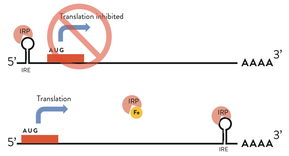
We observed iron deficiency in the brain with aging, hypoxia, & a familial Alzheimer's disease-like mutation.

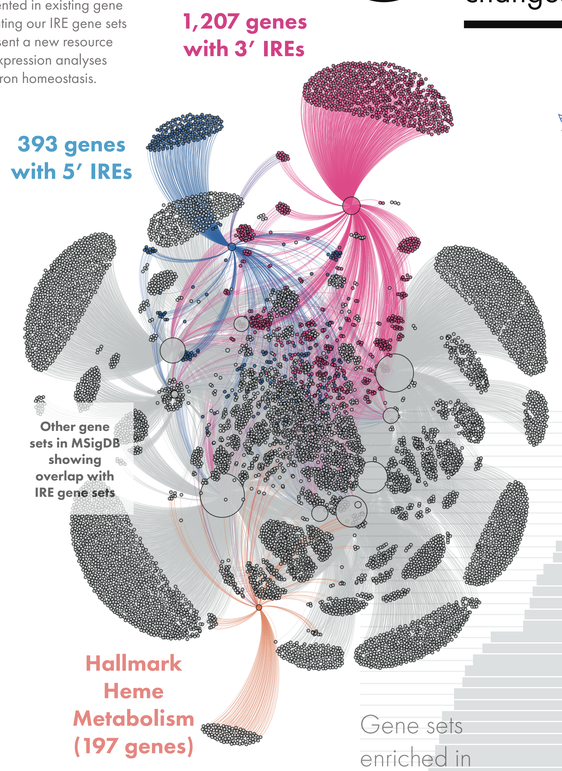
A straightforward approach using RNA-seq data to explore iron homeostasis.

1 Defining IRE gene sets for monitoring iron homeostasis

Under iron-deficient conditions, Iron Responsive Proteins bind to Iron Responsive Elements, which are located in UTRs of genes involved in iron homeostasis. Generally, this results in increased 3′ IRE gene expression and decreased 5′ IRE gene expression[1].



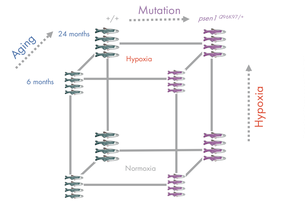
We searched[2] all zebrafish transcript UTRs for IRE stem-loop motifs to form comprehensive sets of genes regulated by 3′ and 5′ IREs. Many genes in these lists are not represented in existing gene sets, indicating our IRE gene sets may represent a new resource for gene expression analyses exploring iron homeostasis.

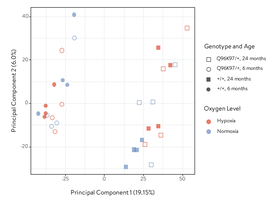


2 Characterising brain gene expression changes in mutants

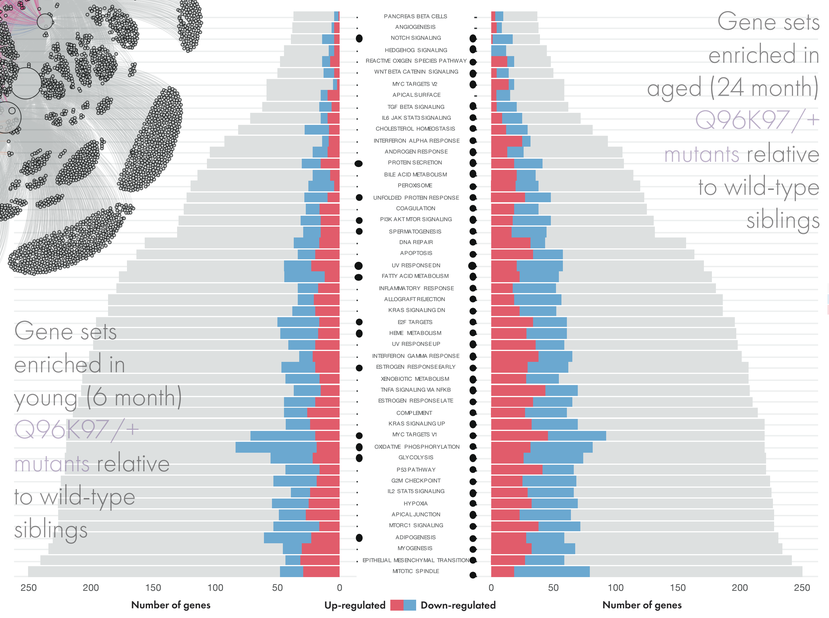
Whole zebrafish brains were subjected to poly-A-enriched RNA-sequencing. Pairwise comparisons between conditions were used to explore effects of the fAD-like mutation, aging, and hypoxia on gene expression.

Principal Component Analysis (PCA) was used to visualise overall similarity between samples. In the PCA plot of ~20,000 expressed genes, the effects of aging appeared to obscure gene expression changes due to genotype or hypoxia.



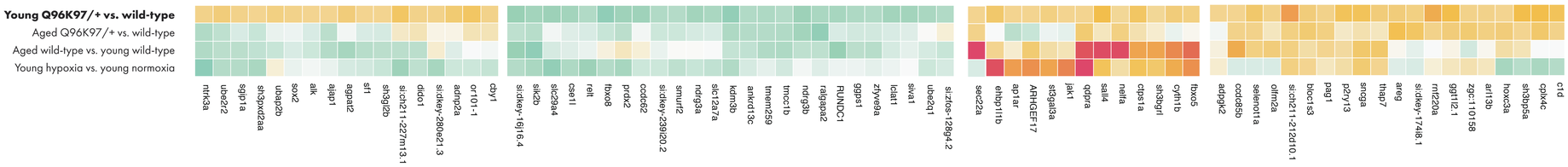


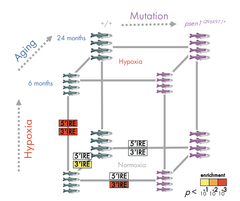
To explore coordinated gene expression changes in Q96K97/+ mutants relative to wild-type siblings, we performed Gene Set Enrichment Analysis on MSigDB Hallmark gene sets[3]. Significant gene sets at 6 months are useful clues for pathways altered earlier in disease pathogenesis (● indicates GSEA enrichment FDR-adj. $p < 0.05$). Up/Down-regulated indicate genes in gene sets that were significantly differentially expressed via *limma*[4] analysis (FDR-adj. $p < 0.05$).

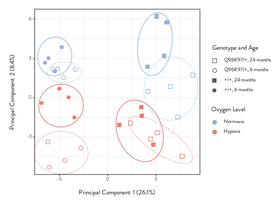


3 Exploring iron deficiency through 3′ IRE gene expression

We focused on iron homeostasis through the IRE genes defined earlier to see whether evidence of an iron deficiency response was present in certain conditions. Together, the Gene Set Enrichment Analysis, PCA, and heatmap of DE genes containing IREs in the young mutant vs. wild-type siblings below all suggest that an 3′ IRE response occurs in aging, hypoxia, and Q96K97/+ mutants, and shared gene expression changes are present. Our findings represent the first evidence in a genetic model of familial Alzheimer's disease supporting a recently-proposed hypothesis that positions disrupted iron homeostasis as a key effect of familial Alzheimer's disease mutations[5].







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