

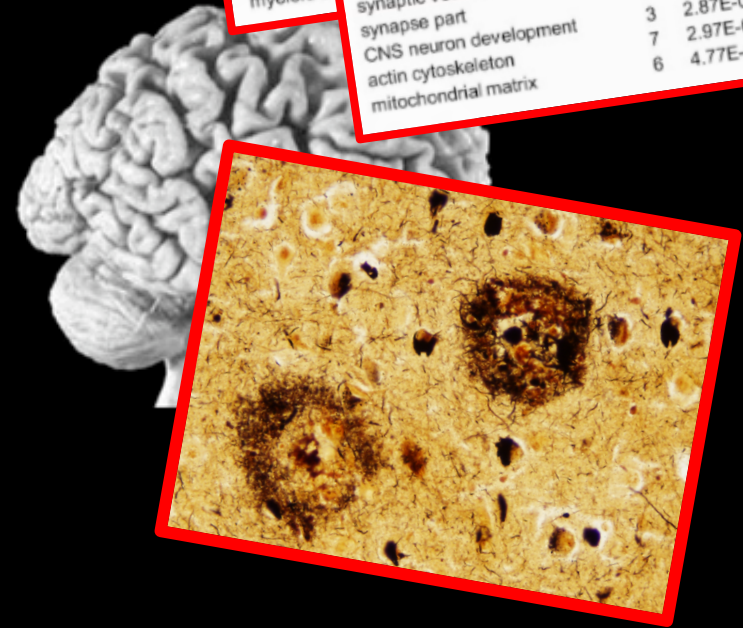
# Bioinformatic analysis of familial Alzheimer's disease-like zebrafish

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University of Adelaide



Healthy



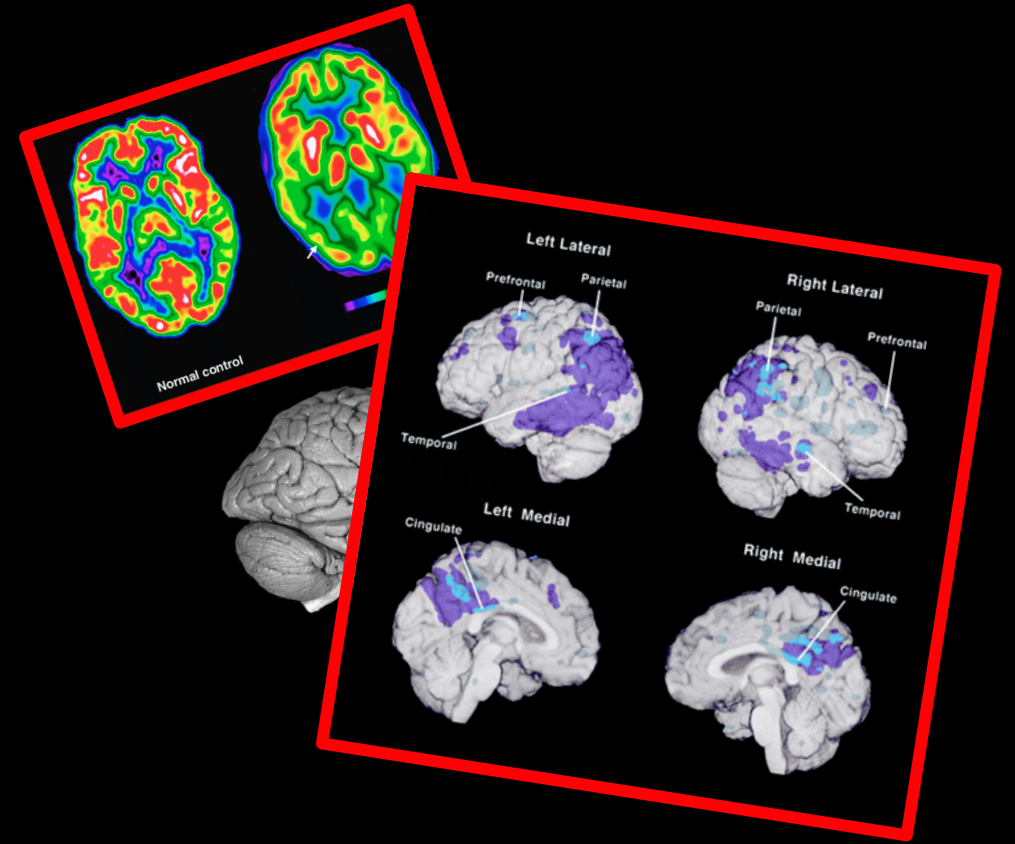
Alzheimer's

Upregulated by AD	#	PValue
cell adhesion	10	8.05E-04
response to wounding	7	1.17E-03
adherens junction	4	7.10E-03

Downregulated by AD	#	PValue
precursor metabolites & energy	18	7.47E-10
organelle envelope	11	4.66E-05
microtubule-based process	29	9.92E-04
ribonucleotide binding	4	9.95E-04
synaptic vesicle membrane	8	5.60E-03
synapse part	3	2.87E-02
CNS neuron development	7	2.97E-02
actin cytoskeleton	6	4.77E-02
mitochondrial matrix		



