From Division of Neurogeriatrics
Center for Alzheimer Research
Department of Neurobiology, Care Sciences and Society
Karolinska Institutet, Stockholm, Sweden

THIOREDOXIN-1 IN ALZHEIMER DISEASE

TORBJÖRN PERSSON

Karolinska Institutet

Stockholm 2015

Published by Karolinska Institutet.
Printed by Eprint AB 2015

ISBN 978-91-7676-128-1

Image on the cover page: *The butterfly* – by Paula Merino-Serrais

The picture shows differentiated SH-SY5Y cells stained with antibodies for Phalloidin (green), Map-2 (blue) and Trx80 (red). The staining was visualized by confocal microscopy using the Zeiss (LSM 510 META) confocal laser scanning system.

"Man måste avsluta en påbörjare"

Kenneth "Kenta" Gustafsson

THIOREDOXIN-1 IN ALZHEIMER DISEASE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Torbjörn Persson

Principal Supervisor:
Associate Professor Angel Cedazo-Minguez
Karolinska Institutet
Department of NVS
Division of Neurogeriatrics

Co-supervisor(s):
Associate Professor Lars Tjernberg
Karolinska Institutet
Department of NVS
Division of Neurogeriatrics

Professor Lars-Olof Wahlund
Karolinska Institutet
Department of NVS
Division of Clinical geriatrics

Opponent:
Professor George Perry
University of Texas at San Antonio
Department of Biology

Examination Board:
Professor Elias Arnér
Karolinska Institutet
Department of Medical Biochemistry and Biophysics
Division of Biochemistry

Associate Professor Dagmar Galter
Karolinska Institutet
Department of Neuroscience

Associate Professor Joakim Bergström
Uppsala University
Department of Department of Public Health and Caring Sciences
Division of Molecular Geriatrics

ABSTRACT

Oxidative stress is one of the earliest signs in Alzheimer Disease (AD) brain. In order to protect themselves against oxidative stress, neurons use antioxidants as a defense mechanism. Such an antioxidant is Thioredoxin-1 (Trx1). Previous studies have shown that the levels of Trx1 are reduced in the brains of AD patients. The aim of this thesis was to further examine the function of Trx1 in AD pathogenesis.

In **Paper I** and **III,** the role of Trx1 in the mechanisms behind risk-modulating factors is investigated. The incidence of AD is higher in women than in men and one reason for this is thought to be the post-menopausal lack of estrogen. In addition, estrogen was shown to have neuroprotective effects both *in vitro* and *in vivo*. In **Paper I** we studied the protective effect of estrogen against amyloid-beta (Aβ) toxicity *in vitro*. We found that estrogen is protective via phosphorylation of Protein kinase B (AKT) and inhibition of the Apoptosis signal-regulating kinase 1 (ASK-1) pathway. However, this occurs independently of Trx1 expression. In **Paper III** we investigated the effect of Apolipoprotein E (ApoE) isoforms on Trx1 in the brain. The ApoE isoform ε4 (ApoE4) is the most important genetic risk factor for sporadic AD and it is also associated with increased oxidative stress in the brain. Furthermore, ApoE4 is suggested to have direct toxic effects via apoptosis. We found that presence of ApoE4 causes a reduction in Trx1 levels, both *in vivo*, in hippocampus of ApoE Target Replacement Mice, and *in vitro*, in human primary cortical neurons and neuroblastoma cells. This occurred after leakage of the lysosomal membrane and cytosolic release of Cathepsin D, and it induced apoptotic cell death via activation of the ASK-1 pathway.

Thioredoxin-1 can be truncated into an 80 amino acid long peptide called Thioredoxin-80 (Trx80). In **Paper II** and **IV,** we demonstrate for the very first time that this peptide is present in the brain, mainly in neurons. The levels were reduced significantly in AD patients and this was also seen in the cerebrospinal fluid (CSF). The reduction in CSF was present already in patients with mild cognitive impairment (MCI). Furthermore, we demonstrate that the peptide is generated by α-secretase cleavage of Trx1 and is secreted from cells in exosomes. The peptide inhibits the aggregation of Aβ and prevents its toxic effects both *in vitro* and in a *Drosophila Melanogaster* model of AD. In addition, Trx80 lowers the levels of Aβ, possibly through a mechanism that involves autophagy.

These findings give support to the view that oxidative stress in general, and Trx1 in particular, has a key role in AD pathogenesis. It also presents Trx80 as a completely new player to the field that has potential as a specific biomarker for the disease. In addition, therapeutic strategies based on these two peptides could be a possibility in AD that should be further investigated.

SAMMANFATTNING PÅ SVENSKA

Alzheimers sjukdom (AS) är den vanligaste formen av demens och påverkar flera funktioner i hjärnan varav försämring av minnesförmågan är den mest påtagliga. Sjukdomen är progressiv och bryter långsamt ned hjärnan. Dessvärre finns idag inget botemedel tillgängligt.

Förutom den fruktansvärda börda som sjukdomen innebär för patienterna och deras anhöriga så är det också en stor utmaning för samhället i stort. Idag uppskattas att nästan 47 miljoner människor är drabbade och antalet stiger i takt med att jordens befolkning ökar och att andelen äldre blir allt fler. Kostnaden för demens i världen är beräknad till 800 miljarder dollar årligen och väntas öka till ofattbara 2 biljoner inom 15 år. Detta kräver omedelbara insatser för att förbättra behandling, diagnos och vård av patienter.

Man brukar dela in sjukdomen i två underkategorier, familjär och sporadisk AS. Den familjära formen utgör endast ett par procent av det totala antalet patienter och orsakas av vissa nedärvda mutationer. Resterande del utgörs av den sporadiska formen och man vet ännu inte vad som orsakar sjukdomen hos dessa patienter men flera bidragande orsaker har föreslagits. De tydligaste förändringarna vid sjukdomen är att neuroner och synapser dör vilket får till följd att hjärnan krymper. Dessutom framträder ansamlingar av proteiner, så kallade plack och neurofibrillära nystan. Placken består huvudsakligen av ett felveckat protein som heter amyloid-beta (Aβ). Man tror att detta protein spelar en viktig roll vid sjukdomsutvecklingen och flera studier har visat det har toxiska effekter i hjärnan vid AS.

En annan förändring i hjärnan är oxidativ stress vilket kan detekteras redan tidigt i sjukdomsutvecklingen. Definitionen av oxidativ stress är obalans mellan bildandet av fria syreradikaler och cellens försvar i form av antioxidanter. Detta kan orsakas antingen genom ökad produktion av syreradikaler eller genom en minskning av antioxidanter. En av kroppens viktigaste antioxidanter är Thioredoxin-1 (Trx1). Detta protein finns i princip alla kroppens celler och kan eliminera syreradikaler och återställa skadade proteiner. Dessutom kan det skydda cellerna genom att hämma aktivering av programmerad celldöd, så kallad apoptos. Tidigare studier har visat att proteinet har en skyddande effekt mot de neurotoxiska effekterna som orsakas av Aβ, samt att dess nivåer är minskade i hjärnan hos Alzheimerpatienter. I delarbetena som ingår i denna avhandling har vi vidare studerat vilken roll Trx1 har vid AS.

I **Studie I** och **III** har vi undersökt om Trx1 är involverat i mekanismerna bakom kända faktorer som påverkar risken att drabbas av AS. Kvinnor drabbas i något större utsträckning av sjukdomen än män och en orsak till detta tros vara den brist på östrogen som drabbar kvinnor i samband med klimakteriet. Östrogen har en skyddande effekt på neuroner och tidigare

experiment i cellkulturer har visat att det också kan hämma Aβ-toxicitet. Dessutom har det visat sig att östrogen ökar nivåerna av Trx1. I **Studie I** ville vi därför undersöka om östrogen hämmar Aβ-toxicitet genom att öka mängden Trx1. Resultaten vi erhöll visade att de skyddande effekterna sker genom aktivering av en specifik östrogenreceptor men dock oberoende av ökad mängd Trx1.

En annan faktor som påverkar risken att drabbas av AS är genvarianten ε4 av Apolipoprotein E (ApoE4), som bärs av cirka 15 % av befolkningen. Individer som har enkel genuppsättning av ApoE4 har ungefär tre gånger högre risk att drabbas, medan hos de som har dubbel uppsättning ökar risken med nästan 15 gånger. Apolipoprotein E4 har associerats med ökad oxidativ stress i hjärnan hos Alzheimerpatienter och dessutom har det föreslagits att proteinet kan ha direkt skadliga effekter på neuroner. I **Studie III** har vi studerat hur Trx1 påverkas av ApoE4 i hjärnan. Till vår hjälp använde vi möss, där musens ApoE ersatts med humant ApoE. Vi fann att de möss som bar på ApoE4-varianten hade lägre nivåer av Trx1 i hjärnan. På samma sätt minskade nivåerna då vi behandlade odlade neuroner med ApoE4. I dessa neuroner försökte vi därefter förstå mekanismen bakom minskningen och fann att ApoE4 orsakar en destabilisering av en struktur inne i cellerna som kallas lysosomer. Denna destabilisering gjorde också att enzymet Cathepsin D läckte ut från lysosomerna. Detta enzym kan bryta ner Trx1 vilket kan vara anledningen till att mössen och de odlade cellerna har lägre nivåer av Trx1 i närvaro av ApoE4. Dessutom såg vi att ApoE4 orsakade aktivering av programmerad celldöd. Med dessa resultat presenterar vi en ny mekanism för hur ApoE4 kan orsaka oxidativ stress och celldöd.

Thioredoxin-1 består av 105 aminosyror. Denna kedja kan klyvas och bilda en 80 aminosyror lång peptidkedja som kallas Thioredoxin-80 (Trx80). Tidigare rapporter om denna molekyl har huvudsakligen behandlat dess roll i immunförsvaret. Huruvida peptiden finns i hjärnan har dock varit okänt. I **Studie II** och **IV** visade vi för första gången att Trx80 finns i hjärnan, främst i neuroner, och att nivåerna är kraftigt minskade hos Alzheimerpatienter. Denna minskning var påtaglig även i ryggmärgsvätska och kunde detekteras redan hos patienter med mild kognitiv svikt, vilket är ett förstadie till AS. När vi jämförde patienter med mild kognitiv svikt som inom två år utvecklade AS med sådana som ej utvecklade sjukdomen fann vi att de som senare utvecklade AS hade lägre nivåer av Trx80 initialt jämfört med de som var stabila. Detta tyder på att Trx80 skulle kunna användas som en diagnostisk och prognostisk markör för sjukdomen.

Man har tidigare inte vetat vilket enzym som generar denna peptid. I **Studie II** visar vi att ett enzym som kallas α-sekretas kan klyva Trx1 till Trx80. Vi visar dessutom att peptiden kan hindra Aβ från att klumpa ihop sig vilket därmed hämmar dess toxiska effekter. Detta kunde vi se i både cellkulturer och i bananfluga. Därtill fann vi att celler med höga nivåer av Trx80 hade minskade

nivåer av Aβ. Liknande upptäckt gjorde vi i hjärnan hos bananflugorna. De bananflugor som hade höga nivåer av Trx80 hade minskad ansamling av Aβ i hjärnan. Dessa bananflugor hade också förbättrad rörelseförmåga och ökad livslängd. Till sist fann vi att peptiden kan utsöndras från celler inuti små strukturer som kallas exosomer. Man vet sedan tidigare att även Aβ finns i dessa strukturer och man tror att Aβ på så sätt kan spridas från cell till cell och därigenom bidra till att sjukdomen sprids i hjärnan. Med tanke på de resultaten som beskrivits ovan är det tänkbart att Trx80 i normala fall kan hindra detta men inte vid AS då nivåerna av Trx80 är låga.

Dessa resultat ger ytterligare stöd för uppfattningen att oxidativ stress i allmänhet, och Trx1 i synnerhet, har en nyckelroll vid AS. De presenterar också Trx80 som en helt ny aktör med potential som specifik biomarkör för sjukdomen. Dessutom tyder detta på att terapeutiska strategier, baserade på dessa två peptider, kan vara en möjlighet vid AD som bör utredas vidare.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to using their Roman numerals.

I. Mateos L, **Persson T**, Katoozi S, Gil-Bea FJ, Cedazo-Minguez A. Estrogen protects against amyloid-β toxicity by estrogen receptor α-mediated inhibition of Daxx translocation. *Neurosci Lett*. 2012 Jan 11;506(2):245-50.

II. Gil-Bea F*, Akterin S*, **Persson T***, Mateos L, Sandebring A, Avila-Cariño J, Gutierrez-Rodriguez A, Sundström E, Holmgren A, Winblad B, Cedazo-Minguez A. Thioredoxin-80 is a product of alpha-secretase cleavage that inhibits amyloid-beta aggregation and is decreased in Alzheimer's disease brain. *EMBO Mol Med*. 2012 Oct;4(10):1097-111. *These authors contributed equally to this work.

III. **Persson T**, Lattanzio F, Calvo-Garrido J, Rubio-Rodrigo M, Sundström E, Maioli S, Sandebring A, Cedazo-Minguez A. Apolipoprotein E4 enhances lysosomal Cathepsin D release, Thioredoxin-1 degradation, and apoptosis. *Manuscript*

IV. **Persson T**, Calvo-Garrido J, Perez-Gonzalez R, Gerenu G, Poska H, Levy E, Presto J, Cedazo-Minguez A. Thioredoxin-80, a peptide secreted in exosomes with protective effects in a *Drosophila* model of Alzheimer disease. *Manuscript*

Other related publications

Persson T, Popescu BO, Cedazo-Minguez A. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? *Oxid Med Cell Longev*. 2014;2014:427318.

Tajeddinn W, **Persson T**, Maioli S, Calvo-Garrido J, Parrado-Fernandez C, Yoshitake T, Kehr J, Francis P, Winblad B, Höglund K, Cedazo-Minguez A, Aarsland D. 5-HT1B and other related serotonergic proteins are altered in APPswe mutation. *Neurosci Lett*. 2015 May 6;594:137-43.

CONTENTS

1 INTRODUCTION 1
1.1 Alzheimer Disease 1
1.1.1 Neuropathology 1
1.1.2 Amyloid-beta 2
1.1.3 Tau 3
1.1.4 Apolipoprotein E 4
1.1.5 Other risk factors 6
1.1.6 Cell death in Alzheimer disease 8
1.2 Oxidative Stress 10
1.2.1 Oxidative stress in Alzheimer disease 11
1.3 Thioredoxin-1 14
1.3.1 Thioredoxin-1 in neurodegeneration 15
1.4 Thioredoxin-80 16
1.5 Brain neuroprotection and cognitive reserve 18
2 AIMS 21
Specific aims 21
3 METHODOLOGICAL CONSIDERATION 23
3.1 Experimental models 23
3.1.1 Cell lines or primary cultures 23
3.1.2 ApoE Targeted Replacement Mice 24
3.1.3 Drosophila Melanogaster 24
3.2 Experimental methods 25
3.2.1 Cell viability assay 25
3.2.2 Analysis of ASK-1 activation 25
3.2.3 In vitro experiments using Aβ and ApoE 26
4 RESULTS & DISCUSSION 28
4.1 Thioredoxin-1 in relation to risk/protective factors for Alzheimer disease 28
4.1.1 Estrogen protection against Aβ neurotoxicity occurs independently of Trx1 expression. 28
4.1.2 Apolipoprotein E4 causes a reduction in TRX1 levels and activation of apoptosis via lysosomal leakage. 29
4.2 Thioredoxin-80 in Alzheimer Disease 30
4.2.1 Thioredoxin-80 is cleaved by α-secretase and is decreased in AD brain. 30
4.2.2 Thioredoxin-80 protects against Aβ neurotoxicity *in vitro* and *in vivo*. 31
5 CONCLUDING REMARKS AND FUTURE PERSPECTIVES 33
6 ACKNOWLEDGEMENTS 37
7 REFERENCES 41

LIST OF ABBREVIATIONS

AD	Alzheimer disease
Aβ	Amyloid-beta
ABAD	Aβ-binding alcohol dehydrogenase
AKT	Protein kinase B
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Arg	Arginine
ASK-1	Apoptosis signal-regulating kinase 1
BDNF	Brain-derived neurotrophic factor
CSF	Cerebrospinal fluid
Cys	Cysteine
E2	17β -estradiol
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
ETC	Electron transport chain
Daxx	Death-domain associated protein
FAD	Familial Alzheimer disease
FDG	Fluorodeoxyglucose
FTD	Frontotemporal dementia
Gly	Glycine
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione S-transferase
GWAS	Genome-wide association studies
HDL	High density lipoprotein
HNE	4-hydroxynonenal
ICC	Immunocytochemistry
IHC	Immunohistochemisty
JNK	c-Jun N-terminal kinase
LAMP-2	Lysosome-associated membrane protein-2
LDL	Low density lipoprotein
LMP	Lysosomal membrane permeabilization
LTP	Long-term potentiation
MAPK	Mitogen activated protein kinase
MCI	Mild cognitive impairment
MDA	Malondialdehyde
MetS	Metabolic syndrom
MnSOD	Manganese superoxide dimutase
MVB	Mulitvesicular bodies
NFT	Neurofibrillary tangle
NGF	Nerve growth factor
PBMC	Peripheral blood mononuclear cell
PiB	Pittsburg compound B
PET	Positron emission tomography
Pro	Proline
PUFA	Polyunsaturated fatty acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SORL1	Sortilin related-receptor 1
TAMs	Thioredoxin-80-activated monocytes
ThT	Thioflavin T
TR	Targeted Replacement
Trx	Thioredoxin
TrxR	Thioredoxin reductase
Trx80	Thioredoxin-80
TXNIP	Thioredoxin-interacting protein
UAS	Upstream activating sequence
VLDL	Very low density lipoproteins
WB	Western Blot

1 INTRODUCTION

Since the beginning of medical science in the ancient Egyptian and Greek societies, copious discoveries have been made for the benefit of humanity. Despite all these innovations and breakthroughs, no one has yet found a way to prevent us from aging. Unfortunately, not all of us are lucky enough to expect a healthy and active life when we get older and some may even face burdensome disorders. Such a disorder is Alzheimer Disease. The heavy burden of this disease is not only carried by the patients, who might experience how themselves and the world around them are changing uncontrollably, but also by family and friends who see their loved-ones fade away.

1.1 ALZHEIMER DISEASE

Alzheimer Disease (AD) is a neurodegenerative disorder that affects cognition, memory and behavior. It is the most common form of dementia with almost 47 million people affected worldwide. With an increasing and aging population, the number is expected to reach more than 130 million by year 2050 [1]. The disease is progressive and eventually fatal and today there is no cure available. Not only is there an urgent need for a curative treatment for all patients but also for society at large. The global cost for society is more than 800 billion US dollars annually and in only 15 years the cost is predicted to reach a staggering 2 trillion US dollars! This calls for an immediate action, not only to find better treatments but also to identify prevention strategies, develop new ways to diagnose patients at an earlier stage and to improve care for affected individuals.

