Brain transcriptome analysis of a familial Alzheimer’s disease-like mutation in the zebrafish presenilin 1 gene implies effects on energy production

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Abstract
To prevent or ameliorate Alzheimer’s disease (AD) we must understand its molecular basis. AD develops over decades but detailed molecular analysis of AD brains is limited to postmortem tissue where the stresses initiating the disease may be obscured by compensatory responses and neurodegenerative processes. Rare, dominant mutations in a small number of genes, but particularly the gene PRESENILIN 1 (PSEN1), drive early onset of familial AD (EOFAD). Numerous transgenic models of AD have been constructed in mouse and other organisms, but transcriptomic analysis of these models has raised serious doubts regarding their representation of the disease state. Since we lack clarity regarding the molecular mechanism(s) underlying AD, we posit that the most valid approach is to model the human EOFAD genetic state as closely as possible. Therefore, we sought to analyse brains from zebrafish heterozygous for a single, EOFAD-like mutation in their PSEN1-orthologous gene, psen1. We previously introduced an EOFAD-like mutation (Q96_K97del) into the endogenous psen1 gene of zebrafish. Here, we analysed transcriptomes of young adult (6-month-old) entire brains from a family of heterozygous mutant and wild type sibling fish. Gene ontology (GO) analysis implies effects on mitochondria, particularly ATP synthesis, and on ATP-dependent processes including vacuolar acidification.

Keywords: Alzheimer’s disease, Presenilin 1, Mutation, Transcriptome, Brain, ATP synthesis, Mitochondria, Vacuolar acidification, Zebrafish, Genome editing

Background
AD is the most common form of dementia with severe personal, social, and economic impacts. Rare, familial forms of AD exist caused by autosomal dominant mutations in single genes (reviewed by [1]). The majority of these mutations occur in the gene PRESENILIN 1 (PSEN1) that encodes a multipass integral membrane protein involved in intra-membrane cleavage of numerous proteins [1].