Healthy



Before Alzheimer's

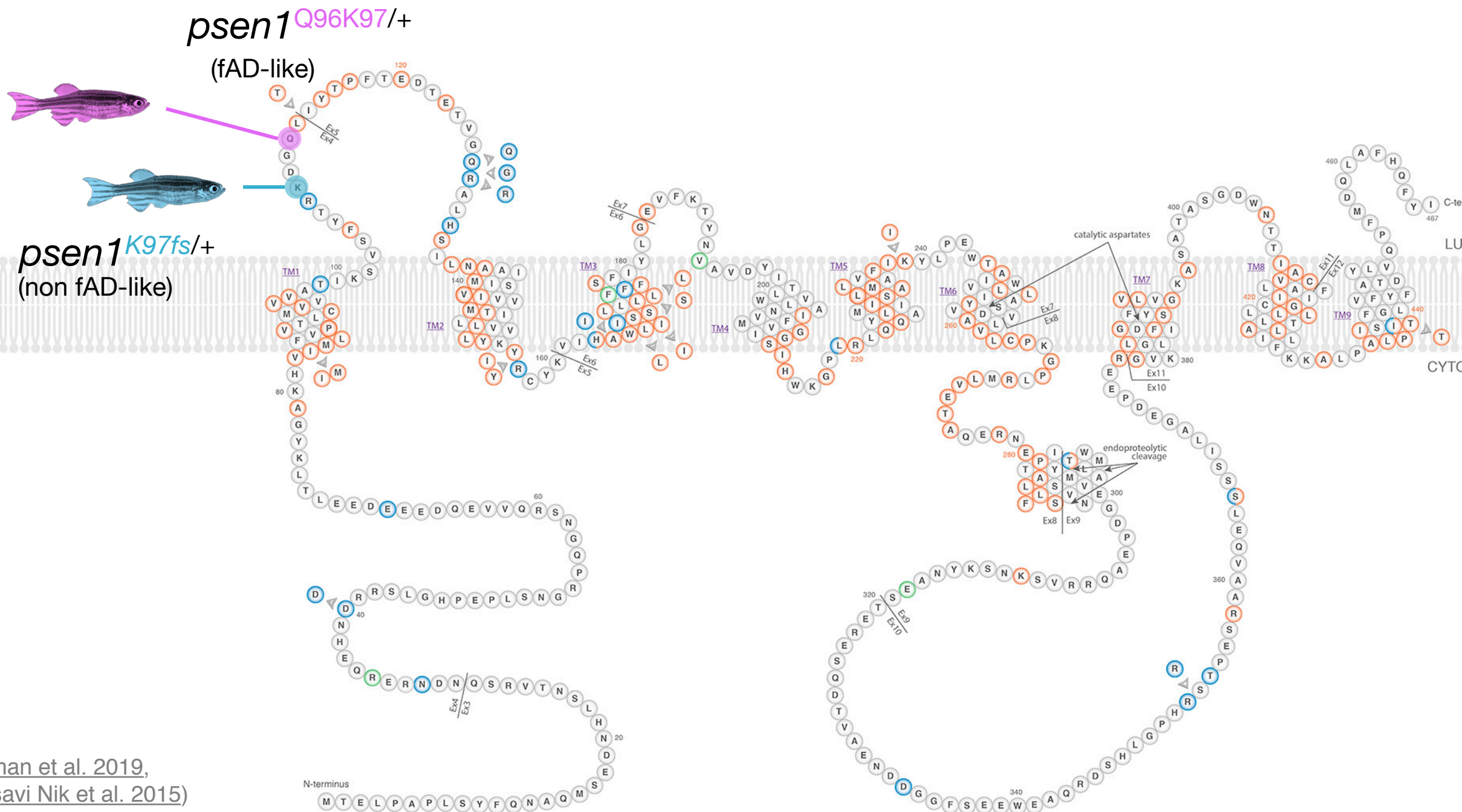


Healthy



**(Modelling genetic  
state of familial AD)**

Alzheimer's-like



(Newman et al. 2019,  
Moussavi Nik et al. 2015)