1.1.1 Neuropathology

The disease is characterized by altered cholinergic function and loss of synapses and neurons in the cerebral cortex and parts of the subcortical areas. In addition, brain accumulation of amyloid-beta (Aβ) peptides and hyperphosphorylated tau, leading to the formation of plaques and neurofibrilliary tangles (NFT) respectively, are other markers of the disease [2,3]. Along with these signs, the brains of individuals with AD also show activation of inflammatory pathways [4] with activated microglia and reactive astrocytes, often in association with Aβ plaques [5]. Many of these primary pathologies emerge years before the first signs of cognitive dysfunction. In early stages, the Aβ deposits are mainly found in the basal parts of the frontal, temporal and occipital lobes of the neocortex, later these can also be found in the allocortex including the hippocampus and finally spreading to subcortical areas [6]. The tau aggregates on the other hand are formed initially in locus coeruleus in the brainstem followed by the entorhinal cortex, the hippocampal formation and finally also throughout the neocortex [7]. The majority of patients also have vascular

changes in the brain such as cerebral amyloid angiopathy (CAA), which is a condition where protein deposits build up in the walls of blood vessels, and this can lead to cerebral ischemia [8]. Another major sign is increased oxidative stress in the brain, which is given a deeper review in a separate section below (1.2).

1.1.2 Amyloid-beta

The characteristic plaques in AD brain consist mainly of Aβ peptides. These peptides are generated through sequential cleavage of the Amyloid Precursor Protein (APP) by the β-secretase enzyme and the γ-secretase complex. This cleavage sequence can generate Aβ peptides that ranges from 39 to 43 amino acids [9], however, the two most abundant species has 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acids [10]. The latter one is more hydrophilic and prone to form amyloid aggregates and is the major component of the amyloid plaques [11]. The familial forms of AD (FAD), are all caused by mutations in genes related to the production of Aβ; APP, Presenilin-1 (PS-1) and Presenilin-2 (PS2), where the two latter constitute the catalytic subunit of the γ-secretase complex [12]. More than 200 mutations have been identified in these genes [13]. The APP gene is located on chromosome 21, which exists in three copies in people with Down's syndrome. These individuals have an overproduction of Aβ peptides and they also develop Alzheimer-like pathology early in life. Despite this, it is not clear how Aβ contributes to the disease or what the physiological role of the peptide is. Several studies have shown that Aβ is neurotoxic and different mechanisms have been proposed. Administration of Aβ directly into rat brain caused both excitotoxicity [14] and synaptic dysfunction [15]. Studies have also shown how Aβ can interact with components both inside the cell and on the plasma membrane leading to cellular dysfunction and cell death. For example, the mitochondrial enzyme Aβ-binding alcohol dehydrogenase (ABAD), was demonstrated to interact with Aβ inside the mitochondria in both AD patients and transgenic mice, causing mitochondrial dysfunction [16]. On the cell surface, many membrane proteins were shown to interact with Aβ leading to direct toxicity [17]. In addition, several studies have linked Aβ to the generation of free radicals and oxidative stress [18-20]. Between cleavage of APP to the formation of plaques, the monomeric Aβ misfolds and forms dimers, oligomers, protofibrils and mature fibrils in a sequential manner [21,22]. In the early 90's, the amyloid hypothesis was presented, which states that it is the Aβ species that are neurotoxic and the driving force behind the disease, with the formation of NFTs and cell death being secondary events [23]. During the last two decades, the Alzheimer research community has debated which one of the Aβ entities is the one mediating the neurotoxic effects. The dimers, oligomers and protofibrils have all been shown to have toxic effects in different studies [24] [25,26]. However, there are also arguments against the amyloid hypothesis. First of all, the amyloid hypothesis is mainly based on FAD that is caused by deterministic genes. This cannot explain the sporadic form of

AD, which accounts for more than 95% of all Alzheimer cases. Interestingly, a recent study in a mouse model showed how some known PS-1 mutations led to abolished protease activity and impaired brain function, independently of Aβ [27]. The neuropathological signs of AD, plaques and tangles, start and spread differently in the brain. If Aβ were the driving force one would expect the two pathologies to follow the same pattern of spreading yet the grade of cognitive impairment is actually more correlated with NFTs than plaques. In addition, people can have plaque pathology in the brain without any signs of dementia [28]. Despite the fact that it was almost thirty years since Aβ was discovered, its physiological function is still not known. However, it has been suggested that Aβ can be involved in control of synaptic activity [29] and that it even can have antioxidant-, neuroprotective- or anti-microbial function [30-32].

The Amyloid Precursor Protein can also be cleaved in a way that does not generate the Aβ peptides. This occurs when APP is initially cleaved by α-secretase instead of β-secretase and it is this pathway, the non-amyloidogenic, which dominates in the healthy brain [12]. The most studied α-secretases belongs to the A Disintegrin And Metalloproteinase domain-containing protein (ADAM) family [33]. They are transmembrane proteolytic enzymes that perform ectodomain shedding of other transmembrane proteins such as APP [34]. One of the most studied proteins in the ADAM family is ADAM10. Besides APP, ADAM10 together with γ-secretase also cleave the Notch protein [35], which is involved in embryogenesis and neurodevelopment. In fact, ADAM10 knockout mice are embryonically lethal. The APP cleavage by ADAM10 occurs constitutively but can be also be regulated through activation of intracellular signaling mediators such as protein kinase C and MAPK [36,37]. ADAM17 is another member of the ADAM family that is considered to have a more regulated α-secretase activity [38].

1.1.3 Tau

The other major protein accumulation found in AD brain, neurofibrillary tangles (NFT) are made up of hyperphosphorylated tau protein. These lesions can be seen in other neurodegenerative diseases as well such as frontotemporal dementia (FTD). The exact role of tau in these diseases is not known, but it is likely a combination of a toxic effect, and a lost physiological function as the protein aggregates [39]. Tau is mainly expressed in neurons and has six different isoforms [40]. They are involved in the stabilization of microtubules. One way to regulate tau is by phosphorylation, an event that is increased in AD [41]. When tau gets hyperphosphorylated it can destabilize the protein leading to dissociation from the microtubule and aggregation of tau into filaments that make up the NFTs. The formation of these tangles is correlated with the severity and progression of the disease [42]. The protein has several phosphorylation sites and the different epitopes are correlated with different stages of aggregated

tau [43]. Furthermore, different modulations of tau have been shown to have neurotoxic effects, such as phosphorylations at certain residues, truncation, oligomerization and formation of the NFTs [44].

1.1.4 Apolipoprotein E

The human gene for Apolipoprotein E (*ApoE*) is located on chromosome 19 and exists principally as three different alleles named ε2, ε3 and ε4 (*ApoE2, 3* and *4*). The ε3 is the most common and has a global frequency of 78% whereas ε2 and ε4 has 8% and 14% respectively [45]. Apolipoprotein E4 is the major genetic risk factor for sporadic AD. Individuals who carry one copy of the ε4 allele have an approximately three times higher risk while those with two copies have an almost 15 times higher risk for developing AD. However, there are variances between different ethnic populations. The ε4 allele is also associated with earlier age of onset, both for familial and sporadic AD. The mean age of onset for the sporadic cases are 84 years in non-carriers and 68 years in ε4 homozygotes [46]. The risk of developing AD is likely a result of the interactions between genetic and environmental risk factors. Both epidemiological and experimental studies have shown that apoE4, in combination with life-style risk factors, can amplify the risk and cause more severe damage than the individual risk factors alone [47,48].

The differences between the isoforms are located at position 112 and 158 in the amino acid sequence of ApoE. These amino acids are etiher cysteine (Cys) or arginine (Arg) in the following arrangement: apoE2 (Cys112, Cys158), apoE3 (Cys112, Arg158), apoE4 (Arg112, Arg 158) [49,50]. The protein consists of two major domains, a receptor-binding domain and a lipid-binding domain [51]. One of the main functions of ApoE is to bind lipoprotein and transport them from the site of production to its target destination. The main source of ApoE is the liver but it is also present in high amounts in the brain. [52] It is synthetized by glial cells and it transports cholesterol to neurons for uptake. The brain is rich in cholesterol and is a main component of cell membranes and myelin sheets. Consequently, ApoE is also important for neuronal repair after brain injury [53]. The secreted ApoE is internalized through interactions with members of the low-density lipoprotein (LDL) receptor family [54,55], which are more abundant in neurons compared to glial cells [56]. The ApoE3 and ApoE4 isoforms have an equal binding capacity to the lipoprotein receptors while the capacity for ApoE2 is poor [49]. The ε2 allele is linked to the genetic disorder type III hyperlipoproteinemia and the reduced binding capacity to the receptor is thought to be a causative factor for this disease. There are also differences in lipid preference between the two isoforms. Apolipoprotein E2 and E3 prefer smaller lipoproteins enriched in phospholipids (HDLs) while ApoE4 favors the larger ones with high triglyceride content (VLDLs). Even though the lipidation state is important for the receptor preference of ApoE, it has been

demonstrated that lipid binding is not required for internalization into the cells. In addition, lipid-free ApoE prefers lipoprotein receptor-related protein (LRP) over the LDL receptor [57]. Furthermore, ApoE4 is rather unstable compared to the other isoforms and can form a so-called molten globule state where the hydrophobic core of the protein is more exposed [58]. How ApoE4 contributes to a greater risk of AD is not clear and hypotheses including both a gain of toxicity and loss of function have been suggested. [59] Some of them are described below and a summary is found in Table 1.

The gene dosage of *ApoE4* was negatively correlated with the number of dendritic spines in the hippocampus, moreover in ApoE4 Targeted Replacement (TR) mice the excitatory synaptic acitivity was reduced compared to ApoE3 mice [60]. This suggests that ApoE4 mediates AD risk through **synaptic dysfunction**. Other studies using mice models have shown that ApoE4 is associated with impaired **lipid metabolism,** by reduced neuronal uptake and lower levels of cholesterol in the brain [61,62], and with defective **neurogenesis** by weakened maturation of newborn neurons in the hippocampus [63]. All these are examples of "loss of function", where normal processes in the brain are disturbed.

Table 1 - Suggested roles of ApoE4 in AD pathogeneis

Loss of function	References	Gain of function	References
Synaptic dysfunction	*60*	Atrophy	*64, 65*
Lipid metabolism	*61, 62*	Tau	*66, 67*
Neurogenesis	*63*	Aβ aggregation	*68*
Aβ clearance	*69*	Oxidative stress	*70 - 72*
		Neurotoxity	*73 - 77*

On the 'gain of function' side are examples of higher **brain atrophy** in the hippocampus and cortex of ε4 carriers [64,65]. There are also associations between ε4 genotype and the classical hallmarks for AD, Aβ and tau. A truncated version of ApoE induces **tau phosphorylation** in brains of transgenic mice and tangle-like inclusions in neuronal cell cultures [66,67]. In addition ApoE4 is associated with **increased aggregation** and **reduced clearance** of **Aβ**. A study using Pittsburg compound B (PiB) positron emission tomography (PET) scans revealed an association between ε4 gene dose and fibrillar Aβ in several brain areas of cognitively normal subjects [68]. Furthermore, a study in mice models expressing the human variants of ApoE, showed by using

in vivo microdialysis, that the genotype affects the clearance of Aβ and the efficiency was lowest for the ApoE4-expressing mice [69]. Moreover, there is a correlation between ApoE4 and **oxidative stress**. AD patients who were ε4 carriers had increased oxidative stress and reduced antioxidant activity in the hippocampus compared to non-carriers [70]. A similar effect was seen in human ApoE4 mice, where markers of oxidative stress were increased in the cortex, especially in females. Notably, these mice had lower estrogen levels in the brain [71]. *In vitro*, it was also demonstrated that ApoE4 worsens Aβ induced oxidative damage to synaptosomes [72]. A deeper review of oxidative stress in general and its role in AD is found in section 1.2. Finally, the ApoE4 peptide can have **neurotoxic** effects, either by causing direct cell death or by mediating the toxic effects of Aβ [73,74]. This has been shown with both full-length ApoE4 and a truncated version [75]. The toxicity has been suggested to occur through different mechanism e.g. mitochondrial dysfunction and increase in intracellular calcium levels [76,77]. In **Paper III**, a new mechanism for ApoE4 mediated neurotoxicity with a clear link to oxidative stress is presented.

1.1.5 Other risk factors

There are many other identified risk factors for AD besides *ApoE*, both genetic and environmental. One of the acknowledged risk genes for the disease is the *SORL1* gene that codes for the sortilin receptor-related protein (SORL1) [78]. A proposed mechanism for SORL1 in AD pathophysiology is through regulation of endocytic trafficking of APP containing vesicles. Interestingly, SORL1 can also function as a receptor for ApoE [79]. With the use of Genome-wide association studies (GWAS) more genes were discovered including *CLU*, *CR1* and *PICALM* [80]. In 2013, an even larger GWAS study was conducted with 17,000 AD cases and more than double the amount in controls. In this study, eleven new susceptibility loci for AD were identified [81]. Many of the candidate genes at these loci are linked to immune response and inflammation.

There are many environmental and life-style factors that are linked to increased risk for AD. The main one is aging, even though one can argue whether it is an environmental or life-style factor. Anyhow, the risk for dementia increases as we age. In western Europe, in the age group 60 - 64, the prevalence of dementia is 1,6%, and increases gradually for each sequential age group. For people above 90 years of age, the prevalence is 43% [82]. Family history is another important aspect. People with a first-degree relative of dementia, have a higher risk of developing AD. This has likely to do with a combination of other genetic and environmental risk factors [83]. As in many other diseases, the diet plays an important role in preventing or contributing to the development of AD. A low intake of certain nutrients such as vitamins and antioxidants is linked to an elevated risk of the disease, while a moderate intake of unsaturated fats and a so-called

Mediterranean diet might be protective [84]. Alcohol abuse and tobacco smoking are examples of life-style factors that have been linked to increased risk for AD [85,86]. Other types of diseases or medical conditions can also predispose individuals to develop AD. Such a condition is diabetes mellitus. A population-based twin study concluded that diabetes increases the risk of AD. Intriguingly, the risk was stronger if the diabetes onset occurred before 65 years of age [87]. Similar results have also been seen regarding high blood pressure and high plasma levels of cholesterol. Hypertension in mid-life increases the risk of AD later in life [88]. Regarding cholesterol levels, the results are conflicting but principally high plasma levels of cholesterol in mid-life increases the risk for AD, while the situation is opposite in older individuals [89]. However, this does not say that increasing cholesterol late in life could protect individuals from developing AD. The last three mentioned risk factors are all directly or indirectly linked to the metabolic syndrome (MetS), which is a cluster of conditions that also includes obesity, and it is important challenge for public health worldwide. Few longitudinal studies have been conducted in order to investigate AD risk by the combined MetS factors. In a study from 2009, no association was found between MetS at baseline and risk for AD within the 4-year follow-up time [90]. However, all of the participants in the study were above 65 years of age, which could explain why no association was found. Apart from the risk factors, there are also environmental influences that are considered to be protective such as physical- and social activity and higher levels of formal education [91].

Highly relevant to this thesis work, is also the fact that the prevalence of AD is higher in women than in men, and a proposed reason is the estrogen deficiency in post-menopausal women [92]. In fact, reduced levels in CSF of the most abundant form of estrogen, 17β -estradiol (E2), are associated with more Aβ in the brain of female AD patients. [93] Estrogen also had neuroprotective effects both in *in vitro*- and *in vivo* models of AD [94,95]. It has also been demonstrated in post-mortem tissue that female AD patients are deficient in mitochondrial estrogen receptor (ER)β [96].

A hypothesis for the mechanism behind estrogen neuroprotection is via defense and improvement of the mitochondria, followed by a reduction in ROS formation, and/or activation of the antioxidant defense system [97,98] A study from 2003 demonstrated that estrogen induced the expression of Trx1 and suggested that it could play an important role in the neuroprotective mechanism [99]. In **Paper I,** this is investigated further. With this knowledge, clinical trials have been conducted using estrogen-containing hormone therapy as a treatment for AD patients. Unfortunately, they have been unsuccessful [100]. However, none of the trials were done to evaluate estrogen as a prevention strategy in younger individuals. Furthermore, women above 65 who got post-menopausal hormone therapy had an increased risk for brain atrophy [101]. This marks the importance of finding the right target groups in the design of clinical trials.

1.1.6 Cell death in Alzheimer disease

The loss of neurons was mentioned earlier as one of the key features of AD pathogenesis. Many toxic triggers have been suggested as a potential cause of cell death such as Aβ, tau, apoE4 and oxidative stress. Unfortunately, there are no therapeutic options available today in order to rescue dying neurons, and the mechanisms behind cell death in AD are not fully elucidated. Cell death is classically divided into two separate categories; necrosis and apoptosis but there are examples of mechanisms that are separate from these two e.g. "dark neurons" that can be formed when neurons lose their communication with other neurons through loss of synapses [102]. Necrosis has been considered as a form of uncontrolled cell death that involves loss of membrane integrity, cell swelling, lysosomal leakage, random DNA fragmentation and lysis. It is often also accompanied with a significant inflammatory response [103]. This has also been seen merely as a random event and a consequence of accidental insult but the view has changed and it seems as if the necrosis process can be regulated as well [104,105]. Necrosis has been proposed as a possible mechanism of cell death in AD. A morphologic and biochemical characterization of hippocampal post mortem section in brains from patients with FAD, showed the typical pattern of necrotic cell death [106]. In addition, the glutamatergic neurotransmission is impaired in AD patients and when glutamate accumulates it can induce necrosis or apoptosis depending on the concentration [107].

Apoptosis, the other archetypal mechanism of cell death, is considered to be a more controlled or physiological mechanism and is often referred to as programmed cell death. The classical view of apoptosis has been that it is induced by a physiological stimulus, followed by membrane blebbing, shrinkage of the cell, non-random fragmentation of DNA and formation of apoptotic bodies that is engulfed by phagocytes. In addition, this view states that the lysosomal compartments generally are kept intact and that no inflammatory response is provoked [103]. Inside the cells there are certain proteins and pathways that can mediate the signal for apoptosis, such as p53, MAPK, Bax, Bcl-2, cathepsins and caspases. The latter ones are a family of cysteine proteases that are important in the end-stages of several apoptotic pathways. Caspase-3 is activated in the very last stage and is considered to be the executioner of these pathways [108].

There are several signs of apoptotic cell death in AD and several studies using TUNEL assay to detect DNA damage have shown positive staining in neurons and glia in post-mortem tissue from AD brains, especially in the hippocampal region [109 110]. Interestingly, these studies found little correlation between the DNA damage and the amyloid plaques. A TUNEL assay labels the terminal end of nucleic acids and is commonly used to detect apoptotic cell death. However, as

fragmentation of DNA occurs also in necrotic cell death, results from TUNEL assays need to be carefully interpreted in combination with additional structural analysis. Other major signs of apoptosis in AD are increased activation of caspases, including caspase 3, 6 and 8 [111-113].

The marked separation of apoptosis and necrosis in neuronal cell death in AD has been questioned [114]. The end-stage of apoptosis is rather fast and can be completed within 24 hours. This would imply that only a small fraction of all neurons die every day considering the long duration of the disease progression. This corresponds with the lack of apoptotic bodies seen in AD brain. However, the number of neurons with apoptotic features is much higher. If all these neurons complete their cell death process, the brain would be short of neurons at a much earlier stage, making pure apoptosis unlikely as the main cause of cell death in AD. Likely, an alternative mechanism is dominant that involves characteristics from both necrosis and apoptosis. Furthermore, activation of an apoptotic pathway does not always lead to cell death but the process can be reversed [115].

