Investigation of Expression of Alzheimer Disease Related Genes in Peripheral Blood and their Diagnostic Implications

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia in older adults. With the continuous expansion of the aging population, AD is becoming a major health challenge. However, peripheral markers that could predict risk of AD are lacking.

The aim of the present study is to evaluate the gene expression profiling of peripheral leukocytes in Chinese subjects with AD and explored its potential of clinical application. Behavioral phenotypes of cognitive performance and neuropsychiatric assessment were also investigated in association with gene expression in AD. Persons with mild cognitive impairment (MCI), as an at-risk state between normal aging and clinical dementia, was also assessed in consideration that the information may provide a better understanding of the mechanisms involved in clinical progression of AD.

The development of AD results from the complex interaction between genetic predisposition and environmental factors. Expression profiling (transcriptome) allows for reflections of acute/current cellular activities and body response in a summative way. Previous studies have reported differential gene expression in AD brain. The altered regulation involved in several processes includes apoptosis, inflammatory response, mitochondria function, and synaptic plasticity. However, it is impossible to obtain brain tissues in a clinical setting. Peripheral blood cells seem to be a potential surrogate tissue

with a reasonable correlation in gene expression with brain. Moreover, recent studies in AD and other psychiatric disorders revealed that some of the gene expression signature happening in brain could be found by analysis of peripheral blood cells. Also, expression profiling of blood has been found potentially clinical useful in various immune and neurological diseases, therefore we would like to explore the potential of expression profiles as a peripheral marker for AD, a disease with neurological and immunological interaction.

In the study, the profile of 12 target gene expression levels in peripheral blood cells were determined in 96 AD, 145 MCI and 167 normal controls (NC) by quantitative real-time RT-PCR. The genes were identified with (i) high expression in blood and brain; (ii) differential expression between AD and control; (iii) AD related candidate genes. Then, a list of genes were selected including CTSB, CTSD, DDT, ITPKB, NDUFA6, NRD1, PIN1, SNX2, TSC1, UQCRC1, CNR2, GSTM3. Seven genes were found to be differentially expressed between AD and NC group, with upregulation of CTSB, CTSD, DDT, TSC1 and UQCRC1, and downregulation of ITPKB and PIN1 in AD patients. Expression levels of two genes were increased in the MCI compared with NC group, including CTSB and CTSD. In addition, an upregulation of CTSD, UQCRC1, NRD1 and downregulation of ITPKB were observed in AD subjects in comparison to the MCI group (Mann-Whitney U test, $p<0.05$). After adjusting for confounding factors of age, gender, education level, ApoE4 status and the total CIRS score, expression level of any single gene was not associated with the classfication of AD or MCI

(Logistic regression, $p>0.05$). A five gene biomarker panel, including DDT, ITPKB, PIN1, TSC1 and UQCRC1 was identified with logistic regression analysis. The function of $\text{Logit}(P)= \ln(P/(1-P))= b_0+b_1\text{Ratio}_{\text{DDT}}+b_2\text{Ratio}_{\text{ITPKB}} + b_3\text{Ratio}_{\text{PIN1}} +b_4 \text{Ratio}_{\text{TSC1}}+b_5\text{Ratio}_{\text{UQCRC1}}$ were defined as the probability of a subject to be diagnosed as "AD" or "MCI" by using 5-gene biomarker panel. ROC analysis showed that the AUC for the 5-gene biomarkers panel in differentiating between AD and NC, between MCI and NC, between AD and MCI were 0.79 (95% CI, 0.72-0.86; $p<0.001$), 0.61 (95% CI, 0.53-0.69; $p=0.007$) and 0.68 (95% CI, 0.60-0.76; $p<0.001$) respectively. The 5-gene combination was found to discriminate AD subjects from normal controls with good sensitivity and specificity of 70.7% and 86.7% respectively at an optimal cut-off point of 0.486. Low sensitivity (42.4%) and acceptable specificity (76.2%) were observed at a cut-off threshold of 0.505 when differentiating MCI from NC subjects. Between AD and MCI subjects, gene combination showed a sensitivity of 61% and specificity of 73.7% at a cut-off value of 0.496.

Several genes including CTSD, DDT, NDUFA6, TSC1 and UQCRC1 were found in association with the cognitive and psychiatric symptoms, indicating the role of genetic factors in moderating the presence of cognitive and NP profiles in demented individuals.

The genes identified in this study were involved in processes implicated in neurodegeneration, including protein isomerization (PIN1), calcium disequilibrium and mitochondria insufficiency (ITPKB and UQCRC1),

increased inflammatory response (DDT), apoptosis (CTSB and CTSD) and neurogeneration (NRD1 and TSC1).

In conclusion, gene expression profiling in blood may have potential to be an adjuvant marker for early detection of AD. Expression marker panel is more informative than single gene expression signature. Further validation in prospective studies will substantiate its clinical application and explore its potential to differentiate AD from other dementias and to predict the progression from MCI to AD.

摘 要

阿爾茨海默氏痴呆症（Alzheimer's disease, AD）是老年痴呆中最常見的一種。隨着人口結构的老年化，AD已成為主要的保健和社會問題之一。

本課題旨在研究AD患者外週血基因表達的變化，探討其潛在的臨床價值。並探索基因表達與疾病定量表型如AD患者認知和精神行為症狀評分的關係。輕度認知功能障礙（Mild cognitive impairment, MCI）作為老年痴呆與正常老化之間的中間狀態，也被納入研究，以希望能夠更好地了解AD的發病機制。

環境和遺傳因素在AD的發病過程中起着重要的作用。基因表達譜水平是機体對外界環境和內在遺傳因素變化的綜合反映。過往研究發現AD患者腦部基因表達失調，主要涉及的生物學功能包括凋亡、炎症反應，綫粒体功能紊亂及突觸可朔性等。然而，活体提取腦組织在實際操作中不可行，因此選用外周血白細胞作為探針，通過研究其基因表達差異從而間接反應中樞神經系統細胞功能的變化可能是一條化繁為簡的思路。首先，外週血的基因表達譜與腦組织有高度相關性。其次，研究證實外週血基因表達能夠反映AD和其他精神疾病患者的腦基因表達譜變化。此外，外週血基因表達譜已經在一些神經及免疫疾病的臨床實踐中加以應用，因此，我們相信它也應該適用與AD，因為神經及免疫機制的相互作用是AD發病機制中重要的一環。

本研究采用以下生物信息學方法篩選候選基因：（1）在腦部和外週血均為高表達；（2）曾報道在AD有差異性表達；（3）曾報道與AD相關的候選基因。12個候選基因被選出，包括CTSB、CTSD、DDT、ITPKB、NDUFA6、NRD1、PIN1、SNX2、TSC1、UQCRC1、CNR2和 GSTM3。運用實時熒光定量RT-PCR方法對96例AD患者，145例MCI患者和167例正常老年人的外週血白細胞進行基因表達檢測。結果顯示，與正常老年人比較，AD患者的CTSB、CTSD、DDT、TSC1、UQCRC1表達上調，ITPKB和PIN1表達下調。MCI患者的CTSB和CTSD基因表達水平顯著升高。與MCI患者比較，AD患者的CTSD, UQCRC1和NRD1水平升高，但ITPKB水平下降 (Mann-Whitney U test, $p<0.05$)。控製混淆因素如年齡、性別、教育水平、其他軀体疾病評分、ApoE4攜帶狀態後，单个基因表達水平對AD或MCI的發病未見顯著作用（Logistic regression, $p>0.05$）。

本研究采用基與條件參數估計的逐步Logistic回歸后退法篩選得到5個對于AD發病具有顯著意義的基因—DDT、ITPKB、PIN1、TSC1和UQCRC1。該5個基因組成一個生物標記組合，以評價其對AD或MCI的診斷價值。函數$Logit(P)= \ln(P/(1-P))= b_0+b_1Ratio_{DDT}+b_2Ratio_{ITPKB}+ b_3Ratio_{PIN1}+b_4Ratio_{TSC1}+b_5Ratio_{UQCRC1}$被定義為應用該5-基因組合標記預測每個樣本患AD或MCI的概率。ROC分析顯示5-基因組合生物標記用于鉴别AD与NC，MCI与NC，AD与MCI之間的曲線下面积分别爲0.79（95% CI, 0.72-0.86; $p<0.001$）, 0.61（95% CI, 0.53-0.69; $p=0.007$）和 0.68（95% CI, 0.60-0.76; $p<0.001$）。在臨界值爲0.486時，基因組合生物標記鉴别AD与

NC的敏感度和特異度分別為70.7%和86.7%。臨界值爲0.505時，基因組合生物標記鑒別MCI与NC的敏感度和特異度分別為42.4%和76.2%。臨界值爲0.496時，基因組合生物標記鑒別AD与MCI的敏感度和特異度分別為61%和73.7%。

結果顯示CTSD、DDT、NDUFA6、TSC1和UQCRC1等基因表達水平与AD患者認知及精神行為症狀評分相關，提示基因在神經退化過程中出现的認知障礙及精神症狀中起一定的作用。

差異表達基因主要涉及多個生物學過程，包括蛋白異搆（PIN1），鈣失衡及綫粒體功能紊亂（ITPKB和UQCRC1），炎症反應（DDT），凋亡（CTSB和CTSD）及神經再生（NRD1和TSC1）。

本課題數據說明了外週血基因表達譜水平可以作為診斷AD的輔助標記物，並且基因標記物組合比單個基因標記更有生物學意義。進一步的前瞻性研究將有助與驗證其臨床應用價值，並探討基因表達標記是否有助於鑒別不同痴呆類型及預測MCI病人向AD的轉化。

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Role of Authors

The thesis represents a component of a prospective study to evaluate clinical and genetic risk factors for cognitive decline in a cohort of Chinese older persons with no clinical dementia in Hong Kong. The clinical team is responsible for subject recruitment, obtaining informed consent, cognitive and clinical assessment. I am responsible for the formulating the research idea for this thesis, literature review, preparation of protocol, laboratory assessment, data analysis and writing up of this thesis.

Publications

1. **Fu BY**, Ma SL, Tang NL, Tam CW, Lui VW, Chiu HF, Lam LC. Cholesterol 24-hydroxylase (CYP46A1) polymorphisms are associated with faster cognitive deterioration in Chinese older persons – a 2 year follow up study. *The International Journal of Geriatric Psychiatry*. 2009, 24(9):921-6.

2. Fan HP, Di Liao C, **Fu BY**, Lam LC, Tang NL. Interindividual and interethnic variation in genome wide gene expression: insights into the biological variation of gene expression and clinical implications. *Clin Chem*. 2009, 55(4):774-85.

3. **Fu Y**, SH Hu, LCW Lam. A selected review of recent biological psychiatric research in China. *East Asian Arch Psychiatry*. 2010, 20: 44-50.

4. Fu Y, He F, Tang NL, Tam CW, Lui VW, Chiu HF, Lam LC. NEDD9 gene polymorphism influences the risk of Alzheimer's disease and cognitive function in Chinese older persons. (Manuscript in preparation)

5. **Fu Y**, Ma SL, Tang NL, Tam CW, Lui VW, Chiu HF, Lam LC. Cyclooxygenase 2 (COX-2) gene polymorphism and the risk of mild cognitive impairment in a Southern Chinese community. 9th World Congress of Biological Psychiatry 2009.

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid β
ACE	Angiotensin
AchEI	Acetylcholinesterase
ACT	Antichymotrypsin
ADAS-Cog	Alzheimer's disease Assessment Scale-Cognitive Subscale
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
BDNF	Brain derived neurotrophic factor
BPSD	Behavioral and psychological symptoms of dementia
CBF	Cholinergic basal forebrain
CDR	Clinical dementia rating
CH25H	Cholesterol 25 hydroxylase
CHRNB2	nAChR β2 encoding gene
CNR1	Cannabinoid receptor 1
CNR2	Cannabinoid receptor 2
COX2	Cyclooxygenase 2
CRP	C reactive protein
CSF	Cerebrospinal fluid
CTF	C-terminal fragments
CTSB	Cathepsin B
CTSD	Cathepsin D
CVFT	Category verbal fluency test
DBM	Deformation based morphometry

DDT	D-dopachrome tautomerase
2-DGE	2-dimensional gel electrophoresis
DLB	Lewy body dementia
DSM-IV	Diagnostic and Statistical Manual of Mental Disorder, 4th edition
FAD	Familiar Alzheimer's disease
18FDG	18fluorodeoxyglucose
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
GSTs	Glutathione S-transferases
ICD-10	International Classification of Diseases, 10th revision
IL-6RC	Interleukia-6 receptor complex
IP3	Inositol 1,4,5-trisphosphate
IP4	Inositol 1,3,4,5-tetrakisphosphate
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B
LC	Liquid chromatography
LCM	Laser capture microdissection
LPO	Lipid peroxidation
MCI	Mild cognitive impairment
aMCI	Amnestic MCI
naMCI	Non-amnestic MCI
MIF	Migration inhibitory factor
MMSE	Mini-mental state examination
NC	Normal controls
NCL	Neuronal ceroid lipofuscinosis
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6
NFTs	Neurofibrillary tangles
NIA	National Institute on Aging

NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorder Association
NMDA	N-methyl-D-aspartate
NPI	Neuropsychiatric Inventory
NRD1	Nardilysin
NSAIDs	Non-steroidal anti-inflammatory drugs
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PET	Positron emission tomography
PHF	Paired helical filaments
PIB	Pittsburgh Compound B
PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PS-1	Presenilin 1
PS-2	Presenilin 2
P-tau	Phosphorylated tau
RBC	Red blood cells
RFLP	Restriction fragment length polymorphism
ROC	Receiver operating characteristics
ROS	Reactive oxygen species
RT	Room temperature
SAD	Sporadic Alzheimer's disease
SELDI	Surfaced enhanced laser desorption/ionization
SNX2	Sorting nexin 2
SPECT	Single photon emission computed tomography
TSC1	Tuberous sclerosis 1
TSC2	Tuberous sclerosis 2
T-tau	Total tau

UQCRC1	Ubiquinol-cytochrome-c reductase core protein1
VBM	Voxel-based volumetry
VD	Vascular dementia
VDBP	Vitamin D binding protein
VLDL	Very low-density lipoproteins

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Chapter 1 General introduction to Alzheimer's disease

Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder characterized by cognitive, behavioral and functional abnormalities. The disease was first described by a German psychiatrist Alois Alzheimer in 1906 and was named after him (Berchtold and Cotman, 1998). The first patient Auguste D developed progressive dementia, accompanied by neuropsychiatric symptoms at her fifties. Dr. Alois Alzheimer examined the postmortem brain of the patient and found cerebral atrophy and pathological hallmarks of senile plaques and neurofibrillary tangles. This observable brain pathology was thought to be associated with malfunctioning cognition and subsequently considered to be characteristics of AD (Tiraboschi et al., 2004, Burns et al., 2002).

AD is a complex neurodegenerative disorder with a wide heterogeneity of clinical symptoms. The neurobiological basis for the clinical heterogeneity remains uncertain, but available evidence supported that the pathogenesis of AD is multifactorial. The interaction of environmental and genetic factors is implicated in the initiation and progression of the disease. Clinical probable AD is diagnosed by using clinical, neuropsychological, and imaging methods. Biomarkers may help to improve the diagnostic sensitivity and specificity and to monitor the disease stage and therapeutic response. The following literature reviews may provide an overview of recent research progress in AD. Further examination and discussion concerning disease biomarkers will be presented in the following chapters.

1.1 Epidemiology

AD has become a major worldwide public health concern. With the steadily increasing of life expectancy and expansion of aging population, the number of person afflicted with AD will inevitably increase. It will bring about enormous medical and financial burden to the society.

