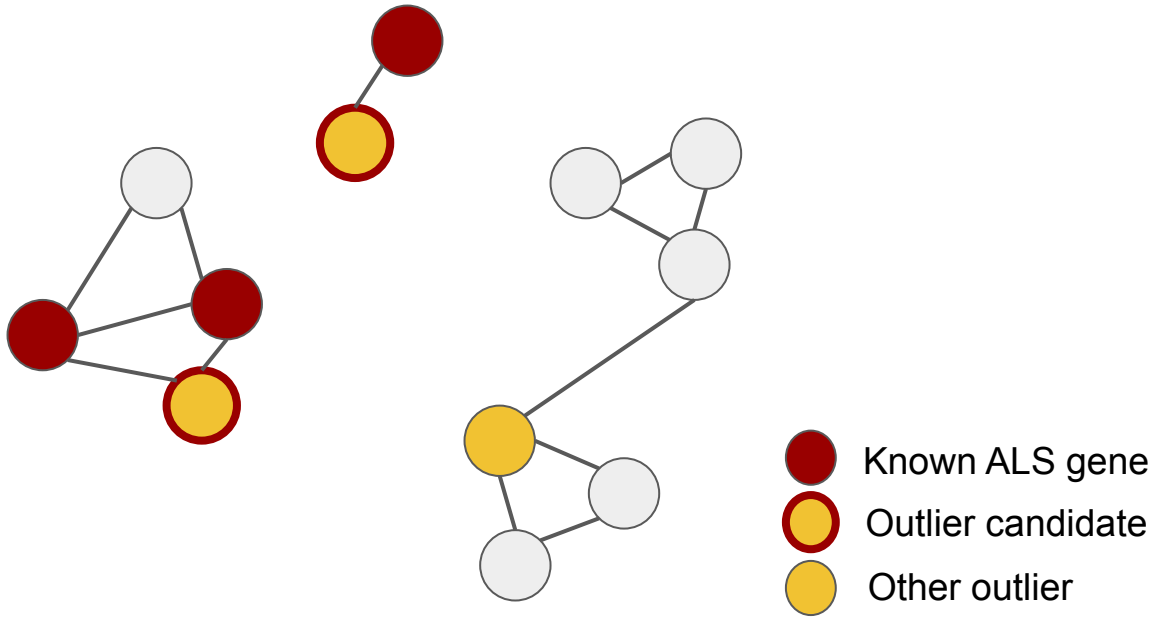
Deep-learning based aberrant splicing predictions[4]



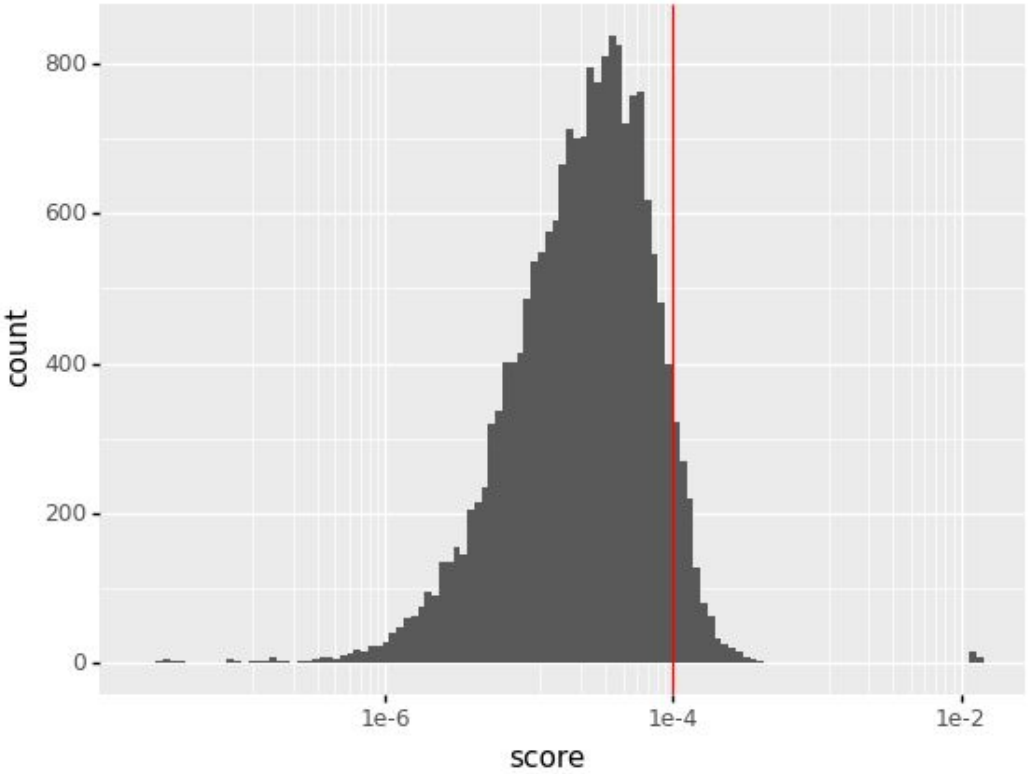
4. MMSplice, Cheng et al. Genome Biology (2019)
5. Karczewski et al. Nature (2020)