Lysosomal impairment was long considered to be a part of necrosis solely. However, nowadays it is clear that it is involved in apoptosis as well. A low amount of stress and physiological stimuli can trigger lysosomal membrane permeabilization (LMP), which releases cathepsins that can activate apoptosis. An overly high stress load can instead cause the lysosomes to rupture with necrotic cell death as a consequence [116,117]. An important protein regulating cell death is the lysosomal protease Cathepsin D. It can activate apoptosis through the cleavage of Bid, induction of mitochondrial dysfunction and the release of cytochrome c followed by further activation of caspases. [118,119]. Furthermore, Cathepsin D has been identified with both β-secretase-like acitivity and a role in Aβ clearance [120,121]. These functions are possibly reflected in the fact that cathepsin D is found in amyloid plaques [122] and there is a correlation between a Cathepsin D polymorphism and the amount of Aβ deposited in these accumulations [123]. In addition, Cathepsin D can degrade Trx1 and thereby, disrupt the inhibition of the ASK-1 pathway, another road to apoptosis [124]. This pathway mediates the signal through c-Jun N-terminal kinase (JNK) and p38 mitogen activated kinase (MAPK) [125] with subsequent translocation of death-domain associated protein (Daxx) from the nucleus to the cytosol [126]. The pathway can be activated by several factors such as tumor necrosis factor (TNF), endoplasmic reticulum stress and oxidative stress. With relevance to AD, it has also been found that Aβ can activate ASK-1 through oxidative stress [19,127], and that tau can be phosphorylated by p38 MAPK [128]. In addition, gene expression profiling studies showed increased expression of the Daxx gene in the hippocampus of AD patients. [129,130]. In **Paper I** and **III,** the role of Trx1 and the inhibition of the ASK-1 pathway are elucidated further in relation to two risk factors for AD; estrogen and ApoE4.

1.2 OXIDATIVE STRESS

The term "oxidative stress" was devised 30 years ago [131] and is defined as the imbalance between the formation of reactive oxygen/nitrogen species (ROS/RNS) and the ability of the cell to counteract them by its antioxidant defense. ROS is mainly formed during oxidative phosphorylation by the electron transport chain (ETC) at the inner membrane of the mitochondria. Here, energy is converted from NADH and $FADH_2$ to ATP via transport of electrons through specific protein complexes. Finally, these electrons react with oxygen and hydrogen ions to form water [132]. During this process, electrons can "leak" and react with oxygen, forming superoxide anions ($O_2^{\bullet -}$). In further reactions, they can form hydroxyl ions (OH-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), where the latter one is the most reactive [133]. When $O_2^{\bullet -}$ reacts with nitric oxide (NO) it forms RNS in the form of peroxynitrite ($ONOO^-$). It can then react further to generate other forms of RNS such as nitrogen dioxide ($NO_2^{\bullet}$) and nitrosoperoxycarbonate ($ONOOCO_2^-$). Transition metals are also involved in the production of ROS. They have changeable oxidation states and can catalyze both reduction and oxidation reactions. For example, hydrogen peroxide can react with ferrous ions (Fe^{2+}) to generate hydroxyl radicals in the so-called Fenton reaction [134]. Certain enzymes can also generate ROS in order to mediate cellular signaling [135], and immune cells produce ROS/RNS as a way to activate the innate immune response [136]. However, when the production of ROS/RNS is excessive or the antioxidant defense is insufficient, the cell is in a state of oxidative stress, which is potentially harmful to all macromolecules of the cell.

When the DNA strand gets oxidized it can affect transcription and replication of genes. The nucleoside guanosine can be oxidized by $OH^{\bullet}$ forming 8-hydroxyguanosine (8-OH-dG) and is used as s biomarker of oxidative stress [137]. In a similar way, RNA bases can become oxidized [138], which can lead to breakage of the nucleotide chain or ribosomal dysfunction [139]. The nuclei appears to be rather resistant to oxidation [140], which can explain why RNA is considered to be more susceptible to oxidation compared to DNA. Modifications of DNA are more likely leading to irreversible changes in the cell, making the need for compartmentalized protection higher.

The lipids of the cell membranes are also sensitive to oxidation. The most susceptible of the fatty acids are the polyunsaturated ones (PUFA). When they are attacked by $OH^{\bullet}$ they get peroxidized forming isoprostanes [141]. Another way of lipid modification is the formation of reactive aldehydes such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA). These aldehydes are dangerous in a sense, as they can react with proteins and nucleic acids disturbing their function [142]. Direct oxidation of proteins can occur at several different sites causing different types of changes such as backbone fragmentation, side-chain oxidation, loss of activity,

unfolding and misfolding [143]. The amino acids are sensitive to oxidative stress, e.g. methionines and cysteines are easily oxidized and this is considered to be a type of post-translational modification [144,145]. The thiol (-SH) group of the cysteine residue can form sulfenic- (-SOH), sulfinic- ($-SO_2H$) and sulfonic ($-SO_3H$) acid when oxidixed. In addition, it can form disulfides with other cysteines, which can cause a dramatic conformational change of the protein. In general, these changes are reversible but there are examples of irreversible modifications as well e.g. when cysteines covalently bind fumarate or dicarbonyl groups forming S-carboxymethylcysteine (CMC) or S-(2-Succinyl)cysteine (2-SC) respectively [146,147]. Carbonyl products are usually formed when threonine, arginine, lysine and proline get oxidized. They can be formed in reactions with the lipid aldehydes mentioned above and are often used as markers of protein oxidation [148]. As the mitochondrion is a major site for the generation of ROS it is also susceptible to oxidative damage. The DNA coding for the mitochondrial proteins are located within the mitochondria itself making them extra vulnerable. In addition, mitochondria is the site of formation of biologically available iron by iron/sulfur clusters [149]. Hence, impairment of macromolecules within the mitochondria can cause even more ROS formation and eventually lead to cell death [150].

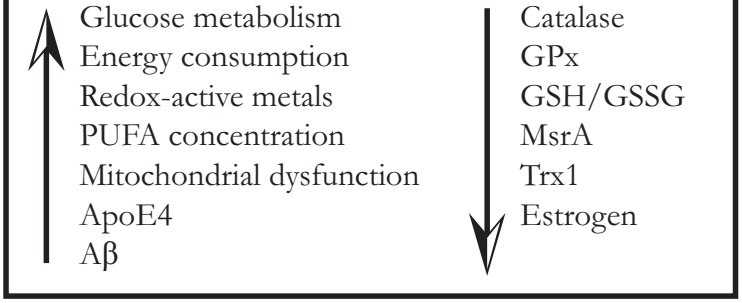
Luckily, the cells have a versatile defense system against oxidative damage, in the form of antioxidants. Some of them are exogenous, coming from our dietary intake, including different vitamins and polyphenols. Many of these are essential to cellular function. However, an excessive intake of exogenous antioxidants can instead have a pro-oxidant effect, giving a double-edged sword character to these dietary compounds [151]. The other types of antioxidants are endogenous and are synthesized by the cells themselves. They can be both enzymatic and non-enzymatic. Examples of non-enzymatic compounds are lipoic acid, coenzyme Q10 and the most abundant one, glutathione (GSH). Glutathione can scavenge oxygen radicals directly or act as a substrate for the enzymatic antioxidants glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) [152]. There are many other enzymatic antioxidants, all with specific functions. Superoxide dismutase (SOD) catalyzes the conversion of superoxide anions to H_2O_2 and O_2. Furthermore, catalase converts the generated H_2O_2 to water and oxygen [153]. Another important antioxidant, Thioredoxin-1 (Trx1), which is the main topic of this thesis, will be reviewed in a specific section below (see 1.3).

1.2.1 Oxidative stress in Alzheimer disease

One major sign of aging is increased oxidative stress and there is a wide spread theory saying that oxidative stress is the answer to why we age [154]. This theory states that aging is driven by the accumulation of oxidative damage. Evidence supporting this theory shows that some animal

models with extended longevity due to genetic alteration or calorie restriction, also had reduced oxidative stress burden. This has also been observed in the brain. One rat strain that has higher longevity than common rats and lives longer without any disease symptoms also showed conserved antioxidant function in brain [155]. Increased oxidative stress in combination with a decreased antioxidant defense is also seen in the aging human brain [156]. The brain is vulnerable to oxidative stress due to several reasons. First of all, the metabolism of the brain is relatively high with 20% of all oxygen and 25% of all glucose being consumed by cerebral functions [157]. Moreover, the brain is an organ with a high amount of PUFA that are more sensitive to oxidation [158] and the levels of redox active metals are also high, which can render an even further increased ROS production [159]. With this in mind, the brain has relatively low levels of antioxidants in comparison with other tissues, especially catalase and GPx, the two most important enzymes in the detoxification of hydrogen peroxide [160]. The fact that neurons in the adult brain are post-mitotic and are generally not replaced also contributes to the vulnerability of this organ.

Proposed factors contributing to oxidative stress in AD brain.



In AD, the signs of oxidative stress are prominent and affects all parts of the cell. Studies on lipid peroxidation showed how the levels of both isoprostanes and HNE were increased in early stages of the disease [161,162]. The levels were also higher when comparing with other neurological disorders [163]. Oxidative damage is also evident when analyzing the nucleic acids of the cell. The levels of 8-hydroxyguanine were increased in AD compared to control, in areas of the brain that are predominantly affected by AD pathology [164]. The same was observed in a study of oxidative protein modifications [165]. Interestingly, the changes were observed early in the disease progression, in patients with mild AD and the levels did not differ in the later stages of the disease. Modifications of proteins by oxidative stress has been linked to neurodegeneration via protein misfolding. When protein-disulphide isomerase gets nitrosylated, its chaperone activity is inhibited, which can cause accumulation of misfolded proteins that is seen in AD and other neurodegenerative disorders [166].

The changes in AD brain stated above can also be detected in the cerebrospinal fluid (CSF). Increased levels of oxidated lipids, DNA, and proteins, have all been detected in samples from AD patients [167-169]. The latter was also negatively correlated with the Mini–Mental State Examination (MMSE) score, a test examining cognition. When it comes to the analyses of plasma and serum, the results have been ambiguous and a study in rats showed that there is no correlation between markers of lipid peroxidation in the brain and plasma [170]. This suggests that markers of oxidative stress in the blood do not mirror the oxidative damage in brain. Also when RNA oxidation was analyzed in both CSF and blood, no correlation was found [171].

Not only is the oxidative damage higher in AD brain, there is also an impairment in the antioxidant defense that is more severe than what is observed for the aging brain. In affected brain regions of AD patients, the ratio between reduced and oxidized glutathione is lower compared to controls [172]. However, both forms of glutathione are individually increased compared to controls, which could reflect a compensatory mechanism where more GSH is produced in order to resist the increased oxidation. There are also examples of enzymatic antioxidants having reduced levels and/or activities in the AD brain e.g. Catalase [173], Methionine sulfoxide reductase (MsrA) [174], GPx [175] and Trx1 [19]. On the contrary, there are enzymes showing increased levels in the brain, for example Manganese superoxide dismutase (MnSOD), which is a protein localized to the mitochondria [176]. In AD brains, this enzyme is increased in neurons in several regions of the hippocampus. Since the role of MnSOD is to detoxify $O_2^{\bullet -}$, this general increase is likely a compensatory mechanism for the increase in oxidative stress. Interestingly, the increase was smallest in the CA1 region, the region that is most affected by AD pathology.

The Aβ peptides that are excessively produced in AD brains may also have a connection with oxidative stress. Aβ can cause increased production of ROS [127], via reduction of redox active metals [177], and mitochondrial dysfunction [178]. Furthermore, the triple-transgenic mouse model that carries mutations associated with familial AD have increased lipid peroxidation in the brain before any signs of plaque pathology [18]. In another AD mouse model overexpressing a double mutant of APP, induction of oxidative stress increased the levels of $A\beta_{42}$ and worsened the plaque load [179]. Cell experiments have also shown that oxidative stress can induce production and accumulation of Aβ [180,181]. These studies demonstrate that the cause/effect relationship between oxidative stress and Aβ works in both directions. The question is, which of the two is the primary event in AD pathogenesis. It has been reported that oxidative damage is the earliest event of the disease [182]. In FAD, the inherited mutations are undoubtedly the causing factor but oxidative stress probably plays a role in the disease progression. In the sporadic cases however, oxidative stress could instead be the driving force.

1.3 THIOREDOXIN-1

More than 50 years ago, in March 1964, researchers from Karolinska Institutet and Uppsala University showed for the first time how they managed to isolate and characterize Thioredoxin (Trx) from *E.coli* [183]. They write: *"The biological function of the protein described in this paper is dependent on the cyclic reduction-oxidation of a single S-S group of the compound, and the name thioredoxin therefore seems to be appropriate"*. Even though the function of the protein was not clear at this time, the scientist suggested that it was functioning as an electron donor for ribonucleotide reductase and that Thioredoxin reductase (TrxR), at that time with a different name, can catalyze the reduction of Trx. These statements turned out to be true and since then, the human thioredoxin family has shown to play an important role in human physiology and has been implicated in several major diseases. Trx1 was previously known as adult t-cell leukemia factor but in the late 1980's it was identified as human homologue of Trx [184]. There are three variants of Trxs in humans; Trx1, which is the most studied, a mitochondrial form called Trx2 and SpTrx that are predominantly expressed in spermatozoa [185]. All these variants contain an active site that is conserved through evolution and consists of the amino acids –Cysteiene-Glycine-Proline-Cysteine- (Cys-Gly-Pro-Cys). This is the site where the oxidoreductase reaction is occurring. In this reaction, two electrons are transferred from the cysteine residues in the active site of Trx to a substrate, e.g. an oxidized protein. Consequently Trx becomes oxidized in this reaction and needs to be reactivated. This is achieved by TrxR as stated by Laurent el al. in the very beginning of the Trx history [183]. In this reactivation reaction, TrxR receives electrons from NADPH and utilizes FAD as a co-factor [186]. See Fig. 1 for a schematic representation of the combined reaction.

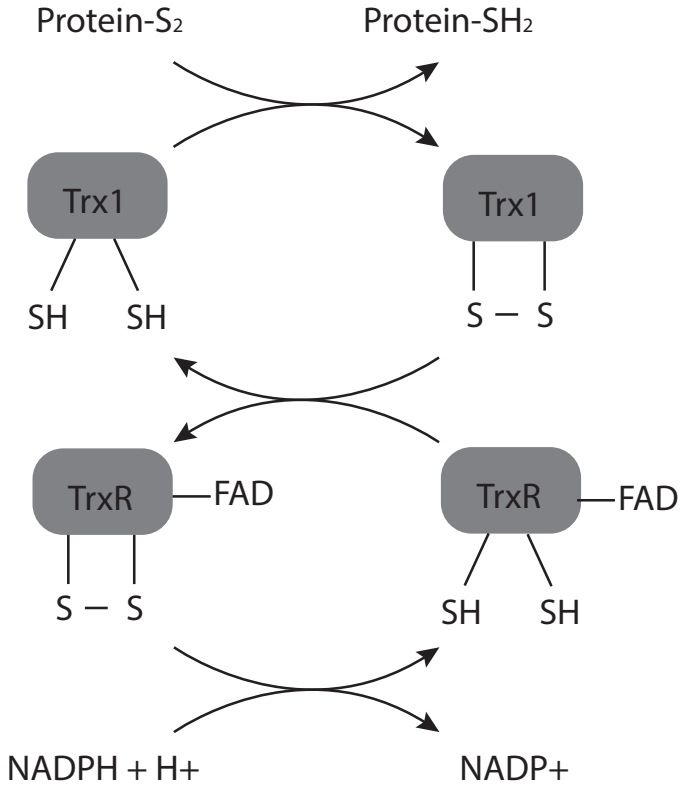


Figure 1 - A schematic representation of how electrons are transferred from NAPH to the oxidized protein (Protein-S_2) via TrxR and Trx1.

There is a plethora of features described for Trx1 and its importance for cellular functions is reflected in the fact that homozygous Trx1 knockout mice are embryonically lethal [187,188]. As mentioned above, it is involved in DNA replication as a hydrogen donor for ribonucleotide

reductase. It is also a part in the regulation of several important transcription factors such as NFκB and p53.[189,190]. In addition, it has been attributed with a chemokine role in inflammation [191] and has anti-apoptotic properties via inhibition of the ASK-1 pathway and inactivation of caspase 3 [192,193]. However, the main function described for Trx1 is in the protection of proteins against oxidative damage, both directly by reducing protein disulfides, and indirectly through activation of other antioxidant proteins such as peroxiredoxin and methionine sulfoxide reductase [185,194,195]. The activity of Trx1 can be regulated by the Thioredoxin-interacting protein (TXNIP). It binds the active site and thereby inhibits the reducing function. It resides normally in the nucleus but can reach the cytosol to interact with Trx1 upon oxidative stress [196,197].

Increased levels of Trx1 has been linked to many types of cancers but its role is controversial. Since cancer cells are under oxidative stress, it is possible that the increase in Trx1 levels is merely a response mechanism. However, since Trx1 has an anti-apoptotic function it could potentially stimulate tumor development. In addition, many cancer therapies rely on the production of ROS to kill cancer cells. Therefore, inhibition of Trx1 has been suggested as a treatment [198]. On the other hand, Trx1 can protect against DNA damage that otherwise could be carcinogenic [199].

1.3.1 Thioredoxin-1 in neurodegeneration

Trx1 is a ubiquitous protein that is expressed in virtually all tissues of the human body. However, expression in the brain is rather low compared to other organs [200]. This could explain why the Trx1 system in brain is sensitive to disturbances and why it is implicated in many neurodegenerative disorders. Several studies have been done in order to determine the levels of Trx1 in AD brains. In the earliest one, Trx1 was detected mostly in the white matter, especially in glial cells, and the levels were higher in AD compared to non-neurological cases [201]. Later, when areas of the grey matter were analyzed more in detail, the levels of Trx1 was shown to be decreased in all brain regions studied, especially in the amygdala, the hippocampal region and parts of the temporal lobe. The same studies also showed an increased activity of TrxR in all regions, with statistically significant differences in the amygdala and cerebellum [202]. Results from my lab showed similar results using immunohistochemistry (IHC). The immunoreactivity of Trx1 was reduced in neurons in the frontal cortex and hippocampus of AD patients. Interestingly, the opposite was seen for cells with an astrocyte-like profile [19]. In addition, another study showed reduction in hippocampal Trx1 levels already in patients with amnestic mild cognitive impairment (MCI), which is a pre-stage to AD [203]. However, a recent report showed no differences in Trx1 levels when using IHC on hippocampal sections. According to the authors, the localization of the protein differed with more cytosolic, and less nuclear staining in AD brains compared to control [204]. There are also a number of experimental studies that have

analyzed the role of Trx1 in AD pathogenesis. Many of them have linked Trx1 in the protection against Aβ toxicity. Both Trx1 treatment in rat primary cultures and overexpression of Trx1 in neuroblastoma cells protected from Aβ-induced reduction in cell viability [19,202]. It was also demonstrated that Aβ could cause neuronal cell death through generation of ROS, oxidation of Trx1 and activation of ASK-1 [19,127]. Furthermore, there are examples of indirect protection, where known neuroprotective compounds upregulate Trx1. This was seen for Omega-3 and S-nitrosoglutathione. They both showed neuroprotection against Aβ cytotoxicity in combination with increased Trx1 levels [205,206]. In addition, a certain neuronal murine cell line, that was resistant against the damaging effect of Aβ, showed increased expression of Trx1. There a fewer links between Trx1 and the other major neuropathological hallmark for AD, the NFTs. However, one *in vitro* study demonstrated how cysteine oxidation of tau could impair its ability to promote microtubule assembly, and how addition of Trx1 could restore this function [207]. In this thesis, the role of Trx1 in AD pathogenesis is further investigated.

