The analysis of Presenilin processing and activity with a focus on its implications for Alzheimer's disease pathogenesis using *Danio Rerio* as a model organism

Lachlan Wilson

Supervised by Michael Lardelli and Joan Kelly

Submitted for partial fulfilment for the degree of Doctor of Philosophy (PhD)

Discipline of Genetics

School of Molecular and Biomedical Science

The University of Adelaide University SA

AUSTRALIA

July 2013

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed

…… ……Date……

Table of Contents

List of Publications contributed to during Ph.D candidature

A zebrafish melanophore model of amyloid beta toxicity.

Morgan Newman, Lachlan Wilson, Giuseppe Verdile, Esther Camp

Ralph Martins and Michael Lardelli

Zebrafish, 2010, Vol7, No2 155-9.

The BACE1-PSEN-AβPP regulatory axis has an ancient role in response to low oxygen/oxidative stress.

Seyyed Hani Moussavi Nik, Lachlan Wilson, Morgan Newman, Kevin Croft, Trevor A Mori, Ian Musgrave and Michael Lardelli

Journal of Alzheimer's Disease, 2011,

The Development of an *in vivo* γ-Secretase Assay using Zebrafish Embryos.

Lachlan Wilson and Michael Lardelli

Journal of Alzheimer's Disease, 2013,

Acknowledgements

I would like to give my thanks and appreciation to:

Michael Lardelli, for providing me with this magnificent opportunity. Your knowledge, wisdom and continual patience with me throughout my studies has been so very appreciated. You have been a continuous inspiration for the scientific process and the virtues of a critical thinking lifestyle.

Morgan Newman, for being a continuous support and always being ready to assist me and my incessant questions. You have been there from the start to finish, from my third year placement in the lab till now. Thank you.

Past and present members of the Genetics discipline for their support and advice over the last few years. Particularly Simon Wells and Jai Denton for the advice, feedback and for reminding me that no matter how long it takes you can get it done. It kept me going in times of serious doubt.

Hani Mousavanik, you made everyday coming into the lab something to look forward to. Always laughing, and seeing the funny side of life. Our conversations, both intellectual and social have been enlightening and a treasure. You are a dear friend of mine, I will miss not coming into the lab everyday to share in your company.

To Mum and Dad thank you for being there through thick and thin. I know I haven't always been the easiest person and I thank you for your constant support. I couldn't have made it in any capacity without the opportunities that you have given me. The education, the love, understanding and continuous support. Thank you for letting me be me.

Garth, I know you hate my sentimentality and romanticism, so I will keep it stark. I would not be here, or the person I am without you. This, and all the things I have done, would not have occurred without you in my life.

Finally, my friends for providing timely distractions and listening to me rabble on about this and that.

Without these people (science related or not), none of this would have been possible.

This thesis is for Garth. My Brother. My Best Friend. My Constant.

Abstract

Aberrant proteolytic processing of AMYLOID BETA PRECURSOR PROTEIN (AβPP) may result in an imbalance between production and clearance of the amyloid-β (Aβ) peptide proteolytic product and promote neuronal dysfunction and death. β-site amyloid-β A4 precursor protein-cleaving enzyme 1 (BACE1) with γ -secretase are responsible for the cleavage of AβPP to produce Aβ peptide. Presenilin proteins form the catalytic core of γ-secretase complexes. *PRESENILIN1* (*PSEN1*) is the major locus for mutations causing familial Alzheimer's disease (FAD) and is also mutated in Pick disease of brain, familial acne inversa and dilated cardiomyopathy. It is a critical facilitator of Notch signalling. The zebrafish, *Danio rerio*, is a versatile vertebrate model for investigating the molecular bases of Alzheimer's disease (AD) pathology. It possesses genes orthologous to human *PSEN1* and *PSEN2*, and the genes *appa* and *appb* that are duplicates of an ancestral *AβPP* orthologue).

This thesis primarily utilizes zebrafish as a system to investigate AD pathogenesis.. **Chapter I** describes an assay in which the level of a γ-secretase substrate (a modified form of Appa protein) is observed in zebrafish embryos by western immunoblotting relative to a co-expressed protein not subject to γ-secretase activity. Prior to the development of this assay there existed no *in vivo* assay appropriate for directly monitoring γ-secretase activity. The assay was subsequently used to analyse the effects on γ-secretase activity of blocking translation of zebrafish *psen1* and/or *psen2*. **Chapter II** explores various truncations of human PSEN1 (or zebrafish Psen1) protein that have differential effects on Notch signalling and cleavage of zebrafish Appa (a paralogue of human AβPP). Different truncations can suppress or stimulate

Notch signalling but not Appa cleavage and vice versa. The results show that the truncated protein potentially translated from these transcripts incorporates into stable Psen1-dependent higher molecular weight complexes and suppresses cleavage of Appa but not Notch signalling. In contrast, the truncated protein potentially produced by the P242LfsX11 acne inversa mutation has no effect on Appa cleavage but, unexpectedly, enhances Notch signalling. The results suggest novel hypotheses for the pathological mechanisms underlying AD. **Chapter III** investigates truncated isoforms of PRESENILIN known to form naturally. In particular a truncated PSEN2 isoform "PS2V" has been previously identified. PS2V is formed by exclusion of exon 5 from *PSEN2* transcripts leading to a frameshift after exon 4 sequence and a premature stop codon. This truncates the ORF/protein after PSEN2's first transmembrane domain. The K115Efx10 mutation in *PSEN2* is the only completely truncating mutation of the *PRESENILIN* genes that is thought to cause AD. K115Efx10 is especially interesting since, if expressed, it would generate a truncated protein very similar to PS2V and would be expected to boost Aβ production. Zebrafish possess an isoform of Psen1 that has a similar role to PS2V and zebrafish Psen1 truncated after exon 4 sequence behaves in a similar manner to PS2V. We have modeled human and zebrafish PS2V and K115Efx10-like mutations in zebrafish to investigate their effect on gene expression profiles, γ-secretase activity and complex constitution.