To date, more than 35 million people worldwide were estimated to suffer from AD and it may be 115 million in 2050. The prevalence of AD increases rapidly with age and doubles every 5 years (Jorm et al., 1987). Age specific prevalence meta-analysis have suggested approximate rates under 1% for dementia in persons aged 60-65, rising to about 20% in persons aged above 85 (Jorm et al., 1987, Hofman et al., 1991, Ritchie et al., 1992). In China, it was estimated that 1.7% of people aged 60-65 and 10.2% of those over the age of 80 will be affected with AD (Tang Z, 2003). The prevalence rate of AD in Hong Kong rises from 1.6% to 14% in the age groups of 75-79 and 85-89 (Chiu et al., 1998).

Similar trends have also been found in the age specific incidence of dementia and AD. The incidence rate for "mild" AD in Europe rose from 2.5 per 1000 person-years in 65-69 years old to 46.1 in those aged 85-89 years compared with U.S. rates of 6.1 to 74.5 and East Asian rates of 0.7 to 39.7 cases per 1000 person-years (Jorm et al., 1987). In China, the incidence of dementia was 3.4‰ in the age group of 60-69 and 33.8‰ in elderly above 80, demonstrating that age is an important modulating factor for AD (Shen YC, 1994).

1.2 Clinical features and diagnosis

Progressive deterioration in memory and cognitive function is the core features of dementia syndrome. Besides, behavior and functional deficits are common concurrent manifestations during disease progression. As the pathological mechanism of AD still remains unclear, clinical features play an indispensable role in the diagnosis of AD. To date, the diagnosis of clinical probable AD is made with high accuracy by using clinical, neuropsychological, and imaging methods (McKhann et al., 1984).

1.2.1 Clinical symptoms

The onset of AD is insidious and early symptoms are often not recognized. When disease progressed, a patient with AD may present a progressive decline in cognitive, behavioral and motor functions.

1.2.1.1 Cognitive symptoms

Memory loss is usually the first cognitive symptoms recognized. In early stage, patients frequently complain of "forgetfulness". Typically, memory change is characterized by short-term memory loss but relative preservation of remote memory. Patients have difficulties in learning new information and remembering recent events. Cognitive dysfunction is detectable by neuropsychological tests such as impaired delay recall in episodic memory tests and reduced verbal fluency.

As forgetfulness increase, other signs of impairment in remaining cognitive domains become evident. Visuospatial disorientation is common. Language

disturbances include word-finding difficulty, naming impairment and diminished speech output. Patients have difficulties in completing complex tasks. Judgment and problem solving are also impaired. The overall cognitive functions deteriorated during moderate AD and patients presented a uniformly poor performance in comprehensive cognitive assessment. As dementia become more severe, only memory fragments and very limited cognitive functions remain. Affected patients failed to recall the names of spouse or children and kept simple social interaction.

1.2.1.2 Behavioral and psychological symptoms

Behavioral and psychological symptoms of dementia (BPSD) are common and significant symptoms of AD, contributing most to the burden of care and faster institutionalization. It is defined as symptoms of disturbed perception, thought content, mood or behavior that frequently occur in patients with dementia (IPA, 1998). BPSD are simply grouped into two symptom clusters: (1) Behavioral symptoms, usually identified on the basis of observation of the patient, including physical aggression, screaming, restlessness, agitation, wandering, culturally inappropriate behaviors, disinhibition. Agitation is a frequent complaint from the caregivers of demented elderly. Evidence showed that the subtypes of agitation were correlated with the degree of cognitive impairment (Cohen-Mansfield, 1996). Patients may also report sleep abnormalities such as nocturnal awakening, excessive daytime sleepiness and shorting of sleep duration. Other diurnal rhythm abnormalities and nocturnal confusion are also found. (2) Psychological symptoms, assessed through interviews with patients and relatives, including delusions, hallucinations, apathy, anxiety and depressive mood. Typical themes of delusions are theft, persecution, abandonment and infidelity. Visual hallucination and

abnormal perception are also common in moderate dementia. Apathy and depressive mood may occur in 40-50% of Subjects (Lyketsos et al., 2000, Wragg and Jeste, 1989).

Clusters of behavior and psychological symptoms may present concurrently, or some remit and recur. Affective symptoms are more likely to occur in the earlier stage, while agitated and psychotic features are frequent with moderate impaired. At more severe stage, however, these symptoms become less evident because of deteriorated physical and neurological condition (IPA, 1998).

1.2.1.3 Functional deficits

Functional deficits are another important presentation of AD, contributing to disability and poor quality of life. Cognitive function is an important determining factor for function performance. Other factors such as physical activity, social interaction and behavior disturbance may influence the rate of functional deterioration.

Functional impairment is partial in the early stage of AD. Patients are able to carry out basic daily activities and could live independently. With the deterioration of cognitive function, the ability of carrying out the instrument activities is affected and patients have difficulties in preparing meals, personal hygiene and need the care assistance. When disease progressed to severe stage, the basic self-maintenance abilities such as bathing, eating and toileting were impaired, and the patient becomes completely dependent.

1.2.2 Classifications of AD

AD was classified into two groups: familial AD (FAD) and sporadic AD (SAD). Familiar AD is an autosomal dorminant disorder with onset age before 65 years. A few causative mutations had been identified: Amyloid precursor protein (APP), presenilin 1 (PS-1) and presenilin 2 (PS-2) genes. However, FAD is rare with the prevalence below 0.1% (Harvey et al., 2003). Sporadic AD accounts for over 95% of cases. As a multigenetic inheritance disorder, SAD is likely to be resulted from an interaction of genetic and environmental factors.

1.2.3 Clinical diagnosis

There are three commonly used diagnostic criteria for AD: (1) the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorder Association (NINCDS-ADRDA) (McKhann et al., 1984), (2) the Diagnostic and Statistical Manual of Mental Disorder, 4^{th} edition (DSM-IV)(APA, 1994) and (3) the International Classification of Diseases, 10^{th} revision (ICD-10)(WHO, 1992). The NINCDS-ADRDA criteria are among the most adopted in the diagnosis of AD and have shown good reliability and validity (Blacker et al., 1994). According to this framework, clinical diagnosis is mostly represented by "probable AD" and "possible AD". A definitive diagnosis of AD requires postmortem histological examination, which is regarded as the gold standard. "Probable AD" is diagnosed when dementia is characterized by gradual onset and progression, having deficits in two or more cognitive domains, excluding other brain or systemic disorders that could cause dementia, and in the absence of disturbance in consciousness. A diagnosis of "Possible AD" is based on the

observation of clinical dementia symptoms but in the presence of other systemic or neuropsychiatric disorder which is capable of causing dementia but not thought to be the course of current dementia. According to NINCDS-ADRDA criteria, AD could not be diagnosed until the patients have apparent clinical manifestation, but treatment are often most effective in early stage of AD. From this perspective, additional disease markers would be of great value as diagnostic tools aiding in early detection of AD, as well as prediction of incipient Alzheimer's disease. Recent progress in using new biomarkers as diagnostic tools of AD will be discussed in the chapter 3.

1.3 Pathogenesis and hypotheses

AD is a complex disorder in which multiple pathogenic mechanisms may be involved in leading to a common phenotype. Genetic factors and programmed cell death were considered to the primary pathogenic events, because it appeared 30-40 years before the onset of disease. The presence of amyloid plaques, neurofibrillary tangles, synaptic and neuronal loss, is major hallmarks of AD. Neuroinflammattory reactions, oxidative stress response, alterations in calcium homeostasis, deficit of neurotrophic factors, mytochondria dysfunction and many other neurochemical changes are also involved in the pathogenesis of the disease (Cacabelos, 2007).

1.3.1 Amyloid plaques

One of the main histopathological features of AD is the deposition of Amyloid plaques in brain. The plaques were always found in frontal and temporal cortex, hippocampus and amygdale in typical AD. Amyloid β(Aβ) peptide is the primary

component of senile plaques and is generated from Amyloid precursor protein (APP).

APP is a single membrane-spanning protein ubiquitously expressed in many cell and tissue types including endothelia, glia and neurons of the brain. It has at least three major protein isoforms such as APP770, APP751 and APP695. APP695 was predominant in brain and achieved great attention in research on AD. As a G-protein-coupled receptor, APP mediated the downstream G-protein activation and inhibition and subsequently linked to pathways involved in neural activity and synaptic plasticity (Okamoto et al., 1995). Other roles of APP in cell adhesion (Breen et al., 1991), neuronal differentiation and long-term potentiation were also reported (Cacabelos, 2007). Studies showed that APP plays a crucial role in pathogenesis of AD and mutations in APP gene accounts for rare familiar AD (Chartier-Harlin et al., 1991, Neve et al., 2000).

APP is proteolypic processed by the action of enzymes α-,β- and γ-secretases and generate peptides with different properties. α-secretase cleaves the Aβ domain of APP at position 16/17 and leads to the production of soluble α APP and C-terminal fragments (CTF) C83. β-secretase acts at the extracellular site of APP and releases soluble βAPP as well as CTF C99. Both soluble α APP and βAPP do not participate in the plaque formation. CTF C99 was further cleaved by γ -secretase at position 40 or 42 to generate two peptides of 40 and 42 amino acid length, known as Aβ40 and Aβ42. Aβ40 is the main component of Aβ while Aβ42 only accounts for 5-15%, but its oligomeric form are more neurotoxic. Although the exact properties of Aβ still remains uncertain, the central role of Aβ42 in AD has

been established through extensive studies. Among the research, the deposition of Aβ42 in brain is an early and typical feature of all forms of AD (Younkin, 1998). Genetic study showed various FAD-associated mutations in APP, PS-1 and PS-2 genes lead to an increase in the production of $A\beta_{42}$ (Borchelt et al., 1996, Ishii et al., 1997). Recent data indicated that a reduction of the ratio of $A\beta_{42}$ / $A\beta_{40}$ correlates with the risk, onset and progression of AD (Fukuyama et al., 2000, Lewczuk et al., 2004). The aggregation of $A\beta_{42}$ showed neurotoxic properties and cause neuronal degeneration partly due to disruption of calcium homeostasis and oxidative stress damage (Varadarajan et al., 2000, Reddy et al., 2004). It may also initiate inflammatory reaction which contributes to neuronal death (Salomon et al., 1992, Yates et al., 2000). Based on this evidence, "Amyloid cascade hypothesis" was proposed by Hardy and Allsop in 1991, and it will be discussed in the following chapters.

1.3.2 Neurofibrillary tangles

Another pathological characteristic of AD is Neurofibrillary tangles (NFTs). NFTs are intracellular accumulations of abnormal paired helical filaments (PHF) with tau protein as the major component. Tangles are found throughout the AD brain especially entorhinal region, hippocampus, cortex and their density correlate with the degree of neuronal loss. NFTs are not unique for AD. They are also found in other neurodegenerative disorders such as Pick's disease, Parkinson's disease and progressive supranuclear palsy. The formation of NFTs is thought to be associated with a collapse of the microtubule network, disturbances of axoplasmic transports, synapse loss, neuritic atrophy, and neuronal death (Delacourte et al., 1999).

Tau is a microtubule associated protein encoded by MAPT gene located in chromosome 17. Tau interacts with tubulin and modulates the stability and assembly of microtubules in neuronal axons. Microtubules are important cytoskeleton components functioning in cell motility, transport, shape and mitosis. Six tau isoforms were generated by alternative splicing of exon 2, 3 and 10 and have different affinity to the microtubule binding domain. In AD brain, tau is hyperphosphorylated and does not bind microtubules. The free tau then associates with other cellular components to aggregate and form PHF. The accumulation of PHF eventually results in NFTs and subsequent synapse and neuronal loss (D'Souza and Schellenberg, 2005, Avila et al., 2004). In this case, hyperphosphorylated tau should be toxic, but the concept of tau cytotoxicity is increasingly being challenged by a number of evidence supporting for the involvement of tau in neuronal protection for oxidative damage (Nunomura et al., 1999b, Li et al., 2007).

1.3.3 Hypotheses of AD pathogenesis

Various hypotheses have been proposed to clarify the molecular pathogenesis of AD, of which the two most favored are "Amyloid cascade hypothesis" and "Tau and tangle hypothesis". Advocates of both hypotheses have argued about which lesion is priority to the other or whether either lesion is sufficient and necessary for dementia (Mudher and Lovestone, 2002). Although these two hypotheses offer a broad framework to explain AD pathogenesis, there are some puzzling observations indicating that these hypotheses are not complete. A collection of other related theories involved in the pathology of AD including "Oxidative stress hypothesis" and "neuroimmunological hypothesis" have been proposed as well.

1.3.3.1 Amyloid cascade hypothesis

Amyloid cascade hypothesis was first proposed in 1991(Hardy and Allsop, 1991). It centers on a dysregulation in APP processing occurs early in the disease, resulting in increased production of $A\beta_{42}$, which is the major component of amyloid plaques. The deposition of $A\beta_{42}$ triggers and induces all the subsequent pathology including tau aggregation, synapse dysfunction and neuronal loss.

There is a wealth of evidence to support the amyloid hypothesis. The most obvious is that AD causing mutations in APP, PS-1 and PS-2 genes predispose to amyloid deposition, either by increasing $A\beta_{42}$ production or enhancing $A\beta_{42}$ aggregation (Borchelt et al., 1996, Ishii et al., 1997). Furthermore, the facts that mutations in tau (FTDP-17) give rise to tangles formation but not plaques proved that tau pathology are likely to occur after amyloid pathology (Poorkaj et al., 1998, Hutton et al., 1998). Moreover, studies showed that Aβ could induce neuronal degeneration both in vitro and in vivo and its metabolism was involved in the pathogenic role of genetic variability at apoE locus (Sanan et al., 1994, LaFerla et al., 1996, Frautschy et al., 1998). Further, growing evidence indicated genetic variance in Aβ metabolism and clearance contributes to the risk of late-onset AD (Wood et al., 2007, Lahiri et al., 2005). Taken together, these findings supported the notion that Aβ aggregation is the primary event in AD, and the subsequent disease process result from an imbalance between Aβ production and Aβ clearance.

Although there is no alternative hypothesis explaining AD pathology with as much experimental support as the amyloid hypothesis, there are some observations that the hypothesis cannot fully explain. The severity of amyloid deposits in brain

did not correlate well with the degree of cognitive impairment (Arriagada et al., 1992). Moreover, transgenic mice undergo progressive amyloid deposition but do not show neuronal loss and neurofibrillary tangles formation as predicted by hypothesis (Hsiao et al., 1995, Irizarry et al., 1997). Furthermore, a recent clinical trial on $A\beta_{42}$ immunization revealed a positive effect on amyloid plaque clearance, but no effect on cognitive function and long-term survival (Holmes et al., 2008) . These findings seem to challenge the primary role of Aβ in the development of AD. At this moment, however, they still cannot provide compelling reasons to abandon the hypothesis, and more extensive studies are required to clarify the exact role of Aβ in the etiology of AD.

1.3.3.2 Tau and tangles hypothesis

Although the majority of researchers accepted amyloid cascade hypothesis, a small group advocated that neurofibrillary tangles and tau protein are the causative factor in AD. Tangles are comprised of hyperphosphorylated tau, which aggregate and lead to the formation of PHFs. Hyperphosphorylated tau could not bind the microtubules and subsequently result in the degradation of cytoskeleton, axon transport disturbance and cell death (Gray et al., 1987).