Thioredoxin-1 also has a neuroprotective role after ischemic stroke. Studies on rats showed how experimentally induced ischemia, through middle cerebral artery occlusion, diminished Trx1 in the ischemic region while the levels were increased in the penumbra. This was seen in combination with increased survival of cells in the penumbra while the ischemic region was more susceptible to cell death [208]. Furthermore, experimentally increased Trx1 levels led to decreased brain damage in mice models of cerebral ischemia. This was seen after both intravenous and intraperitoneal injections of recombinant Trx1 and in overexpressing mice [209-211].

1.4 THIOREDOXIN-80

Trx1 can become truncated at the C-terminal generating an 80 amino acid long peptide called Thioredoxin-80 (Trx80) [212,213] (Fig. 2). This peptide was known before as eosinophil cytotoxicity-enhancing factor and was discovered in the supernatant of human peripheral blood mononuclear cell (PBMC). [214,215]. When Trx1 gets truncated it looses one helix and one strand which theoretically exposes the inner hydrophobic area. However, the active site and all structural cysteines are still left in the sequence but the remaining N-terminal peptide does not maintain the oxidoreductase activity of Trx1. Until we published our results in **Paper II**, the enzyme responsible for cleaving Trx80 was unknown. There is a limited amount of studies on Trx80 and the majority of the work is done on macrophages and monocytes. In these cells, the peptide was reported to be present mainly at the cell surface facing the extracellular environment and also secreted into plasma [216,217]. There is a rather big variance between individuals in the Trx80 levels in plasma, and the levels do not correlate with the levels of Trx1 [217].

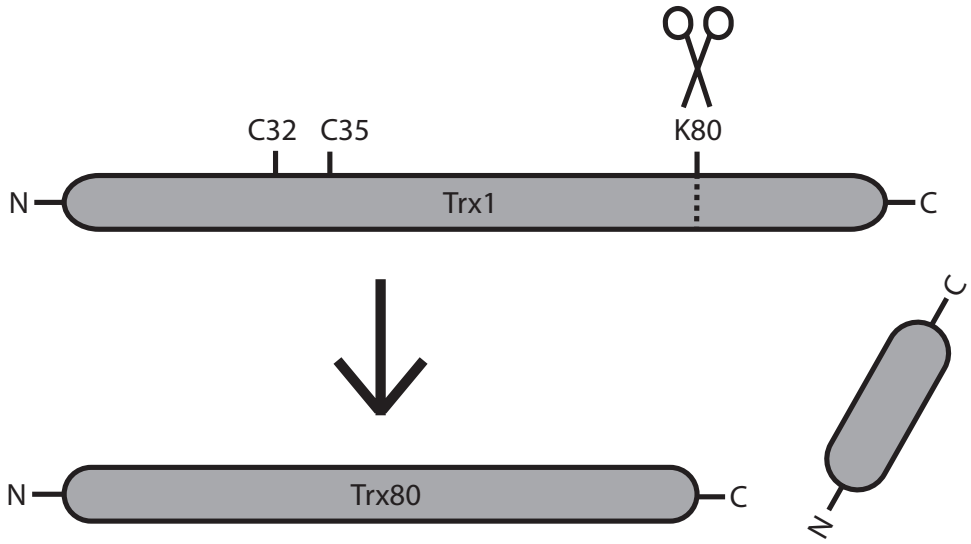


Figure 2 – TRX80 is generated via enzymatic cleavage at a lysine residue at postion 80 in the amino acid sequence of Trx1. The active site and all structural cysteines remain after truncation (not shown) but the peptide looses its reductive capacity.

Most reports of the function of the peptide are related to the immune system. Interestingly, the function can differ dramatically between Trx80 and Trx1. When exogenous Trx1 was applied to human macrophages carrying the HIV virus, it inhibited the expression of the virus, while Trx80 enhanced the production [218]. In a similar way, Trx1 and Trx80 had opposite effects in activating/inhibiting the complement cascade [219]. Furthermore, a study on Peripheral blood mononuclear cells (PBMC) showed that Trx80 had a mitogenic effect that was not seen for Trx1. In addition, Trx80 increased the production of the cytokines Interleukin-12 (IL-12) and Interferon-gamma (IFN-γ) in these cells [220]. Monocytes can be activated and differentiated by Trx80, and it has been described as a new cell type called Thioredoxin-80-Activated Monocytes (TAMs). The differentiation occurs via activation of MAP kinase pathways and these cells have been shown to inhibit the replication of intracellular pathogens [221,222]. Regarding diseases, Trx80 has been linked to both atherosclerosis and rheumatoid arthritis. A recent study showed how the peptide promoted differentiation of macrophages into the M1 phenotype and how it severed vessel lesions after intraperitoneal injections in a mouse model of artherosclerosis [223]. Synoviocytes from rheumatoid arthritis patients released Trx80 after stimulation with inflammatory cytokines [224]. However, whether Trx80 is found in the brain or not has been entirely unknown. **Paper II** and **IV** show the first results on Trx80 in the brain and its implications in AD.

1.5 BRAIN NEUROPROTECTION AND COGNITIVE RESERVE

The most frequently used drugs to treat AD today are cholinesterase inhibitors and an NMDA antagonist. They are used in mild to moderate AD and delays symptoms for a limited time. Unfortunately, these drugs are only symptomatic and they do not stop the disease progression. A large amount of clinical trials have been conducted in order to find a disease-modifying treatment but have so far been unsuccessful [225]. Many of the trials have had Aβ as a target, by lowering its production, increasing its clearance or inhibiting its aggregation. However, they have all failed since the primary end-point was not met and/or the treatment had adverse side effects [226]. The reason for the lack of effects in these trials has partially been attributed to the fact that the study population was not optimal with respect to both age and pathology. A lesson learnt from this has been that a treatment is more probable to be successful if it is given in an early stage of the disease. In order to tackle this issue, improved diagnostics is needed. However, AD is a heterogeneous disorder with many factors contributing to the disease. This makes single-target strategies questionable. Perhaps neuroprotective strategies in combination with therapies that directly target the disease mechanisms would be a better option to find a cure. At the same time, preventive approaches against neurodegeneration are also important in order to lower the incidence and delay the age of onset. Since many environmental risk factors are identified, the options for such an approach are potentially numerous. In a double-blind randomized controlled trial, aged individuals were given a multi-domain intervention including diet, physical training, cognitive exercise and vascular risk monitoring for 2 years. The control group was given general health advice. The outcome of the study showed that the group receiving the intervention improved or maintained their cognitive function [227].

An example of a neuroprotective and preventative strategy is the use of antioxidants [228]. Disappointingly, none of the trials performed have shown any clear improvement in patients, or a preventative effect in patients at risk. However, many of them consisted of treatments with a single compound, which can explain the lack of effect since exogenous antioxidants often have selective areas in the cells where they are protective. Interestingly, a study using a mix of several antioxidants and other nutrients that were given to healthy individuals without dementia showed an improvement in cognitive testing. This supports the idea of a multifaceted treatment approach. However, when using exogenous and dietary antioxidant, the effect on ROS will not only occur locally, which can lead to an unnecessary depletion of oxidants in areas not affected by the disease. Therefore, activation of endogenous antioxidants could be a more suitable approach for neuroprotection. Support from this are seen in experimental models of Parkinson disease, AD and cerebral ischemia [19,211,229]. There are also other approaches with neuroprotection as a focus. Insulin has neuroprotective effects both *in vivo* and *in vitro*. In a pilot trial, insulin was

administered nasally in healthy subjects, MCI- and AD patients. The healthy subjects showed improvement in attention and memory tasks and the MCI/AD patients also showed improvements in memory tasks, cerebrospinal fluid markers and in fluorodeoxyglucose (FDG)-PET analysis. [230]. Estrogen, which was mentioned previously, is another example of a neuroprotective hormone that has been used in clinical trials [100]. Furthermore, growth factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) hold great potential as protective and stimulating agents in AD brains. The latter one has been tested in a small study using encapsulated delivery of NGF producing cells to the basal forebrain. Of the six patients participating in this study, two showed a positive result on cognitive tests [231,232]. These responders also showed less brain shrinkage and a better status of CSF biomarkers at follow-up. Interestingly, Trx1 has been reported to be important for NGF mediated signaling and neurite outgrowth [233]. Regarding BDNF, several studies have shown beneficial effects in *in vivo* models of AD [234].

On top of the preventative, neuroprotective and mechanistic treatment comes also the concept of cognitive reserve. This suggests that individual differences exist in neural networks and processes behind cognitive functions that make some individuals more prone to brain damage than others [235]. This is linked to AD since individuals can have widespread AD pathology with few clinical symptoms of the disease [236]. Studies have also shown that factors such as education and leisure activities can reduce the risk of dementia suggesting that the cognitive reserve is modifiable [237]. Cognitive reserve is also related to brain reserve, which essentially means the size of the brain and number of neurons. These two concepts are likely important in deciding the symptomatic manifestation of a pathological or damaging insult to the brain. To understand the neural basis of cognitive reserve is important in order to find new therapeutic options in AD.

2 AIMS

The brain is sensitive to oxidative stress and this is reflected in the brains of AD patients and could be a driving force of the disease. The patients also exhibit a reduction in the levels and activities of several antioxidant proteins, including Trx1. The general aim of this thesis was to investigate the role of Trx1 in AD using a molecular and cell biology approach in order to deeply understand the underlying mechanisms of the disease.

SPECIFIC AIMS

- To investigate the role of Trx1 in the mechanisms behind factors that modulate the risk for developing AD (**Paper I** and **III**)
- To investigate the production of Trx80 in the brain and its potential role in AD pathogenesis. (Paper **II** and **IV**)
- To investigate the influence of Trx1 and Trx80 on Aβ effects (**Paper I**, **II**, **IV**).

3 METHODOLOGICAL CONSIDERATION

This thesis covers a cell and molecular biology approach aiming to understand mechanisms in the human brain. For this, several models have been used. All of them have advantages and limitations and in the following section some of them will be discussed. A more detailed description of all methods and models can be found in the individual papers (**Paper I-IV**).

3.1 EXPERIMENTAL MODELS

To study the mechanisms underlying AD it would be ideal to do experiments on actual patients carrying the disease. For obvious ethical and practical reasons this is not possible and therefore it is crucial to find the optimal model for what you are analyzing. When choosing the right model several factors have to be taken into consideration including: ethics, species, time, price, availability and the possibilities to do experimental modifications. The corresponding ethical committees have approved all the work done on human and animal samples in the studies.

3.1.1 Cell lines or primary cultures

In all of the studies presented in this thesis, a neuroblastoma cell line has been used (SH-SY5Y). This is a simple but excellent model for studies of cellular mechanisms, with high reproducibility that allows for genetic manipulation in a convenient way, either through overexpression or silencing of genes. In addition, it is easy to obtain a large amount of these cells which some experiments require while no ethical permit is needed. However, this is a cancer cell line that is immortalized and is originally derived from a tumor. The neurons in the brain are typically post-mitotic, which is not the case for neuroblastoma cells, and in many situations they will not behave the same. Hence, the data obtained should be carefully interpreted and extrapolation to what occurs in the human brain cannot be done directly. However, primary neuronal cells derived from embryos of rodents can be used instead and is done in this thesis work as well, although to a limited extent (**Paper II**). These cells reflect the human brain more, even though they are isolated embryonic cells and are no longer part of such a complex organ as the brain. In addition, differences exist between species that have to be taken into consideration. To overcome this problem, human primary cultures derived from elective routine, first trimester abortions are used in two of the studies presented (Paper **II** and **III**). They mirror even more what is happening in the human brain. However, the access to these cells is limited and they can only be obtained in small amounts. There is also a major ethical aspect that has to be taken into

consideration when using this material and it is not uncontroversial. In **Paper II**, we also used mixed human primary cultures with both neurons and glial cells. These cultures are more complex and demanding but provide the interplay between different cell types, which mirrors the situation in the brain further.

3.1.2 ApoE Targeted Replacement Mice

In **Paper III** we use ApoE TR mice in order to study the effect of human ApoE isoforms on Trx1 levels in the hippocampus. These mice express human ApoE3 or ApoE4 under the control of the endogenous murine ApoE regulatory sequences [48]. This model offers a well-designed *in vivo* system that allows us to analyze the effect of physiological levels of ApoE in the brain, in the same temporal and spatial pattern as murine ApoE. This is suitable especially since the human and mouse ApoE promotors differ significantly [238] and there is a clear advantage to use this model compared to "knock-in" or "knock-out" models. Nonetheless, the human ApoE gene still differs from mouse ApoE and there is no mouse equivalent of the human isoforms. In addition, the ApoE4 TR mice have lower ApoE levels in the brain compared to ApoE3 TR mice. However, this is also seen in humans, where a reduction in hippocampal ApoE is proportional to ApoE4 allele dose [239].

3.1.3 Drosophila Melanogaster

In **Paper IV** we use a transgenic fly model in order to investigate the effect of Trx80 on Aβ induced neurotoxicity. This model has some strong advantages in experimental research. It is relatively cheap and easy to handle. It has a short generation time, allowing for fast production of new genotypes when crossing flies. It also ensures a significant amount of replicates to work with. The *Drosophila Melanogaster* genome has about 17,000 genes and has many human analogs [240]. This makes it a simple but relevant model for studying human diseases. The flies have four chromosomes including the sex chromosome. When crossing flies, a few balancer chromosome fly lines are used. Flies homozygous for the balancer are not viable. The balancers also carry a physical marker such as curly wings or distorted eyes, which makes it possible to distinguish genotypes without genetic screening [241]. In order to express the transgenes we use a GAL4/UAS system [242]. Briefly, this system uses an upstream activating sequence (UAS) that is placed upstream of the gene of interest. This sequence has a GAL4 binding site that controls the expression of the gene. By crossing a fly line containing the UAS sequence plus the gene of interest (responder) with a line having tissue specific expression of GAL4 (driver) one achieves selective expression in certain tissues. We use a driver line called ElavC155 that expresses the transgenes in all types of neurons. However, the expression of GAL4 is temperature dependent with more expression at higher temperatures. This demands a good control of temperature in the

fly incubators to minimize variability [243]. In our model, we overexpress two peptides that are not present in normal flies. This has to be taken into account when interpreting results.

3.2 EXPERIMENTAL METHODS

As with experimental models it is important to find the appropriate method for what you want to study. It can be a qualitative, quantitative or semi-quantitative method. In the latter one, the experimenter does not obtain a real value of what is measured but rather a value that has to be related to others. In this thesis work, we have used many different methods and a few of them are discussed below.

3.2.1 Cell viability assay

In order to study what effect a certain influence has on cell viability we have used cell viability assays, primarily an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. The MTT assay relies on the decrease, or absence of the activity of mitochondrial succinyldehydrogenases and other reductases. Dead cells do not have any mitochondrial activity and apoptotic cells or cells under stress also have a reduced activity. A membrane-permeable tetrazolium salt, which is a substrate for succinyldehydrogenases, is added to the cultures. Living and healthy cells then produce a purple-colored and water-insoluble formazan salt, which can be dissolved in DMSO. The absorbance of the solution is proportional to the mitochondrial enzymatic activity, and indirectly to the viability of the cells in the culture [244]. Hence, a reduction in signal in the MTT assay does not necessarily mean more cell death but could rather be a reduced acitivity of the mitochondrial enzymes. A similar kind of assay is the resazurin assay used in **Paper IV**. In this assay, a rezasurin salt is converted to another formazan by the same type of enzymes as for the MTT assay. However, this formazan is fluorescent and water-soluble making this assay an improvement over the MTT assay. In **Paper III**, we use a method that measures the formation of mono- and oligonucleosomes. Since DNA cleavage is a sign of apoptotic cell death, this assay indirectly measures the levels of apoptosis, which is more specific than just measuring cell death in general.

3.2.2 Analysis of ASK-1 activation

In **Paper I** and **III,** we have analyzed the effect of estrogen and ApoE on activation of the ASK-1 pathway. We have previously used a vast amount of commercially available antibodies in order to detect endogenous ASK-1, either directly by Western Blot (WB) or via immunoprecipitation of the protein to increase the concentration. However, all of these antibodies have failed to detect the ASK-1 in non-overexpressing conditions. Therefore, we have used Daxx as an indirect measure of ASK-1 activation. This protein is located downstream of ASK-1 and translocates

from the nucleus to the cytosol when the pathway is activated [245,246]. We have analyzed this event both by separation of cell lysates into nuclear and cytosolic fractions, and by immunocytochemistry.

3.2.3 In vitro experiments using Aβ and ApoE

In all the papers of this thesis work, we have performed treatments of cells with Aβ. As mentioned above, it is debated which of the Aβ species: dimers, oligomers or fibrils, is the one mediating the neurotoxic effects. In our experiments we have mainly used recombinant Aβ that has been aged for 24-48h. This kind of preparation generates a mixture of different aggregation states [26]. Due to the fact that Aβ exists in several different aggregation forms it is difficult mimic the true physiological concentration in the brain. The plaques and its proximity obviously have a high concentration while other areas have lower. In addition there differences also exist between anatomical areas of the brain. In our experiments we have mainly studied the neurotoxic effects of Aβ. Therefore we have used a concentration of 10 μM, since in our model this generated a consistent decrease in cell viability of approximately 40%. Furthermore, we have also used Aβ enriched fractions from human brain in our experiment on cell viability in **Paper II**. The effect from this type of Aβ was similar to the recombinant preparation.

In **Paper III,** we treat cells with recombinant ApoE peptide. In the brain, neurons take up ApoE that is mainly synthetized by astrocytes. Therefore, we used exogenous treatment of ApoE in neuroblastoma cells and human primary neurons, in order to study its neurotoxic effects. ApoE binds members of the low-density lipoprotein (LDL) receptor on the surface of the neurons and can then be internalized. The lipidation state of ApoE plays an important role for the receptor preference, however lipid binding is not required for ApoE binding to the receptors and internalization into cells [55]. We have used 100 nM of ApoE in these experiments, which is also considered to be the physiological concentration [247].

4 RESULTS & DISCUSSION

In the following chapter, the main findings of this thesis will be summarized and discussed. Details about results and methods can be found in the individual papers.

4.1 THIOREDOXIN-1 IN RELATION TO RISK/PROTECTIVE FACTORS FOR ALZHEIMER DISEASE

There are many factors, both environmental and genetic, that affect the risk of developing AD. Some of them will increase the risk while others are protective. Previous studies have demonstrated how Trx1 is decreased in AD brain. Therefore, we wanted to study the role of Trx1 in the mechanism behind some of the factors that modulates the risk for AD (**Paper I** and **III**).