# Compare between groups

to look for gene expression differences



**Age:** 6 months  
***psen1*:** +/+  
(n=4)



**Age:** 6 months  
***psen1*:** Q96K97/+  
(n=4)

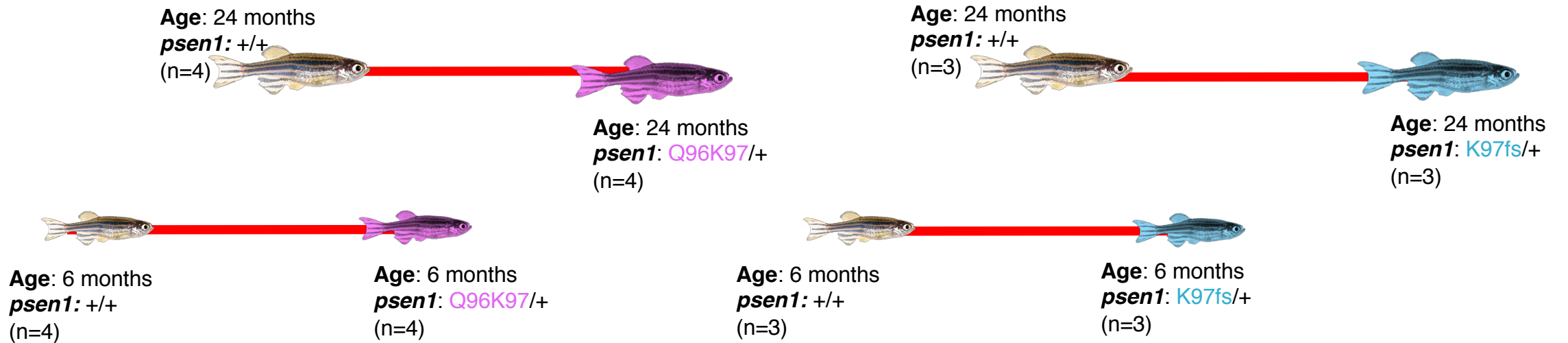


**Age:** 6 months  
***psen1*:** +/+  
(n=3)

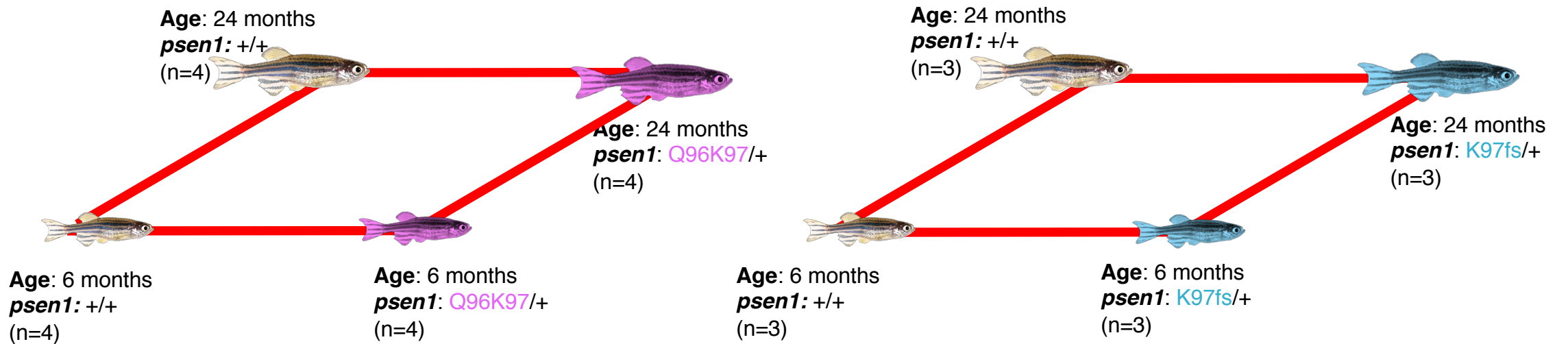


**Age:** 6 months  
***psen1*:** K97fs/+  
(n=3)

# Compare between groups to look for gene expression differences

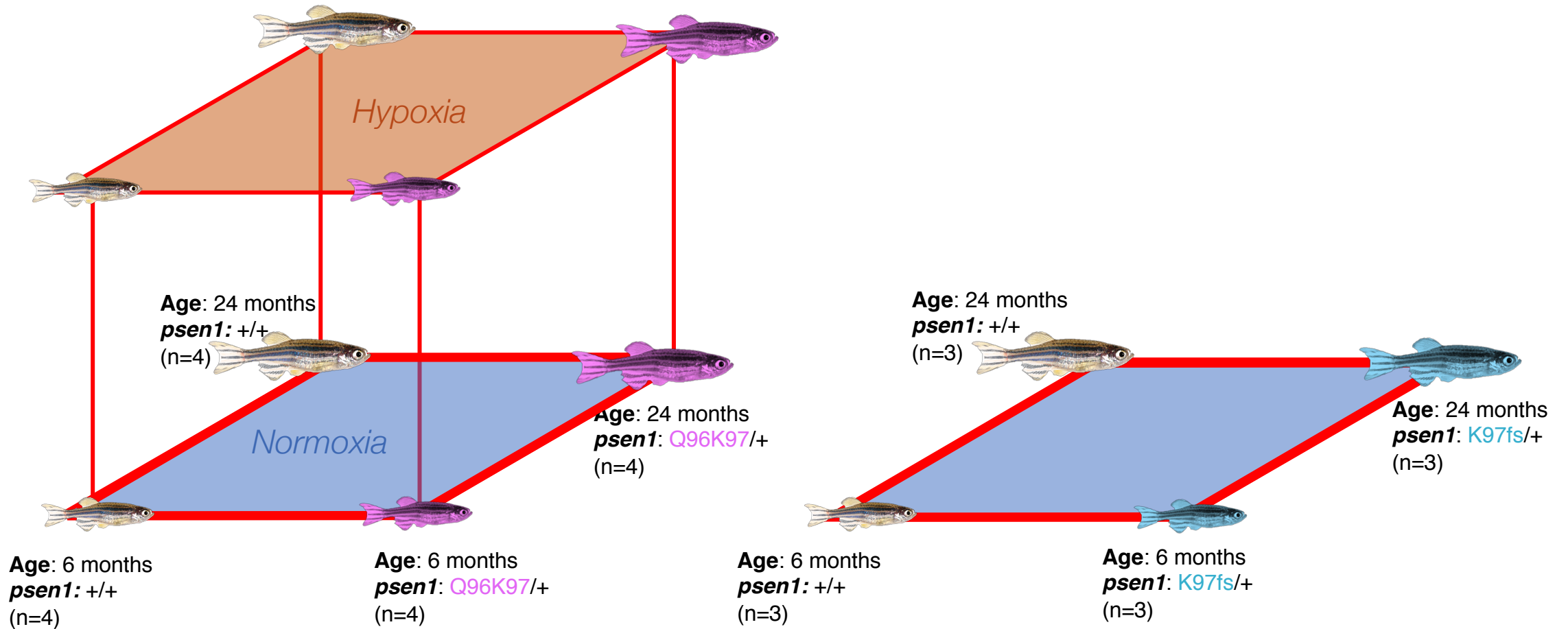


# Compare between groups to look for gene expression differences

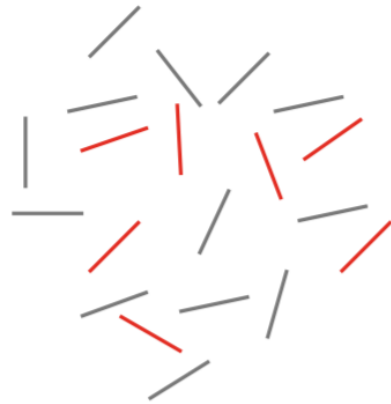




# Compare between groups to look for gene expression differences

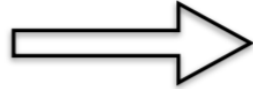