The hypothesis was mainly supported by the fact that the density of neurofibrillary tangles correlated more closely with the degree of cognitive impairment than amyloid deposition (Arriagada et al., 1992). Moreover, evidence showed that the process of tangle formation starts with increased phosphorylation and then aggregation of tau in those areas known to be critical for memory (Braak and Braak, 1998, Braak et al., 1994). Further, the defects in tau may cause impaired

axonal transport and synapse damage in animal models of tauopathy (Yoshiyama et al., 2007, Chee et al., 2006). Finally, the discovery of tau mutations (FTDP-17) has consistently demonstrated that tau hyperphosphorylation are sufficient to produce neurodegeneration without amyloid plaques (Hutton et al., 1998). The evidence that FTDP-17 induced tangles but not plaques formation as well as mutations in APP led to both tangle and plaques formation (Goate et al., 1991) have demonstrated that tau pathology occurs after amyloid deposition, which has been debated between advocates of "tau hypothesis" and "amyloid hypothesis" for many years.

Although diverse risk factors such as Aβ, reactive oxygen species (ROS), inflammatory cytokines, iron overload and mitochondria dysfunction are involved in the pathogenesis of AD, tau hyperphophylation was considered as the final common way of various harmful molecular and cellular processes that eventually leads to neuronal degeneration (George Perry, 2009). The exact mechanism in regulating tau phosphorylation still remains elusive. Recent observation indicated that innate immune system and inflammation played an important role in triggering pathological phosphorylation (Orellana et al., 2005, Rojo et al., 2008). Tau pathology and cytoskeleton dysfunction converge as the final common result of diverse danger signals pathway to induce neurodegeneration as well as cognitive impairment.

Another concern about the neurotoxic role of tau pathology, based on the recent experiment data that challenged the notion of primary toxicity, was raised: (1) NFTs are encountered in viable neurons until late stage disease; (2) neurons with NFTs contain normal content and structure of microtubules; (3) profound NFTs

exist in cognitively intact elderly sometimes; (4) a number of evidence supported that phosphorylated tau is not toxic, but function as a response to oxidative stress damage and promote neuronal survival (Castellani et al., 2008, Morsch et al., 1999, Nunomura et al., 1999b). In order to clarify whether tau aggregation represent a harmful or physiological process, more study to explore aggregation process and further use of aggregation agonists and inhibitors will be useful.

Although the details of tau hypothesis still lack and skepticism about the hypothesis exists, the importance of tau pathology in AD has been fully demonstrated and received increasing interests. Based on ongoing research, the tau hypothesis together with other underlying hypothesis will be improved better to uncover the detail mechanism of AD etiology.

1.3.3.3 Oxidative stress hypothesis

Oxidative stress is defined as the imbalance between the production and removal of reactive oxygen species (ROS). In normal condition, ROS production is tightly controlled by antioxidants as well as antioxidant enzymes (Yu, 1994). When ROS levels exceed the antioxidative capacity of a cell, oxidative stress occur causing molecular damage, promoting cell adaptation and leading to the loss of cell biological function (Su et al., 2008).

Oxidative stress has been well documented to be an early event in AD and play an important role in initiating the disease through several cell signal pathways (Su et al., 2008). Mitochondria dysfunction is one of the major sources of oxidative stress. In AD, damaged mitochondria and deficiency in key enzymes responsible for

oxidative metabolism have been observed (Castellani et al., 2002, Gibson et al., 1998, Chandrasekaran et al., 1994). AD neurons had a significant higher percentage of the completely damaged mitochondria compared with elderly control (Hirai et al., 2001). Some sporadic mutations in mitochondria DNA were identified and unique to AD (Coskun et al., 2004). Moreover, ApoE4 fragment was reported to show toxicity to mitochondria function in AD brain (Harris et al., 2003, Chang et al., 2005).

Other sources of oxidative stress including Aβ, metal abnormity, and astrocytes/microglia have also been implicated (Su et al., 2008). It is note worthy that some studies found Aβ exerted its toxicity by impairing mitochondria function or by generating different ROS (Reddy et al., 2004, Varadarajan et al., 2000, Behl et al., 1994), but others argued that amyloid plaques and NFTs formation may function as neuronal adaptations to chronic oxidative stress (De Kloet, 2004, Nunomura et al., 1999a). Based on these findings, it was hypothesized that oxidative stress, as an upstream pathphysiological process in AD, had dual effect, not only causing the subsequent cell degeneration and death but also provoking compensatory response to protect cells against ROS damage. The dynamic balance between oxidative damage and compensatory response may result in the pathological presentation of AD.

1.3.3.4 Inflammatory hypothesis

It has been widely accepted that inflammatory plays an important role in the pathogenesis of AD. Amyloid plaques were surrounded with a wide variety of inflammatory related proteins and clusters of activated microglia and astrocytes

(Yates et al., 2000, Smits et al., 2002). Neuropathological studies showed increased expression of cyclooxygenase-2 (COX2) as well as microglia and astroglial activation in different stage of AD pathology (Hoozemans et al., 2006, Webster et al., 2006). Recent positron emission tomography (PET) studies indicated that amyloid deposition and microglia activation can be detected in half of the subjects with MCI (Okello et al., 2009). Cytokines such as IL-1, IL-6 and α2-macroglobulin were elevated in AD brain, suggesting a persistent inflammatory response (Bauer et al., 1992, Wood et al., 1993). Moreover, genetic studies showed that certain polymorphisms of the inflammatory cytokines such as IL-1, IL-6 and TNF- α were risk factors for AD (Paradowski et al., 2008, Deniz-Naranjo et al., 2008, Laws et al., 2005, Ma et al., 2005). Further, the inflammatory hypothesis was supported by the epidemiological studies that showing long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces the risk of AD (Simmons et al., 2004, de Craen et al., 2005).

In AD brain, not only astrocytes and microglial, but also neurons could produce inflammatory mediators including chemokines, cytokines and prostaglandins (Smits et al., 2002, Kempermann and Neumann, 2003). Inflammatory mediators influence neuronal and synaptic function via diverse mechanisms, including regulation of neurotransmission, neurotransmitters receptors, and synaptic efficacy. Besides their direct cytotoxic effects, inflammatory cytokines could induce the synthesis of NO and hydrogen peroxide which increase neuronal sensitivity to oxidative stress (Bal-Price and Brown, 2001, Brown and Bal-Price, 2003). Moreover, cytokines such as TNF- α and IL-1 β could induce the generation and aggregation of Aβ (Rogers et al., 1999, Sheng et al., 2003). The presence of Aβ

can in turn provoke the secreting of various cytokines (Salomon et al., 1992, Yates et al., 2000). The interaction between cytokines and Aβ form a vicious cycle, further exaggerating chronic inflammation and neuronal toxicity.

Despite a wealth of evidence have suggested that up-regulated inflammatory response can be responsible for synapse dysfunction and neuronal degeneration, others argued that the role of inflammation in AD is not simple as previously assumed but is a mixture of both detrimental and beneficial. The end result of inflammatory response depends on the profile of the secreted inflammatory and anti-inflammatory cytokines and other factors of the immediate environment (Vaca and Wendt, 1992, Dhandapani et al., 2003, Smits et al., 2001). Although the exact role of inflammation in the pathology of AD and its mechanisms in terms of the cells including microglia and astrocytes and inflammatory mediators are still not clear, it has been widely accepted that inflammatory response can be an important component for the pathogenesis and progression of the disease.

1.4 Risk factors

1.4.1 Environmental risk factors

Age is the most significant risk factor for AD. The prevalence of AD increases rapidly with age and doubles every 5 years (Jorm et al., 1987).

A higher prevalence of AD was observed in woman than in man across different ethnics (Gao et al., 1998). Apart from confounding factors of life spans and educational attainments, the drop in estrogen levels after menopause may

explain the female preponderance in AD, because estrogen is a putative protective factor against oxidative injury and Aβ induced toxicity (Fitzpatrick et al., 2002).

Education is considered to be a protective factor for cognitive decline (Chibnall and Eastwood, 1998, Shadlen et al., 2006). It was hypothesized that individuals with high levels of education may have more synapse reserve and offer higher resilience towards development of clinical symptoms in face of AD and tau pathology (Roe et al., 2007).

Growing evidence showed that physical activity may reduce the risk of dementia and is positively correlated with cognitive function among older people (Yaffe et al., 2001, Heyn et al., 2004). The protective effect of physical activity might be a result of the reduced vascular risk and obesity, improvement of overall health status and enhanced positive behavior (Barnes et al., 2007).

Physical health is found to significantly influence the development of AD. Epidemiology studies have identified positive associations between hypertension, hypercholesterolaemia, stroke and an increased risk of AD. Type 2 Diabetes is related to insulin resistance, which is involved in the formation of Aβ and inflammatory response in brain and subsequently increase the risk of AD (Craft, 2006, Sun and Alkon, 2006). Infection has also been reported to increase the risk of dementia among the elderly (Dunn et al., 2005). The meta-analysis have shown that a history of depression is a risk factor for AD in later life (Ownby et al., 2006, Jorm, 2001).

Besides, a history of head trauma, exposure to toxins and metals and high intake of saturated fat were identified as risk factors for AD as well (Solfrizzi et al., 2006, Lye and Shores, 2000, Otsuka et al., 2002).

1.4.2 Genetic risk factors

Several causative mutations in APP, PS-1 and PS-2 genes have been identified in familiar early-onset AD. In sporadic late-onset AD, however, only Apolipoprotein E (ApoE) has been widely accepted as genetic risk factor.

APP gene is the first gene found to be associated with inherited susceptibility to AD. It is located on chromosome 21 and encodes amyloid precursor protein, which is metabolized by β- and ɣ -secretase to produce $A\beta_{42}$. The missense mutations at codon 670/671 (Swedish mutation) (Mullan et al., 1992), codon 692 (Flemish mutation) (Hendriks et al., 1992), codon 716 (Eckman et al., 1997) and 717 (Chartier-Harlin et al., 1991) of the APP gene has been reported to be pathogenic. Most of the pathogenic mutations close to α - , β- or ɣ -secretase sites, were reported to influence the processing of APP and lead to an increased $A\beta_{42}$ production (Mullan et al., 1992).

The amyloid cascade hypothesis is also supported by the discoveries that AD related mutations in PS-1 and PS-2 genes enhance the production of $A\beta_{42}$ (Lee et al., 1997) The PS-1 gene on chromosome 14 encodes an integral membrane protein of 467 amino acids. The function of presenilin proteins is still unknown. More than 160 mutations in the presenilin genes have been identified in families with autosomal-dominant AD. Mutations in PS-1 accounted for most of familiar AD

(Raux et al., 2005). Mutations in PS-2 gene also give rise to AD. The PS-2 gene on chromosome 1 encodes a transmembrane protein of 448 amino acids. Several missense mutations in PS-2 gene have also been found in family with early-onset AD (Bird et al., 1988, Rogaev et al., 1995). Multiple mechanisms have been involved in the etiological effect of mutations in preselinin genes such as inducing the production of $A\beta_{42}$ (Lee et al., 1997) , provoking apoptosis and down-regulating the important signal pathway of unfolded protein response (Katayama et al., 1999).

The only well replicated genetic risk factor for sporadic late-onset AD is the apolipoprotein E gene (ApoE) polymorphisms. ApoE gene is located on chromosome 19 and has three alleles (E2, E3 and E4) with E4 allele being a risk factor and E2 allele being protective. Compared with subjects with no E4 allele, the risk of AD is 2-3 fold increasing in those with one E4 allele and about 12 fold increasing in person with two E4 alleles (Roses, 1996). The ApoE4 allele is also associated with an earlier onset age of AD (Asada et al., 1996, Craft et al., 1998). The differential effects of ApoE isoforms on the production and clearance of Aβ has contributed to the underlying mechanism of this increased risk. In addition, other potential mechanisms such as differential modulation of tau phophorylation, synaptic plasticity and neuroinflammation have been also implicated (Kim et al., 2009). Various lines of evidence suggested that ApoE plays an important role in neuronal degeneration process. ApoE was found to be involved in the aggregation and clearance of Aβ *in vitro* (Sanan et al., 1994, Strittmatter et al., 1993). Human studies demonstrated a positive correlation between amyloid deposition and ApoE4 allele dose (Rebeck et al., 1993, Schmechel et al., 1993, Nathan et al., 1994). ApoE is important for the maintenance of synaptic plasticity and function. The inhibitory

of ApoE4 on neurite outgrowth was secondary to its deleterious effect on cytoskeleton (Nathan et al., 1994). In addition, inflammatory response is also modulated by ApoE, and ApoE4 isoform has more robust pro-inflammatory activity than ApoE3 (Guo et al., 2004). Besides the indirect potential harmful effects, ApoE4 may exert direct neurotoxicity. The 29 kDa carboxyl-terminal-truncated fragment of apoE4 has been shown to induce cytoskeletal disruptions and mitochondrial dysfunction (Mahley et al., 2006, Mahley et al., 2007).

Although ApoE4 was proved to increase the risk of AD, it is not sufficient and necessary for the development of AD. In the past twenty years, more than 500 genes have been reported as potential risk factors for AD by candidate gene approach (Papassotiropoulos et al., 2002b, Reiman et al., 2007, Kawamata and Shimohama, 2002, Miller et al., 2007, Dickerson et al., 2005), but none has been consistently demonstrated to influence disease risk (Yamauchi K, 1999, Saunders et al., 1993). Recent literature based on genome-wide association (Locascio et al., 2008, Dickerson et al., 2005, Guerreiro et al., 2010) and meta-analysis (Di Bona et al., 2008, Dickerson et al., 2005) suggested more than 20 genes not related to apoE4 allele may be associated with significant risk effect. Some examples included angiotensin I converting enzyme1 (ACE), cholesterol 25-hydroxylase (CH25H), nAChR β2 encoding gene (CHRNB2), GRB2 associated binding protein 2.

1.5 Management

The present pharmacological treatment of AD would not prevent progression of the disease, but has beneficial effects on clinical symptoms. Acetylcholinesterase inhibitors (AChEI) are used for the treatment of mild to moderate dementia in clinic.

AChEI enhance the action of acetylcholine, thus offering symptomatic improvements to cognitive symptoms (Doody et al., 2001, Krishnan et al., 2003). Another approved drug named memantine is a low affinity noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist. Memantine improves symptoms of AD patients with moderate to severe impairment (Danysz et al., 2000, Rohrbacher et al., 1994, Orgogozo et al., 2002). Epidemiology studies demonstrated a beneficial effect of long-term use of NSAIDs in the prevention of AD (McGeer et al., 1996, in t' Veld et al., 2001, Hughes et al., 1982)). However, clinical trials produced negative results (Hughes et al., 1982, Group et al., 2007).

Psychotropic medication may be used for management of distressing neuropsychiatric and behavioral symptoms including psychosis, depression, impulsivity and agitation.

Recent progress in AD therapeutics also focuses on modulation of Aβ formation and aggregation, as well as accelerating its clearance (Citron, 2004, Siemers et al., 2007). Immunotherapy targeting the amyloid peptide is also a novel approach to disease treatment. Both active immunisation with N-terminal Aβ fragments and passive immunisation with anti-Aβ monoclonal and polyclonal antibodies are now in phase II trials (Rafii and Aisen, 2009).

Besides pharmacological management of AD, non-pharmacological options are important adjuvants to the management of this complex disorder.

Chapter 2 The concept of Mild cognitive impairment

Mild cognitive impairment (MCI) is an at-risk state between normal aging and clinical dementia. Persons with MCI have some degree of cognitive decline greater than expected for an individual's age and education level but function independently (Gauthier et al., 2006). The amnestic subtype of MCI (aMCI) with memory deficits was consistently shown to increase the risk of AD (Petersen et al., 1999). Over the last ten years, a growing number of studies of MCI focused on epidemiology, clinical features, neuropathology, predictive biomarkers and treatment.