4.1.1 Estrogen protection against Aβ neurotoxicity occurs independently of Trx1 expression.

Estrogen is a factor that is considered to have a protective effect against AD. The incidence of the disease is higher in women than in men and one reason for this is thought to be the post-menopausal lack of this hormone. In addition, several evidences from *in vitro* and *in vivo* studies have shown that estrogen has neuroprotective effects against Aβ induced toxicity. Since Trx1 inhibits Aβ toxicity *in vitro*, we wanted to test if the protection of estrogen was mediated by Trx1 (**Paper I**). As a model, we used SH-SY5Y cells treated with "aged" $A\beta_{42}$ together with 17β-estradiol (E2) or agonists specific for either ERα or ERβ. We found, using MTT assay that E2 protected against the reduction in cell viability caused by Aβ, and it prevented the cytosolic translocation of Daxx from the nucleus to the cytosol, a downstream event of ASK-1 activation, as seen by immunocytochemistry (ICC) and nuclear fractionation. Furthermore, E2 induced phosphorylation of Extracellular signal-regulated kinase (ERK) and Protein kinase B (AKT), two events that is linked to estrogen-mediated neuroprotection [248]. In addition, E2 also increased the expression of Trx1. However, when using selective agonists, the effect on cell viability and Daxx translocation was only seen for the ERα-agonist. Moreover, treatment with this agonist induced the phosphorylation of AKT but not ERK, and it did not affect the expression of Trx1. From these results, we concluded that activation of AKT was the most important mechanism for the E2 protection against Aβ toxicity in SH-SY5Y cells and that the protection occurred independently of Trx1 expression.

4.1.2 Apolipoprotein E4 causes a reduction in TRX1 levels and activation of apoptosis via lysosomal leakage.

Apolipoprotein E4 is the most important genetic risk factor for AD and several mechanisms for how ApoE4 contributes to the disease development has been proposed. It has been suggested that ApoE4 has direct injurious effects on the brain, either via activation of apoptosis or through mediation of Aβ toxicity. Furthermore, AD patients have increased oxidative stress in the brain and this is worsened in ApoE4 carriers. Therefore, we wanted to study the effects of ApoE on Trx1 in the brain (**Paper III**). To do this, we used transgenic mice expressing human isoforms of ApoE, and different cell models treated with recombinant ApoE isoforms. We discovered that the Trx1 levels were decreased in the hippocampus of ApoE4 mice compared to ApoE3 mice. A similar effect was seen in SH-SY5Y cells and human primary cortical neurons after 5h treatment with ApoE4. In the ApoE4 mice, the mRNA expression of Trx1 was instead increased while it was not affected *in vitro*. This suggests that the reduction in Trx1 levels were due to degradation. Since lower Trx1 levels would imply less inhibition of ASK-1, we wanted to investigate how ApoE4 affected cell viability and the subcellular localization of Daxx. We found that 24h treatment with ApoE4 caused a reduction in cell viability and increased levels of apoptosis. This was accompanied by a cytosolic translocation of Daxx suggesting an activation of the ASK-1 pathway. This was supported by the fact that overexpression of TRX1 and other endogenous ASK-1 inhibitors, including DJ-1 and Glutaredoxin-1, inhibited the ApoE4 induced reduction in cell viability. However, the treatment did not affect the redox status of TRX1, which previously was shown as a mechanism behind Aβ induced activation of ASK-1 [19]. Instead, ApoE4 caused a disruption of lysosomes and a leakage of the lysosomal protease Cathepsin D into the cytosol. This was seen using both co-localization studies of Cathepsin D and the lysosomal marker LAMP-2, and fractionation of cell lysates into cytosolic and microsomal fractions. It has previously been shown that ApoE4 is taken up into lysosomes and that it can destabilize membranes via formation of a so-called molten globule structure [58,249]. It has also been reported that Cathepsin D degrades Trx1, and lysosomal leakage of Cathepsin D can activate other apoptotic pathways as well [119,124]. Hence, presence of ApoE4 leads to a reduction in Trx1 levels and activation of apoptosis, via destabilization of the lysosomal membrane and leakage of Cathepsin D. This is a new mechanistic explanation as to why ApoE4 confers increased risk for AD. However, it is unlikely that ApoE4 carriers have constantly leaking lysosomes and activated apoptotic pathways. However, these individuals might be extra sensitive to other insults that can destabilize the lysosomal membrane such as Aβ. The significance of our findings in an *in vivo* context of neurodegeneration should be further investigated.

4.2 THIOREDOXIN-80 IN ALZHEIMER DISEASE

The results presented above involved Trx1. However this protein can be truncated, generating an 80 amino acid long peptide called Trx80. This peptide lacks the oxidoreductase capacity of the full-length protein and its function differs dramatically in many occasions. Until our first published results, all studies on Trx80 in humans had been dealing with its role in the periphery and nothing was known about Trx80 in the brain. In Paper **II** and **IV,** we have analyzed Trx80 in the brain and explored its possible role in AD.

4.2.1 Thioredoxin-80 is cleaved by α-secretase and is decreased in AD brain.

In **Paper II** we first performed IHC and WB analyses of cortex and hippocampus from human brain and discovered the presence of Trx80. The staining by IHC was mainly seen in pyramidal and bipolar neurons but it was also detected in glia cells when analyzing human primary cells. In pure neuronal cultures, we found that Trx80 was present in both the soma and neurites. In WB analyses, where peptides are separated according to size, we found that the Trx80 band was mainly migrating at approximately 30 kDa. The predicted size of Trx80 is rather 10 kDa but we could by a number of different analyses, including gene overexpression and silencing, confirm that the 30kDa band indeed was representing Trx80. It is likely that the peptide is present in brain in an aggregated form. Furthermore, we could also detect Trx80 in the media from cultivated cells. In **Paper IV,** we did additional investigations on the secretion of Trx80 and discovered that both Trx80 and the full-length protein Trx1 were present in exosomes purified from human brain. By using immuno electronmicroscopy on neuroblastoma cells we could detect Trx80 intracellularly in vesicular structures resembling multivesicular bodies (MVB). The vesicular localization was already suggested in **Paper II,** in our aim to find the enzyme responsible for cleavage of Trx1 to Trx80. We then observed, using ICC that Trx80 was co-localizing with the enzyme ADAM17 in vesicular structures in the cytoplasm. This enzyme is a metalloprotease and has α-secretase activity, meaning it can cleave APP without generating Aβ species. When using modulators of ADAM17 and ADAM10, which is another α-secretase, we could see that the levels of Trx80 and Trx1 were changed. Thus, we concluded that Trx80 could be generated by α-secretase. The general α-secretase activity is decreased in AD brain [250] and so are the levels of Trx1. Consequently we also found a drastic decrease in Trx80 levels in AD brains. This decrease was also seen in the CSF, and the reduction was detectable already in samples from MCI patients. Interestingly, there was a significant decrease in MCI patients that progressed to AD within 2 years, compared to those that were stable. This suggests that Trx80 has potential as a diagnostic and prognostic biomarker for AD.

4.2.2 Thioredoxin-80 protects against Aβ neurotoxicity *in vitro* and *in vivo*.

Thioredoxin-1 has a neuroprotective effect against Aβ neurotoxicity as mentioned above. Therefore we wanted to test if Trx80 had the same effect. In **Paper II**, we treated neuroblastoma cells with "aged" $A\beta_{42}$ and analyzed the effect on cell viability as in **Paper I**. We found that cells overexpressing Trx80 were protected against the $A\beta_{42}$ induced toxicity. The protection was also seen when $A\beta_{42}$ was aged together with the Trx80 peptide but not when the peptide was co-treated with already aged $A\beta_{42}$. From these results, we speculated that Trx80 could stop the amyloid formation of $A\beta_{42}$. To test this hypothesis, we used a Thioflavin T (ThT) assay. The fluorescence of ThT is enhanced when it binds to amyloid fibrils. We found that monomeric $A\beta_{42}$ quickly formed amyloid fibrils when incubated in solution but this was inhibited by co-incubation with Trx80. From the amino acid sequence of Trx80 and the crystal structure of Trx1 we performed *in silico* analyses to determine the hydrophobicity and aggregation profile of Trx80. From this we identified a hydrophobic region in the core of the Trx1 structure that is prone to aggregation. This region is shielded by an alpha helix in the Trx1 structure but would likely be exposed after truncation (Fig. 3). Furthermore, this region has a sequence (KLVVV) with similar properties as the sequence in $A\beta_{42}$ that is responsible for its aggregation (KLVFF).

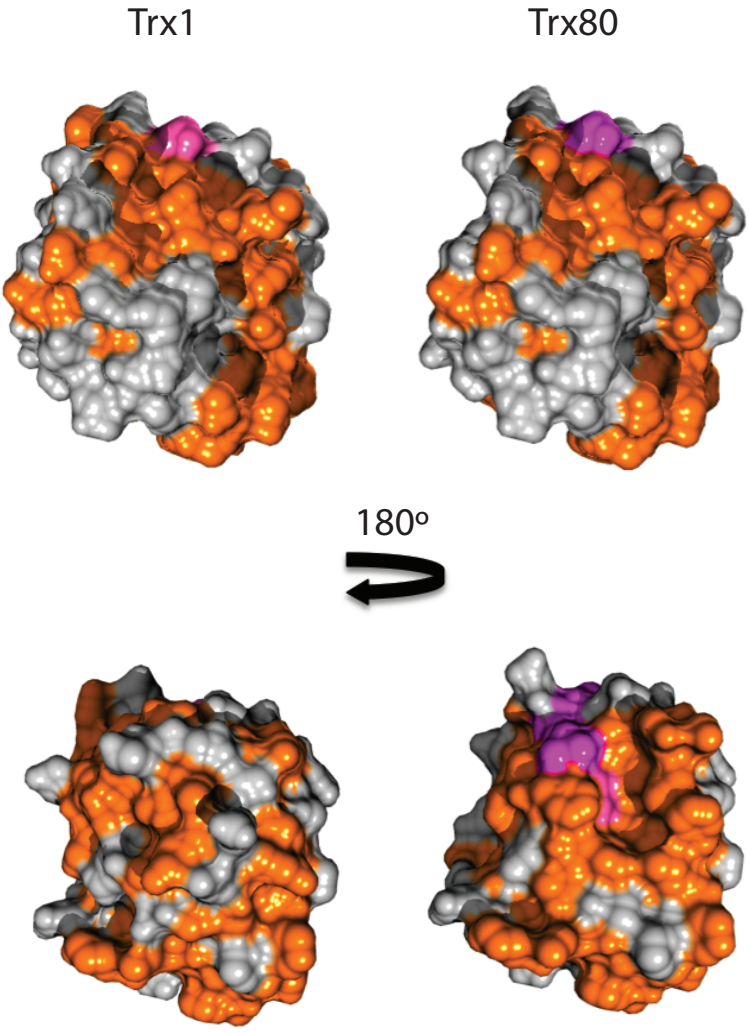


Figure 3 - Surface representation of Trx1 and Trx80. Hydrophobic residues are shown in orange. Pink residues represent the KLVVV sequence. The size of the hydrophobic surface increases after cleavage.

We hypothesized that these regions are involved in the Aβ-Trx80 interaction. In **Paper IV**, we examined this further *in silico* with a protein-protein docking analysis of Trx80 and the crystal structures of $A\beta_{40}$ and $A\beta_{42}$. This showed that Trx80 likely interacts with both Aβ species and the indicated region is expected to be part of the interaction. However, when we changed two amino acids in the Trx80 sequence by point mutations and overexpressed it in neuroblastoma cells, we did not observe any loss of protection against Aβ toxicity. This could mean that other amino acids are involved in the interaction or that Trx80 protects against the toxicity independently of Aβ binding.

These studies mainly explain the effect of Trx80 on extracellular Aβ but not the intracellular content. Using ICC analyses, we measured the levels of $A\beta_{40}$ and $A\beta_{42}$ in SH-SY5Y cells overexpressing Trx80. The results show that both species were reduced intracellularly in these cells compared to control. We also saw that the overexpressing cells had an increased staining of LAMP-2. Furthermore, by WB we discovered that levels of LC3-II were increased in these cells. LAMP-2 is not only a lysosomal marker but it also positively correlates with chaperon-mediated autophagy, and the levels of LC3-II reflect the formation of autophagosomes. This suggests that Trx80 promotes the autophagy machinery, which could be the reason for lower Aβ levels in Trx80 overexpressing cells.

Next, we wanted to know how Trx80 affects Aβ in an *in vivo* model. We used a transgenic *Drosophila Melanogaster* expressing $A\beta_{42}$ in the CNS. By removing the brain from the head of the flies and staining them with an antibody for $A\beta_{42}$, we found that $A\beta_{42}$ accumulated in the brain. This was accompanied by a reduction in the lifespan and impaired locomotor activity, measured by a climbing assay. However, when the flies also expressed Trx80 there were clearly less $A\beta_{42}$ accumulation in the brains. These flies also had the same life span as wild-type flies and the locomotor activity was restored. This shows that Trx80 also protects against the neurotoxic effects of $A\beta_{42}$ *in vivo*. Since Trx80 reduced the levels of $A\beta_{42}$ in these flies similarly to what was observed in cells, it is possible that autophagy is involved in the removal of $A\beta_{42}$. This however needs to be further investigated in this model as well.

In summary, Trx80 is present in the brain and is generated by α-secretases. It is located intracellularly in MVB-like vesicles and is secreted in exosomes. The levels of the peptide are decreased in the brain and CSF of AD patients. In addition, it interacts with Aβ and inhibits its polymerization and toxic effects both *in vitro* and *in vivo*. Furthermore, it lowers the intracellular levels of Aβ, possibly through a degradation mechanism involving autophagy. Together this suggests that Trx80 could be used as a specific biomarker for AD and that therapeutic strategies based on Trx80 have potential.

5 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The work presented in this thesis has been focused on the role of Trx1 in AD. The findings in the studies support the notion that Trx1 has an important function in AD pathogenesis and it also adds a completely new player to the field, namely the cleavage product, Trx80.

The hypothesis in **Paper I** was that Trx1 is involved in the mechanism behind estrogen neuroprotection against Aβ. However, our findings demonstrate that the protection occurs independently of Trx1 expression. Estrogen has been tried as a preventative and therapeutic strategy for AD, however, without any clear beneficial outcome. To find the right target group seems to be important if strategies based on estrogen should work. However, estrogen will increase the expression of Trx1 and this could increase the risk of tumor development. This has to be taken into consideration when investigating the estrogen approach of neuroprotection.

The presence of ApoE4 is associated with increased oxidative stress in AD patients. In **Paper III,** a mechanism is presented that shows how ApoE4 leads to lower levels of Trx1. Since Trx1 is a major antioxidant protein, this could partially explain why ApoE4 carriers are subjected to higher oxidative stress. In addition, the study explained how ApoE4 could be neurotoxic via lysosomal leakage and activation of apoptosis. Thus the possibility that individuals carrying ApoE4 are extra sensitive to other insults such as Aβ, or other factors causing oxidative stress, should be investigated. Support of this can be found in epidemiological studies that have shown that ApoE4 in combination with environmental risk factors multiplies the risk of developing AD. A lack of also Trx1 implies a lower inhibition of the ASK- pathway and an increased susceptibility to the activation of apoptosis.

In Paper **II** and **IV**, the presence of Trx80 in the brain is described for the first time. In addition, the studies describe the location, generation and secretion of the peptide. This substantially adds to the existing knowledge about Trx80. Furthermore, we found that the levels were dramatically decreased in the brains and CSF of AD patients. We also show that Trx80 inhibits polymerization of Aβ and protects against its neurotoxic effects, both *in vitro* and *in vivo*. Moreover, we demonstrate that Trx80 lowers the levels of Aβ and propose a mechanism where Trx80 stimulates autophagic degradation of Aβ.

The physiological role of Trx80 could be as a chaperone for Aβ, keeping it in a native condition and mediating its secretion in exosomes or degradation in lysosomes via autophagy (Fig. 4, top). However, in a pathological situation where Trx80 levels are depleted, Aβ can start to aggregate into toxic species that can spread to other neurons via exosomes, and thereby spread the pathology (Fig. 4, bottom).

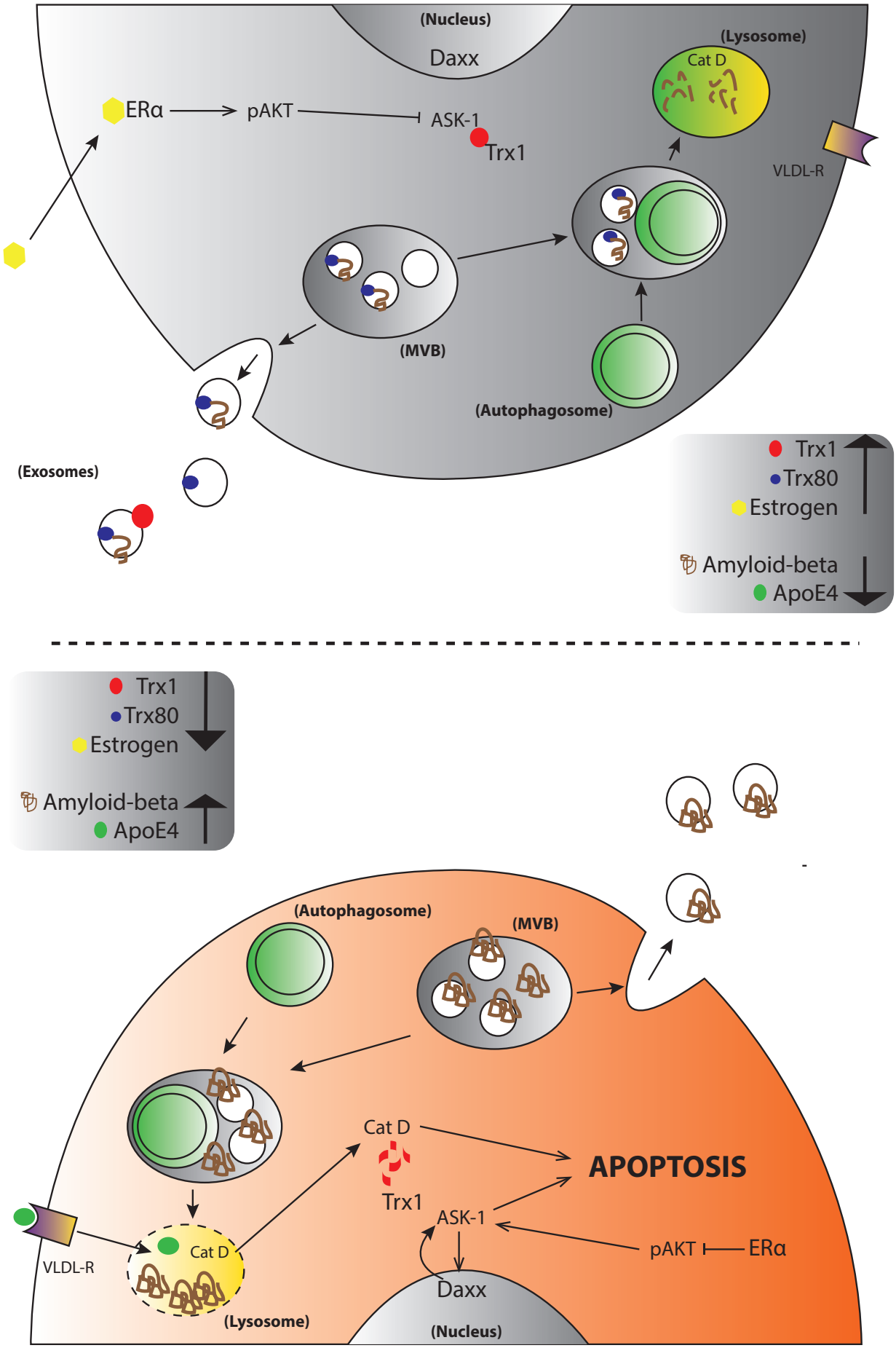


Figure 4 – Summary of results presented in this thesis and suggested mechanisms.