Gene network analysis - level 1:
Outliers in the network vicinity of ALS genes as new candidates

STRING https://string-db.org/
was used as a gene network.



Modeling network vicinity with random walks



Vicinity to ALS genes modeled as the probability of visiting the gene by random walks starting from an ALS gene.

Genes with a prob. Larger than 10^{-4} were considered interesting.

Results

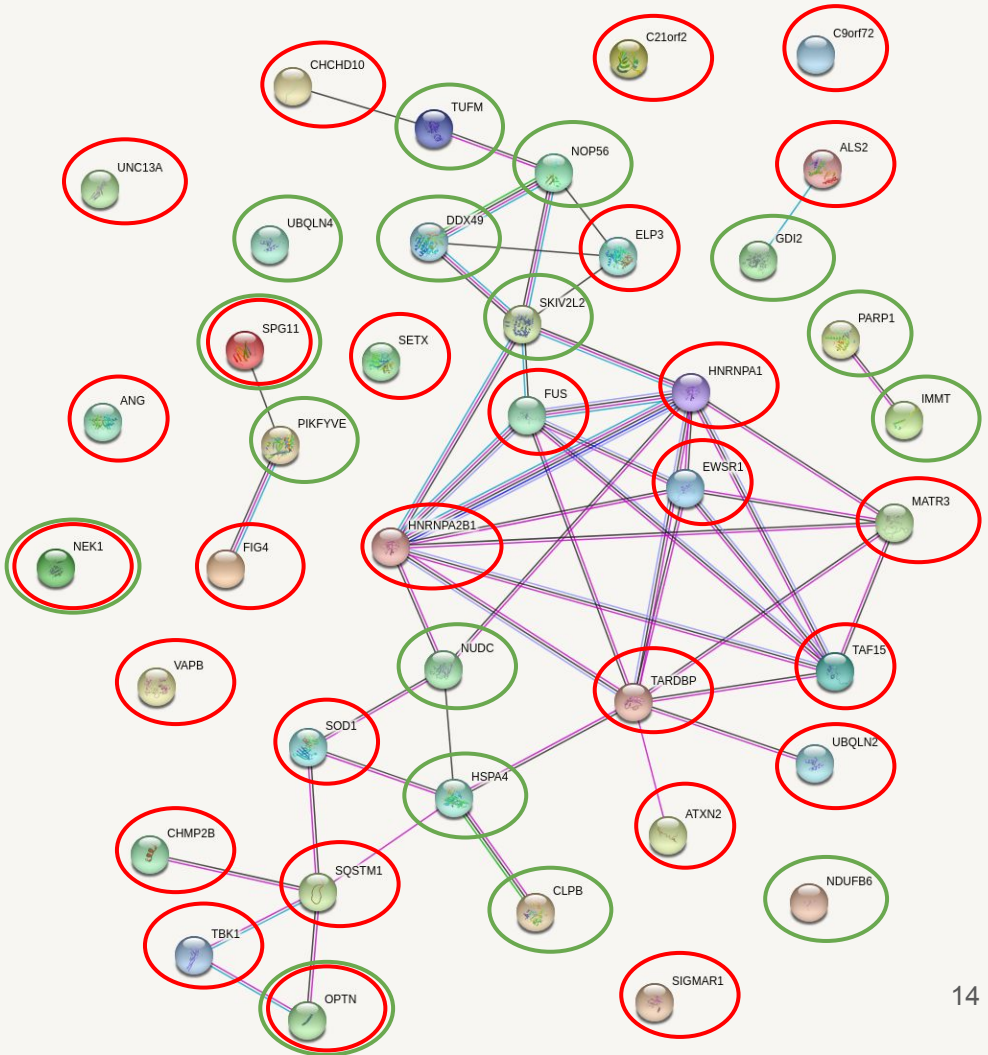
Network of known ALS genes and expression **outlier genes** containing a rare deleterious **variant** and a high **PPI score**.