2.1 Definition

In 1999, a group of experts put forward a diagnostic criteria outline for MCI (Petersen et al., 1999). It focused on MCI as a prodromal stage of AD represented by the impairment of memory domain. In 2003, the revised criteria for MCI were proposed (Petersen, 2004).

2.2 Epidemiology

The prevalence of MCI varied greatly, ranging from 3% to 24% due to different criteria among studies (Unverzagt et al., 2001, Ganguli et al., 2004, Manly et al., 2008, Plassman et al., 2008). More than half of MCI patients were shown to progress to dementia within five years, with the progression rate of 10%-15% per year compared with the rate of 1%-2% in cognitive intact controls (Petersen et al., 2001, Tierney et al., 1996, Petersen et al., 1999). In China, a recent epidemiology

study has reported that the prevalence rate of MCI for the population aged 60 years and older was 8.9% with a higher prevalence in rural areas (15.8%) (Tang Z et al., 2007).

2.3 Clinical features and diagnostic algorithm

Persons with MCI may present with a variety of symptoms. "Forgetfulness" is the frequent complaint from the patients or their relatives. Other cognitive domains including language, executive function and visuospatial skills may also been affected in some subjects. The prevalence of neuropsychiatric features in MCI intermediate between that of normal aging and dementia (Geda et al., 2004, Hwang et al., 2004).

An algorithm based on Peterson's criteria (Petersen, 2004) may be used to identify the subtypes of MCI (Petersen and Negash, 2008). Through taking history and careful neuropsychological assessment, the clinician needs to determine that the patient is neither normal aging nor dementia. If the memory is impaired for age and education, which could be identified by the office memory tests such as a delay recall of list learning scoring, the condition of amnestic MCI (aMCI) could be considered. aMCI as a prodromal stage of AD have attracted a lot of research interests. If memory domain is not affected and the subjects have impairment of other cognitive domains, this is regarded as non-amnestic MCI (naMCI). Once the subtype of MCI is determined, the subsequent question is to decide whether single domain or multiple domains of cognitive functions were impaired. The diagnosis of single domain aMCI or naMCI is assumed when only single domain such as memory or one of other domains including language, executive function, or

visuospatial skills was involved. Similarly, multiple domain aMCI or naMCI refers to impairments in at least two cognitive domains. The non-amnestic subtypes with presumed degenerative etiology have a higher likelihood of progression to non-AD dementia, such as frontotemporal dementia and dementia with Lewy bodies (Petersen and Negash, 2008).

2.4 Neuropathology

Studies have shown that early AD pathology is underway in persons with MCI. Neurofibrillary pathology and amyloid deposition were identified in the medial temporal lobe and neocortex (Markesbery et al., 2006, Bennett et al., 2005). Most subjects, however, did not have sufficient neuritic plaque pathology to meet the neuropathological criteria of AD (Jicha et al., 2006). Another study has also demonstrated that MCI patients appeared to have intermediate pathology between that of normal aging and very early AD, in spite of the contribution of both degenerative and vascular factors (Bennett et al., 2005). Moreover, recent neuroimaging studies have observed progressive loss of gray matter as MCI converted to AD as well (Karas et al., 2004).

2.5 Factors modulating progressive cognitive decline in MCI

MCI is a heterogeneous syndrome that runs variable trajectories. Some persons with MCI may improve; others may progress to clinical dementia. Hence, looking for markers capable of predicting disease course is a recent focus in MCI research. ApoE4 allele is reported to be associated with MCI transition to dementia (Cosentino et al., 2008, Mosconi et al., 2004), but the results are not consistent. The severity of cognitive impairment is associated with disease progression (Visser et

al., 1999). The presence of behavioral and psychological symptoms, including depression was a variable predictor of progression to dementia (Copeland et al., 2003). Additionally, a number of neurchemical and neuroimaging biomarkers were found having predictive value in MCI patients. Low levels of $A\beta_{42}$ and high levels of tau protein in cerebrospinal fluid (CSF) may predict a higher probability of progression to AD (Riemenschneider et al., 2002, Hampel et al., 2004, Andreasen et al., 2003). Hippocampal atrophy identified by MRI are also able to predict progression to AD with about 80% accuracy (Wang et al., 2006, Jack et al., 1999). Further, the role of novel neuroimaging methods such as 18FDG-PET and PIB in tracking the course of MCI are under active investigation (Drzezga et al., 2005, Jack et al., 2008).

2.6 Early intervention

Several clinical trials have been undertaken to test whether using drugs for AD could also delay the progression of MCI to clinical dementia (Petersen et al., 2005, Feldman et al., 2007, Thal et al., 2005). From a large randomized controlled clinical trial involved donepezil and vitamin E in a 769 subjects with aMCI, donepezil group was demonstrated to reduce the risk of developing AD for the first 12 months. However, over the 36 months of study, there was no significant difference in the probability of progression to AD among the donepezil, vitamine E and placebo group (Petersen et al., 2005, Doody et al., 2009). Cumulatively, there is no standard pharmacological treatment for MCI. On the other hand, non-pharmacological management such as frequent participation in cognitively stimulating activities, less dietary fat consumption and active social interaction were recommended to protect against cognitive decline and lower the risk of AD (Gauthier et al., 2006).

Chapter 3 Biomarkers for AD and MCI

3.1 Introduction

Current diagnosis of AD depends on the combination of clinical, neuropsychological, and imaging assessments. The definitive diagnosis still requires postmortem pathological examination. The identification of biomarkers allows for more accurate and earlier diagnosis. Additionally, biomarkers facilitate differential diagnosis of AD from other degenerative disorders. More importantly, biomarkers may be of value in tracking disease progression and evaluating therapeutic response.

The current consensus criteria of biomarkers for AD were proposed by National Institute on Aging (NIA) in 1998. According to the criteria, an ideal biomarker 1) should detect the fundamental CNS pathophysiology of AD and be validated in neuropathologically confirmed cases, 2) should have a diagnostic sensitivity >85% for detecting AD and a specificity >75% for distinguishing between other dementias, 3) should detect any beneficial effects of disease modifying therapy, 4) should be reliable, reproducible, non-invasive, simple to perform, and inexpensive, and 5) be confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals (Frank et al., 2003).

Although a variety of biomarker candidates for AD have been identified, there has been no single biomarker that completely fulfills the NIA criteria. There are

limitations to the consensus of an ideal situation. Current diagnosis of AD are based on clinical assessment, control group are likely to include some people with pathological but not clinical AD. Postmortem validation of diagnosis, however, is not practical. Lack of standard protocols including clinical assessment, sampling process and statistical analysis also contributes to the methodological variability. On the other hand, AD is a complex disorder with a diversity of neuropathological and etiological factors. Moreover, the severity of AD pathological lesions does not reflect the severity of clinical symptoms or the rate of disease progression (Craig-Schapiro et al., 2009, Song et al., 2009). In spite of these limitations, a large number of advances have been achieved in identification of potential biomarkers for AD, and its at risk state of MCI. In this chapter, three main approaches of biomarkers research in AD including neurochemical testing, genetic testing and neuroimaging measurements were reviewed and their potential use in clinical practice is discussed.

3.2 Cerebrospinal fluid and blood biomarkers

Cerebrospinal fluid (CSF) is a good resource for research into neurodegenerative disorders, but its clinical application is limited by the complex and invasive nature of the procedure. Blood contacts with every cell in the body and is capable of reflecting physical and pathological changes of every organ including the central nervous system. Blood is thought to be another suitable source of AD biomarkers as well. Until now, a large number of potential biomarkers for AD have been studied in CSF and blood.

3.2.1 Amyloid precursor protein

Amyloid precursor protein (APP) is sequential cleaved by β- and γ -secretase to produce Aβ peptide and soluble β-sAPP. If APP processing is abnormal in AD brain, it may lead to the changing of the level of sAPP, which may serve as a diagnostic biomarker. Results of studies measuring CSF total sAPP, α -sAPP and β-sAPP levels in AD compared to healthy control and MCI patients are contradictory, ranging from a decrease (Van Nostrand et al., 1992, Henriksson et al., 1991), no significant change (Chong et al., 1990) to an increase (Ghiso et al., 1989, Weidemann et al., 1989). Taken together, these inconsistent results do not support sAPP as a useful biomarker for AD.

3.2.2 $A\beta_{40}$ and $A\beta_{42}$

Amyloid plaques are one of pathological hallmarks of AD. $A\beta_{42}$, as the major component of plaques, as well as other Aβ species are widely studied to be potential biomarkers of AD.

3.2.2.1 Aβ in CSF

Early studies on total Aβ in CSF showed that it is not well correlated with the diagnosis of AD (Lannfelt et al., 1995, Southwick et al., 1996). Recent studies more focus on two major species: $A\beta_{42}$ and $A\beta_{40}$. The majority of studies demonstrated a decreased level of $A\beta_{42}$ in CSF of AD patients (Andreasen et al., 1999, Andreasen et al., 2001, Clark et al., 2003, Engelborghs et al., 2008, Lewczuk et al., 2004, Blennow et al., 2001). The specificity to distinguish patients with AD from controls has varied from 42% to 88%, with the sensitivity varying from 72% to 100%. A few

studies report increased (Jensen et al., 1999, Csernansky et al., 2002) or no change (Csernansky et al., 2002, Fukuyama et al., 2000). It has also been reported that CSF $A\beta_{42}$ levels were lower in AD patients with ApoE4 allele. The sensitivity of $A\beta_{42}$ measurement in AD was 83.6% for ApoE4 carriers but 54.2% in E4 non carriers (Tapiola et al., 2000).

In addition, decreased CSF $A\beta_{42}$ was also reported to predict conversion to AD in MCI patients (Andreasen et al., 2003, Hampel et al., 2004, Hansson et al., 2006). Moreover, the CSF concentration of $A\beta_{42}$ was observed to decrease from mild to more severe dementia (Jensen et al., 1999, Riemenschneider et al., 2002). These evidences suggested that CSF $A\beta_{42}$ may be useful in tracking disease course. However, decreased CSF $A\beta_{42}$ level are not specific for AD. Normal and decreased levels of $A\beta_{42}$ were reported in Parkinson's disease (Motter et al., 1995, Ida et al., 1996). Half of the patients with vascular dementia (VaD) had decreased CSF $A\beta_{42}$ (Hulstaert et al., 1999). In lewy body dementia (DLB) and frontotemporal dementia (FTD), low levels of $A\beta_{42}$ proteins have also been detected (Kanemaru et al., 2000, Sjogren et al., 2000). Therefore, CSF $A\beta_{42}$ appears to be not a good biomarker for differentiation diagnosis of AD.

Extensive studies in animal and human have shown an inverse relationship between CSF $A\beta_{42}$ and the amount of plaques in brain (DeMattos et al., 2002, Strozyk et al., 2003). This hypothesis was further supported by the recent findings that increased plaque load with positive Pittsburgh Compound B (PIB) signal in PET is associated with lower $A\beta_{42}$ values (Fagan et al., 2006, Fagan et al., 2007).

However, contradictory studies also found (Kanemaru et al., 2000, Sjogren et al., 2002).

In a recent study, the ratio of $A\beta_{42}$/ $A\beta_{40}$ is significantly decreased in MCI patients converting to AD, compared to cognitive stable MCI patients and those developing other dementia (Hansson et al., 2007). The evidence points to the usefulness of the $A\beta_{42}$/ $A\beta_{40}$ ratio as a predictive biomarker for AD.

3.2.2.2 Plasma Aβ level

Aβ peptide is generated in the CNS and transported to peripheral vascular system through blood-brain barrier (Zlokovic, 2004) and also secreted by platelets in blood (Chen et al., 1995). Levels of Aβ in plasma were influenced by a number of factors such as AD-related pathology, age, cerebrovascular disease, liver catabolism and renal excretion (Lopez et al., 2008). It has been reported that Aβ levels in plasma are 100 fold lower than CSF (Scheuner et al., 1996). The early cross-sectional studies demonstrated that plasma $A\beta_{42}$ and $A\beta_{40}$ levels are similar in AD patients and controls (Fukumoto et al., 2003, Kosaka et al., 1997, Tamaoka et al., 1996), indicating plasma Aβ levels may not be a potential diagnostic marker.

Recent longitudinal studies, however, suggested that plasma $A\beta_{42}$ seems to be a potential marker for disease progression. Mayeux et al reported that baseline plasma $A\beta_{42}$ levels were significantly higher in those who developed AD than those who did not (Mayeux et al., 1999, Pomara et al., 2005). Besides, low plasma levels of $A\beta_{42}$ and $A\beta_{40}$ were reported to predict rapid cognitive decline (Locascio et al., 2008). However, the findings are still inconsistent (Lopez et al., 2008, Hansson et

al., 2008).

In summary, decreased CSF $A\beta_{42}$ levels appears to be a potential biomarker in distinguishing AD from non-demented controls, but is limited by difficulty in differentiating from other dementias. The ratio of $A\beta_{42}/A\beta_{40}$ in CSF has been suggested to be superior to each alone in facilitating AD diagnosis. Declining plasma $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ levels may be related to disease progression, but the research reports are conflicting. In comparison with CSF, blood is easy to obtain and more applicable for routine practice, however, the levels of blood composition are affected by various systemic changes and it is still not clear to what extent changes in plasma Aβ levels could reflect the pathological alteration in the brain.

3.2.3 Tau proteins

Another pathological hallmark of AD is the presence of neurofibrillay tangles. They consisted of PHF, derived from hyperphosphorylated microtubule associated protein tau. At least 30 phosphorylation sites exit on tau protein. In AD brain, tau is abnormally hyperphosphorylated and loses the function of binding to microtubules, causing cytoskeletal dysfunction, axonal atrophy and synapse loss. Total tau (T-tau) and phosphorylated tau (P-tau) are released and can be measured in the CSF.

3.2.3.1 Total Tau

T-tau has been widely studied as a diagnostic tool in AD (Blennow and Hampel, 2003, Blennow, 2004, Galasko, 1998). The most consistent finding is that significant increase in CSF t-tau protein in AD. The specificity varied from 65% to 86%, with the sensitivity varying from 40% to 86% (Blennow et al., 2001). Age

was related with t-tau, therefore, the age effect should be considered (Sjogren et al., 2001b).

Elevated t-tau in CSF are also found in other dementias such as VaD, FTD and DLB (Sjogren et al., 2001b, Kanemaru et al., 2000, Blennow and Hampel, 2003). CSF t-tau was reported to distinguish AD from other dementia with a sensitivity of 81% but a specificity of only 57%, indicating that it may not be a marker for the distinguishing AD from other dementias (Parnetti et al., 2000).

An increase of T-tau has also been suggested to predict developing AD in MCI patients. A recent study has shown that 88% of MCI patients had elevated CSF T-tau and/or low CSF $A\beta_{42}$ levels at baseline (Andreasen et al., 2001). Another follow up study has found that high baseline T-tau concentration could discriminate MCI subjects who developed AD from those who did not with 90% sensitivity and 100% specificity (Arai et al., 1997). CSF T-tau remained elevated for up to two years in mild to moderate AD.

3.2.3.2 Phosphorylated Tau

Although there is no doubt that tau phosphorylation differs in AD, there is no consensus whether there exist any phosphorylation sites that are unique for AD. Several ELISA methods have been developed to specifically detect phosphorylation of tau at different epitopes, such as the threonine 181+231, threonine 181, threonine 231+serine 235, serine 199, threonine 231, and serine 396+404 (Blennow, 2004). By using all of these different methods, a moderate to marked increase in CSF P-tau has been observed with the mean sensitivity to discriminate AD from non-demented

controls is 81%, at a specificity level of 91% (Blennow, 2004). As P-tau is more specifically in reflecting the phosphorylation state of tau and subsequent tangles formation in AD, it may be more appropriate to differentiate AD from other dementias (Buerger et al., 2002b, Sjogren et al., 2001a, Parnetti et al., 2001, Buerger et al., 2003, Blennow, 2004).