# RNA-seq analysis



Libraries of reads prepared from RNA extracted from zebrafish brains

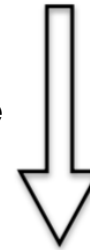
1. Trim adapters, quality filtering,



2. Align reads to reference zebrafish genome assembly



3. Count reads aligning to gene



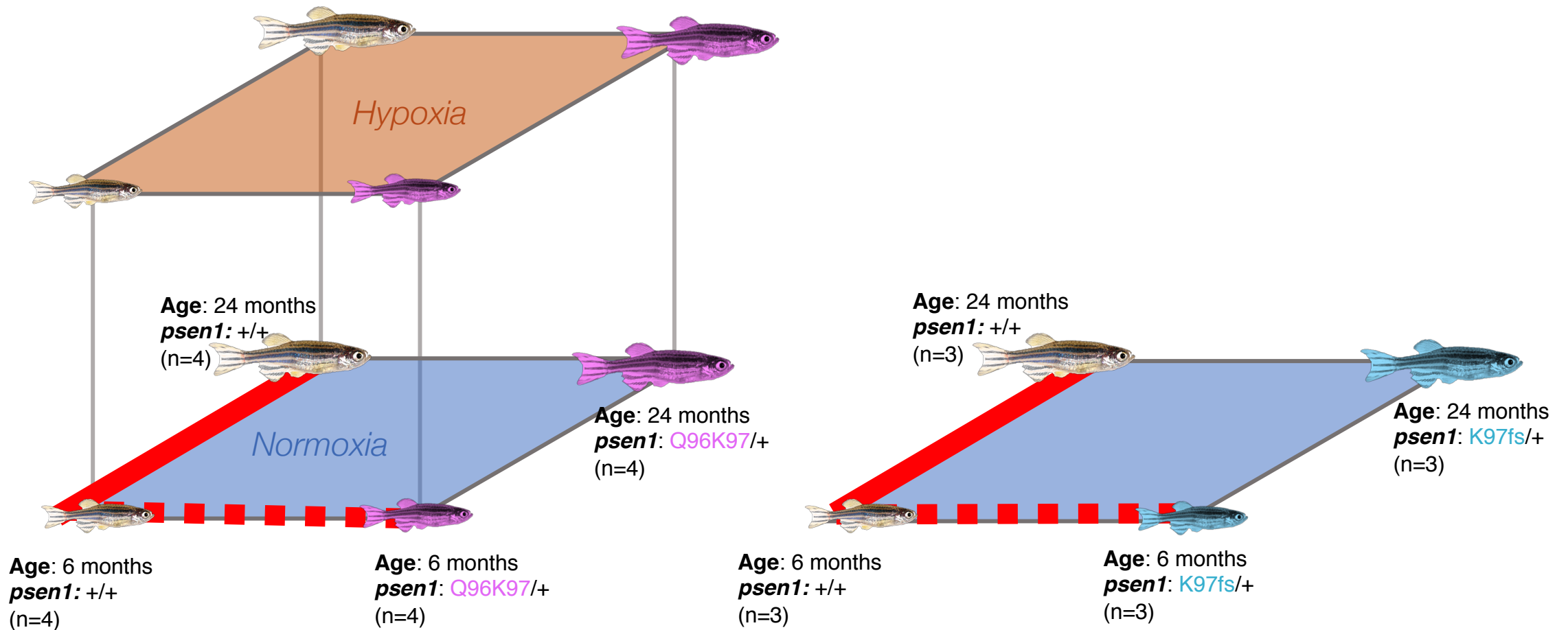
	Sample 1	Sample 2	Sample 3
<b>Gene A</b>	6	3	2
<b>Gene B</b>	13	4	12
.	.	.	.
.	.	.	.
.	.	.	.
<b>Gene X</b>	.	.	.

Gene-by-sample matrix  
~20,000 genes

4. Moderated *t*-test approach to determine genes which are differentially expressed between conditions (FDR-adjusted  $p$ -value  $< 0.05$ ).

5. Test whether biological functions / pathways are over-represented in the DE genes (FDR-adjusted  $p$ -value  $< 0.05$ ).