Known ALS gene

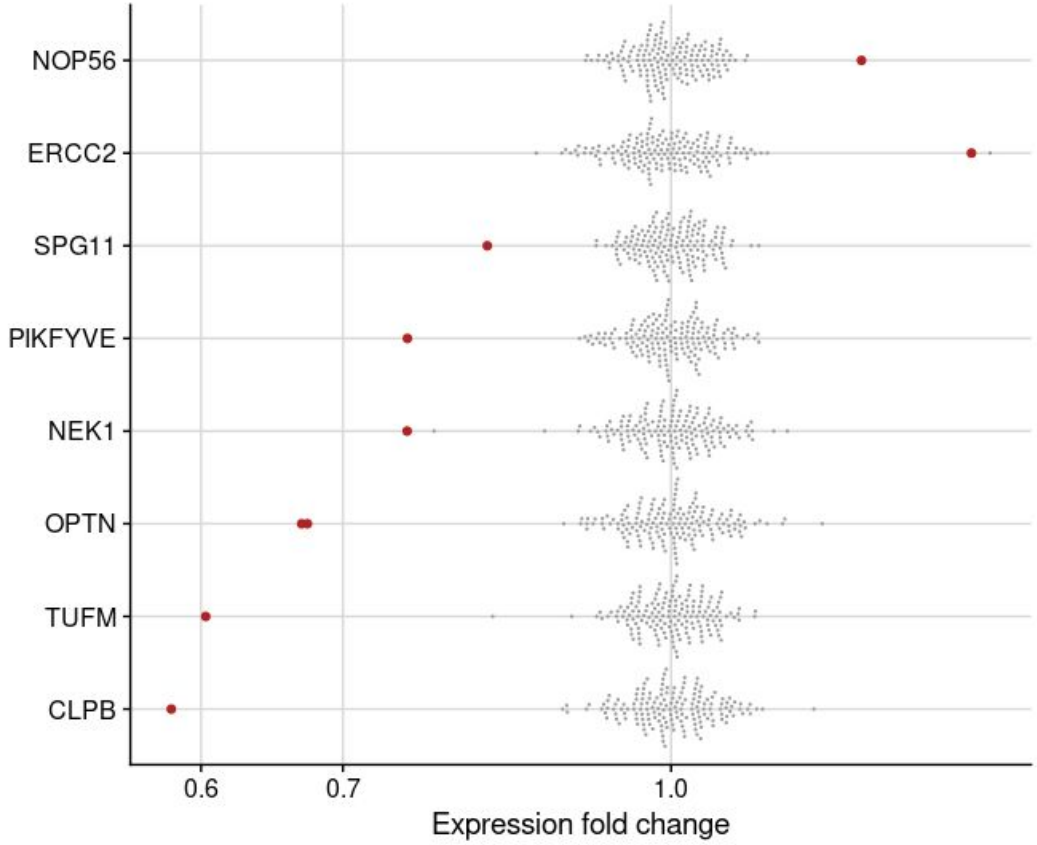
expression outlier

1. We found 16 expression outliers interacting with known ALS genes.

2. Some of them e.g. PIKFYVE connect known ALS genes



Identification of known genes and new interesting candidates



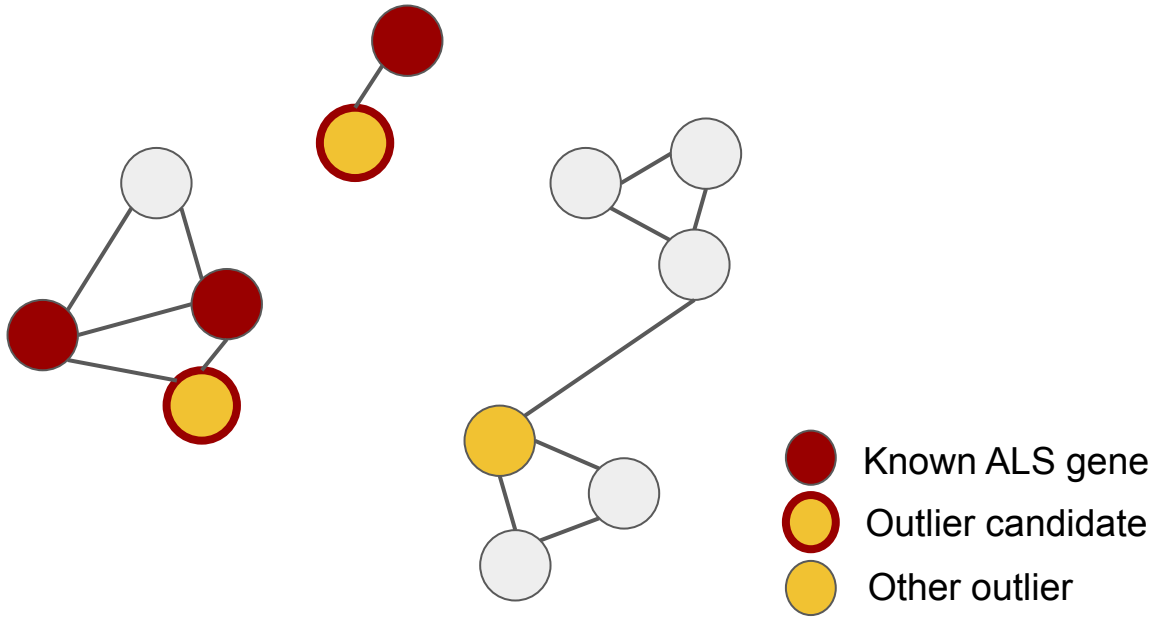
Identification of known genes and new interesting candidates

Aberrantly expressed genes containing a rare high impact variant.
The genes are either **known to cause ALS** (according to ALSoD) or **associated to other relevant diseases**. All genes are close to the established ALS genes in the gene network.

