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Abbreviations

aa Amino acid
ABCA1 ATP-binding cassette transporter 1
AChEi Acetylcholinesterase inhibitor
AD Alzheimer’s disease
ADIT Alzheimer’s disease innovative drug target
AICD APP intracellular domain
AMPARs α-aminoadenosine-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPS Ammonium persulfate
ANOVA Analysis of variance
AP Alkaline phosphatase
APH-1 Anterior pharyx-defective 1
APLP1 APP-like proteins 1
APLP2 APP-like proteins 2
ApoE Apolipoprotein
APP Amyloid protein precursor
ATP Adenosine-5′-triphosphate
AVE Anterior visceral endoderm
Aβ beta-amyloid peptide
BACE-1 beta-site amyloid precursor protein cleaving enzyme 1
BBB Blood-brain-barrier
Bp Base pairs
BrdU Thymidine analog bromodeoxyuridine
BSA Bovine serum albumin
°C Degrees Celsius
C1P Ceramide-1-phosphate
CA1-2-3 Cornu Ammonis 1-2-3
CAA Cerebral amyloid angiopathy
CamKII Calmodulin-dependent protein kinase II
CamKII-α Calmodulin-dependent protein kinase II
cDNA Complementary DNA
CLU Clusterin
CNS Central nervous system
COX Cyclooxygenase
DAPI 4′,6-diamidino-2-phenylindole
DDT Dithiothreitol
DEPC Diethyl pyrocarbonate
DG Dentate gyrus
DKK1 Dickkopf-1
DMEM Dulbecco’s modified Eagle’s medium
DMSO Dimethyl sulfoxide
DS Down Syndrome
DTT Dithiothreitol
E Embryonic day
EDTA Diaminoethanetetra-acetic acid disodium salt
EGFP Enhanced green fluorescent protein
EOAD Early-onset Alzheimer’s disease
EU European Union
FBS Foetal bovine serum
FP6  | Framework program 6
FTDP-17 | Frontotemporal Dementia with Parkinsonism-17
G418 | Geneticin selective antibiotic
GABA<sub>γ</sub> | γ-aminobutyric acid
GFAP | Glial fibrillary acidic protein
GM-CSF | Granulocyte-macrophage colony-stimulating factor
GNDF | Glial Cell derived Neurotrophic Factor
G-PCR | G-protein coupled receptor
GSAP | γ-secretase activating proteins
GSK3-α | Glycogen synthase kinase 3 alpha
GSK3-β | Glycogen synthase kinase 3 beta
GWAS | Genome-wide associated studies
hCMV | Human cytomegalovirus
HD | Huntington’s disease
HTS | Hit-to Lead Phase
IHC | Immunohistochemistry
IL-1 | Interleukin 1
IL-6 | Interleukin 6
IL-8 | Interleukin 8
Kb | Kilo base
LB | Luria-Bertani
LIF | Leukaemia inhibitor factor
LOAD | Late-onset Alzheimer’s disease
LTP | Long term potentiation
mES | Mouse Embryonic stem cells
MHCII | Major histocompatibility complex class II
mM | Millimolar
mM | Millimolar
mRNA | Messenger ribonucleic acid
MWM | Morris water maze
NF-κβ | Nuclear factor kappa beta
NFTs | Neurofibrillary tangles
ng | Nanogram
NGS | Normal goat serum
NMDARs | N-methyl D-aspartate
NSAID | Nonsteroidal anti-inflammatory drugs
p | Probability value
PAGE | Polyacrylamide gel electrophoresis
PBS | Phosphate buffered saline
PBST | PBS with Tween-20
PCR | Polymerase chain reaction
PD | Parkinson’s disease
PFA | Paraformaldehyde
PGCs | Primordial germ cells
PHF | Paired helical filament
PICALM | Phosphatidylinositol binding clathrin assembly protein
PKA | Protein kinase A
PP-2A | Protein phosphatase 2
PS1 | Presenilin 1
<table>
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<td>Subiculum</td>
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Abstract

AD is thought to be caused by an abnormal production and aggregation of amyloid-beta (Aβ) peptide. Consequently, precluding the generation of Aβ has been considered as a strategy for AD treatment. Pharmacological compounds reducing Aβ formation have been developed and tested in preclinical and clinical trials and the often promising results obtained in preclinical trials have not been successful reproduced in clinical trials.

With this in mind in 2005 the European Framework Program 6 funded the ADIT project (Design of Small Molecule Therapeutics for the Treatment of Alzheimer Disease Based on the Discovery of Innovative Drug Targets) and this study has been part of this large collaborative study which involved eight institutions across Europe.

The main aim of the ADIT project was to identify new druggable targets for AD drug discovery. The project involved a screen for novel candidate AD target genes, performed by Siena Biotech and involved a number of other collaborating laboratories with roles in validating these targets. Validated targets were those genes: induced in response to Aβ treatment of cultured neurons and with demonstrable neurotoxic activity; induced in the brains of AD patients and in brains from an existing mouse model of AD; deemed most tractable as targets of small molecule inhibitors. Two targets, DKK-1 and S1P3, fulfilled these requirements.

Drug discovery relies on animal models and the aim of this thesis was to develop transgenic mice for the selected targets and to investigate their role in AD pathology. Animal models were generating by pronuclear injection.

Preliminary findings suggest that S1P3 may contribute to the inflammatory process seen in AD. Chronic neuroinflammation is a common characteristic of AD and it may be responsible of the neuronal loss seen in AD. GFAP immunohistochemistry on brains of the S1P3 mice revealed a strong astrocytotic process particularly evident in the hippocampus (mainly in the dentate gyrus) Upregulation of GFAP is commonly accompanied by astrocyte proliferation and activation which leads to the production of pro-inflammatory and cytotoxic cytokines, as well as toxic molecules. A large body of evidence suggests that by transforming from a basal to a reactive state, astrocytes neglect their neurosupportive functions, thus rendering neurons vulnerable to neurotoxins, including proinflammatory cytokines and reactive oxygen species. The S1P3 mouse model represents a model for acquiring more insights into mechanisms of Aβ-mediated toxicity in AD and a target for preventing astrocyte activation.

The characterization of the DKK-1 mouse model is still in its infancy, nevertheless preliminary analysis have already demonstrated that is up-regulation cause Glycogen synthase kinase-3β
(GSK3-β) activation which in turn hyperphosphorylate tau. It is known that hyperphosphorylation of tau is responsible for NFTs formation and DKK-1 inhibition might prevent this process.