A wide variety of transgenic models of AD have been created and studied. These are aimed at reproducing histopathologies posited to be central to the disease process, i.e. amyloid plaques and neurofibrillary tangles of the protein MAPT [2]. However, analysis of the effects on the brain transcriptome of the transgenes driving a number of these mouse models showed little concordance with transcriptomic differences between human AD brains and age-matched controls [3] (although a recent study asserts that this lack of concordance for the popular “5XFAD” transgenic mouse model is due to previous failure to analyse the effects of its transgenes in a variety of genetic backgrounds [4]). We posit that, in the absence of an understanding of the molecular mechanism(s) underlying AD, the most objective approach to modeling this disease (or, at least, modeling its genetic form, EOFAD) is to create a genetic state as similar as possible to the EOFAD state in humans. Mouse “knock-in” models of EOFAD mutations were created over a decade ago and showed subtle phenotypic effects but not the desired histopathologies (e.g. [5, 6]).
| Gene Ontology Term | Ontology | Total Genes | DE Genes | p-value   | FDR p-value |
|--------------------|----------|-------------|----------|-----------|-------------|
| ATP biosynthetic process | BP       | 29          | 7        | 3.48987E-08 | 0.00041     |
| ribonucleoside triphosphate biosynthetic process | BP       | 49          | 8        | 9.41317E-08 | 0.00045     |
| nucleoside triphosphate biosynthetic process | BP       | 54          | 8        | 2.06555E-07 | 0.00060     |
| purine nucleoside triphosphate biosynthetic process | BP       | 41          | 7        | 4.46237E-07 | 0.00060     |
| purine ribonucleoside triphosphate biosynthetic process | BP       | 41          | 7        | 4.46237E-07 | 0.00060     |
| hydrogen transport | BP       | 60          | 8        | 4.783E-07  | 0.00060     |
| proton transport   | BP       | 60          | 8        | 4.783E-07  | 0.00060     |
| energy coupled proton transport, down electrochemical gradient | BP       | 27          | 6        | 5.89038E-07 | 0.00060     |
| ATP synthesis coupled proton transport | BP       | 27          | 6        | 5.89038E-07 | 0.00060     |
| transport          | BP       | 2072        | 48       | 2.11748E-06 | 0.00165     |
| purine nucleoside monophosphate biosynthetic process | BP       | 54          | 7        | 3.09019E-06 | 0.00172     |
| purine ribonucleoside monophosphate biosynthetic process | BP       | 54          | 7        | 3.09019E-06 | 0.00172     |
| hydrogen ion transmembrane transport | BP       | 54          | 7        | 3.09019E-06 | 0.00172     |
| ribonucleoside triphosphate metabolic process | BP       | 133         | 10       | 3.8448E-06  | 0.00178     |
| establishment of localization | BP       | 2123       | 48       | 4.20295E-06 | 0.00182     |
| ATP metabolic process | BP       | 109         | 9        | 5.50772E-06 | 0.00230     |
| nucleoside triphosphate metabolic process | BP       | 140         | 10       | 6.08925E-06 | 0.00245     |
| cation transport   | BP       | 452         | 18       | 6.61154E-06 | 0.00258     |
| monovalent inorganic cation transport | BP       | 219         | 12       | 1.10729E-05 | 0.00392     |
| ribonucleoside monophosphate biosynthetic process | BP       | 65          | 7        | 1.08944E-05 | 0.00392     |
| nucleoside monophosphate biosynthetic process | BP       | 68          | 7        | 1.47269E-05 | 0.00492     |
| purine ribonucleoside triphosphate metabolic process | BP       | 125         | 9        | 1.68142E-05 | 0.00546     |
| purine nucleoside triphosphate metabolic process | BP       | 126         | 9        | 1.79263E-05 | 0.00552     |
| transmembrane transport | BP       | 654         | 21       | 2.93288E-05 | 0.00797     |
| purine nucleoside monophosphate metabolic process | BP       | 136         | 9        | 3.2951E-05  | 0.00837     |
| purine ribonucleoside monophosphate metabolic process | BP       | 136         | 9        | 3.2951E-05  | 0.00837     |
| energy coupled proton transmembrane transport, against electrochemical gradient | BP       | 35          | 5        | 5.20342E-05 | 0.01106     |
| ATP hydrolysis coupled proton transport | BP       | 35          | 5        | 5.20342E-05 | 0.01106     |
| ATP hydrolysis coupled transmembrane transport | BP       | 35          | 5        | 5.20342E-05 | 0.01106     |
| ATP hydrolysis coupled ion transmembrane transport | BP       | 35          | 5        | 5.20342E-05 | 0.01106     |
| ATP hydrolysis coupled cation transmembrane transport | BP       | 35          | 5        | 5.20342E-05 | 0.01106     |
| ion transport      | BP       | 737         | 22       | 5.61478E-05 | 0.01152     |
| localization       | BP       | 2621        | 52       | 6.0913E-05  | 0.01207     |
| ribonucleoside monophosphate metabolic process | BP       | 147         | 9        | 6.06496E-05 | 0.01207     |
| nucleoside monophosphate metabolic process | BP       | 150         | 9        | 7.09445E-05 | 0.01360     |
| single-organism localization | BP       | 819         | 23       | 9.51294E-05 | 0.01738     |
| single-organism transport | BP       | 776         | 22       | 0.000119082 | 0.02109     |
| ribonucleotide biosynthetic process | BP       | 129         | 8        | 0.000143028 | 0.02423     |
| ribose phosphate biosynthetic process | BP       | 129         | 8        | 0.000143028 | 0.02423     |
| vacuolar acidification | BP       | 11          | 3        | 0.000246582 | 0.04101     |
| ribonucleotide metabolic process | BP       | 220         | 10       | 0.000281352 | 0.04506     |
| proton-transporting two-sector ATPase complex, proton-transporting domain | CC       | 25          | 6        | 3.59375E-07 | 0.00060     |
| proton-transporting two-sector ATPase complex | CC       | 45          | 7        | 8.65692E-07 | 0.00078     |
| mitochondrial membrane | CC       | 285         | 15       | 1.42199E-06 | 0.00119     |
However, at that time, researchers did not have access to RNA-Seq technology. To the best of our knowledge, transcriptome analysis of the EOfAD mutation knock-in mouse models was never performed.