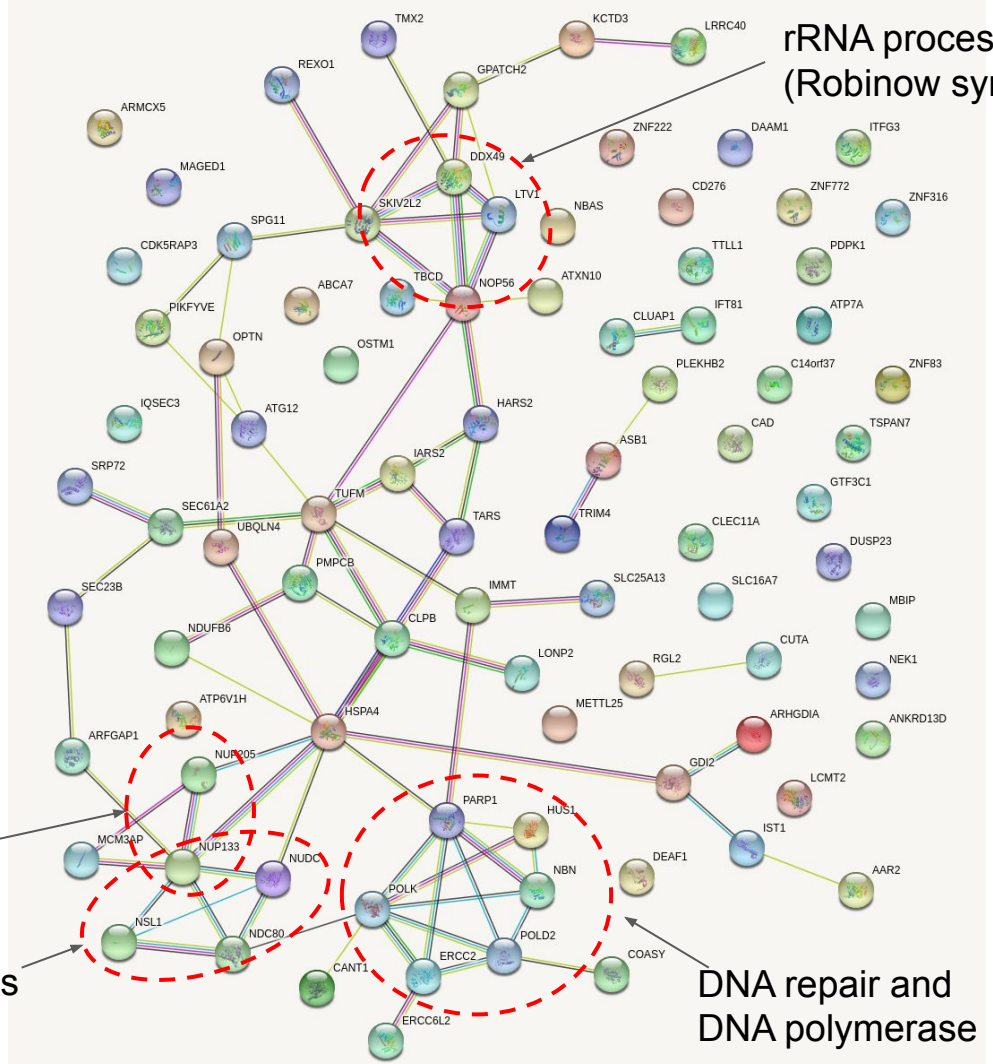
Sample	Gene	fold change	PPI score	Variant	Consequence	ClinVar	Comment
CASE.NEUEK191WYC	*NEK1*	0.75	1.22E-02	chr4:169424645:G>A	stop		Definitive ALS gene
CASE.NEUBK117YXL	*OPTN*	0.67	1.23E-02	chr10:13122390:C>A	stop		Definitive ALS gene
CASE.NEUZT557DHF	*OPTN*	0.67	1.23E-02	chr10:13112464:T>TAG	frameshift		Definitive ALS gene
CASE.NEUVX902YNL	*SPG11*	0.82	1.23E-02	chr15:44620189:C>A	splice donor	likely pathogenic	Tenuous ALS gene, variant predicted to cause aberrant splicing
CASE.NEULD354RZB	*NOP56*	1.23	1.61E-04	chr20:2655751:G>A	splice region		Variant predicted to cause aberrant splicing. Gene related to Ataxia.
CASE.NEUTA689LN5	*TUFM*	0.60	1.06E-04	chr16:28844814:G>A	stop	uncertain significance	Mitochondrial disease gene
CASE.NEUGW326BRV	*CLPB*	0.58	1.30E-04	chr11:72302312:G>A	stop	pathogenic	Mitochondrial disease gene
CASE.NEUME498PCJ	*PIKFYVE*	0.75	1.54E-04	chr2:208352730:A>AT	frameshift		Linked to neurodegeneration
CASE.NEURR881FKY	*ERCC2*	1.38	5.86E-05	chr19:45364832:CCTCA>C	splice donor	likely pathogenic	Causes neurological symptoms, e.g. spasticity and reflex abnormalities, and skin manifestations

Gene network analysis - level 2: Clusters of outliers as new candidates

STRING https://string-db.org/ was used as a gene network.