P-tau may be useful to predict cognitive decline in MCI. A longitudinal study showed the CSF P-tau levels were higher in persons with MCI than those in healthy controls and high P-tau was correlated with the conversion to clinical AD (Buerger et al., 2002a).

In conclusion, T-tau, as a marker reflecting neuronal degeneration and damage, is not specific for AD. P-tau protein, however, is superior to T-tau in distinguishing AD because P-tau is more specific in reflecting AD pathology.

3.2.4 Isoprostanes

Oxidative stress has been considered as an important event in the pathogenesis of AD. Lipid peroxidation (LPO) is one of manifestations in oxidative damage. Isoprostanes, as end-products of LPO, has been studied in AD. Isoprostanes is generated from peroxidation of arachidonic acid (Morrow and Roberts, 1997), circulate in plasma and excreted in urine (Morrow et al., 1992). Several studies have found an increased F2-isoprostanes in the frontal and temporal cortex of AD compared to control and FTD brains, suggesting a specificity for AD (Pratico et al., 1998, Yao et al., 2003b). Other studies have demonstrated the CSF F2-isoprostanes levels are elevated in autopsy-verified as well as living individuals diagnosed with

AD (Grossman et al., 2005, Montine et al., 1999a, Pratico et al., 2000). The baseline increased F2-isoprostanes levels in CSF were reported to distinguish subjects who developed AD or MCI with 100% accuracy over one and two years follow-up (De Leon et al., 1997, Quinn et al., 2004). CSF F2-isoprostanes has been shown to correlate with brain weight, degree of cortical atrophy, as well as dementia severity (Pratico et al., 2000). In addition to sporadic AD, elevated levels of F2-isoprostanes could also be found in preclinical FAD mutation carriers (Ringman et al., 2008), early Huntington's disease, stroke and meningoencephalitis (Montine et al., 2004, Montine et al., 1999b).

Results have also been more inconsistent with others showing no significant change (Feillet-Coudray et al., 1999, Irizarry et al., 2007, Montine et al., 2000, Bohnstedt et al., 2003) in AD. The discrepancy may be partly due to different selection criteria among studies, because several systemic factors such as smoking or other oxidative stress related conditions may affect the peripheral levels of F2-isoprostanes (Craig-Schapiro et al., 2009).

F2-isoprostanes seems to be a useful biomarker in AD, not only in CSF, but also in plasma and urine. Additional studies are needed to confirm these results in larger cohorts of MCI and AD patients.

3.2.5 Inflammatory markers

Inflammatory response is an important component for the pathogenesis and progression of the disease. Antichymotrypsin (ACT), one of the serine proteinase inhibitors, is colocalized with Aβ in senile plaques and plays a pivotal role in

inflammation (Abraham et al., 1988). Studies showed that ACT level were increased in the brain of AD patients (Abraham et al., 1988) . In terms of CSF and serum ACT levels, however, the results are controversial (Brugge et al., 1992, Hinds et al., 1994, Harigaya et al., 1995, Matsubara et al., 1990) (Furby et al., 1991, Lanzrein et al., 1998, Pirttila et al., 1994). More recent studies with large sample size and more homogeneous study subjects, however, have demonstrated an increase ACT levels in AD (Licastro et al., 2000) (DeKosky et al., 2003, Engelhart et al., 2004, Nielsen et al., 2007). These evidence points to the significance of using ACT as a screening marker in AD. Elevated levels of serum or CSF ACT are also found in other dementia such as VaD and DLB (Engelhart et al., 2004, Nielsen et al., 2007), suggesting it may be not an effective instrument to facilitate differential diagnosis of AD.

Other inflammatory related molecules such as cytokines, the soluble Interleukia-6 receptor complex (IL-6RC), C-reactive protein (CRP) and C1Q have also been investigated in AD. Nevertheless the results are highly inconsistent. The discrepancy was found in the studies of candidate molecules including IL6, IL-6RC, CRP, C1Q, IL-4, IL-8 and IL-10 (Blum-Degen et al., 1995, Jia et al., 2005).

In summary, the results in inflammatory mediators are inconsistent, but they have advanced our understanding the role of inflammation in the pathogenesis of AD.

3.2.6 Homocysteine

Homocysteine is derived from methionine and could be recycled into methionine or converted to cysteine. Folate and vitamin B12 are needed for the conversion of homocysteine to methionine (Blennow et al., 1995), and vitamin B6 is required for the conversion to cysteine (Leboeuf, 2003). Deficiency of folate, vitamin B12 or vitamin B6 may lead to high homocysteine levels (Kabir et al., 1994), a risk factor of developing cardiovascular disease, cognitive impairment, AD and bone weakness (Sachdev, 2005). It has been reported that plasma homocysteine levels greater than 14 umol/L almost doubled the risk of AD (Seshadri et al., 2002). In another study, higher levels of homocysteine were associated with cognitive decline in older subjects (Morris et al., 2001). But this positive result was denied by a large community study with 702 elderly subjects (Kalmijn et al., 1999). Moreover, a recent MRI study suggested that increased homocysteine increase the risk of cerebrovascular disease independent of AD (Miller et al., 2002). This may lead to a question of whether elevated homocysteine protein increases the risk of AD directly or through cerebrovascular disease.

Homocysteine was also related with cognitive deterioration in MCI patients. A three-year follow up study showed that subjects who converted from MCI to AD had higher baseline plasma homocysteine levels than non-converters (Gabryelewicz et al., 2007); A recent longitudinal study have shown that cognitive intact controls who developed AD had a baseline higher plasma homocysteine levels than those who developed MCI or those remaining stable (Blasko et al., 2008).

Homocysteine seems to be a strong and independent risk factor for cardiovascular disease, stroke, cognitive impairment and AD. Elevated levels of homocysteine have become an important consideration in risk assessment of AD.

3.2.7 Markers for cholesterol and lipoprotein metabolism

Cholesterol metabolism pathway is involved in the pathogenesis of AD. It has been hypothesized that enhanced removal of cholesterol occurs in AD brain. Researches correlating serum cholesterol with AD have found contradictory results. A longitudinal study showed that high serum cholesterol at midlife is a risk factor for AD (Notkola et al., 1998). Another 21 year follow-up study also found that high midlife cholesterol appears to be a risk factor for more severe cognitive impairment in later life (Solomon et al., 2007). However, one population-based study failed to find the relationship between cholesterol and AD (Romas et al., 1999). In addition, the oxysterols in CSF and serum could be measured as reflection of brain elimination of cholesterol. 24S-Hydroxycholesterol is one of oxysterols investigated in AD. Most studies showed that 24S-Hydroxycholesterol was not an ideal biomarker for AD (Papassotiropoulos et al., 2002b).

ApoE is another candidate biomarker for AD. It is a major component of very low-density lipoproteins (VLDL), and plays an important role in cholesterol transport. The levels of ApoE in CSF and serum have been compared between AD patients and healthy controls. However, the results are conflicting with some studies showing enhancement while others reporting reduction of ApoE levels (Lindh et al., 1997, Yamazaki et al., 1990, Hesse et al., 2000). Moreover, the alteration of ApoE levels is independent of ApoE4 allele. Taken together, the levels of ApoE in CSF

and serum seem not meeting the criteria for a useful AD biomarkers.

3.2.8 Proteomics

Proteomics technology provided a high throughout approach to search for novel biomarkers in neurodegenerative disorders. It allows for studying the whole protein profiling in complex sample mixtures such as CSF and blood as well as the proteins functional modification and activities. The accurate quantification also allows for the comparison of different proteomes among groups. Over the last several years, a growing number of studies have adopted this technology to look for biomarkers in AD.

Finehout et al. have identified a panel of 23 spots in CSF that could be used to differentiate AD and non-AD patients with a total sensitivity of 93% and a total specificity of 90% (Finehout et al., 2007). These proteins are related to the transport of beta-amyloid, the inflammatory response, proteolytic inhibition, and neuronal membrane proteins. Other studies have detected more than 100 potential proteins, including brain-derived neurotrophic factor (BDNF), IL-8, vitamin D binding protein (VDBP) and apoE, as potential CSF biomarkers for AD (Abdi et al., 2006, Zhang et al., 2008). Carrette and his colleagues detected five differentially expressed polypeptides in the CSF of AD patients compared to healthy controls. A combination of the five polypeptides achieved a specificity of 100% and sensitivity of 60% in identifying AD (Carrette et al., 2003).

Additional proteomics studies in AD have been performed to demonstrate the presence of novel biomarkers in plasma or serum. One study have identified several

potential plasma biomarkers including, vitamin D-binding protein, heavy chain-related protein, cAMP-dependent protein kinase catalytic subunit alpha 1 (Liao, 2007). Another study has found four potential biomarker peaks that could distinguish AD from control subjects and PD by using the serum albumin-bound fraction (German et al., 2007).

Proteomics analysis has also been used to investigate the conversion from MCI to AD, but there is limited data at present. Simonsen et al. reported seventeen potential protein biomarkers that were differentially expressed in the MCI patients who developed AD (Simonsen et al., 2007). In addition, a recent study has utilized proteomics methods to evaluate treatment efficacy. They found that a number of plasma proteins expression were correlated with the change of ADAS-Cog score in rosiglitazone groups compared with placebo (Akuffo et al., 2008). Further validation work is still in progress.

In summary, proteomic analysis, being a discovery driven approach, allows for identifying large numbers of novel biomarkers without bias and potentially lead to new hypothesis. The new identified biomarkers need to be validated independently by different groups to confirm the specificity and sensitivity of detected proteins as biomarkers.

3.3 Genetic biomarkers

Genetic factors are important in the development of AD. Hence, genetic information is considered to be useful in the diagnosis of AD. In familiar AD, genetic tests for APP and presenilin gene mutations play a significant role in

diagnosis. For Sporadic AD, however, the identification of risk genes is difficult because it is a complex disorder with both genetic and environmental etiological factors. Moreover, multiple genes with small effects each are likely to contribute to the disease development.

ApoE is the only confirmed risk gene in SAD. However, researches into the usefulness of ApoE4 in the diagnosis of AD have achieved low sensitivity and specificity, suggesting ApoE genotyping could not be used alone as a diagnostic tool. When used in combination with clinical criteria, it may increase the specificity of diagnosis (Mayeux et al., 1998). Furthermore, ApoE genotyping are also used in clinical trials for balancing risk factors among different groups.

Apart from ApoE, a series of potential risk genes have been reported in AD by hypothesis driven approach (Papassotiropoulos et al., 2002b, Reiman et al., 2007, Kawamata and Shimohama, 2002, Miller et al., 2007, Dickerson et al., 2005), but the results are difficult to replicate. Recent studies using genome-wide association technology have identified several genes in association with the risk of AD. The potentials of utilizing these genetic variances as diagnostic tools in AD are unknown at this moment. However, the concept of "genetic profiling" that incorporating established genetic susceptibility loci into a diagnostic or predictive test for AD was proposed with the coming of post-GWAS era (Sleegers et al., 2010).

3.4 Neuroimaging biomarkers

3.4.1 Structure imaging

Hippocampal atrophy is the best established structural biomarker for AD, particularly for early diagnosis. It has been demonstrated that the atrophy rate is 3%-7% per year in AD patients while a maximum of 0.9% in age matched controls (Raz et al., 2004). Significant atrophy of hippocampus could be identified by MRI in preclinical AD and predict later progression with about 80% accuracy (Wang et al., 2006, Jack et al., 1999). The advance of automated analysis programs has improved the procedures by reducing the measurement time (Csernansky et al., 2005, Hsu et al., 2002). Although automated programs seem to be superior to manual protocols, further validation studies are needed to confirm its application.

Entorhinal cortex is thought to be affected in the early stage of AD. Studies have shown that volumetry of the entorhinal cortex provided a predictive value in MCI patients (Hsu et al., 2002, Du et al., 2001), but it may not offer benefit for AD patients at late stage (Xu et al., 2000, Teipel et al., 2006). The laborious measurement and lack of automated protocols restrict this method for research, rather than on clinical development of this biomarker in early diagnosis of AD.

In addition to manual volumetric methods, various automated data-driven methods are being investigated in detecting structure changes in AD and MCI subjects such as the whole brain volumetry, voxel-based volumetry (VBM), deformation-based morphometry (DBM) and cortical thickness (Teipel et al., 2007, Lerch et al., 2008).

3.4.2 Functional imaging

Brain activity changes have been widely investigated in functional magnetic resonance imaging (fMRI) studies for the development of a marker of early diagnosis of AD. Nevertheless, the results are contradictory partly due to the differences in clinical assessment, the types of the tasks and data analysis methods across different studies (Dickerson et al., 2005, Celone et al., 2006, Machulda et al., 2003, Sperling et al., 2003). Another new approach is to explore the functional connection between regions of the activated network. A preliminary study in MCI patients has shown widespread changes in functional connectivity of the fusiform gyrus to other visual processing areas and areas within the ventral and dorsal visual pathways (Bokde et al., 2006). As functional connectivity across activated network is considered as an important factor influencing cognitive function, it merits further research for seeking novel markers of AD and MCI.

Positron emission tomography (PET) with 18fluorodeoxyglucose (18FDG) is used to study cortical metabolism. This marker still requires well validated and it is too expensive for routine practice (Hampel et al., 2008). By using 18FDG-PET, the decreased metabolism in temporoparietal (Hoffman et al., 2000, Sakamoto et al., 2002), posterior cingulate (Minoshima et al., 1997, Nestor et al., 2003), hippocampal complex, medial thalamic regions, and mamillary bodies (Nestor et al., 2003) were observed in AD brain. Additional studies have also found the metabolism deficit was an indicative of predicting conversion from MCI to AD (Chetelat et al., 2005, Drzezga et al., 2005, Mosconi et al., 2004). 18FDG-PET was used in several monocenter studies to demonstrate the effect of cholinergic treatment in metabolic pattern of AD patients (Potkin et al., 2001). Recently, novel

markers have been developed to image the receptor binding of specific transmitters or image acetylcholinesterase activity or image amyloid plaques in vivo by PET (Hampel et al., 2008). One of the most extensively studies is using PIB to trace amyloid plaques in AD brain. PIB imaging may be suitable for affirming the clinical diagnosis of AD in preclinical stage (Jack et al., 2008, Klunk et al., 2007, Pike et al., 2007). Further evaluation of its sensitivity to cognitive changes with time still waits validation.

Another neuroimaging technique named single photon emission computed tomography (SPECT) has been utilized to detect decreased regional cerebral perfusion in discriminating AD patients from normal controls (Burns et al., 1989, Harris et al., 1998, Montaldi et al., 1990) and also in predicting MCI conversion to AD (Huang et al., 2007, Borroni et al., 2006).

In summary, imaging biomarkers have attracted special interests in recent years. Hyppocampal volumetry and 18FDG-PET have still stood out as standard in the early diagnosis of AD. Automated methods are in progress to explore its applicability as biomarkers. The development of functional imaging such as amyloid imaging technology has propelled imaging biomarkers to the frontier and laid a path to follow in the future.

3.5 Combination of biomarkers

As each biomarker has its own distinctive characteristics, a combination of different biomarkers may help to optimize the clinical potential in diagnosis and disease monitoring.

