No observed effect on brain vasculature of Alzheimer’s disease-related mutations in the zebrafish presenilin 1 gene

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Abstract
Previously, we found that brains of adult zebrafish heterozygous for Alzheimer’s disease-related mutations in their presenilin 1 gene (psen1, orthologous to human PSEN1) show greater basal expression levels of hypoxia responsive genes relative to their wild type siblings under normoxia, suggesting hypoxic stress. In this study, we investigated whether this might be due to changes in brain vasculature. We generated and compared 3D reconstructions of GFP-labelled blood vessels of the zebrafish forebrain from heterozygous psen1 mutant zebrafish and their wild type siblings. We observed no statistically significant differences in vessel density, surface area, overall mean diameter, overall straightness, or total vessel length normalised to the volume of the telencephalon. Our findings do not support that changes in vascular morphology are responsible for the increased basal expression of hypoxia responsive genes in psen1 heterozygous mutant brains.

Keywords: Zebrafish, Vasculature, Confocal laser scanning microscopy, 3D reconstruction

Introduction
The dominant hypothesis of Alzheimer’s disease (AD) pathogenesis is the amyloid cascade hypothesis (ACH) [1], which postulates the amyloid β peptide (Aβ) as initiating a pathological process resulting in neurodegeneration and dementia (reviewed in [2]). An alternative to the ACH is the vascular hypothesis [3], asserting that age-related cerebral vascular abnormalities induce AD pathologies by limiting nutrient and oxygen delivery to produce hypoxic stress, a neural energy crisis and, consequently, neurodegeneration. Significant evidence supports the vascular hypothesis of AD (reviewed in [4]).

Rare, inherited forms of AD are caused by dominant mutations in a small number of genes (early-onset familial AD, EOAd). Most EOAd cases are due to heterozygous mutations in the gene presenilin 1 (PSEN1) that obey a “reading-frame preservation rule” [5]. Mutations allowing production of a transcript(s) with an altered coding sequence but, nevertheless, utilising the original stop codon cause EOAd while mutant alleles coding only for truncated proteins do not. We previously generated knock-in models in zebrafish with each of these types of mutant psen1 allele: K97Gfs, a frameshift mutation encoding a truncated protein similar to the human PS2V isoform that is increased in sporadic, late onset AD [6], and Q96_K97del: an EOAd-like, reading-frame-preserving deletion of two codons [7].

We recently observed in normoxic adult zebrafish brains that heterozygosity for either of the above two mutations causes increased basal expression levels of hypoxia responsive genes (HRGs, genes with expression regulated by a master regulator of the transcriptional response to hypoxia: hypoxia-inducible factor 1 (HIF1)). This implied that the heterozygous psen1 mutant fish brains were already under some form of hypoxic stress [8], possibly due to changes in vasculature, as have been observed in...
transgenic mice expressing human PSEN1 EOAD mutation-bearing transgenes in neurons [9]. Therefore, we examined the effects on forebrain vasculature with age of heterozygosity for the K97Gfs and Q96_K97del mutations of psen1 by exploiting the flI1:GFP transgene that labels zebrafish endothelial cells [10].
Methods
Single zebrafish heterozygous for either psen1 mutation were mated with single fish bearing the fli1::GFP [10] transgene. GFP-fluorescent progeny were selected to form families of siblings either wild type or heterozygous for the psen1 mutant alleles (Fig. 1a). We used n = 4 brains of each sibling genotype at 6 months (young adult) and 24 months (aged) of age for tissue clearing using the PACT method [11]. Briefly, PACT involves infusing and crosslinking the brain with an acrylamide-based hydrogel. Then, light scattering lipids are passively removed by incubating the brain with a detergent, allowing light to penetrate deep into the tissue [11, 12]. We imaged the telencephalons (thought to be the region loosely equivalent of the prefrontal cortex in humans) using an Olympus FV3000 confocal microscope, and performed 3D image analysis using Imaris v9.1 (Bitplane) (Fig. 1b). For a detailed description of methods, see Additional File 1.

Results and conclusion
No statistically significant differences between sibling genotypes at each age were observed for any of the measured parameters (see Fig. 1). This does not support that the increased basal levels of HRGs observed previously in our zebrafish psen1 mutants are due to vascular changes. However, subtle changes to vasculature due to psen1 genotype may be too small to detect using this method and further experimentation using a larger number of biological replicates may increase statistical power to detect changes to these measured parameters. Alternatively, other factors such as altered γ-secretase activity [13] and/or cellular ferrous iron levels [14] may influence HIF1-α activity to affect basal HRG expression.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13041-021-00734-5.

Additional file 1. Detailed description of sample preparation, imaging and 3D image analysis.

Additional file 2. Quantified values used to produce the graphs in Fig. 1.

Abbreviations
ACH: Amyloid cascade hypothesis; AD: Alzheimer’s disease; Aβ: Amyloid beta; EOfAD: Early-onset familial Alzheimer’s disease; HIF1: Hypoxia-inducible factor 1; HIF1-α: Hypoxia-inducible factor 1, alpha subunit; HRG: Hypoxia response gene; PSEN1: Presenilin 1.

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Authors’ contributions
KB performed the experiments, MN and ML conceived the project, CN provided advice and access for 3D image analysis. All authors read and contributed to the final manuscript.

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Availability of data and materials
The quantified values used to produce the graphs in Fig. 1 can be found in Additional File 2. Raw microscopy images from the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate
Work with zebrafish was conducted under the auspices of the University of Adelaide Animal Ethics Committee (permit numbers: S-2017-073 and S-2017-089) and Institutional Biosafety Committee (permit number 15037).
Consent for publication
Not applicable.

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

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