Further outliers with rare deleterious variants interact with each other indicating new implicated pathways



Discussion / outlook

- These new candidate genes could expand the understanding of pathways involved in the etiology of ALS

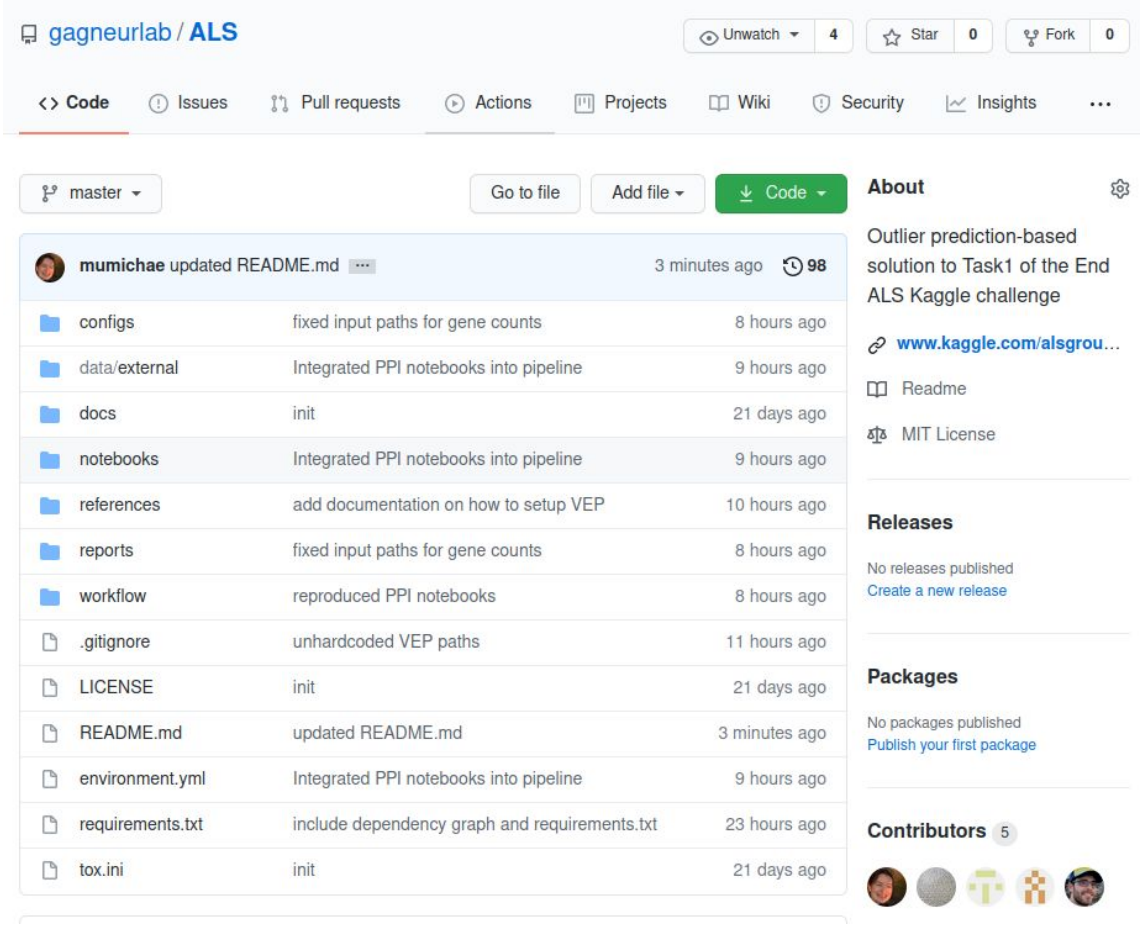
- Future analysis would include:
  - Replicating the findings looking at WGS of the entire ALS dataset (other patients with damaging variants in the same genes)
  - Multi-omics outlier analysis : ATAC-seq, splicing, proteomics
  - Functional follow-ups