# 1. "Accelerated aging" in young adult mutants

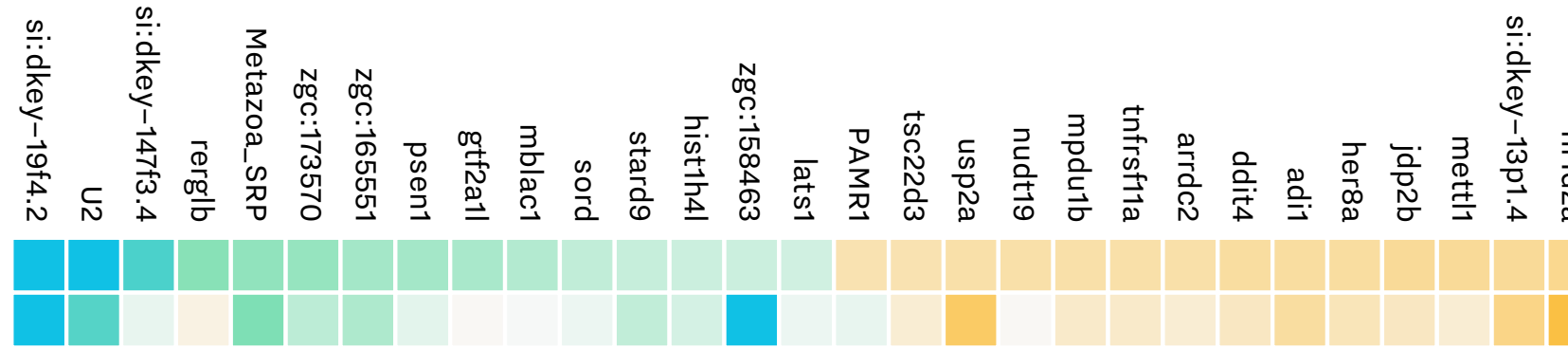


# 1. “Accelerated aging” in young adult mutants

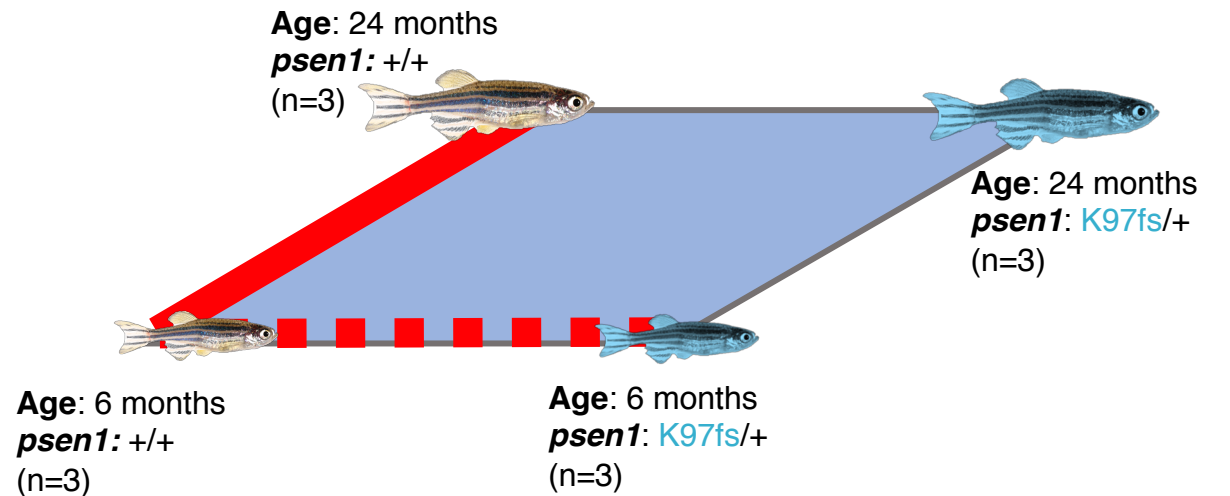
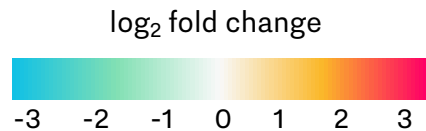
COMPARISON

**6-month mutant vs. 6-month wildtype**

**24-month wildtype vs. 6-month wildtype**

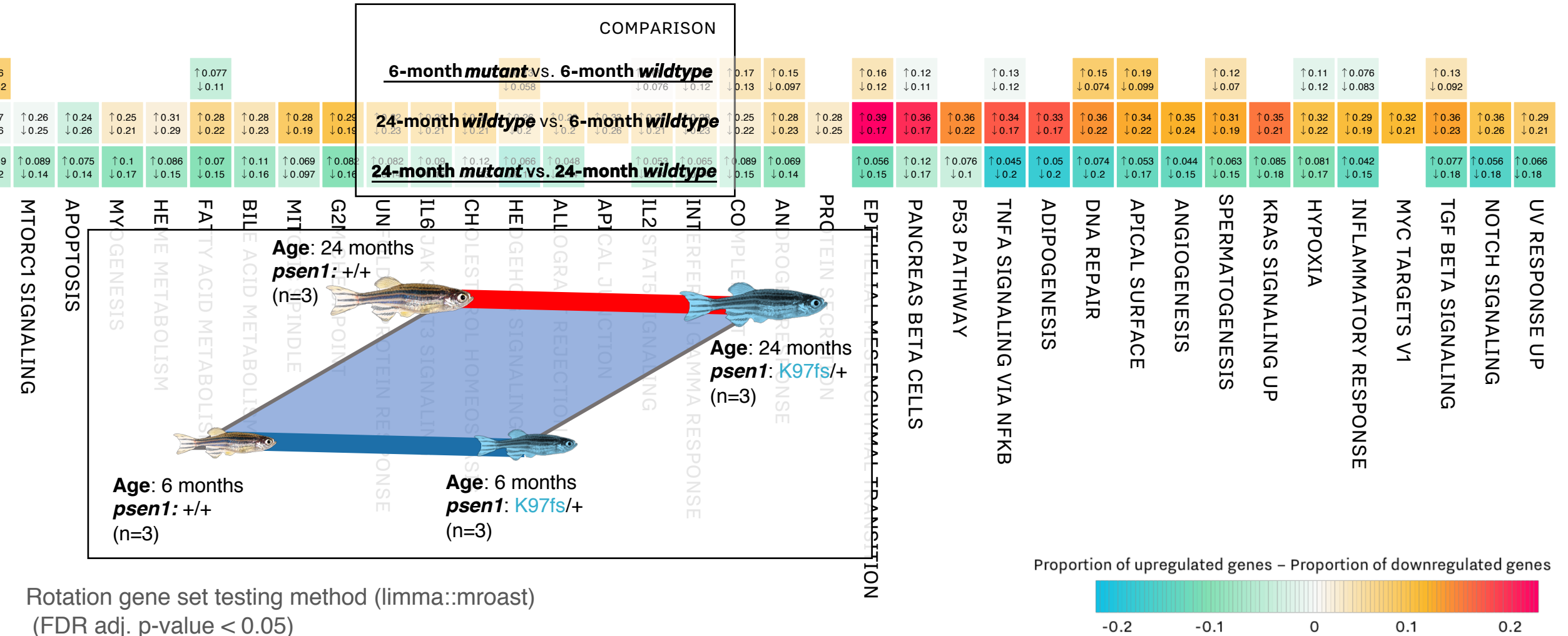


Differentially expressed genes between K97fs/+ and +/+ at 6 months (FDR adj.  $p$ -value < 0.05)