Inside the neurons, Aβ starts to accumulate in the vesicles where it is generated. The lysosomes will try to degrade the increased misfolded Aβ but when the load is too high the lysosomes will fail to function properly and might start to leak. In the presence of ApoE4, this condition will be worsened and Cathepsin D will leak out into the cytosol. This will induce a number of apoptotic pathways including degradation of Trx1 and activation of the ASK-1 pathway. A reduction in Trx1 levels will likely also to lead to less Trx80 and a vicious circle ensues. On top of this, in a state of chronic oxidative stress, the amount of reduced and active Trx1 will be even lower which makes the ASK-1 pathway even more susceptible to activation (Fig. 4, bottom).

The scenario above highlights the importance in maintaining the levels of Trx1 in neurons and therefore opens up new therapeutic opportunities in AD. One strategy would be to increase the levels of Trx80 in the brain. Since α-secretase can cleave Trx1 to Trx80, activators of these enzymes would generate more Trx80. However, this strategy has some major obstacles. First, the activity of α-secretases is not confined to Trx1 but has various other substrates as well. Therefore, activation of these enzymes could lead to several unwanted off-effects. Second, more α-secretase cleavage would lead to less full-length protein and less inhibition of ASK-1 and lower protection against oxidative stress. Finally, if the activators are administered in a way that it also increases Trx80 levels in the periphery, it will likely cause inflammation due to its pro-inflammatory effects on macrophages. Other options would be to find ways to directly deliver Trx80 into the brain or to generate Trx80-like peptides that do not have a pro-inflammatory effect but is still capable of interacting with Aβ.

Another opportunity would be to increase the levels of Trx1 however this strategy also has drawbacks. Since the cell also uses ROS as a signaling mechanism it is not desirable to deplete them if the cell is not under oxidative stress. Furthermore, Trx1 is linked to many types of cancers and an increase in Trx1 levels could increase the risk of tumor development. However, neurons are generally post-mitotic and therefore less prone to proliferate uncontrollably. Therefore, a selective increase of Trx1 in neurons could be a better option. Delivery of peptides directly into neurons is an alternative but this is also problematic due to the combination of the large molecular weight and two specific hurdles, the blood-brain barrier and the plasma membrane. Gene therapy by viral vectors could be an option but this method needs improvement, especially regarding safety. Stimulation of the neuronal expression of Trx1 would be a more plausible possiblity and in addition, one would also expect an increase in Trx80 levels. I believe this last approach holds potential and suggest that it should be further investigated. Interestingly, physical exercise increased the level of Trx1 in rat brain [251], suggesting that not only pharmacological interventions should be considered. However, AD is a heterogeneous disorder with many factors that contribute to the disease development and progression. Therefore, a

multimodal strategy is more likely to achieve success in curing or preventing AD. There is also heterogeneity between patients that perhaps demands different treatments for different subgroups of patients. The results in **Paper III** suggest that ApoE4 carriers might be such a subgroup.

A few of the explanations suggested for Trx80 and Trx1 function in this thesis work needs to be further analyzed. To achieve this, a genetic model with reduced levels of Trx80 is preferred. A classic knockout approach is not possible due the fact that the peptide is generated via enzymatic cleavage and not transcription and translation. However, with the new CRISPR/Cas9 tools available it is possible to do genomic point mutations. By mutating the cleavage site on Trx1, one can generate a model that completely lacks Trx80. For such a model to be optimal one must ensure that the point mutation does not affect the redox activity of Trx1. It is not known if Trx80 is present in rodents. However, both human Trx1 and α-secretases have homologues in mice and rats, thus Trx80 is likely present in these animals. A rodent Trx80 "knock-out" model is therefore a possibility and would contribute significantly to the understanding of Trx80 function.

Is Trx80 involved in other neurodegenerative disorders? Does the peptide have anti-aggregant effects against other amyloidogenic peptides? Can it be used as a specific biomarker to set diagnosis and prognosis of AD? How is Trx80 affected by risk/protective factors? Many questions still remain and hopefully this thesis work will bring more interest to Trx80 as an important factor in the brain, so these questions can be answered.

The population in the world is growing and elderly people make up an increasing share. This inevitable fact together with the immense impact AD has on patients, relatives and society demands urgent action from all parts of society. Many attempts to find a disease-modifying treatment has failed but our knowledge about the underlying mechanisms is increasing and I am optimistic that a cure will be found in the near future, as long as the extent of the challenges are taken seriously.

6 ACKNOWLEDGEMENTS

This thesis work would have been impossible without the support from colleagues, friends and family. I would like to acknowledge and thank some of these people below.

Associate Professor **Angel Cedazo-Minguez**, my main supervisor. First I have to thank you for bringing me to your group and accepting me as a Ph.D. student. You have given me freedom to grow as a researcher and guided me when I needed it. Some of your best skills are your creativity and optimism, and the research group you created probably has the best atmosphere I will ever experience. You are great a co-worker and friend and I hope we will collaborate in the future as well.

My co-supervisor, associate professor **Lars Tjernberg,** for your great mix of humor and seriousness when asking me about my projects. Even though we have not interacted so much regarding my work, I have always that felt that I had your support. Every time we discuss science you give me ideas and suggestions and sometimes those talks have made big changes in the actual projects.

Professor **Lars-Olof Wahlund**, my other co-supervisor, for accepting me as a Ph.D. student. I admire your work and what you have contributed to the center. I also enjoy your attitude and the nice discussions we had during lunch.

Professor **Bengt Winblad** for creating a top-class center in Alzheimer research and for your true passion in the field. You are an inspiration and it is an honor to be a part of your team. I am also very grateful for the support you have given me for continuing doing research abroad and for giving me this opportunity.

Professor **Gunnar Norstedt** and **Amilcar Flores-Morales** for accepting me a master student at KI and for introducing me to medical science.

Professor **Thomas Sakmar** for your inspirational talk during the NVS day and for giving me the opportunity to work in your lab. I am looking forward to the new adventure.

Mattias Vesterlund for teaching me how to work in the lab during my master thesis and for your great taste in music. What you taught me then, I teach master students today.

Professor **Sophia Hober** for being my mentor. I appreciated our meetings a lot and how you always had time for me despite your busy schedule.

All the co-authors in my papers especially; **Francisco Gil-Bea, Susanne Akterin**, **Javier Avila-Cariño, Angel Gutierrez-Rodriguez, Arne Holmgren, Rocio Perez-Gonzalez, Efrat Levy and Jenny Presto** for all your great contribution to the work on Thioredoxin-80.

Erik Sundström for all your contribution to my work, the interesting courses and for being aware of the beauty in routines. **Eva-Britt Samuelsson** for helping me with the human primary cultures.

Helen and **Erik Hermansson** for all your help and for teaching me about flies, **Kerstin** for helping me with ordering and for creating a neat working environment.

All senior scientists at Division of Neurogeriatrics, especially **Helena** for nice discussions in the cell lab and for your nice and warm attitude, **Maria** for your great work as a director of doctoral education at NVS and for helping us in the PhD Student Council, **Homira** for nice early morning chats and your struggle to improve the PhD seminars, **Janne** for your valuable

comments to our Trx80 projects and for letting me use your fly lab, **Dag** for fruitful collaboration and for refreshing my Norwegian, **Ronnie** for all your help and the quick chats in the corridors.

Bitti, **Lotta** and **Lena** for your joyful spirit in the "glass box".

The administrators for invaluable help with all types of issues, especially **Gunilla**, **Inga-Lill**, **Eva** and **Anna J**.

To all the present group members: **Mustafa,** we have been on this journey together and you have made it a lot easier. You have made every new person in the group feel welcome, including me on my first day. Thanks for the inspiring discussions about politics and science in the office and for putting up with me when I joke about milk and chocolate. Thanks also for all the work you did to improve the PhD student council and for proofreading my thesis. I am privileged to have you as a friend. **Anna S** for "holding my hand" when I was new in the lab and for doing it again when I was writing my thesis. It was truly great having you back in the group and you are a very intelligent and funny person that I admire a lot. **Silvia**, for your great attitude and for being normal when everyone else was insane. Thanks also for all the help in the lab and for sharing thoughts about parenthood. **Gorka**, for giving a new meaning to the word "always". Thanks also for all the help with the flies and for nice discussions about being a father. **Paula**, for all the laughs in the office and for teaching me about brain anatomy. Now I have finally reached the end of the beginning, but I have to stay alert, just in Bieber. **Patricia,** for all your help in the Trx80 project and for always supporting me and cheering me up when I need it. **Cristina,** for sharing my taste in humor. **Raul**, for your constant smile and all the movie and tv-serie suggestions. **Laura M** for teaching me new methods in the beginning and for nice collaboration in our papers. **Laura P** for being the new master of our apartment and for the partying in Almázan. **Christa** for nice discussions about long-distance running. **Yi** and **Erik Hansson** for helping me in the end of my thesis work.

I also want to acknowledge former members of the group, especially: **Javi,** for all your contributions to my thesis work. You are a true scientist! Thanks also for all our talks about the most important thing in life, football. **Bea,** for sharing the first confusing months in the lab and for all the fun in Barcelona. **Francesca** and **Marta** for your work on the ApoE paper and for all your positive energy. **Elena P, Elena C, Maria, Inci, Nihan, Lin, Shirin, Monica, Manuel, Lucia, Raquel** and all other former group member for contributing to the nice atmosphere in the ACM group.

I am also grateful for all the former and present colleagues and friends at Dept of NVS. I would especially like to acknowledge **Erik Hjorth, Per-Henrik and Erik Westman** for all deep and competent discussions we have had during Friday meetings, **Walid** for nice collaboration and for being the best sheriff in town, **Lisa** for the nice discussions and complaining-sessions in the lab, **Heela** for being so creative, smart and sometimes lovely naïve, **Carlos** for helping me with the thesis printing process and a special thanks also to **Anna L, Louise, Linn, Jennie, Michael, Annelie, Johanna, Antonio, Simone, Jolanta, Daniele, Carlos, Camilla, Joana, Bernadette, Cata, Nuno, Alina, Mahmod, Daniela, Ning, Aleks, Märta, Xiuzhe, Minqin, Alex, Gefei, Nina, Henrik, Jojje, Anton, Oihan, Médoune, Kirsten, Quipin, Huei-Hsin, Kostas, Mohammed,** and all other colleagues at Dept. of NVS.

Jag vill också rikta ett stort tack till alla vänner utanför jobbet. Från och med nu hoppas jag kunna ta igen lite förlorat umgänge.

Johan A, för du är en fantastisk vän. Ditt stöd är ovärderligt och de 10 senaste åren i Stockholm hade varit betydligt tristare utan dig. Tack också för all "pepp" under luncherna de sista veckorna av skrivande, **Daniel,** för din unika personlighet som jag uppskattar enormt och värdesätter högt. Tack också för orienteringsrundorna, Bajenmatcherna och för att du ser det vackra i att

vara manisk ibland, **Johan S** för din humor, energi och för att du förgyller allt häng i Stockholm, **Jens** för din värme, underfundighet och stora empati. Tillsammans är ni fyra ypperliga vänner!

Anders, för vår tid i Prag och för att du visade att man kan skapa nära vänner i ”vuxen” ålder, **Pawel** för ditt mentorskap och för att du delade allt lidande på Söderstadion med mig. Nu går vi mot nya tider! **Christian** för Bajenmatcher, middagar i Kransen, och för Allan-tiden. **Jonas** för att du sa ja till vår vänskap på KTH och för att du delat med dig av livet som doktorand.

Till sist vill jag tacka min älskade familj:

Farmor för att du fick mig att flytta till Stockholm och för allt stöd under pluggtiden och åren som doktorand. Jag är stolt över att just du är min farmor. **Nina** för alla roliga resor med familjen, till Nässjö och utomlands, och för din evigt postiva inställning. **Arthur,** för att du kom till oss så lägligt. Jag ser fram emot att lära känna dig mer och leka med dig tills jag storknar.

Andreas, för att du är världens bästa bror. Du har alltid skyddat mig och banat vägen. Jag har alltid sett upp till dig och är väldigt stolt över att vara din bror.

Mamma och **Pappa,** för allt stöd under mina studier och för att ni visat att kärlek och trygghet är viktigare än prestation. Jag vet att ni är stolta och jag hade aldrig nått hit utan er.

Signe, min skatt. Sen du kom har du skapat ett nytt ljus i mitt liv som jag aldrig kunnat föreställa mig. Den kärlek jag känner för dig är obeskrivlig och att varje dag få se glädjen och glöden i dina ögon är ovärderligt. Jag ser fram emot att följa dina äventyr genom livet.

Charlotte, min fru och allra bästa vän. Jag är oerhört lycklig över att få dela livet med Dig och är oerhört tacksam över det stöd Du gett mig. Du har dragit ett tungt lass hemma då jag skrivit avhandlingen, men snart är det över. Du är det bästa som jag vet och jag älskar Dig!

Tack också till alla stiftelser och fonder som gjort detta arbete möjligt:

Swedish Brain Power, Gun & Bertil Stohnes stiftelse, Margaretha af Ugglas stiftelsen, Stiftelsen för Gamla Tjänarinnor, Alzheimerfonden, Stiftelsen för ålderssjukdomar vid Karolinska Institutet, Anders Otto Swärds stiftelse, Insamlingsstiftelsen för Alzheimer och demensforskning (SADF), Knut och Alice Wallenberg stiftelsen, Stiftelsen Sigurd och Elsa Goljes Minne, Alzheimer association (USA), AFA Soria (Spanien), Demensförbundet, National Institute on Aging (USA) och Lundströms minne.

7 REFERENCES

1. Alzheimer's Disease International (ADI). World Alzheimer Report - The Global Impact of Dementia - An analysis of prevalence, incidence, cost and trends, 2015.
2. Selkoe DJ. The deposition of amyloid proteins in the aging mammalian brain: implications for Alzheimer's disease. *Ann Med* 1989; **21**(2): 73-6.
3. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *The Journal of biological chemistry* 1986; **261**(13): 6084-9.
4. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. *Neurobiology of aging* 2000; **21**(3): 383-421.
5. Dandrea MR, Reiser PA, Gumula NA, Hertzog BM, Andrade-Gordon P. Application of triple immunohistochemistry to characterize amyloid plaque-associated inflammation in brains with Alzheimer's disease. *Biotech Histochem* 2001; **76**(2): 97-106.
6. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002; **58**(12): 1791-800.
7. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 2006; **112**(4): 389-404.
8. Weller RO, Nicoll JA. Cerebral amyloid angiopathy: pathogenesis and effects on the ageing and Alzheimer brain. *Neurol Res* 2003; **25**(6): 611-6.
9. Teplow DB. Structural and kinetic features of amyloid beta-protein fibrillogenesis. *Amyloid* 1998; **5**(2): 121-42.
10. Murphy MP, LeVine H, 3rd. Alzheimer's disease and the amyloid-beta peptide. *Journal of Alzheimer's disease : JAD* 2010; **19**(1): 311-23.
11. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* 1994; **13**(1): 45-53.
12. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiological reviews* 2001; **81**(2): 741-66.
13. Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat* 2012; **33**(9): 1340-4.
14. Harkany T, Abraham I, Timmerman W, et al. beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. *Eur J Neurosci* 2000; **12**(8): 2735-45.
15. Walsh DM, Klyubin I, Fadeeva JV, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002; **416**(6880): 535-9.
16. Lustbader JW, Cirilli M, Lin C, et al. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 2004; **304**(5669): 448-52.
17. Verdier Y, Penke B. Binding sites of amyloid beta-peptide in cell plasma membrane and implications for Alzheimer's disease. *Curr Protein Pept Sci* 2004; **5**(1): 19-31.

18. Resende R, Moreira PI, Proenca T, et al. Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. *Free radical biology & medicine* 2008; **44**(12): 2051-7.

19. Akterin S, Cowburn RF, Miranda-Vizuete A, et al. Involvement of glutaredoxin-1 and thioredoxin-1 in beta-amyloid toxicity and Alzheimer's disease. *Cell death and differentiation* 2006; **13**(9): 1454-65.

20. Butterfield DA, Hensley K, Harris M, Mattson M, Carney J. beta-Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. *Biochem Biophys Res Commun* 1994; **200**(2): 710-5.

21. Harper JD, Lansbury PT, Jr. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem* 1997; **66**: 385-407.

22. Lee J, Culyba EK, Powers ET, Kelly JW. Amyloid-beta forms fibrils by nucleated conformational conversion of oligomers. *Nat Chem Biol* 2011; **7**(9): 602-9.

23. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992; **256**(5054): 184-5.

24. Shankar GM, Li S, Mehta TH, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 2008; **14**(8): 837-42.

25. Ahmed M, Davis J, Aucoin D, et al. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat Struct Mol Biol* 2010; **17**(5): 561-7.

26. Hartley DM, Walsh DM, Ye CP, et al. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 1999; **19**(20): 8876-84.

27. Xia D, Watanabe H, Wu B, et al. Presenilin-1 Knockin Mice Reveal Loss-of-Function Mechanism for Familial Alzheimer's Disease. *Neuron* 2015; **85**(5): 967-81.

28. Crystal H, Dickson D, Fuld P, et al. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology* 1988; **38**(11): 1682-7.

29. Kamenetz F, Tomita T, Hsieh H, et al. APP processing and synaptic function. *Neuron* 2003; **37**(6): 925-37.

30. Kontush A, Berndt C, Weber W, et al. Amyloid-beta is an antioxidant for lipoproteins in cerebrospinal fluid and plasma. *Free radical biology & medicine* 2001; **30**(1): 119-28.

31. Giuffrida ML, Caraci F, Pignataro B, et al. Beta-amyloid monomers are neuroprotective. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2009; **29**(34): 10582-7.

32. Soscia SJ, Kirby JE, Washicosky KJ, et al. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PloS one* 2010; **5**(3): e9505.

33. Lichtenthaler SF. alpha-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential. *Journal of neurochemistry* 2011; **116**(1): 10-21.

34. Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. *Mol Aspects Med* 2008; **29**(5): 258-89.

35. Hartmann D, de Strooper B, Serneels L, et al. The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Human molecular genetics* 2002; **11**(21): 2615-24.