The most investigated combination is the CSF $A\beta_{42}$ and tau combination (Blennow, 2004, Maddalena et al., 2003). Other studies in MCI patients have demonstrated the combination of CSF t-tau and the ratio of Aβ42/p-tau181 significantly predicted the conversion to AD (Hansson et al., 2006). The $A\beta_{42}$/p-tau_{181} ratio has also been found useful in the differential diagnosis of AD (Le Bastard et al., 2009, Chen LM et al., 2009). Besides, recent studies have shown that a combination of amloid imaging by PIB-PET or hippocampal volumetry by MRI with CSF Aβ and tau has been proposed as the best predictive model at different disease stages (Forsberg et al.). Further validation studies are in progress to test their effectiveness.

Research into biomarkers improves specificity of early diagnosis, and also enhances understanding of pathogenesis of AD. CSF panel of Aβ42, t-tau and p-tau proteins come closest to fulfilling the criteria for a useful biomarker. The combination among different panels of biomarkers may be informative in the comprehensive evaluation of disease process.

Chapter 4 Project approach

4.1 Development of research hypotheses

Numerous efforts have been made to look for predictive markers for AD. However, there are few (other than ApoE4) that are confirmed as risk factors in AD or MCI. AD, as a complex and heterogeneous disease, results from the interaction of environmental and genetic factors. Although there is a genetic predisposition for the development of AD, gene polymorphisms are inborn and do not reflect recent changes in the body. If there are other risk factors that could reflect body response to both static (inherited) and dynamic (environmental) changes, they may be more applicable for AD, a complex disorder with multifactorial nature.

Gene expression profiling allows for reflection of acute/current cellular activities and body response in a summative way. Previous studies have reported differential gene expression in AD brain. The altered regulation involved in several processes includes apoptosis, inflammatory response, mitochondria function, and synaptic plasticity. However, it is impossible to obtain brain tissues in a clinical setting. Peripheral blood cells seem to be a potential surrogate tissue. Recent studies in AD and other psychiatric disorders revealed that some of the gene expression signature happening in brain is also reflected by activities of peripheral blood cells (Sullivan et al., 2006, Liew et al., 2006). Also, expression profiling of blood has been found potentially clinical useful in various immune and neurological diseases (Tang et al., 2004, Tang et al., 2005), therefore, I would like to explore the potential of expression profiles as a peripheral marker for AD, a disease with neurological and immunological interaction. Cognitive performance and neuropsychiatric

assessment were used as phenotypes, because quantitative traits may provide more information than simply using affection status as phenotypes and thus provide more statistical power to detect small polygenic effects. MCI, as an at risk state between normal aging and early dementia, was recruited in the study in consideration that the information may provide a better understanding of the mechanisms involved in clinical progression of AD.

4.1.1 Literature review on transcriptional profiling in AD

To evaluate the molecular events associated with the mechanisms of AD pathogenesis, gene expression profiling (transcriptome) allows an overview of simultaneous activity of genes in multiple cellular pathways in AD. Also, altered gene expression pattern, if validated, has great potential to develop a signature-gene-set marker for clinical application. Microarray-based gene expression profiling provided a powerful tool for rapid and high-throughput measurement of gene expression on a genome-wide scale and represented an unbiased method for exploring novel candidate genes in disease pathogenesis.

Gene expression profiling was analyzed directly on postmortem brain tissues of AD patients (Loring JF et al., 2002, Yao et al., 2003a). Despite it is difficult to compare microarray data across studies, the general conclusion from these and other studies is that AD brains are up-regulated in genes involved in inflammation and apoptosis and down-regulated in synaptic plasticity, signal transduction and gene transcription (Pasinetti, 2001, Hata et al., 2001).

However, gene expression profiles for the brain tissues cannot be

accomplished during life and the possibility of postmortem RNA degradation may confound the interpretation of results in brain. Hence, several studies attempted to analyze gene expression pattern in peripheral blood cells derived from AD patients. Blood cells are easier to obtain and have a reasonable correlation in gene expression with brain. One earlier study, using cDNA microarray on lymphocytes of AD patients and age matched controls, has identified 20 differentially expressed genes related to immune response, apoptosis and blood pressure regulation, with the deduction of defensin and α -2c-adrenoceptor genes confirmed by real-time RT-PCR (Kalman et al., 2005). Another group has found major up-regulated functions in AD blood mononuclear cells are apoptotic and inflammation while down-regulated functions are cytoskeletal maintenance, cellular trafficking, cellular stress response, redox homeostasis, transcription and DNA repair (Maes et al., 2007). A recent study has investigated the profile of 33 genes expression levels in whole blood by quantitative real-time RT-PCR and five genes showed a tendency of correlation to MMSE score (Grunblatt et al., 2009). These results confirmed that some of the features / signature of gene expression profiles happening inside the brain could be found by analysis of peripheral blood cells.

Extensive studies have been conducted to explore gene expression profiling in the transgenic animal model of AD (Wu et al., 2006, Reddy et al., 2004, Dickey et al., 2003, Selwood et al., 2009). Animal models harboring knock-in mutations in genes encoding APP, presenilins or tau may exhibit the development of AD pathologies, such as amyloid deposition, neurofibrillary tangles and synapse loss. Analysis in transgenic animal model showed that regulated genes are implicated in calcium homeostasis, cholesterol transport and uptake, inflammation and

mitochondria energy metabolism (Reddy et al., 2004, Dickey et al., 2003, Selwood et al., 2009).

More recent microarray experiments have focused on single cell or population cell gene expression analysis in AD. Single cell / population cell RNA analysis minimize the contamination of other cell subpopulations and is more applicable for brain tissue analysis, because brain are composed of diverse neuronal and nonneuronal cells. Two methods including laser capture microdissection (LCM) and microaspiration were commonly used to separate target cells from the rest of tissue section (Ginsberg et al., 2004). At the present, research into single-cell expression profiling in AD is still limited. Analysis of NFT-bearing and SPs-bearing CA1 neurons as well as cholinergic basal forebrain (CBF) neurons (Ginsberg et al., 2000, Mufson et al., 2002, Ginsberg et al., 1999) indicated that single cell/population cell transcriptional analysis pattern may be more informative than patterns from regional tissues.

In summary, transcriptional profiling analysis in AD have demonstrated numerous dysregulation events associated with AD and revealed underlying mechanisms of neuronal degeneration. However, whether the identified genes represent a consequence and compensatory response or the cause of the disease still remains elusive. Corroborating with genetic information and proteomic may provide a more global picture of biological process in the future.

4.1.2 Consideration on gene expression profiling in leukocytes

Several groups have reported a high degree of correlation between gene

expression profile of peripheral blood cells and brain (Sullivan et al., 2006, Rollins et al., 2010, Liew et al., 2006). Liew et al demonstrated peripheral blood cells (PB) shared more than 80% of the transcriptome with each of the nine tissues, including brain, colon, heart, kidney, liver, lung, prostate, spleen and stomach (Liew et al., 2006). Sullivan et al found that blood gene expression was significantly similar to multiple CNS tissues, especially several regions of importance in neuropsychiatry including prefrontal cortex, amygdala (Sullivan et al., 2006). A number of neurotransmitter, inflammation cytokines and corresponding receptors have been found expressed in both neuronal cells and peripheral blood cells, indicating a bi-direction communication between blood cells and central nervous system (Gladkevich et al., 2004). Further, blood cells come into contact with every cell in the body and its expression signature may reflect body's response to various physical, pathological and environmental changes (Liew et al., 2006).

Alteration of peripheral gene expression signatures has been demonstrated in a wide range of diseases, including hypertension, chronic fatigue disease, cancer, neuronal injuries, juvenile arthritis, and transplantation (Liew et al., 2006). More recent studies have reported that neuropsychiatric disorders, such as schizophrenia, bipolar disorder and Huntington's disease could be distinguished by specific PB gene expression profiles (Tsuang et al., 2005, Borovecki et al., 2005). Others studied PB transcriptome in AD, neurofibrobmatosis, epilepsy, Parkinson disease and Down syndrome (Tang et al., 2004, Tang et al., 2005, Grunblatt et al., 2009).

In previous studies of association between PB transcriptome and neurological disease, three major blood components were analyzed, including (1) whole blood

sample on PAXgene tube, by Trizol and (2) leukocytes after lysis of RBC and (3) peripheral blood mononuclear cells (PBMC) separated by gradient centrifugation. In general, handling of whole blood would be easier and closer to routine clinical setting, but the inclusion of red cell RNA is associated with high noise and reduced responsiveness in transcriptome analysis (Debey et al., 2004, Feezor et al., 2004). Study of PBMC also gain an insight into the transcriptome of PB cells, however, excessive procedure for isolation of mononuclear cells may involve a time delay before RNA stabilization and lead to a gene expression changes not related to the disease status or clinical response under study (Whitney et al., 2003). Leukocytes, as a mixture of mononuclear cells and neutrophil-rich fraction, are easily obtained in the layer of buffy coat between sedimented red blood cells and the plasma after centrifugation. Leukocytes expressed various cytokines, neurotropic factors, and receptors for neurotransmitters and hormones, which undergo the same regulatory mechanisms as those in the brain (Hori et al., 1991). Moreover, mRNA expression profiling derived from leukocytes was demonstrated to be more responsive than those from whole blood due to the absence of hemoglobin mRNA (Feezor et al., 2004). Hence, peripheral leukocytes may be a potential surrogate tissue to reflect changing in the brain.

4.1.3 Bioinformatic approach based target genes selection (Diagram 4.1 and Table 4.1)

In this study, target genes are selected based on the bioinformatic approach. 964 differentially expressed genes in AD were identified from published dataset (Maes et al., 2007, Kalman et al., 2005, Colangelo et al., 2002). 2183 Alzheimer's related genes list were generated by GeneCards website and SABioscience AD

array. Both two genes lists produced an overlapping of 134 differentially expressed genes in AD. These genes were further selected based on the criteria of previously reported differentially expressed in AD brain or transgenic model $\geqslant$ 2 times (Blalock et al., 2004, Dickey et al., 2003, Reddy et al., 2004, Emilsson et al., 2006, Chano et al., 2007). From the 44 surviving genes, we selected 10 genes that are highly expressed in both brain and blood (Gene Expression Omnibus) (Diagram 4.1). Additional two genes SNX2 and CNR2 were recently reported to be upregulated in peripheral blood of AD patients and showed a tendency to correlate with MMSE score (Grunblatt et al., 2009). Both genes are highly expressed in both blood and brain. In the present study, 12 candidate genes were selected to be validated by quantitative real-time RT-PCR (Table 4.1).

Diagram 4.1 The diagram for bio-informatics approach to gene selection

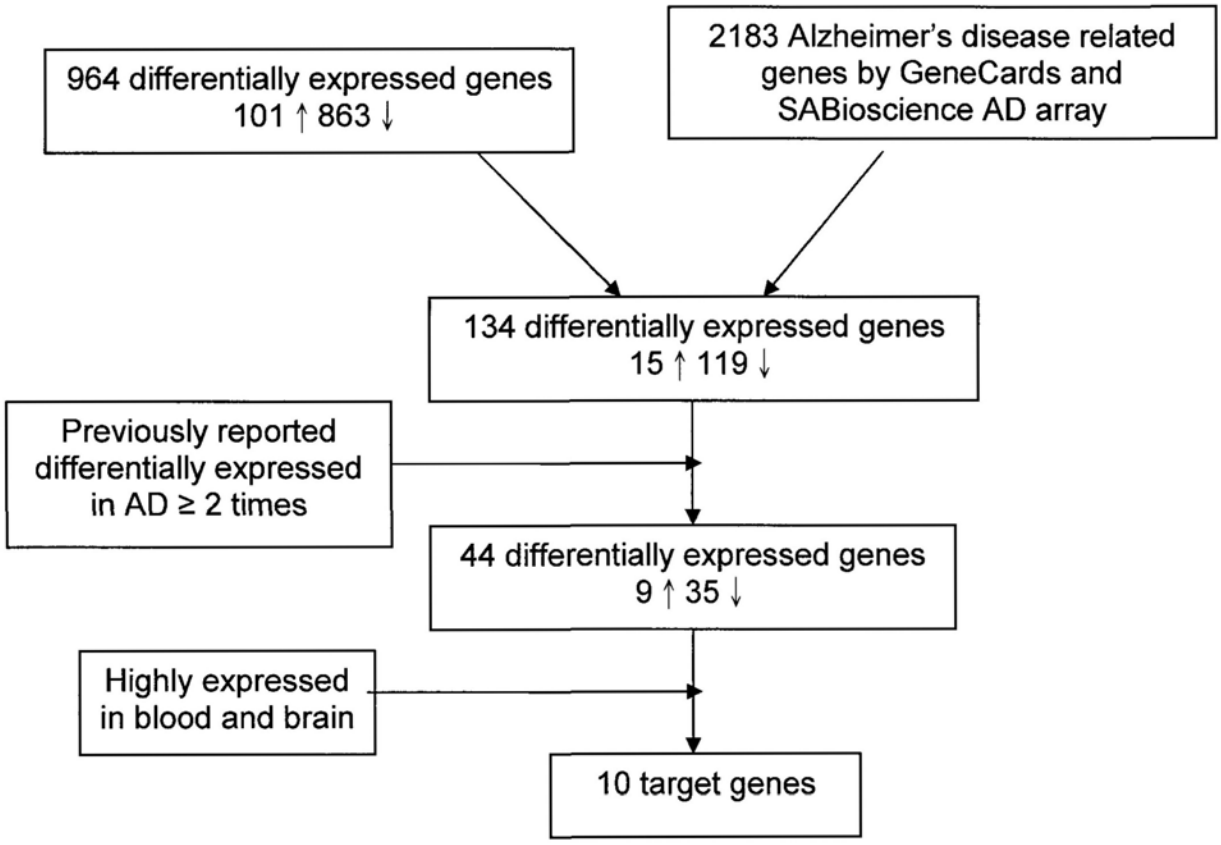


Table 4.1 A list of 12 candidate genes selected to be examined by QRT-PCR

Gene symbol	Gene name	Chromosome	Function by oncology
CTSB	Cathepsin B	8p22	Apoptosis, Endopeptidase activity
CTSD	Cathepsin D	11p15.5	Apoptosis, Endopeptidase activity
DDT	D-dopachrome tautomerase	22q11.23	Immune response, Melanin biosynthesis
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	1q42.13	Calmodulin binding, Inositol phosphate metabolism
PIN1	Peptidylprolyl cis/trans isomerase, NIMA-interacting 1	19p13	Isomerase activity, Cell cycle
TSC1	Tuberous sclerosis 1	9q34	Brain development, Cell proliferation
NRD1	Nardilysin	1p32.2-p32.1	Cell proliferation
UQCRC1	Ubiquinol-cytochrome creductase core protein I	3p21.3	Mitochondria electron transport
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6	22q13.2-q13.31	Mitochondria electron transport
GSTM3	Glutathione S-transferase mu 3	1p13.3	Detoxification, Antioxidative stress
SNX2	Sorting nexin 2	5q23	Protein transport
CNR2	Cannabinoid receptor 2	1p36.11	Immune response, Synaptic transmission

4.1.3.1 CTSB gene

Cathepsin B (CTSB) is a lysosomal cysteine proteases presented in lysosomes. The protease is recognized as a multi-functional enzyme involved in diverse biological processes, including proteins turnover, antigen processing and presentation, cleaving of membrane-bound proteins, as well as degradation of the extracellular matrix and tissue remodeling (Turk et al., 2000). In addition, the protease is implicated in various pathological conditions, such as tumorigenesis, bone disorder, and pancreatitis (Watson and Kreuzaler, 2009, Halangk et al., 2000, Hopwood et al., 2009). Recent studies have also identified CTSB as a novel β-secretase for the production of $A\beta_{42}$ in AD brain (Hook et al., 2008).