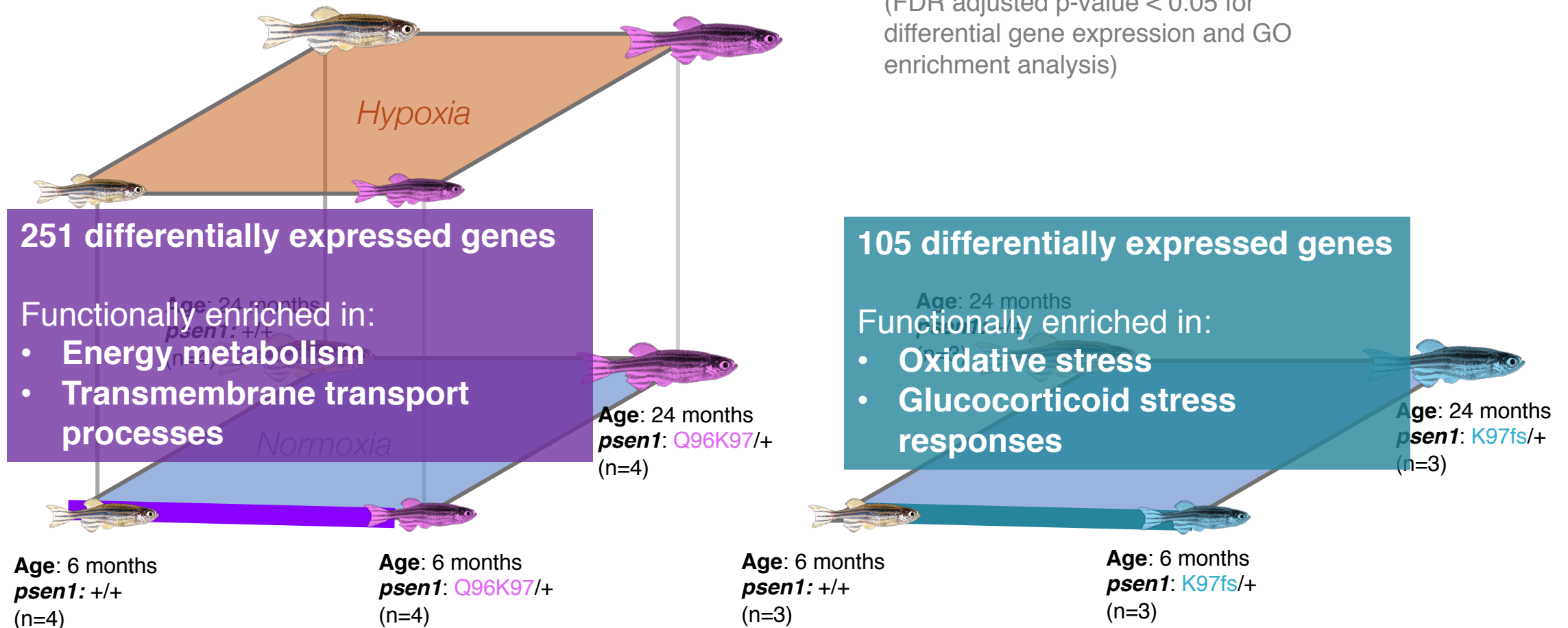


## 2. Gene expression changes in aged mutant brains, mostly distinct from young mutant brains



### 3. Q96K97 and K97fs mutations have different effects on gene expression at 6 months of age

(FDR adjusted p-value < 0.05 for differential gene expression and GO enrichment analysis)



**HYPOTHESIS AND THEORY ARTICLE**

Front. Neurosci., 13 August 2018 | <https://doi.org/10.3389/fnins.2018.00533>



## Dysregulation of Neuronal Iron Homeostasis as an Alternative Unifying Effect of Mutations Causing Familial Alzheimer's Disease

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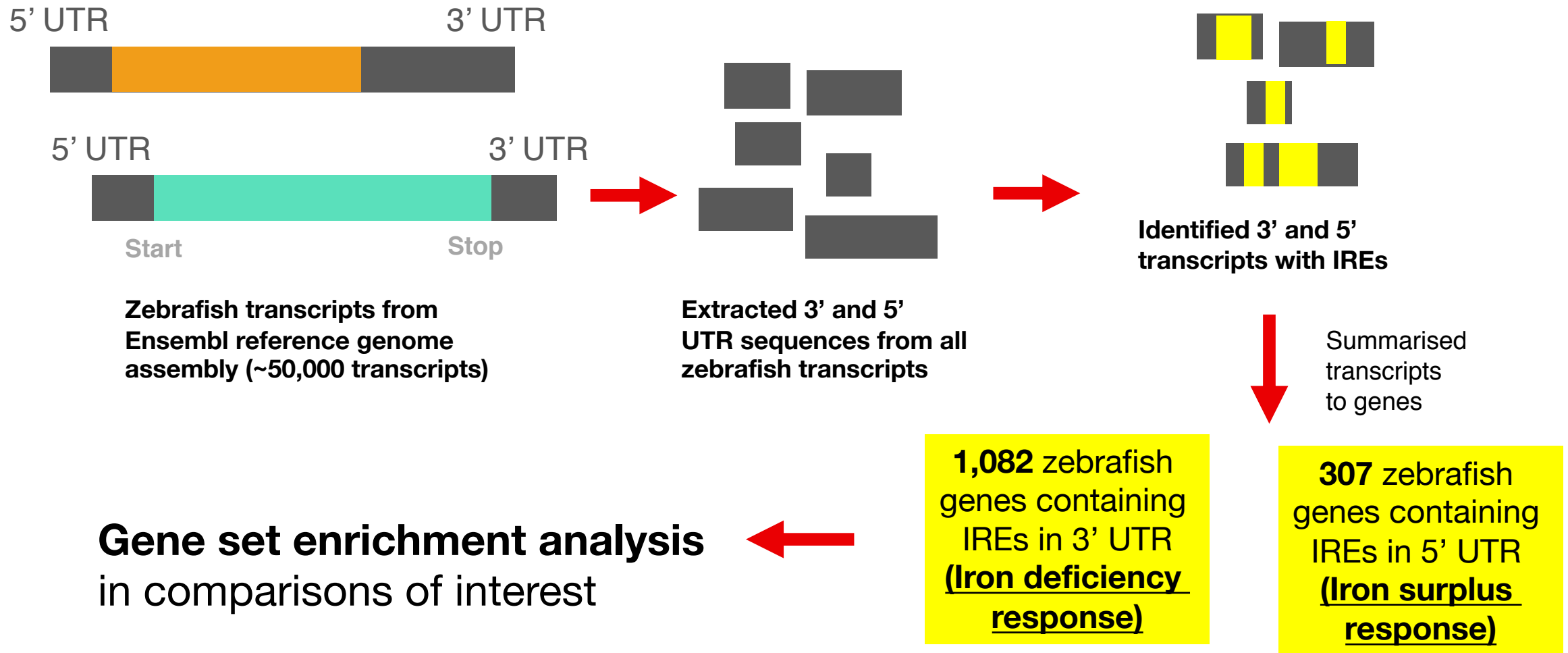
<sup>7</sup>School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Bentley, WA, Australia