36. Lammich S, Kojro E, Postina R, et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America* 1999; **96**(7): 3922-7.

37. Bandyopadhyay S, Goldstein LE, Lahiri DK, Rogers JT. Role of the APP non-amyloidogenic signaling pathway and targeting alpha-secretase as an alternative drug target for treatment of Alzheimer's disease. *Curr Med Chem* 2007; **14**(27): 2848-64.

38. Ludwig A, Hundhausen C, Lambert MH, et al. Metalloproteinase inhibitors for the disintegrin-like metalloproteinases ADAM10 and ADAM17 that differentially block constitutive and phorbol ester-inducible shedding of cell surface molecules. *Comb Chem High Throughput Screen* 2005; **8**(2): 161-71.

39. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci* 2007; **8**(9): 663-72.

40. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* 1989; **3**(4): 519-26.

41. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences of the United States of America* 1986; **83**(13): 4913-7.

42. Congdon EE, Duff KE. Is tau aggregation toxic or protective? *Journal of Alzheimer's disease : JAD* 2008; **14**(4): 453-7.

43. Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol* 2002; **103**(1): 26-35.

44. Farias G, Cornejo A, Jimenez J, Guzman L, Maccioni RB. Mechanisms of tau self-aggregation and neurotoxicity. *Curr Alzheimer Res* 2011; **8**(6): 608-14.

45. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA : the journal of the American Medical Association* 1997; **278**(16): 1349-56.

46. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261**(5123): 921-3.

47. Kivipelto M, Rovio S, Ngandu T, et al. Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: a population-based study. *Journal of cellular and molecular medicine* 2008; **12**(6B): 2762-71.

48. Maioli S, Puerta E, Merino-Serrais P, et al. Combination of apolipoprotein E4 and high carbohydrate diet reduces hippocampal BDNF and arc levels and impairs memory in young mice. *Journal of Alzheimer's disease : JAD* 2012; **32**(2): 341-55.

49. Rall SC, Jr., Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. *The Journal of biological chemistry* 1982; **257**(8): 4171-8.

50. Weisgraber KH, Rall SC, Jr., Mahley RW. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *The Journal of biological chemistry* 1981; **256**(17): 9077-83.

51. Mahley RW, Rall SC, Jr. Apolipoprotein E: far more than a lipid transport protein. *Annual review of genomics and human genetics* 2000; **1**: 507-37.

52. Elshourbagy NA, Liao WS, Mahley RW, Taylor JM. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proceedings of the National Academy of Sciences of the United States of America* 1985; **82**(1): 203-7.

53. Horsburgh K, Graham DI, Stewart J, Nicoll JA. Influence of apolipoprotein E genotype on neuronal damage and apoE immunoreactivity in human hippocampus following global ischemia. *Journal of neuropathology and experimental neurology* 1999; **58**(3): 227-34.

54. Williams KR, Saunders AM, Roses AD, Armati PJ. Uptake and internalization of exogenous apolipoprotein E3 by cultured human central nervous system neurons. *Neurobiology of disease* 1998; **5**(4): 271-9.

55. Ruiz J, Kouiavskaia D, Migliorini M, et al. The apoE isoform binding properties of the VLDL receptor reveal marked differences from LRP and the LDL receptor. *Journal of lipid research* 2005; **46**(8): 1721-31.

56. Wolf BB, Lopes MB, VandenBerg SR, Gonias SL. Characterization and immunohistochemical localization of alpha 2-macroglobulin receptor (low-density lipoprotein receptor-related protein) in human brain. *The American journal of pathology* 1992; **141**(1): 37-42.

57. LaDu MJ, Stine WB, Jr., Narita M, Getz GS, Reardon CA, Bu G. Self-assembly of HEK cell-secreted ApoE particles resembles ApoE enrichment of lipoproteins as a ligand for the LDL receptor-related protein. *Biochemistry* 2006; **45**(2): 381-90.

58. Morrow JA, Hatters DM, Lu B, et al. Apolipoprotein E4 forms a molten globule. A potential basis for its association with disease. *The Journal of biological chemistry* 2002; **277**(52): 50380-5.

59. Michaelson DM. APOE epsilon4: The most prevalent yet understudied risk factor for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2014; **10**(6): 861-8.

60. Ji Y, Gong Y, Gan W, Beach T, Holtzman DM, Wisniewski T. Apolipoprotein E isoform-specific regulation of dendritic spine morphology in apolipoprotein E transgenic mice and Alzheimer's disease patients. *Neuroscience* 2003; **122**(2): 305-15.

61. Rapp A, Gmeiner B, Huttinger M. Implication of apoE isoforms in cholesterol metabolism by primary rat hippocampal neurons and astrocytes. *Biochimie* 2006; **88**(5): 473-83.

62. Hamanaka H, Katoh-Fukui Y, Suzuki K, et al. Altered cholesterol metabolism in human apolipoprotein E4 knock-in mice. *Human molecular genetics* 2000; **9**(3): 353-61.

63. Li G, Bien-Ly N, Andrews-Zwilling Y, et al. GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. *Cell Stem Cell* 2009; **5**(6): 634-45.

64. Manning EN, Barnes J, Cash DM, et al. APOE epsilon4 is associated with disproportionate progressive hippocampal atrophy in AD. *PloS one* 2014; **9**(5): e97608.

65. Espeseth T, Westlye LT, Fjell AM, Walhovd KB, Rootwelt H, Reinvang I. Accelerated age-related cortical thinning in healthy carriers of apolipoprotein E epsilon 4. *Neurobiology of aging* 2008; **29**(3): 329-40.

66. Brecht WJ, Harris FM, Chang S, et al. Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2004; **24**(10): 2527-34.

67. Huang Y, Liu XQ, Wyss-Coray T, Brecht WJ, Sanan DA, Mahley RW. Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proceedings of the National Academy of Sciences of the United States of America* 2001; **98**(15): 8838-43.

68. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* 2009; **106**(16): 6820-5.

69. Castellano JM, Kim J, Stewart FR, et al. Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med* 2011; **3**(89): 89ra57.

70. Ramassamy C, Averill D, Beffert U, et al. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiology of disease* 2000; **7**(1): 23-37.

71. Shi L, Du X, Zhou H, et al. Cumulative effects of the ApoE genotype and gender on the synaptic proteome and oxidative stress in the mouse brain. *Int J Neuropsychopharmacol* 2014; **17**(11): 1863-79.

72. Lauderback CM, Kanski J, Hackett JM, Maeda N, Kindy MS, Butterfield DA. Apolipoprotein E modulates Alzheimer's Abeta(1-42)-induced oxidative damage to synaptosomes in an allele-specific manner. *Brain research* 2002; **924**(1): 90-7.

73. Cedazo-Minguez A, Huttinger M, Cowburn RF. Beta-VLDL protects against A beta(1-42) and apoE toxicity in human SH-SY5Y neuroblastoma cells. *Neuroreport* 2001; **12**(2): 201-6.

74. Ji ZS, Miranda RD, Newhouse YM, Weisgraber KH, Huang Y, Mahley RW. Apolipoprotein E4 potentiates amyloid beta peptide-induced lysosomal leakage and apoptosis in neuronal cells. *The Journal of biological chemistry* 2002; **277**(24): 21821-8.

75. Marques MA, Tolar M, Harmony JA, Crutcher KA. A thrombin cleavage fragment of apolipoprotein E exhibits isoform-specific neurotoxicity. *Neuroreport* 1996; **7**(15-17): 2529-32.

76. Tolar M, Keller JN, Chan S, Mattson MP, Marques MA, Crutcher KA. Truncated apolipoprotein E (ApoE) causes increased intracellular calcium and may mediate ApoE neurotoxicity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 1999; **19**(16): 7100-10.

77. Chang S, ran Ma T, Miranda RD, Balestra ME, Mahley RW, Huang Y. Lipid- and receptor-binding regions of apolipoprotein E4 fragments act in concert to cause mitochondrial dysfunction and neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* 2005; **102**(51): 18694-9.

78. Rogaeva E, Meng Y, Lee JH, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet* 2007; **39**(2): 168-77.

79. Yamazaki H, Bujo H, Kusunoki J, et al. Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. *The Journal of biological chemistry* 1996; **271**(40): 24761-8.

80. Jun G, Naj AC, Beecham GW, et al. Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. *Arch Neurol* 2010; **67**(12): 1473-84.

81. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013; **45**(12): 1452-8.

82. World Health Organization (WHO) and Alzheimer Disease International (ADI). Dementia: a public health priority, 2012.

83. van Duijn CM, Clayton D, Chandra V, et al. Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case-control studies. *Int J Epidemiol* 1991; **20 Suppl 2**: S13-20.

84. Mi W, van Wijk N, Cansev M, Sijben JW, Kamphuis PJ. Nutritional approaches in the risk reduction and management of Alzheimer's disease. *Nutrition* 2013; **29**(9): 1080-9.

85. Peters R, Poulter R, Warner J, Beckett N, Burch L, Bulpitt C. Smoking, dementia and cognitive decline in the elderly, a systematic review. *BMC Geriatr* 2008; **8**: 36.

86. Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. *Annals of neurology* 1993; **33**(3): 258-66.

87. Xu W, Qiu C, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Mid- and late-life diabetes in relation to the risk of dementia: a population-based twin study. *Diabetes* 2009; **58**(1): 71-7.

88. Qiu C, Winblad B, Fratiglioni L. The age-dependent relation of blood pressure to cognitive function and dementia. *Lancet Neurol* 2005; **4**(8): 487-99.

89. Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease: I. Review of epidemiological and preclinical studies. *Arch Neurol* 2011; **68**(10): 1239-44.

90. Raffaitin C, Gin H, Empana JP, et al. Metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: the Three-City Study. *Diabetes Care* 2009; **32**(1): 169-74.

91. Sindi S, Mangialasche F, Kivipelto M. Advances in the prevention of Alzheimer's Disease. *F1000Prime Rep* 2015; **7**: 50.

92. Tang MX, Jacobs D, Stern Y, et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* 1996; **348**(9025): 429-32.

93. Schonknecht P, Pantel J, Klinga K, et al. Reduced cerebrospinal fluid estradiol levels are associated with increased beta-amyloid levels in female patients with Alzheimer's disease. *Neuroscience letters* 2001; **307**(2): 122-4.

94. Amtul Z, Wang L, Westaway D, Rozmahel RF. Neuroprotective mechanism conferred by 17beta-estradiol on the biochemical basis of Alzheimer's disease. *Neuroscience* 2010; **169**(2): 781-6.

95. Yao M, Nguyen TV, Pike CJ. Estrogen regulates Bcl-w and Bim expression: role in protection against beta-amyloid peptide-induced neuronal death. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2007; **27**(6): 1422-33.

96. Long J, He P, Shen Y, Li R. New evidence of mitochondria dysfunction in the female Alzheimer's disease brain: deficiency of estrogen receptor-beta. *Journal of Alzheimer's disease : JAD* 2012; **30**(3): 545-58.

97. Olivieri G, Novakovic M, Savaskan E, et al. The effects of beta-estradiol on SHSY5Y neuroblastoma cells during heavy metal induced oxidative stress, neurotoxicity and beta-amyloid secretion. *Neuroscience* 2002; **113**(4): 849-55.

98. Nilsen J. Estradiol and neurodegenerative oxidative stress. *Front Neuroendocrinol* 2008; **29**(4): 463-75.

99. Chiueh C, Lee S, Andoh T, Murphy D. Induction of antioxidative and antiapoptotic thioredoxin supports neuroprotective hypothesis of estrogen. *Endocrine* 2003; **21**(1): 27-31.

100. Henderson VW. Alzheimer's disease: review of hormone therapy trials and implications for treatment and prevention after menopause. *J Steroid Biochem Mol Biol* 2014; **142**: 99-106.

101. Espeland MA, Brinton RD, Manson JE, et al. Postmenopausal hormone therapy, type 2 diabetes mellitus, and brain volumes. *Neurology* 2015; **85**(13): 1131-8.

102. Csordas A, Mazlo M, Gallyas F. Recovery versus death of "dark" (compacted) neurons in non-impaired parenchymal environment: light and electron microscopic observations. *Acta Neuropathol* 2003; **106**(1): 37-49.

103. Mohamad N, Gutierrez A, Nunez M, et al. Mitochondrial apoptotic pathways. *Biocell* 2005; **29**(2): 149-61.

104. Liu K, Ding L, Li Y, et al. Neuronal necrosis is regulated by a conserved chromatin-modifying cascade. *Proceedings of the National Academy of Sciences of the United States of America* 2014; **111**(38): 13960-5.

105. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci* 2007; **32**(1): 37-43.

106. Velez-Pardo C, Arroyave ST, Lopera F, Castano AD, Jimenez Del Rio M. Ultrastructure evidence of necrotic neural cell death in familial Alzheimer's disease brains bearing presenilin-1 E280A mutation. *Journal of Alzheimer's disease : JAD* 2001; **3**(4): 409-15.

107. Ankarcrona M, Dypbukt JM, Bonfoco E, et al. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* 1995; **15**(4): 961-73.

108. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007; **35**(4): 495-516.

109. Lucassen PJ, Chung WC, Kamphorst W, Swaab DF. DNA damage distribution in the human brain as shown by in situ end labeling; area-specific differences in aging and Alzheimer disease in the absence of apoptotic morphology. *Journal of neuropathology and experimental neurology* 1997; **56**(8): 887-900.

110. Sugaya K, Reeves M, McKinney M. Topographic associations between DNA fragmentation and Alzheimer's disease neuropathology in the hippocampus. *Neurochem Int* 1997; **31**(2): 275-81.

111. Stadelmann C, Deckwerth TL, Srinivasan A, et al. Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. *The American journal of pathology* 1999; **155**(5): 1459-66.

112. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *The American journal of pathology* 2004; **165**(2): 523-31.

113. Rohn TT, Head E, Nesse WH, Cotman CW, Cribbs DH. Activation of caspase-8 in the Alzheimer's disease brain. *Neurobiology of disease* 2001; **8**(6): 1006-16.

114. Zhu X, Raina AK, Perry G, Smith MA. Apoptosis in Alzheimer disease: a mathematical improbability. *Curr Alzheimer Res* 2006; **3**(4): 393-6.

115. Geske FJ, Lieberman R, Strange R, Gerschenson LE. Early stages of p53-induced apoptosis are reversible. *Cell death and differentiation* 2001; **8**(2): 182-91.

116. Bursch W. The autophagosomal-lysosomal compartment in programmed cell death. *Cell death and differentiation* 2001; **8**(6): 569-81.

117. Turk B, Turk V. Lysosomes as "suicide bags" in cell death: myth or reality? *The Journal of biological chemistry* 2009; **284**(33): 21783-7.

118. Bidere N, Lorenzo HK, Carmona S, et al. Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. *The Journal of biological chemistry* 2003; **278**(33): 31401-11.

119. Appelqvist H, Johansson AC, Linderoth E, et al. Lysosome-mediated apoptosis is associated with cathepsin D-specific processing of bid at Phe24, Trp48, and Phe183. *Annals of clinical and laboratory science* 2012; **42**(3): 231-42.

120. Gruninger-Leitch F, Berndt P, Langen H, Nelboeck P, Dobeli H. Identification of beta-secretase-like activity using a mass spectrometry-based assay system. *Nat Biotechnol* 2000; **18**(1): 66-70.

121. Hamazaki H. Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid protein. *FEBS letters* 1996; **396**(2-3): 139-42.

122. Malik M, Fenko MD, Sheikh AM, Wen G, Li X. A novel approach for characterization of cathepsin D protease and its effect on tau and beta-amyloid proteins. *Neurochemical research* 2011; **36**(5): 754-60.

123. Davidson Y, Gibbons L, Pritchard A, et al. Genetic associations between cathepsin D exon 2 C-->T polymorphism and Alzheimer's disease, and pathological correlations with genotype. *J Neurol Neurosurg Psychiatry* 2006; **77**(4): 515-7.

124. Haendeler J, Popp R, Goy C, Tischler V, Zeiher AM, Dimmeler S. Cathepsin D and H2O2 stimulate degradation of thioredoxin-1: implication for endothelial cell apoptosis. *The Journal of biological chemistry* 2005; **280**(52): 42945-51.

125. Tobiume K, Matsuzawa A, Takahashi T, et al. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO Rep* 2001; **2**(3): 222-8.

126. Khelifi AF, D'Alcontres MS, Salomoni P. Daxx is required for stress-induced cell death and JNK activation. *Cell death and differentiation* 2005; **12**(7): 724-33.

127. Kadowaki H, Nishitoh H, Urano F, et al. Amyloid beta induces neuronal cell death through ROS-mediated ASK1 activation. *Cell death and differentiation* 2005; **12**(1): 19-24.

128. Peel AL, Sorscher N, Kim JY, Galvan V, Chen S, Bredesen DE. Tau phosphorylation in Alzheimer's disease: potential involvement of an APP-MAP kinase complex. *Neuromolecular Med* 2004; **5**(3): 205-18.

129. Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *Journal of neuroscience research* 2002; **70**(3): 462-73.

130. Lukiw WJ. Gene expression profiling in fetal, aged, and Alzheimer hippocampus: a continuum of stress-related signaling. *Neurochemical research* 2004; **29**(6): 1287-97.

131. Sies H. Oxidative stress. London: Orlando : Academic Press; 1985.

132. Koopman WJ, Nijtmans LG, Dieteren CE, et al. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. *Antioxidants & redox signaling* 2010; **12**(12): 1431-70.

133. Pryor WA. Oxy-radicals and related species: their formation, lifetimes, and reactions. *Annu Rev Physiol* 1986; **48**: 657-67.

134. Lloyd RV, Hanna PM, Mason RP. The origin of the hydroxyl radical oxygen in the Fenton reaction. *Free radical biology & medicine* 1997; **22**(5): 885-8.

135. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 2000; **279**(6): L1005-28.

136. Kohchi C, Inagawa H, Nishizawa T, Soma G. ROS and innate immunity. *Anticancer Res* 2009; **29**(3): 817-21.

137. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009; **27**(2): 120-39.

138. Poulsen HE, Specht E, Broedbaek K, et al. RNA modifications by oxidation: a novel disease mechanism? *Free radical biology & medicine* 2012; **52**(8): 1353-61.

139. Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2005; **25**(40): 9171-5.

140. Go YM, Jones DP. Redox compartmentalization in eukaryotic cells. *Biochimica et biophysica acta* 2008; **1780**(11): 1273-90.

141. Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. *Biomarkers* 2005; **10 Suppl 1**: S10-23.

142. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free radical biology & medicine* 1991; **11**(1): 81-128.

143. Headlam HA, Davies MJ. Markers of protein oxidation: different oxidants give rise to variable yields of bound and released carbonyl products. *Free radical biology & medicine* 2004; **36**(9): 1175-84.

144. Hoshi T, Heinemann S. Regulation of cell function by methionine oxidation and reduction. *J Physiol* 2001; **531**(Pt 1): 1-11.

145. Reddie KG, Carroll KS. Expanding the functional diversity of proteins through cysteine oxidation. *Curr Opin Chem Biol* 2008; **12**(6): 746-54.

146. Alderson NL, Wang Y, Blatnik M, et al. S-(2-Succinyl)cysteine: a novel chemical modification of tissue proteins by a Krebs cycle intermediate. *Arch Biochem Biophys* 2006; **450**(1): 1-8.