In humans, AD is thought to develop over decades and the median survival to onset age for EOfAD mutations in human \(PSEN1\) considered collectively is 45 years [7]. Functional MRI of human children carrying EOfAD mutations in \(PSEN1\) has revealed differences in brain activity compared to non-carriers in individuals as young as 9 years of age [8]. Presumably therefore, heterozygosity for EOfAD mutations in \(PSEN1\) causes early molecular changes/stresses that eventually lead to AD.

Transcriptome analysis is currently the most detailed molecular phenotypic analysis possible on cells or tissues. Here we present an initial analysis of the transcriptomic differences caused in young adult (6-month-old) zebrafish brains by the presence of an EOfAD-like mutation in the

### Table 1 GOs enriched for genes differentially expressed between heterozygous mutant and wild type sibling fish brains (Continued)

| Gene Ontology Term                                      | Ontology | Total Genes | DE Genes | p-value  | FDR p-value |
|--------------------------------------------------------|----------|-------------|----------|----------|-------------|
| mitochondrial envelope                                  | CC       | 303         | 15       | 3.0322E-06 | 0.00172     |
| membrane part                                           | CC       | 4868        | 85       | 1.1722E-05 | 0.000403    |
| organelle membrane                                      | CC       | 789         | 24       | 1.84982E-05 | 0.000555    |
| mitochondrial inner membrane                            | CC       | 195         | 11       | 1.97958E-05 | 0.000579    |
| integral component of membrane                          | CC       | 4419        | 78       | 2.52479E-05 | 0.000720    |
| intrinsic component of membrane                         | CC       | 4453        | 78       | 3.77494E-05 | 0.000840    |
| organelle envelope                                       | CC       | 420         | 16       | 3.76291E-05 | 0.000917    |
| envelope                                                | CC       | 422         | 16       | 3.98337E-05 | 0.000950    |
| organelle inner membrane                                | CC       | 215         | 11       | 4.86028E-05 | 0.01106     |
| Cul2-RING ubiquitin ligase complex                       | CC       | 7           | 3        | 5.4156E-05 | 0.01131     |
| proton-transporting ATP synthase complex                 | CC       | 19          | 4        | 6.25883E-05 | 0.01220     |
| mitochondrial membrane part                              | CC       | 117         | 8        | 7.21148E-05 | 0.01360     |
| mitochondrial part                                       | CC       | 404         | 15       | 8.83156E-05 | 0.01639     |
| membrane                                                | CC       | 5379        | 88       | 0.000106964 | 0.01924     |
| vacuolar proton-transporting V-type ATPase, V0 domain   | CC       | 9           | 3        | 0.000127733 | 0.02229     |
| mitochondrial proton-transporting ATP synthase complex, coupling factor F(o) | CC       | 12          | 3        | 0.000325933 | 0.04885     |
| proton-transporting V-type ATPase, V0 domain            | CC       | 12          | 3        | 0.000325933 | 0.04885     |
| ATPase activity, coupled to transmembrane movement of ions, rotational mechanism | MF       | 34          | 7        | 1.1446E-07 | 0.00045     |
| hydrogen ion transmembrane transporter activity          | MF       | 84          | 9        | 6.11883E-07 | 0.00060     |
| ATPase activity, coupled to transmembrane movement of substances | MF       | 98          | 9        | 2.27123E-06 | 0.00166     |
| hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances | MF       | 101         | 9        | 2.92425E-06 | 0.00172     |
| primary active transmembrane transporter activity        | MF       | 104         | 9        | 3.73269E-06 | 0.00178     |
| P-P-bond-hydrolysis-driven transmembrane transporter activity | MF       | 104         | 9        | 3.73269E-06 | 0.00178     |
| cation-transporting ATPase activity                      | MF       | 56          | 7        | 3.96731E-06 | 0.00178     |
| ATPase coupled ion transmembrane transporter activity    | MF       | 56          | 7        | 3.96731E-06 | 0.00178     |
| ATPase activity, coupled to movement of substances       | MF       | 112         | 9        | 6.88692E-06 | 0.00260     |
| active ion transmembrane transporter activity            | MF       | 96          | 8        | 1.72916E-05 | 0.00546     |
| active transmembrane transporter activity                | MF       | 281         | 13       | 2.87859E-05 | 0.00797     |
| proton-transporting ATP synthase activity, rotational mechanism | MF       | 16          | 4        | 3.02121E-05 | 0.00803     |
| transporter activity                                     | MF       | 991         | 25       | 0.000249051 | 0.04101     |
| substrate-specific transmembrane transporter activity    | MF       | 709         | 20       | 0.000263528 | 0.04279     |
| ion transmembrane transporter activity                   | MF       | 660         | 19       | 0.000293184 | 0.04572     |
| substrate-specific transporter activity                  | MF       | 828         | 22       | 0.000297009 | 0.04572     |
| monovalent inorganic cation transmembrane transporter activity | MF       | 264         | 11       | 0.000297217 | 0.04572     |