## Is iron homeostasis disrupted in **Q96K97/+** (fAD-like) zebrafish? Can this be seen in the brain RNA-seq data?

1. Define gene set representing iron homeostasis genes (genes containing Iron Regulatory Elements in their UTRs).
2. Apply gene set enrichment analysis to assess differences in iron homeostasis in the *psen1*<sup>Q96K97/+</sup> fish vs. wild type siblings.



# Defining sets of genes containing Iron Regulatory Elements

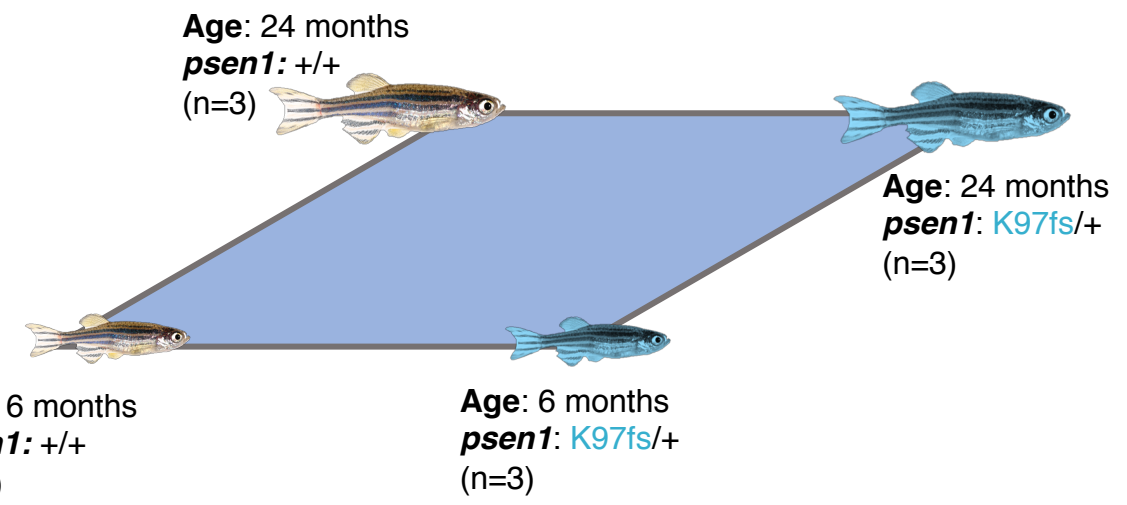
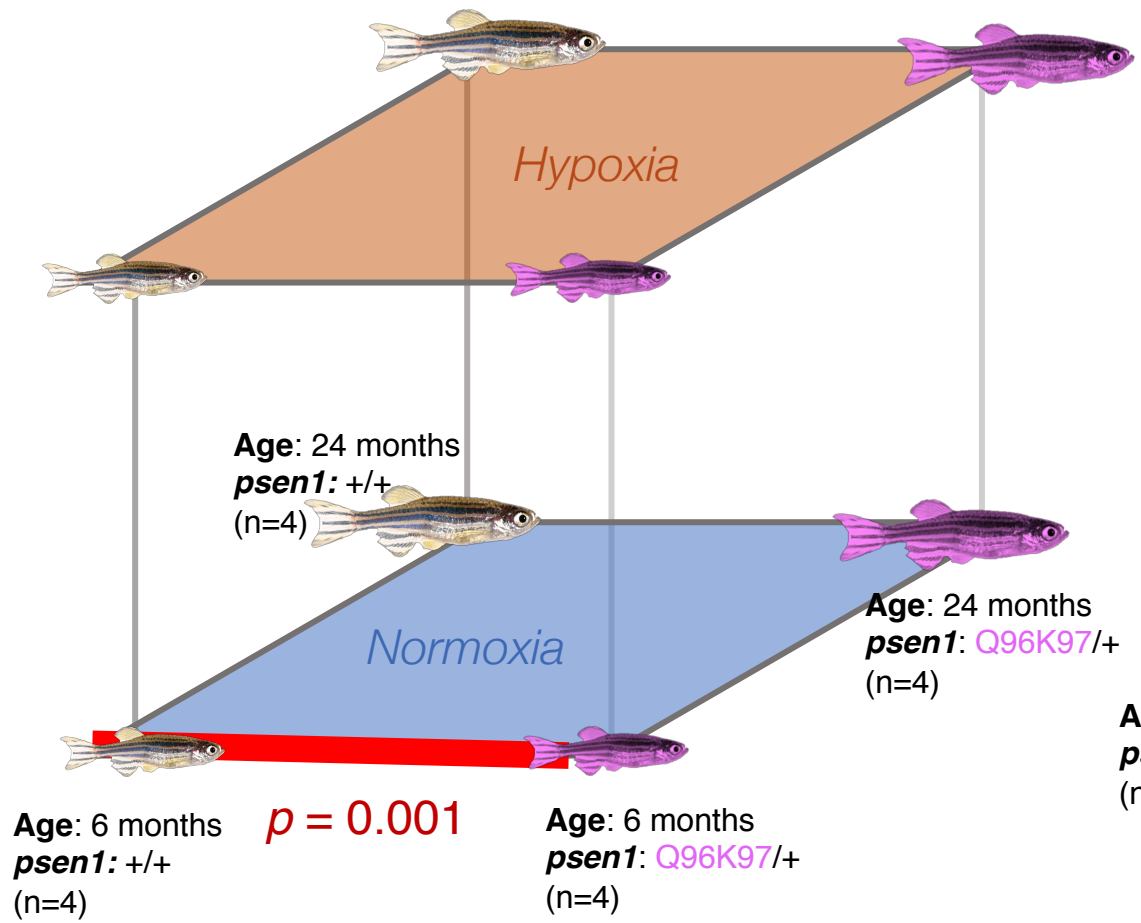