CTSB gene is located on chromosome 8p22 and spans more than 27 kilobases (Fong et al., 1992). The gene was reported to have 13 exons and at least five alternative transcript variants have been found. Increased expression and/or activity of CTSB have been found in various kinds of tumors (Vasiljeva et al., 2006, Watson and Kreuzaler, 2009). In addition, CTSB, as an alternative β-secretase for production of β amyloid peptide in AD has been demonstrated recently (Hook et al., 2008). In comparison with BACE-1 and CTSD, the dominant β-secretases for cleaving mutant Swedish site of APP in constitutive secretory pathway, CTSB efficiently cleaves the wide-type β-secretase site in regulatory secretory pathway. Inhibitors of CTSB improved memory and reduced Aβ in transgenic AD mice expressing the wide-type β-secretase site of APP and suggested the potential of using such inhibitors in AD therapeutics. Decreased mRNA expression of CTSB was observed in PBMC of AD patients (Maes et al., 2007) but increased in hippocampus (Blalock et al., 2004). CTSB was also essential in mediating different

models of apoptosis by activating caspases and/or inducing mitochondria dysfunction (Hwang et al., 2009, Yan et al., 2009). Evidence showed that CTSB may function as a mediator of neuronal death induced by Aβ-activated inflammatory response (Gan et al., 2004). All these findings suggested the role of CTSB in the pathogenesis of AD.

4.1.3.2 CTSD gene

Cathepsin D (CTSD) is a soluble lysosomal aspartic endopeptidase synthesized in rough endoplasmic reticulum as preprocathepsin D and targted to lysosomes as procathepsin D and finally activated as mature CTSD through complex proteolytic cleavage process (Hasilik and Neufeld, 1980). In addition to the function of protein degradation, CTSD was shown to involve in multiple physical and pathological functions. Studies on CTSD knock-out mice revealed that CTSD is not essential for embryonic development but is reqired for postnatal tissue homeostasis (Benes et al., 2008). Mutations that inhibiting CTSD activity cause neuronal ceroid lipofuscinosis (NCL) characterized by severe neurodegeneration, developmental regression, visual loss and epilepsy (Awano et al., 2006). Some studies reported that CTSD directly trigger apoptosis, while others demonstrated that it may act as a mediator of apoptosis (Benes et al., 2008) .

Numerous studies have demonstrated the importance of CTSD in various pathological processes including cancer, AD and atherosclerosis (Thorpe et al., 1989, Cataldo et al., 1995, Duran et al., 2007). Here we focused on reviewing the biological relevance of CTSD in AD. CTSD was demonstrated to be an β-secretase as BACE-1, and CTSD is 280-fold more abundant in human brain than BACE-1

(Hook et al., 2008). In addition, CTSD has been implicated in the processing of ApoE and tau protein (Kenessey et al., 1997, Zhou et al., 2006). An downregulation of CTSD mRNA was observed in PBMC of AD patients (Maes et al., 2007). The gene encoding CTSD is located in the short arm of chromosome 11 (11p15.5) and polymorphisms have been associated with the risk of AD (Papassotiropoulos et al., 2002a, Riemenschneider et al., 2006, Davidson et al., 2006). However, a recent meta-analysis has shown that the CTSD gene polymorphisms is not a major risk factor for AD on a population level (Ntais et al., 2004). The exact mechanism of CTSD in the pathogenesis of AD still awaits further investigation.

4.1.3.3 DDT gene

D-dopachrome tautomerase (DDT) is an enzyme that converts D-dopachrome into 5,6-dihydroxyindole. This protein is shown to be related to the migration inhibitory factor (MIF) in terms of sequence, enzyme activity and structure. Both of DDT and MIF genes were located on chromosome 22 (Weiser et al., 1989). MIF is a cytokine originated from activated T-lymphocytes and macrophage, and plays a pivotal role in response to inflammatory stimuli (Bucala, 1994). It was speculated that DDT may participate in a process of inflammatory reactions and immune responses. Few studies showed that DDT was involved in the biosynthesis of melanin, which has bioactivity as an antioxidant (Hung et al., 2006). In addition, 5,6-dihydroxyindole produced by DDT is a precursor of melanin showing bioactivity as antioxidant (Heiduschka et al., 2007).

Growing evidence supported the role of MIF in the pathogenesis of AD. In APP transgenic mouse model, MIF was found in microglia cells in association with

Aβ plaques. Functional studies demonstrated that Aβ induced cytotoxicity could be reversed by inhibitor of MIF (Bacher et al., 2010). A marked increase of MIF levels was found in the CSF of AD and MCI patients (Popp et al., 2009). As the homology of MIF, DDT may act closely with MIF.

4.1.3.4 ITPKB gene

Inositol 1,4,5-trisphosphate 3-kinase B (ITPKB) is a calcium-dependent inositol kinase, phosphorylating the 3' position of the inositol ring to convert inositol 1,4,5-trisphosphate (IP3) to inositol 1,3,4,5-tetrakisphosphate (IP4). IP3 is essential for triggering the release of calcium from both the intracellular stores and extracellular space (Joseph et al., 1984). Studies in mice lacking ITPKB have demonstrated the importance of ITPKB in B cell and T cell development, selection and activation (Miller et al., 2007). ITPKB also mediate B cell survival through controlling proapoptotic Bim gene expression (Marechal et al., 2007). Alteration of ITPKB may lead to increased conversion of IP3 to IP4 affecting calcium homeostasis in the cells and severe T cell deficiency in mice (Pouillon et al., 2003).

Intracellular calcium dysregulation is one of early pathological events in AD. However, the molecular mechanisms involved in the alteration of calcium signal have not been identified yet. Transcriptional profiling studies in AD brain and peripheral blood have found that ITPKB was downregulated (Emilsson et al., 2006, Maes et al., 2007), indicating the underlying processes regulated by ITPKB in initiating calcium deregulation in AD.

4.1.3.5 PIN1 gene

The peptidyl prolyl *cis/trans* isomerase PIN1 specifically binds phosphorylated Ser/Thr-Pro proein motifs and catalyzes the *cis/trans* isomerization of the peptide bond. Isomerization by PIN1 modulates protein folding, biological activity, stability, and localization of its substrates and subsequently regulates the factors involved in the control of cell proliferation, apoptosis and inflammation (Takahashi et al., 2008). Various transcription factors and regulators have been identified as substrates for PIN1, including AP-1, *p*53, *p*73, ARE-binding proteins et al. Due to a large number of substrates, PIN1 is considered to involve in multiple biological processes, including the regulation of cell cycle, cell signaling and response to DNA damage (Lu et al., 1996, Zhou et al., 1999). Moreover, PIN1 is also associated with pathological conditions, such as cancer, AD and asthma and represents an attractive therapeutic target.

Growing evidence suggested that PIN1 is overexpressed in various tumors and its overproduction correlates with poor clinical outcome (Bao et al., 2004, Ayala et al., 2003). In contrast, PIN1 knockout in mice or cancer cells supress tumor or cell grow by inhibiting certain oncogenic pathways (Wulf et al., 2004, Ryo et al., 2005). These and other results (Lu, 2003, Lu et al., 2006) indicated PIN1 play a pivotal role in cancer development.

On the other hand, the downregulation of PIN1 is associated with the increased risk of AD (Liou et al., 2003). PIN1 interacts with phosphorylated Thr-231 of tau and promotes its dephosphorylation, restoring tau's activity to bind microtubules (Zhou et al., 2000). Decreased levels of PIN1 may impair dephosphorylation of tau

and failed to restore its biological activity. PIN1 also binds with APP at phosphorylted Thr-668 and regulates its metabolism (Akiyama et al., 2005). Overexpression of PIN1 has been shown to reduce Aβ production and PIN1 deficiency leads to a significant increase in βAPPs and Aβ42 (Pastorino et al., 2006, Balastik et al., 2007). Moreover, the findings of PIN1 knockout mice presenting tauopathy and neurodegeneration also suggested that PIN1 might protect against tau hyperphosphorylation and NFT formation (Liou et al., 2003). Recent studies found that the levels or activity of PIN1 were decreased in the brains of AD patients compared with control (Lu et al., 1999). In addition, gene polymorphisms of PIN1 have been reported to be associated with reduced PIN1 levels and increased risk of AD (Wijsman et al., 2004, Segat et al., 2007).

PIN1 isomerase activity is regulated by its posttranslational modifications, including phosphorylation and oxidation. Studies found that PIN1 was subjected to oxidative modification in the hippocampus of MCI and AD patients (Sultana et al., 2006, Butterfield et al., 2006). It was hypothesized that oxidative modification of PIN1 which inhibits the enzyme activity may be a major driving force for the initiation or progression of AD pathogenesis.

4.1.3.6 TSC1 gene

Tuberous sclerosis 1 (TSC1) gene, located on chromosome 9q34, encodes a 130-Kda protein hamartin. Hamartin binds TSC2-encoded protein tuberin to form a functional heterodimer (van Slegtenhorst et al., 1998). Mutations in TSC1 or TSC2 could cause an autosomal dominant disorder of tuberous sclerosis complex (TSC).

The neurological manifestations of TSC include infantile spasms, intractable epilepsy, cognitive disabilities, and autism (Astrinidis and Henske, 2005).

TSC1 or TSC2 encoded proteins modulate cell function via the mTOR signaling cascade and serve as key messengers in regulating cell growth and proliferation (Rosner et al., 2008). In the brain, TSC1 and TSC2 have been implicated in cell body size, dendritic arborization, axonal outgrowth and targeting, neuronal migration and spine formation (Rosner et al., 2008). In AD, hamartin may function as an integrator of the neuronal intermediate filament and the actin cytoskeletal network (Haddad et al., 2002). Tuberin levels were found to be decreased in the brain tissues of AD patients (Ferrando-Miguel et al., 2005). In addition, the alteration of mTOR signaling has been reported in AD brain, and the mTOR signaling activities were also correlated with MMSE scores in AD subjects (Lafay-Chebassier et al., 2005). Recent study showed that unbalanced TSC1 abundance may be associated with mTOR signaling repression and neuronal atrophy (Chano et al., 2007).

4.1.3.7 UQCRC1 gene

Ubiquinol-cytochrome-c reductase core protein1 (UQCRC1) is a component of the ubiquinol-cytochrome c reductase complex (complex III), which is part of the mitochondrial respiratory chain. The mitochondria complex III catalyzes transfer of electrons from coenzyme QH2 to ferricytochrome c with the coupled translocation of protons across the mitochondrial inner membrane.

Overexpression of UQCRC1 was documented to affect mitochondrial

morphology and physiology, leading to mitochondrial dysfunction (Johnston-Wilson et al., 2000). In animal model for Rett syndrome, UQCRC1 overexpression correlated positively with neurological symptom severity and a reduction in respiratory efficiency (Kriaucionis et al., 2006). In humans, UQCRC1 was upregulated in AD peripheral blood and mood disorders brains but downregulated in schizophrenia (Prabakaran et al., 2004, Johnston-Wilson et al., 2000). In addition, it is highly expressed in breast and ovarian tumors (Kulawiec et al., 2006).

4.1.3.8 NRD1 gene

Nardilysin (N-arginine dibasic convertase, NRD1 or NRDc) is a metalloendopeptidase that functions cleave peptides such as dynorphin-A, somatostatin-28, α -neoendorphin and glucagons (Chesneau et al., 1994). It may also enhance the ectodomain shedding of a wide range of membrane proteins, including APP, TNF- α and heparin-binding EGF-like growth factor (HB-EGF) (Hiraoka et al., 2007, Hiraoka et al., 2008, Nishi et al., 2006). As the process of ectodomain shedding is essential for the activation of specific receptors, cytokines and growth factors, NRD1 was considered involved in a wide spectrum of physiological and pathological processes.

NRD1 seems to play specific roles in neuronal development based on the observation that it is predominantly expressed in fetal placenta, heart and brain, but highly expressed exclusively in neural tissues with the development of mouse (Fumagalli et al., 1998). NRD1 deficient mice had impaired motor activities and cognitive deficits with smaller brains and a thin cerebral cortex, suggesting the role

of NRD1 in regulating axonal maturation (Ohno et al., 2009). Further, as a new receptor for HB-EGF, NRD1 was found to promote cellular migration, proliferation and neurogenesis (Nishi et al., 2001, Jin et al., 2002). Finally, NRD1 is involved in reducing the Aβ production by regulating α -secretase activity in cultured cells, suggesting it may be targets for AD (Hiraoka et al., 2007). More recent study showed that NRD1 coexists and interacts with brain-specific protein p42IP4 (Bernstein et al., 2007). The protein p42IP4 was previously reported to be highly expressed in neurons and plaques of AD patients (Reiser and Bernstein, 2002, Reiser and Bernstein, 2004).

4.1.3.9 NDUFA6 gene

Oxidative stress is considered as the major event in the pathogenesis of AD. NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (NDUFA6) is subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I). Complex I catalyze transfer of electrons from NADH to the respiratory chain (Ton et al., 1997). Recent study showed that NDUFA6 gene silencing induces apoptosis of HIV-infected cells and its overexpression reduces apoptosis (Ladha et al., 2005). Microarray studies showed an upregulation of NDUFA6 in PBMC of AD patient's compared to controls (Maes et al., 2007), suggesting that oxidative damage is implicated in the development of AD.

4.1.3.10 SNX2 gene

SNX2 gene on chromosome 5q23 encodes sorting nexin 2, a member of the sorting nexin family. SNX2 is a component of the retromer complex and involved

in intracellular trafficking. The retromer complex functions as retrieving lysosomal enzyme receptors to the trans-Golgi network and processing of the amyloid precursor protein (Seaman, 2005). Human retromer comprised three proteins, including Vps26, Vps29, and Vps35, plus SNX1 and SNX2. Moreover, SNX1 and SNX2 are essential for retromer structure and function (Rojas et al., 2007).

Recent study showed that SNX2 was increased in peripheral blood of AD subjects and inversely correlated with MMSE score. Another studies found downregulation of VPS35 and VPS26 can regulate Aβ levels in cultured cell and retromer deficiency in mice caused hippocampal dysfunction, neurodegeneration and Aβ accumulation (Muhammad et al., 2008, Small et al., 2005).

4.1.3.11 CNR2 gene

Cannabinoids generally refer to a group of secondary metabolites in the cannabis plant, which exert the major pharmacological effects of the cannabis. Endogenous cannabinoids are produced in the bodies of humans and animals. Cannabinoids have a wide range of central and peripheral effects by interacting with cannabinoid receptors, including CNR1 and CNR2 (Lambert and Fowler, 2005). CNR1 are mainly expressed in the CNS, wherever CNR2 are primarily located in peripheral immune cells (Munro et al., 1993). In the brain, CNR2 are widely distributed in microglia, blood vessels but not neurons (Cabral et al., 2008). Cannabinoids induce psychoactive effects mainly through CNR1, but recent studies indicated the potential role of CNR2 as therapeutic targets in the treatment of AD, depression, anxiety disorder, neuropathic pain (Benito et al., 2003, Hill and Gorzalka, 2009, Cheng and Hitchcock, 2007).

CNR1 and CNR2 were found colocalized in senile plaques with the activation of microglia in brain of AD patients. The nitration of both receptor proteins is significantly enhanced suggesting the alteration of function may occur in AD pathogenesis. Administration of mixed CNR1/CNR2 cannabinoid receptor agonist to mice protected against Aβ induced microglia activation, cognitive impairment and neuronal loss. In culture cells, selective CNR2 agonist also counteracted Aβ stimulated microglia activation (Ramirez et al., 2005). Recent studies reported that gene expression of CNR2 was changed in brain tissues and blood of AD patients (Grunblatt et al., 2009, Grunblatt et al., 2007). Further, the transcript levels showed tendency to inversely correlate with the MMSE score (Grunblatt et al., 2009). The role of CNR2 in the neuroprotective effects of cannabinoids has attracted special interests.