147. Zeng J, Davies MJ. Evidence for the formation of adducts and S-(carboxymethyl)cysteine on reaction of alpha-dicarbonyl compounds with thiol groups on amino acids, peptides, and proteins. *Chem Res Toxicol* 2005; **18**(8): 1232-41.

148. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. *The Journal of biological chemistry* 1997; **272**(33): 20313-6.

149. Levi S, Rovida E. The role of iron in mitochondrial function. *Biochimica et biophysica acta* 2009; **1790**(7): 629-36.

150. Ricci C, Pastukh V, Leonard J, et al. Mitochondrial DNA damage triggers mitochondrial-superoxide generation and apoptosis. *American journal of physiology Cell physiology* 2008; **294**(2): C413-22.

151. Bouayed J, Bohn T. Exogenous antioxidants - Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxid Med Cell Longev* 2010; **3**(4): 228-37.

152. Meister A. Glutathione metabolism. *Methods in enzymology* 1995; **251**: 3-7.

153. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci* 2008; **4**(2): 89-96.

154. Edrey YH, Salmon AB. Revisiting an age-old question regarding oxidative stress. *Free radical biology & medicine* 2014; **71**: 368-78.

155. Moyse E, Arseneault M, Gaudreau P, Ferland G, Ramassamy C. Gender- and region-dependent changes of redox biomarkers in the brain of successfully aging LOU/C rats. *Mech Ageing Dev* 2015; **149**: 19-30.

156. Venkateshappa C, Harish G, Mahadevan A, Srinivas Bharath MM, Shankar SK. Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. *Neurochemical research* 2012; **37**(8): 1601-14.

157. Belanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 2011; **14**(6): 724-38.

158. Marszalek JR, Lodish HF. Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. *Annual review of cell and developmental biology* 2005; **21**: 633-57.

159. Bush AI. Metals and neuroscience. *Curr Opin Chem Biol* 2000; **4**(2): 184-91.

160. Marklund SL, Westman NG, Lundgren E, Roos G. Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer research* 1982; **42**(5): 1955-61.

161. Bradley MA, Xiong-Fister S, Markesbery WR, Lovell MA. Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression. *Neurobiology of aging* 2012; **33**(6): 1034-44.

162. Markesbery WR, Kryscio RJ, Lovell MA, Morrow JD. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. *Annals of neurology* 2005; **58**(5): 730-5.

163. Pratico D, V MYL, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 1998; **12**(15): 1777-83.

164. Lovell MA, Soman S, Bradley MA. Oxidatively modified nucleic acids in preclinical Alzheimer's disease (PCAD) brain. *Mech Ageing Dev* 2011; **132**(8-9): 443-8.

165. Hensley K, Hall N, Subramaniam R, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *Journal of neurochemistry* 1995; **65**(5): 2146-56.

166. Uehara T, Nakamura T, Yao D, et al. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 2006; **441**(7092): 513-7.

167. Montine TJ, Beal MF, Cudkowicz ME, et al. Increased CSF F2-isoprostane concentration in probable AD. *Neurology* 1999; **52**(3): 562-5.

168. Lovell MA, Gabbita SP, Markesbery WR. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. *Journal of neurochemistry* 1999; **72**(2): 771-6.

169. Ahmed N, Ahmed U, Thornalley PJ, Hager K, Fleischer G, Munch G. Protein glycation, oxidation and nitration adduct residues and free adducts of cerebrospinal fluid in Alzheimer's disease and link to cognitive impairment. *Journal of neurochemistry* 2005; **92**(2): 255-63.

170. Montine TJ, Quinn JF, Milatovic D, et al. Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. *Annals of neurology* 2002; **52**(2): 175-9.

171. Abe T, Tohgi H, Isobe C, Murata T, Sato C. Remarkable increase in the concentration of 8-hydroxyguanosine in cerebrospinal fluid from patients with Alzheimer's disease. *Journal of neuroscience research* 2002; **70**(3): 447-50.

172. Adams JD, Jr., Klaidman LK, Odunze IN, Shen HC, Miller CA. Alzheimer's and Parkinson's disease. Brain levels of glutathione, glutathione disulfide, and vitamin E. *Mol Chem Neuropathol* 1991; **14**(3): 213-26.

173. Gsell W, Conrad R, Hickethier M, et al. Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type. *Journal of neurochemistry* 1995; **64**(3): 1216-23.

174. Gabbita SP, Aksenov MY, Lovell MA, Markesbery WR. Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *Journal of neurochemistry* 1999; **73**(4): 1660-6.

175. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *Journal of neuropathology and experimental neurology* 2010; **69**(2): 155-67.

176. Marcus DL, Strafaci JA, Freedman ML. Differential neuronal expression of manganese superoxide dismutase in Alzheimer's disease. *Med Sci Monit* 2006; **12**(1): BR8-14.

177. Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. *Ann N Y Acad Sci* 2004; **1012**: 153-63.

178. Takuma K, Yao J, Huang J, et al. ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2005; **19**(6): 597-8.

179. Karuppagounder SS, Xu H, Shi Q, et al. Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model. *Neurobiology of aging* 2009; **30**(10): 1587-600.

180. Misonou H, Morishima-Kawashima M, Ihara Y. Oxidative stress induces intracellular accumulation of amyloid beta-protein (Abeta) in human neuroblastoma cells. *Biochemistry* 2000; **39**(23): 6951-9.

181. Paola D, Domenicotti C, Nitti M, et al. Oxidative stress induces increase in intracellular amyloid beta-protein production and selective activation of betaI and betaII PKCs in NT2 cells. *Biochem Biophys Res Commun* 2000; **268**(2): 642-6.

182. Nunomura A, Perry G, Aliev G, et al. Oxidative damage is the earliest event in Alzheimer disease. *Journal of neuropathology and experimental neurology* 2001; **60**(8): 759-67.

183. Laurent TC, Moore EC, Reichard P. Enzymatic Synthesis of Deoxyribonucleotides. Iv. Isolation and Characterization of Thioredoxin, the Hydrogen Donor from Escherichia Coli B. *The Journal of biological chemistry* 1964; **239**: 3436-44.

184. Tagaya Y, Maeda Y, Mitsui A, et al. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *The EMBO journal* 1989; **8**(3): 757-64.

185. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free radical biology & medicine* 2001; **31**(11): 1287-312.

186. Collet JF, Messens J. Structure, function, and mechanism of thioredoxin proteins. *Antioxidants & redox signaling* 2010; **13**(8): 1205-16.

187. Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *European journal of biochemistry / FEBS* 2000; **267**(20): 6102-9.

188. Matsui M, Oshima M, Oshima H, et al. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 1996; **178**(1): 179-85.

189. Schenk H, Klein M, Erdbrugger W, Droge W, Schulze-Osthoff K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappa B and AP-1. *Proceedings of the National Academy of Sciences of the United States of America* 1994; **91**(5): 1672-6.

190. Ueno M, Masutani H, Arai RJ, et al. Thioredoxin-dependent redox regulation of p53-mediated p21 activation. *The Journal of biological chemistry* 1999; **274**(50): 35809-15.

191. Bertini R, Howard OM, Dong HF, et al. Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. *J Exp Med* 1999; **189**(11): 1783-9.

192. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *The EMBO journal* 1998; **17**(9): 2596-606.

193. Mitchell DA, Morton SU, Fernhoff NB, Marletta MA. Thioredoxin is required for S-nitrosation of procaspase-3 and the inhibition of apoptosis in Jurkat cells. *Proceedings of the National Academy of Sciences of the United States of America* 2007; **104**(28): 11609-14.

194. Stadtman ER, Moskovitz J, Levine RL. Oxidation of methionine residues of proteins: biological consequences. *Antioxidants & redox signaling* 2003; **5**(5): 577-82.

195. Chae HZ, Kang SW, Rhee SG. Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. *Methods in enzymology* 1999; **300**: 219-26.

196. Lu J, Holmgren A. The thioredoxin antioxidant system. *Free radical biology & medicine* 2014; **66**: 75-87.

197. Yu Y, Xing K, Badamas R, Kuszynski CA, Wu H, Lou MF. Overexpression of thioredoxin-binding protein 2 increases oxidation sensitivity and apoptosis in human lens epithelial cells. *Free radical biology & medicine* 2013; **57**: 92-104.

198. Tonissen KF, Di Trapani G. Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy. *Mol Nutr Food Res* 2009; **53**(1): 87-103.

199. Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science* 1983; **221**(4617): 1256-64.

200. Cha MK, Suh KH, Kim IH. Overexpression of peroxiredoxin I and thioredoxin1 in human breast carcinoma. *J Exp Clin Cancer Res* 2009; **28**: 93.

201. Asahina M, Yamada T, Yoshiyama Y, Yodoi J. Expression of adult T cell leukemia-derived factor in human brain and peripheral nerve tissues. *Dementia and geriatric cognitive disorders* 1998; **9**(4): 181-5.

202. Lovell MA, Xie C, Gabbita SP, Markesbery WR. Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. *Free radical biology & medicine* 2000; **28**(3): 418-27.

203. Di Domenico F, Sultana R, Tiu GF, et al. Protein levels of heat shock proteins 27, 32, 60, 70, 90 and thioredoxin-1 in amnestic mild cognitive impairment: an investigation on the role of cellular stress response in the progression of Alzheimer disease. *Brain research* 2010; **1333**: 72-81.

204. Arodin L, Lamparter H, Karlsson H, et al. Alteration of thioredoxin and glutaredoxin in the progression of Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2014; **39**(4): 787-97.

205. Casanas-Sanchez V, Perez JA, Fabelo N, et al. Addition of docosahexaenoic acid, but not arachidonic acid, activates glutathione and thioredoxin antioxidant systems in murine hippocampal HT22 cells: potential implications in neuroprotection. *Journal of neurochemistry* 2014; **131**(4): 470-83.

206. Ju TC, Chen SD, Liu CC, Yang DI. Protective effects of S-nitrosoglutathione against amyloid beta-peptide neurotoxicity. *Free radical biology & medicine* 2005; **38**(7): 938-49.

207. Landino LM, Skreslet TE, Alston JA. Cysteine oxidation of tau and microtubule-associated protein-2 by peroxynitrite: modulation of microtubule assembly kinetics by the thioredoxin reductase system. *The Journal of biological chemistry* 2004; **279**(33): 35101-5.

208. Takagi Y, Tokime T, Nozaki K, Gon Y, Kikuchi H, Yodoi J. Redox control of neuronal damage during brain ischemia after middle cerebral artery occlusion in the rat: immunohistochemical and hybridization studies of thioredoxin. *J Cereb Blood Flow Metab* 1998; **18**(2): 206-14.

209. Hattori I, Takagi Y, Nakamura H, et al. Intravenous administration of thioredoxin decreases brain damage following transient focal cerebral ischemia in mice. *Antioxidants & redox signaling* 2004; **6**(1): 81-7.

210. Wang B, Tian S, Wang J, et al. Intraperitoneal administration of thioredoxin decreases brain damage from ischemic stroke. *Brain research* 2015; **1615**: 89-97.

211. Takagi Y, Mitsui A, Nishiyama A, et al. Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. *Proceedings of the National Academy of Sciences of the United States of America* 1999; **96**(7): 4131-6.

212. Pekkari K, Holmgren A. Truncated thioredoxin: physiological functions and mechanism. *Antioxidants & redox signaling* 2004; **6**(1): 53-61.

213. Silberstein DS, McDonough S, Minkoff MS, Balcewicz-Sablinska MK. Human eosinophil cytotoxicity-enhancing factor. Eosinophil-stimulating and dithiol reductase activities of biosynthetic (recombinant) species with COOH-terminal deletions. *The Journal of biological chemistry* 1993; **268**(12): 9138-42.

214. Balcewicz-Sablinska MK, Wollman EE, Gorti R, Silberstein DS. Human eosinophil cytotoxicity-enhancing factor. II. Multiple forms synthesized by U937 cells and their relationship to thioredoxin/adult T cell leukemia-derived factor. *Journal of immunology* 1991; **147**(7): 2170-4.

215. Dessein AJ, Lenzi HL, Bina JC, et al. Modulation of eosinophil cytotoxicity by blood mononuclear cells from healthy subjects and patients with chronic schistosomiasis mansoni. *Cell Immunol* 1984; **85**(1): 100-13.

216. Elsas PX, Elsas MI, Dessein AJ. Eosinophil cytotoxicity enhancing factor: purification, characterization and immunocytochemical localization on the monocyte surface. *Eur J Immunol* 1990; **20**(5): 1143-51.

217. Pekkari K, Gurunath R, Arner ES, Holmgren A. Truncated thioredoxin is a mitogenic cytokine for resting human peripheral blood mononuclear cells and is present in human plasma. *The Journal of biological chemistry* 2000; **275**(48): 37474-80.

218. Newman GW, Balcewicz-Sablinska MK, Guarnaccia JR, Remold HG, Silberstein DS. Opposing regulatory effects of thioredoxin and eosinophil cytotoxicity-enhancing factor on the development of human immunodeficiency virus 1. *J Exp Med* 1994; **180**(1): 359-63.

219. King BC, Nowakowska J, Karsten CM, Kohl J, Renstrom E, Blom AM. Truncated and full-length thioredoxin-1 have opposing activating and inhibitory properties for human complement with relevance to endothelial surfaces. *Journal of immunology* 2012; **188**(8): 4103-12.

220. Pekkari K, Avila-Carino J, Bengtsson A, Gurunath R, Scheynius A, Holmgren A. Truncated thioredoxin (Trx80) induces production of interleukin-12 and enhances CD14 expression in human monocytes. *Blood* 2001; **97**(10): 3184-90.

221. Pekkari K, Goodarzi MT, Scheynius A, Holmgren A, Avila-Carino J. Truncated thioredoxin (Trx80) induces differentiation of human CD14+ monocytes into a novel cell type (TAMs) via activation of the MAP kinases p38, ERK, and JNK. *Blood* 2005; **105**(4): 1598-605.

222. Cortes-Bratti X, Basseres E, Herrera-Rodriguez F, et al. Thioredoxin 80-activated-monocytes (TAMs) inhibit the replication of intracellular pathogens. *PloS one* 2011; **6**(2): e16960.

223. Mahmood DF, Abderrazak A, Couchie D, et al. Truncated thioredoxin (Trx-80) promotes pro-inflammatory macrophages of the M1 phenotype and enhances atherosclerosis. *J Cell Physiol* 2013; **228**(7): 1577-83.

224. Lemarechal H, Anract P, Beaudeux JL, Bonnefont-Rousselot D, Ekindjian OG, Borderie D. Expression and extracellular release of Trx80, the truncated form of thioredoxin, by TNF-alpha- and IL-1beta-stimulated human synoviocytes from patients with rheumatoid arthritis. *Clin Sci (Lond)* 2007; **113**(3): 149-55.

225. Schneider LS, Mangialasche F, Andreasen N, et al. Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. *J Intern Med* 2014; **275**(3): 251-83.

226. Panza F, Logroscino G, Imbimbo BP, Solfrizzi V. Is there still any hope for amyloid-based immunotherapy for Alzheimer's disease? *Curr Opin Psychiatry* 2014; **27**(2): 128-37.

227. Ngandu T, Lehtisalo J, Solomon A, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 2015; **385**(9984): 2255-63.

228. Persson T, Popescu BO, Cedazo-Minguez A. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? *Oxid Med Cell Longev* 2014; **2014**: 427318.

229. Arodin L, Miranda-Vizuete A, Swoboda P, Fernandes AP. Protective effects of the thioredoxin and glutaredoxin systems in dopamine-induced cell death. *Free radical biology & medicine* 2014; **73**: 328-36.

230. Holscher C. First clinical data of the neuroprotective effects of nasal insulin application in patients with Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2014; **10**(1 Suppl): S33-7.

231. Eriksdotter-Jonhagen M, Linderoth B, Lind G, et al. Encapsulated cell biodelivery of nerve growth factor to the Basal forebrain in patients with Alzheimer's disease. *Dementia and geriatric cognitive disorders* 2012; **33**(1): 18-28.

232. Ferreira D, Westman E, Eyjolfsdottir H, et al. Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. *Journal of Alzheimer's disease : JAD* 2015; **43**(3): 1059-72.

233. Bai J, Nakamura H, Kwon YW, et al. Critical roles of thioredoxin in nerve growth factor-mediated signal transduction and neurite outgrowth in PC12 cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2003; **23**(2): 503-9.

234. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov* 2011; **10**(3): 209-19.

235. Stern Y. Cognitive reserve. *Neuropsychologia* 2009; **47**(10): 2015-28.

236. Katzman R, Terry R, DeTeresa R, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Annals of neurology* 1988; **23**(2): 138-44.

237. Valenzuela MJ, Sachdev P. Brain reserve and dementia: a systematic review. *Psychol Med* 2006; **36**(4): 441-54.

238. Maloney B, Ge YW, Alley GM, Lahiri DK. Important differences between human and mouse APOE gene promoters: limitation of mouse APOE model in studying Alzheimer's disease. *Journal of neurochemistry* 2007; **103**(3): 1237-57.

239. Bertrand P, Poirier J, Oda T, Finch CE, Pasinetti GM. Association of apolipoprotein E genotype with brain levels of apolipoprotein E and apolipoprotein J (clusterin) in Alzheimer disease. *Brain Res Mol Brain Res* 1995; **33**(1): 174-8.

240. Reiter LT, Potocki L, Chien S, Gribskov M, Bier E. A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome Res* 2001; **11**(6): 1114-25.

241. Zheng B, Sage M, Cai WW, et al. Engineering a mouse balancer chromosome. *Nat Genet* 1999; **22**(4): 375-8.

242. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993; **118**(2): 401-15.

243. Duffy JB. GAL4 system in Drosophila: a fly geneticist's Swiss army knife. *Genesis* 2002; **34**(1-2): 1-15.

244. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**(1-2): 55-63.

245. Karunakaran S, Diwakar L, Saeed U, et al. Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by alpha-lipoic acid.

FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2007; **21**(9): 2226-36.

246. Song JJ, Lee YJ. Role of the ASK1-SEK1-JNK1-HIPK1 signal in Daxx trafficking and ASK1 oligomerization. *The Journal of biological chemistry* 2003; **278**(47): 47245-52.

247. Moir RD, Atwood CS, Romano DM, et al. Differential effects of apolipoprotein E isoforms on metal-induced aggregation of A beta using physiological concentrations. *Biochemistry* 1999; **38**(14): 4595-603.

248. Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM. Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 2007; **72**(5): 381-405.

249. DeKroon RM, Armati PJ. The endosomal trafficking of apolipoprotein E3 and E4 in cultured human brain neurons and astrocytes. *Neurobiology of disease* 2001; **8**(1): 78-89.

250. Tyler SJ, Dawbarn D, Wilcock GK, Allen SJ. alpha- and beta-secretase: profound changes in Alzheimer's disease. *Biochem Biophys Res Commun* 2002; **299**(3): 373-6.

251. Lappalainen Z, Lappalainen J, Oksala NK, et al. Diabetes impairs exercise training-associated thioredoxin response and glutathione status in rat brain. *J Appl Physiol (1985)* 2009; **106**(2): 461-7.