These gene sets represent genes involved in iron metabolism / homeostasis

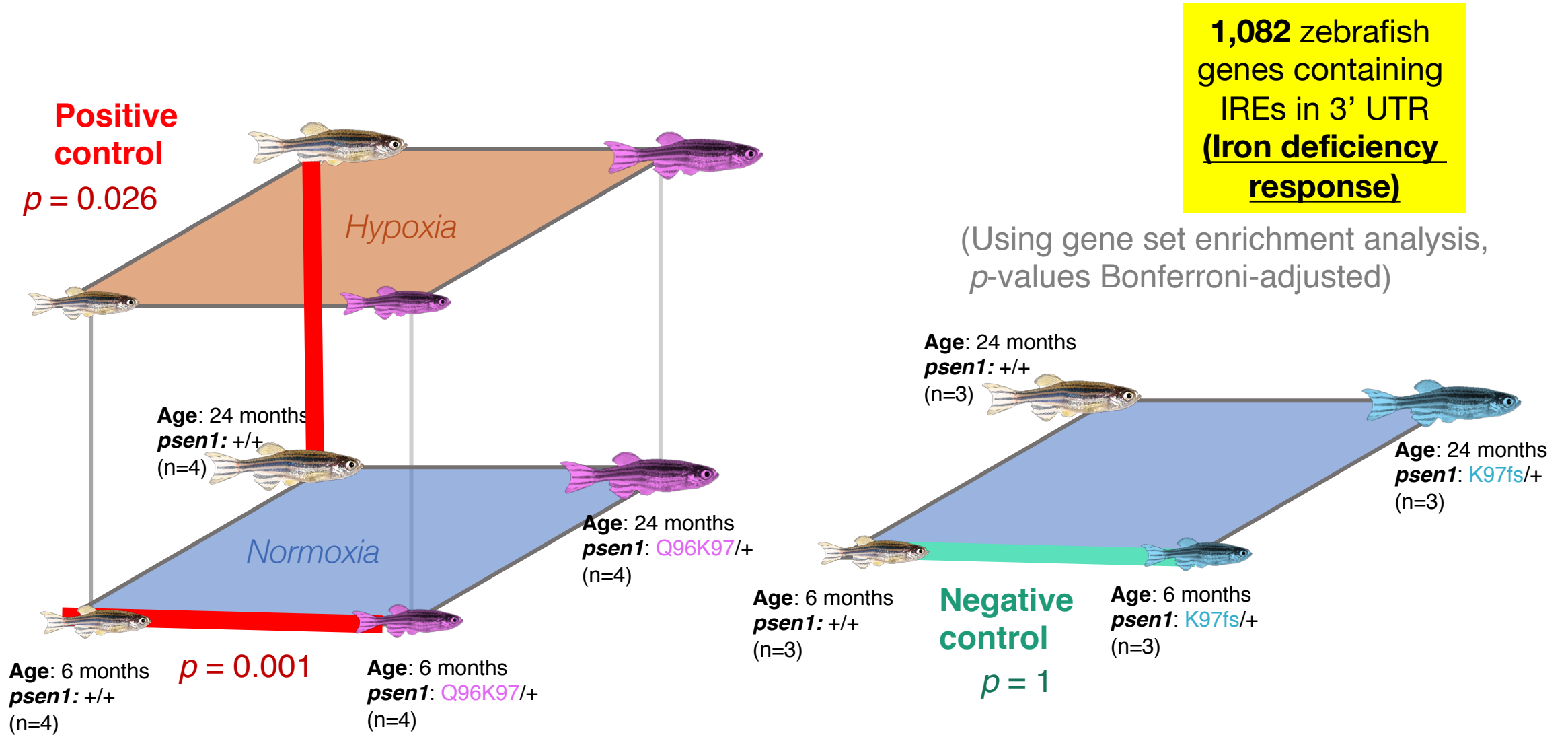
# 4. Evidence of iron deficiency response in Q96K97/+ brains at 6 months

1,082 zebrafish genes containing IREs in 3' UTR (Iron deficiency response)

(Using gene set enrichment analysis, *p*-values Bonferroni-adjusted)



# 4. Evidence of iron deficiency response in Q96K97/+ brains at 6 months



# Summary

1. Analysing RNA-seq data can be an effective approach for detecting **broad patterns of gene expression changes** across the brain, and also more **targeted hypotheses** (with a representative gene set).
2. Gene set enrichment testing shows preliminary evidence to support iron dyshomeostasis in early stages of familial Alzheimer's disease (need to test other zebrafish models/mutations).

# Acknowledgements

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