GOs are grouped by ontology (BP, CC or MF) and ranked by FDR-corrected p-value.
gene \textit{psen1} that is orthologous to the human \textit{PSEN1} gene. GO analysis supports very significant effects on mitochondrial function, especially synthesis of ATP, and on ATP-dependent functions such as the acidification of lysosomes that are critical for autophagy.

Materials and methods

The mutant allele, \textit{Q96\_K97del}, of \textit{psen1} was a byproduct identified during our introduction of the K97fs mutation into \textit{psen1} (that models the K115fs mutation of human \textit{PSEN2} – see [9] for an explanation).

\textit{Q96\_K97del} is a deletion of 6 nucleotides from the coding sequence of the \textit{psen1} gene. This is predicted to distort the first luminal loop of the Psen1 protein. In this sense, it is similar to a number of EO\textit{FAD} mutations of human \textit{PSEN1} [10]. Also, in common with all the widely distributed \textit{EO\textit{FAD}} mutations in \textit{PSEN1}, (and consistent with the \textit{PRESENILIN EO\textit{FAD}} mutation “reading frame preservation rule” [1]), the \textit{Q96\_K97del} allele is predicted to encode a transcript that includes the C-terminal sequences of the wild type protein. Therefore, as a model of an \textit{EO\textit{FAD}} mutation, it is superior to the K97fs mutation in \textit{psen1} [9].

To generate a family of heterozygous \textit{Q96\_K97del} allele (i.e. \textit{psen1}\textit{Q96\_K97del}/+) and wild type (+/+ ) sibling fish, we mated a \textit{psen1}\textit{Q96\_K97del}/+ individual with a +/- individual and raised the progeny from a single spawning event together in one tank. Zebrafish can live for up to 5 years but, in our laboratory, typically show greatly reduced fertility after 18 months. The fish become fertile after around 3 months of age, so we regard 6-month-old fish as equivalent to young adult humans. Therefore we analysed the transcriptomes of entire young adult, 6-month-old fish brains using poly-A enriched RNA-seq technology, and estimated gene expression from the resulting single-end 75bp reads using the reference GRCz11 zebrafish assembly transcriptome [11, 12]. Each zebrafish brain has a mass of approximately 7 mg. Since AD is more prevalent in human females than males, and to further reduce gene expression “noise” in our analyses, we obtained brain transcriptome data from four female wild type fish and four female heterozygous mutant fish. This data has been made publicly available at the Gene Expression Omnibus (GEO, see under Availability of data and materials below).

