The deregulation of Fyn kinase in Alzheimer's Disease

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STATEMENT OF ORIGINALITY

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma 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. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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ACKNOWLEDGEMENT OF AUTHORSHIP

I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publications.

By signing below, I confirm that Goce Taleski contributed to the following publications entitled:

1. *Altered protein phosphatase 2A methylation and Tau phosphorylation in the young and aged brain of methylenetetrahydrofolate reductase (MTHFR) deficient mice*
2. *Methylenetetrahydrofolate reductase deficiency deregulates regional brain amyloid-β protein precursor expression and phosphorylation levels*
3. *The protein serine/threonine phosphatases PP2A, PP1 and calcineurin: A triple threat in the regulation of the neuronal cytoskeleton*
4. *Protein Phosphatase 2A and Tau: an orchestrated 'Pas de Deux'*
5. *Disturbances in PP2A methylation and one-carbon metabolism critically affect Fyn distribution and function* (submitted)

Outlined below are the items that the candidate has contributed towards:

- *Conduction of experiments (1, 2 and 5)*
- *Analysis of results (2 and 5)*
- *Review of literature (3 and 4)*
- *Prepared and organised the figures (2, 3 and 4)*

A/Prof Estelle Sontag

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LIST OF PUBLICATIONS

1. Sontag JM, Wasek B, **Taleski G**, Smith J, Arning E, Sontag E, Bottiglieri T, *Altered protein phosphatase 2A methylation and Tau phosphorylation in the young and aged brain of methylenetetrahydrofolate reductase (MTHFR) deficient mice.* FRONT AGING NEUROSCI, 22;6 (2014)

2. Hoffman A, **Taleski G**, Qian H, Wasek B, Arning E, Bottiglieri T, Sontag JM, Sontag E. *Methylenetetrahydrofolate reductase deficiency deregulates regional brain amyloid-β protein precursor expression and phosphorylation levels.* J ALZHEIMERS DIS, 64(1) (2018)

3. Hoffman A, **Taleski G**, Sontag, E, *The protein serine/threonine phosphatases PP2A, PP1 and calcineurin: A triple threat in the regulation of the neuronal cytoskeleton.* MOL CELL NEUROSCI, 84 (2017)

4. **Taleski G**, Sontag E, *Protein Phosphatase 2A and Tau: an orchestrated 'Pas de Deux'*. FEBS LETT, 592(7) (2018)

5. **Taleski G**, Schumacher D, Su H, Sontag JM, Sontag E. *Disturbances in PP2A methylation and one-carbon metabolism compromises Fyn distribution, neuritogenesis and APP regulation*. J BOL CHEM, 296(100237) (2020)

See Appendix for Publications 1-5.

CONFERENCE ABSTRACTS

Taleski G, Hoffman A, Qian H, Wasek B, Arning E, Bottiglieri T, Sontag JM and Sontag E. *MTHFR deficiency and low dietary folate interact to promote the accumulation of phosphorylated amyloid protein precursor and tau.* The 38th Annual Scientific Meeting of the Australasian Neuroscience Society, Brisbane, Australia, December 2018. Poster presenter.

Bottiglieri T, Hoffman A, **Taleski G**, Qian H, Wasek B, Arning E, Sontag JM and Sontag E. *Methylenetetrahydrofolate reductase (MTHFR) deficiency deregulates regional brain amyloid-b precursor protein and phosphorylation levels.* FASEB Conference on Folic Acid, Vitamin B12 and One-Carbon Metabolism, Nova Scotia, Canada, August 2018.

Sontag JM, Gomez RJ, Hoffman A, **Taleski G**, Mazalouskasb MD, Hanks SK, Frohner I, Ogris E, Wadzinski BE and Sontag E. *At the crossroads between Tyr and Ser/Thr signaling: A new paradigm in the regulation of PP2A by Src kinase.* The American Society for Biochemistry and Molecular Biology 2017 Annual Meeting/ Experimental Biology 2017, Chicago, USA April 2017. The FASEB Journal vol. 31 no. 1 Supplement 771.2, April 2017.

Sontag JM, Wasek B, **Taleski G**, Smith J, Arning E, Sontag E and Bottiglieri T. *Methylation of protein phosphatase 2A and phosphorylation of Tau protein in brain tissue from methylenetetrahydrofolate reductase deficient mice.* FASEB Conference on Folic Acid, Vitamin B12 and One-Carbon Metabolism, Steamboat Springs, Colorado, August 2014.

ORAL PRESENTATIONS AT CONFERENCES

Taleski G, Hoffman A, Qian H, Wasek B, Arning E, Bottiglieri T, Sontag JM, Sontag E. (Newcastle, Australia, 3rd August 2019) "A novel metabolic intervention for Tauopathies" **Centre for Brain and Mental Health Research, Hunter Medical Research Institute, 2019**.

Taleski G, van Hummel A, Ittner L, Sontag JM, Sontag E. (Newcastle, Australia, 2nd August 2018) "MTHFR deficiency and low dietary folate interact to promote the accumulation of phosphorylated amyloid protein precursor and tau" **Centre for Brain and Mental Health Research, Hunter Medical Research Institute, 2018**.