Conclusion

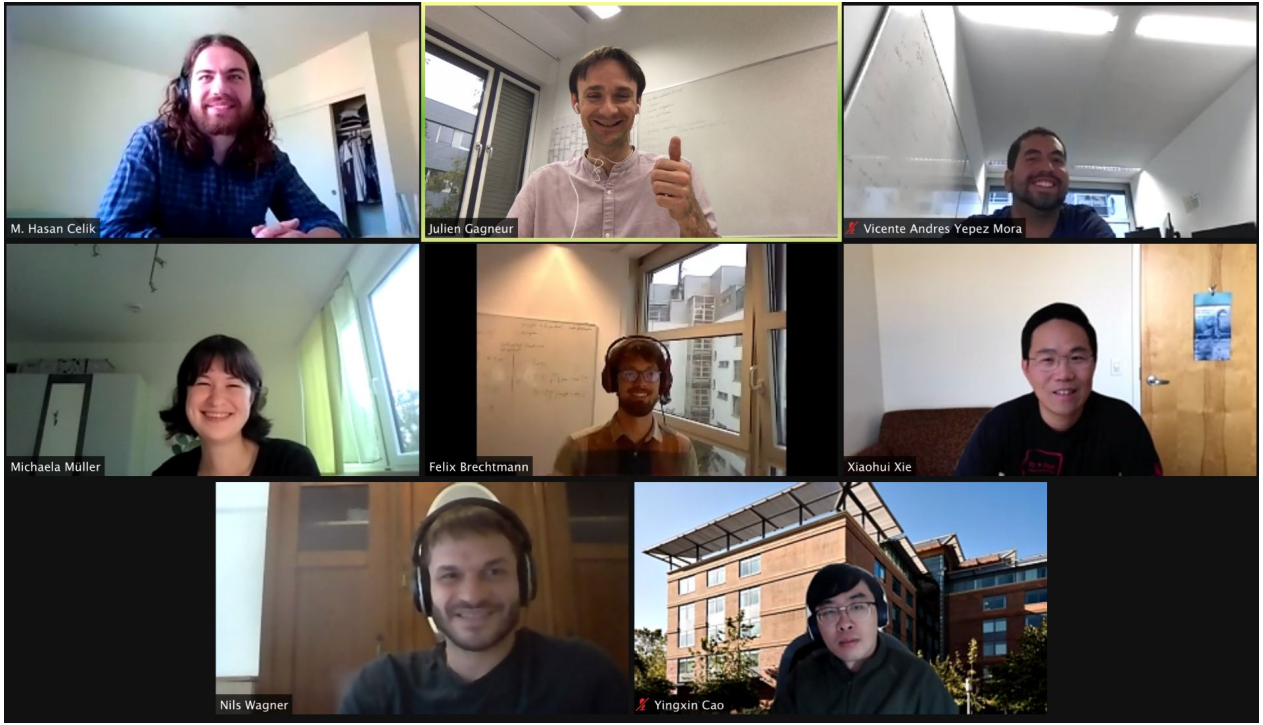
- We found variants associated with aberrant expression for known ALS genes, potentially characterising those affected patients (n = 4)
- We found new high impact variants in further cases in a gene potentially related to ALS, which would improve our catalogue of pathogenic variants
- We found new candidate genes in known pathways
- We found potential new pathways
- Altogether, this gives a potential genetic explanation to 63 (46%) of the patients and further supports a multi-causal view of ALS

Code to reproduce the results

https://github.com/gagneurlab/ALS



The team



Felix Brechtmann[1], Hasan Çelik[2], Julien Gagneur[1], Florian Hölzlwimmer[1], Michaela Müller[1], Nils Wagner[1], Xiaohui Xie[2], Vicente Yépez[1], Michael Zech[1]

[1] Technical University of Munich [2]University of California, Irvine

Appendix

Analysis Workflow

- Reproducible pipeline in Snakemake
- Parallelized and robust
- Main steps:
  - Prepare gene counts
  - OUTRIDER analysis
  - Variant annotation
  - PPI network analysis
  - UMAP on expression space (not shown here)