Results

Differentially expressed genes (DE genes)

Genes differentially expressed between wild type and heterozygous mutant sibling fish were identified using moderated \textit{t}-tests and a false discovery rate (FDR)-adjusted \textit{p}-value cutoff of 0.05 as previously described [9, 13, 14]. In total, 251 genes were identified as differentially expressed (see Additional file 1). Of these, 105 genes showed increased expression in heterozygous mutant brains relative to wild type sibling brains while 146 genes showed decreased expression.

GO analysis

To understand the significance for brain cellular function of the differential gene expression identified in young adult heterozygous mutant brains we used the \textit{goana} function [15] of the \textit{limma} package of Bioconductor software [14] to identify GOs in which the DE genes were enriched at an FDR-corrected \textit{p}-value of less than 0.05. Seventy-eight GOs were identified (Table 1) of which 20 addressed cellular components (CC). Remarkably, most of these CCs concerned the mitochondrion, membranes, or ATPases. Seventeen GOs addressed molecular functions (MF) and largely involved membrane transporter activity, particularly ion transport and ATPase activity coupled to such transport. Forty-one GOs addressed biological processes (BP) and involved ATP metabolism, ribonucleoside metabolism, and transmembrane transport processes including vacuolar acidification (that has previously been identified as affected by \textit{EO\textit{FAD}} mutations in \textit{PSEN1} [16]). Overall, our GO analysis indicates that this \textit{EO\textit{FAD}}-like mutation of zebrafish \textit{psen1} has very significant impacts on cellular energy metabolism and transmembrane transport processes.

Additional file

Additional file 1: Genes differentially expressed between heterozygous mutant and wild type brains at 6 months. Lists the genes identified as differentially expressed between the brains of heterozygous \textit{psen1}\textit{Q96\_K97del/+ }mutant fish and the brains of their wild type siblings at an age of 6 months. Genes are ranked according to FDR-corrected \textit{p}-value. Only genes with a FDR-corrected \textit{p}-value less than 0.05 are shown. “FC” denotes fold change. “DE” denotes differential expression. For \textit{DE}, “1” denotes increased expression in the mutant and “-1” denotes decreased expression in the mutant. (XLSX 39 kb)

Abbreviations

AD: Alzheimer’s disease; ATP: Adenosine triphosphate; BP: Biological process (GO term); CC: Cellular component (GO term); DE: genes; EO\textit{FAD}: Early onset familial Alzheimer’s disease; FDR: False discovery rate; GEO: Gene Expression Omnibus; GO: Gene ontology; MAPT: ASSOCIATED PROTEIN TAU (human protein); MF: Molecular function (GO term); mg: Milligrams; MRI: Magnetic resonance imaging; PSEN1: PRESENILIN 1 (human gene); PSEN1: PRESENILIN 1 (human protein); \textit{psen1}: presenilin 1 (zebrafish gene); Psen1: Presenilin 1 (zebrafish protein)

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Availability of data and materials
The datasets generated and/or analysed during the current study are available in the GEO repository (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE126096. psen1Q96_K97del mutant zebrafish are available upon request. However, due to Australia’s strict quarantine and export regulations, export of fish involves considerable effort and expense and these costs must be borne by the party requesting the fish.

Authors’ contributions
MN conceived the project, sought funding, generated the psen1Q96_K97del mutant zebrafish, identified the genotype of individuals, and isolated mRNA from zebrafish brains. NH processed the RNA-seq data and performed bioinformatics analysis to identify DE genes and GOs. SP supervised the work of NH and performed data quality checks. ML conceived the project, sought funding, coordinated the project, and drafted this research report. All authors contributed to interpretation of data and to reviewing and editing drafts of the submitted manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study was conducted under the auspices of the Animal Ethics Committee of the University of Adelaide, under permits S-2014-108 and S-2017-073.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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