LIST OF AWARDS

2018

Travel Grant
Hunter Medical Research Institute (HMRI)

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2016

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
APP	Amyloid Precursor Protein
Aβ	Amyloid beta
CNS	Central nervous system
CP13	Antibody against Tau phosphorylated at Ser202
FTLD-Tau	Frontotemporal Lobar Degeneration-Tau
Fyn^{CA}	Constitutively active Fyn
GAPDH	Glyceraldehyde-3-phosphate-dehydrogenase
HET	*Mthfr* heterozygous knockout genotype
Hcy	Homocysteine
HTL	Homocysteine thiolactone
MCI	Mild cognitive impairment
Mthfr	Gene encoding methylenetetrahydrofolate reductase
MTHFR	Methylenetetrahydrofolate reductase
NFT	Neurofibrillary tangle
NULL	*Mthfr* homozygous knockout genotype
P-Tau	Hyperphosphorylated Tau
PHF-1	Antibody against Tau phosphorylated at Ser396 and Ser404
PP2A	Protein phosphatase 2A
PP2A/Bα	Protein phosphatase 2A holoenzyme containing Bα subunit
SAM	*S*-adenosylmethionine
SAH	*S*-adenosylhomocysteine
SFK	Src family kinase
TAU58/2	Transgenic mice overexpressing human Tau-P301S
THcy	Total homocysteine (homocysteine and derivatives)
WT	Wild-type

ABSTRACT

Sporadic Alzheimer's Disease (AD) is the prevailing form of dementia worldwide. Neuropathologically, this debilitating disorder is characterised by deposition of senile amyloid beta (Aβ) plaques and Tau-containing neurofibrillary tangles (NFTs). Aβ peptides are produced from the proteolytic processing of amyloid precursor protein (APP). There is strong experimental evidence that pathological accumulation of Aβ oligomers triggers a degenerative cascade mediated by Fyn kinase and abnormally phosphorylated Tau (p-Tau) proteins; this ultimately promotes synaptic and memory deficits in AD. The deregulation of Tau also prompts its self-aggregation into paired helical filaments that can aggregate further into NFTs. Significant clinical failures of Aβ-centric therapeutics for AD have recently shifted the industry towards development of Tau-targeting approaches that are currently under clinical investigation. However, the sole targeting of either Aβ or p-Tau may fail to address the underlying disease mechanisms, established risk factors, and complexity of this disorder. In an attempt to disentangle some of these issues, we considered the contribution of prevalent modifiable risk factors associated with AD, more specifically, low plasma folate levels and elevated plasma total homocysteine levels. Folate and homocysteine play a key role in one-carbon metabolism, a series of metabolic pathways that control cellular methylation potential. The integrity of one-carbon metabolism is critical for formation of methylation-dependent Protein Phosphatase 2A (PP2A) enzymes, which are major signalling mediators that become downregulated in AD-affected brain regions. Methylated PP2A holoenzymes are the predominant phosphatases that dephosphorylate p-Tau at many Ser/Thr epitopes. They also dephosphorylate APP phosphorylated at the Thr668 residue, which is linked to enhanced APP amyloidogenic processing. Here, we first demonstrated that both APP phosphorylation at Thr668 and Tau phosphorylation at AD-like epitopes are enhanced in various brain regions from mouse models with disturbances to one-carbon metabolism. These changes were associated with a concomitant reduction in methylated PP2A enzyme levels and increased activity of the Tau/APP kinase, GSK-3β. These findings support the involvement of altered one-carbon metabolism in AD pathogenic processes. Since Fyn kinase regulates both APP and Tau *via* Tyrosine (Tyr) phosphorylation, and becomes aberrantly activated in AD, we also deemed it important to address the impact of one-carbon metabolism and PP2A methylation on Fyn regulation. Our studies show that one-carbon metabolism and/or methylated PP2A enzymes regulate the distinct subcellular distribution of Fyn in neuronal cellular and *ex vivo* models. Fyn compartmentalisation was closely associated with its function in neurite outgrowth. Furthermore, we found that Fyn interacted with methylated PP2A enzymes, which may play a role in its targeting. In mice with altered one-carbon metabolism, the net activity and total protein expression levels of Fyn were dramatically altered in a brain region-specific manner; notably, Fyn was hyperactive in the cortex of these mice. Moreover, under conditions of increased Fyn signalling, we identified site-specific Tyr phosphorylation of PP2A catalytic

subunit (PP2Ac), which could have downstream ramifications for both APP and Tau regulation. Tyr phosphorylation of PP2Ac influenced physiological Fyn targeting and Fyn-dependent neurite outgrowth in N2a neuroblastoma cells. Together, our findings support the existence of a reciprocal functional interaction between Fyn and PP2A. Lastly, we demonstrated that a methyl group donor-based intervention ameliorated behavioural impairments, improved spatial memory, and reduced brain p-Tau accumulation in an AD-like mouse model. The mechanisms of action of this intervention likely included Fyn downregulation and the upregulation of methylated PP2A enzymes. Thus, the collective experimental work presented in this thesis unveils novel regulatory mechanisms of significance to AD pathogenesis. Besides opening up new avenues for AD research, our findings should also guide the development of rational therapeutics for delaying AD onset and/or disease progression.