4.1.3.12 GSTM3 gene

Glutathione S-transferases (GSTs) are identified as a family of detoxification enzymes, protecting against oxidative stress and chemical stress induced by peroxidised lipids, xenobiotics and toxins (Hayes and Pulford, 1995). The cytoplasmic GSTs are composed of eight classes, including alpha, kappa, mu, omega, pi, sigma, theta and zeta and GSTM3 belonging to mu class (Hayes et al., 2000). The mu class proteins function in defensing against oxidative stress, carcinogens, and environmental toxins. Gene encoding GSTM3 is located on chromosome 1p13.3 and known to be highly polymorphic (Tetlow et al., 2004). Mutations in GSTM3 gene were found to increase the risk of cancer, hypertension and AD (Hong et al., 2009, Golka et al., 2008, Singh et al., 2008, Delles et al.,

2008).

Numerous studies have attempted to investigate the role of GSTs in the oxidative damage of AD pathogenesis. Lovell et al. have found that GSTs activity decreased in AD amygdala, hippocampus, and parietal lobe with accentuating of oxidative burden (Lovell et al., 1998). Other studies showed a significant reduction in transcript levels of GSTM3 in AD hippocampus and PBMC (Blalock et al., 2004, Maes et al., 2007). GSTM3 subunit, as the second most abundant GST subunit in brain, received special attention with the evidence that it accumulated in plaques and tangles in AD affected hypocampal region (Tchaikovskaya et al., 2005). In addition, functional mutations in GSTM3 have been reported to alter protein expression/activity and increased the risk of AD in Caucasians (Maes et al., 2010, Tetlow et al., 2004, Hong et al., 2009). Although it is still not clear whether the alteration of GSTM3 is an initiating event contributing to disease pathology or the consequence or response to the damage condition in AD, the role of this enzyme is essential for the redox pathophysiology of AD.

4.2 Research objectives and hypotheses

Research Objectives:

Based on those candidate genes selected above, the present project aims to explore the following objectives:

i) To identify genes that are differently expressed in AD and MCI

ii) To find sets of expression gene biomarkers that would be useful in the classification of AD and MCI

iii) To identify gene markers that are associated with the clinical phenotypes of AD, including cognitive and NP symptoms.

Research hypotheses are formulated and set as follow:

i) There would be differences in expression profiles between AD, MCI and NC for the candidate genes investigated.

ii) Expression gene markers would be able to differentiate between AD, MCI and NC groups.

iii) Gene markers would be associated with clinical phenotypes of AD.

Chapter 5 Methodology

5.1 Study samples

AD and MCI subjects were recruited from the psychogeriatric services, residential homes and social centers of older persons from the New Territories East region of Hong Kong. Subjects with normal cognition were recruited from the same centers for comparison. All subjects were examined by a qualified psychiatrist for clinical diagnosis of dementia. Severity of cognitive impairment was assessed by using Clinical Dementia Rating (CDR) (Hughes et al., 1982). Subjects were invited for participation if they satisfied the recruitment criteria as detailed in the following section.

5.1.1 Inclusion and exclusion criteria

For inclusion criteria, subjects with AD had to satisfy the NINCDS-ADRDA criteria for possible or probable AD (McKhann et al., 1984). MCI patients had to meet the criteria as follow: presence of subjective memory complaints, a delayed recall of list learning scoring at =< 1.5 SD compared to educational matched subjects with CDR 0, and a global CDR of <1. Cognitively intact controls should be assessed clinically with CDR of 0. To obtain a more homogeneous group of subjects with AD, only those who were aged over 65 at onset were recruited. Three groups of subjects were also required to be ambulatory and active, capable of understanding instruction for assessment and living with an informant who could give reliable collateral history for behavior assessment. In addition, all the subjects had to be of Chinese ethnicity.

For exclusion criteria, subjects with a history of head injury, or with known neuropsychiatric disorders apart from AD, were excluded. Subjects with an unstable physical condition, including unstable diabetes mellitus, poorly controlled hypertension, dysregulation of thyroid and other endocrine functions, known vitamin B12 and folate deficiency, sepsis, neurosyphilis and other chronic illness affecting cognitive function were also excluded. Significant sensory deficits are regarded as exclusion criteria as well. Written informed consent was obtained from the subjects and/or their caregivers and this study was approved by the institutional ethical review board.

5.1.2 Blood sample processing

Peripheral blood (12ml) was collected into two EDTA tubes. The blood samples were separated into plasma, buffy coat and red blood cells (RBCs) layers by centrifugation at 4000 rpm for 10 minutes. Plasma was discarded and RBCs were ruptured with lysis buffer (1.6 mM EDTA, 10 mM $KHCO_3$ and 153 mM NH_4Cl, PH 7.4). Each mixture was centrifuged at 6500 rpm for 5 minutes to yield a pellet of leukocytes. The total genomic DNA was extracted from the pellet of leukocytes by DNA extraction kit according to manufacturer's instruction (Roche, U.S.). RNA was extracted with TRIzol Reagent based on manufacturer's instruction (Invitrogen; Carlsbad, CA).

5.2 Assessment tools

5.2.1 Clinical staging

Clinical Dementia Rating (CDR) was used to evaluate the severity of AD. The CDR is standard instrument for the diagnosis and evaluation of the overall level of severity of dementia (Hughes et al., 1982, Morris et al., 2001). It is a five-point scale to characterize six domains of cognitive and functional performance, including memory, orientation, judgment & problem solving, community affairs, home & hobbies and personal care (Morris, 1993). The overall CDR comprises five stages of severity, ranging from normal cognition to severe dementia. A CDR of 0 represents normal cognition; a CDR of 0.5 represents questionable dementia; a CDR of 1 represents mild dementia; a CDR of 2 represents moderate dementia; a CDR of 3 represents severe dementia. Postmortem examination and multicenter studies have demonstrated good reliability and validity of the CDR (Morris et al., 1997, Morris et al., 1988).

5.2.2 Cognitive assessment

5.2.2.1 Cantonese version of the Mini-Mental State Examination (MMSE)

The MMSE is a widely used tool for the screening of cognitive impairment. It is a brief 30-point questionnaire test examining orientation, memory, attention, language, and construction skills. Since its introduction by Folstein (Folstein et al., 1975), the MMSE has been validated in different population and across different

centers. In Hong Kong, a validated Cantonese version has been developed and the cutoff score for dementia is adjusted according to educational levels (Chiu HFK et al., 1994). For subjects having received two or more years of education, the cutoff score is 22; for subjects with 1 to 2 years of education, the cutoff score is 20; for subjects with no formal education, the cutoff score is 18 or below (Chiu et al., 1998). In this study, the MMSE was assessed in AD, MCI and NC groups to estimate the global cognitive function.

5.2.2.2 Chinese version of the Alzheimer's disease Assessment scale-Cognitive subscale (ADAS-Cog)

The Alzheimer's disease Assessment Scale cognitive subscale (ADAS-Cog) is a popular test designed for the evaluation of cognitive deficits in subjects with AD and is a relatively sensitive indicator of dementia progression (Rosen et al., 1984). The ADAS-Cog has also become a standard instrument used to demonstrate cognitive efficacy in AD drug clinical trials (Mohs et al., 1983). The ADAS-Cog is composed of subscales that examine list learning, delayed recall, naming, ideational apraxia, construction, and recognition. The total possible score is 70, with increasing scores indicating a greater severity of impairment. A locally validated Chinese version for the screening of subjects with AD has been developed (Chu et al., 2000) and adopted to assess cognitive function in the present study.

5.2.2.3 Delayed recall of list learning test

The test was used to examine episodic memory function. The subjects were read a list of ten words three times for immediate recall as part of the ADAS-Cog

test. After 10 min, the subjects were asked to recall the word list (delayed recall).

5.2.2.4 Category verbal fluency test (CVFT)

Verbal fluency tests are widely used as a measure of executive functions and language, and can also be used to evaluate semantic memory. The CVFT has been modified and validated in Hong Kong, showing a useful screening tool for dementia in Chinese elderly. For CVFT used in the study, subjects were asked to generate examples in three categories of living objects in one minute. The categories were 'animals', 'fruit', and 'vegetables'. The number of examples that were generated in three categories was combined to form a CVFT score (Mok et al., 2004, Chiu HF et al., 1997).

5.2.3 Behavior and NP assessment

Neuropsychiatric Inventory (NPI) is a tool for assessment of behavior and NP disturbances in patients with dementia and other neuropsychiatric disorders. It has been widely used because it covers a broad range of symptoms and shows good reliability and validity. The Chinese version of NPI has been validated in Hong Kong (Leung et al., 2001).

The NPI is based on a semi-structure interview with a caregiver who is familiar with the patient. It comprises 10 groups of NP symptoms and 2 groups of neurodegenerative symptoms. The symptoms groupings are delusions, hallucinations, aggressivity, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, night-time behaviors and appetite disturbances. For each group, a screening question is asked to if the behavioral change is present

or absent. If the answer is positive, the group is explored with the more specific sub-questions. If the sub-questions confirm the screening question, the severity and frequency of the behavior are determined according to the criteria provided for each symptom group and a symptom core computed from the product of severity and frequency. The maximum score for each symptom group is 12 and the maximum NPI score is 144.

5.2.4 Medical comorbidity assessment

Growing evidence suggested that medical comorbidity plays an important role in modulating the onset and progression of dementia. High prevalence of medical comorbidity is observed in AD patients and the presence of medical comorbidity is correlated with greater cognitive impairment (Doraiswamy et al., 2002). In the present study, a questionnaire with a physical illness checklist was used to document the number of medical conditions in all subjects. As a global indicator, the total numbers of medical comorbidity in three groups were compared.

5.2.5 ApoE genotyping

ApoE genotyping was performed by polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP). Genomic DNA was subjected to PCR with the following primers: 5'-GCA CGG CTG TCC AAG GAG CTG CAG GC-3' and 5'-GGC GCT CGC GGA TGG CGC TGA G-3'. PCR reaction was carried out in a total volume of 25ul comprising 10mM of each primer pair, 2mM $MgCl_2$, 0.6 unit AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), 0.2mM dNTP and PCR reaction buffer (10mM Tris-HCL, PH 8.3; 50mM KCL). Samples were denatured at 96℃ for 15 minutes followed by 35 cycles of

96℃ for 30 seconds, 54℃ for 45 seconds and 72℃ for 30 seconds, and then a final extension at 72℃ for 7 minutes. The amplified product of 246 bp was digested with 5 units of HhaI (New England Biolabs, UK) and then electrophoresed on 12% polyacrylamide non-denaturing gel with ethidium bromide.

5.2.6 Gene expression validation by Quantitative real-time PCR

5.2.6.1 RNA extraction

Total RNA was extracted by TRIzol reagent according to manufacturer's instruction (Invitrogen; Carlsbad, CA). After lysing cell pellet with 1 ml TRIzol reagent at room temperature (RT) for 5 minutes, 0.2ml bromochloropropane (BCP, Sigma-Aldrich) was added in the tube. The samples were shaken for 15 seconds and incubate them at RT for 3 minutes. Subsequently samples were centrifuged at 11500 g for 15 minutes at 4℃. Following centrifugation, the upper aqueous phase were transferred and the RNA precipitated by mixing with 0.5 ml isopropyl alcohol. Samples were incubated at RT for 10 minutes and centrifuged at 11500g for 10 minutes at 4℃. The supernatant were removed and the RNA pellet was washed with 1 ml of 75% ethanol. Samples were mixed by vortexing and centrifuged at 7300g for 5 minutes at 4℃. The supernatant were removed and the RNA pellet was air dried. RNA were dissolved in RNase-free water and incubated for 10 minutes at 58℃.

The quantity of RNA was determined by absorption at 260 nm. Purity of the total RNA was evaluated by the 260/280 nm ratio with values between 1.7 and 2. Integrity and quality of each RNA sample were estimated by visualization of clear

18S and 28S ribosomal RNA bands and the 28S band was two times bright as 18S after electrophoresis of 1 μg RNA in 1.5 % agarose gel.

5.2.6.2 cDNA synthesis

After treated with DNase (Turbo DNA-free Kit, Ambion), RNA was reversed transcribed to cDNA by using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Each reverse transcription run included five RNA samples without adding reverse transcriptase. A serial dilution of reference cDNA generated from a normal blood RNA pool.

5.2.6.3 Quantitative real-time PCR

Real-time PCR was performed with LightCycler 480 SYBR Green I Master (Roche, U.S) on 96 well iCycler thermal cycler (Bio-Rad Laboratories). Primers that flank the exon-exon junctions were designed by Primer3 and shown in Table 5.1. Relative expression of target genes was normalized against normalization factor and determined by efficiency corrected Ct model (Pfaffl, 2001): ratio= $(E_{target})^{\Delta CPtarget\ (calibrator\text{-}sample)} / (E_{reference})^{\Delta CPreference\ (calibrator\text{-}sample)}$ The normalization factor for each sample was calculated as geometric mean of relative quantities of three house-keeping genes GAPDH, RPS18, RPL11. CP value of calibrator sample was calculated by the standard curve equation for an assigned concentration for each gene. Standard curve of CP versus concentration was plotted to get the efficiency for each PCR reaction. This efficiency (E) can be obtained by the formula $10^{-(1/slope)}$, acceptable range 1.8–2.2. Each PCR run included a no-template control and a sample without reverse transcriptase. All measurements were performed in duplicate and averaged Ct value obtained was used for calculation. PCR products

were electrophoresed on 4% agarose gel to confirm only one gene-specific amplicon is amplified.

Table 5.1 Forward and reverse primers of each gene used for QRT-PCR

	Forward primer	Reverse primer
CTSB	5'-ggacaagcactacggatacaa-3'	5'-ggtacactcctgacttgtagagca-3'
CTSD	5'-ctggaccaggtggaggtg-3'	5'-ggatcatgtactcgccctga-3'
DDT	5'-ctccatcctgggcaaacct-3'	5'-actacgccgatggaggagat-3'
ITPKB	5'-cctgtgtgatggactgcaag-3'	5'-ggtccacctcgatcatcttc-3'
PIN1	5'-ctggtgaagcacagccagt-3'	5'-ctcccgacttgatcttctgg-3'
TSC1	5'-caagcacctcttttgccttc-3'	5'-caagcacctcttttgccttc-3'
UQCRC1	5'-tggctttcaagggaacaaag-3'	5'-ccacagctttcggcagat-3'
NRD1	5'-cttgatagatgggcgcagtt-3'	5'-aggccttgcaagttgatattc-3'
NDUFA6	5'-ccgaacactgtgcaccaa-3'	5'-cgatctttcccttaatgacca-3'
SNX2	5'-tggaaagttcagagctgccta-3'	5'-ttttcttcaaaccatgcatcc-3'
CNR2	5'-caacacaacccaaagccttc-3'	5'-ctgtcttctggggaccactc-3'
GSTM3	5'-ccgaacactgtgcaccaa-3'	5'-cgatctttcccttaatgacca-3'
GAPDH	5'-tgcaccaccaactgcttagc-3'	5'-ggcatggactgtggtcatgag-3'
RPL11	5'-ttccaaagctagatacactgtcaga-3'	5'-ccgcacctttagacccttct-3'
RPS18	5'-gaggatgaggtggaacgtgt-3'	5'-tcttcagtcgctccaggtct-3'

5.2.6.4 Quality control

Amplification efficiency for each primer pair was determined using a serial dilution of reference cDNA generated from a normal blood RNA pool to ensure that values were within linear range. Between-batch variation coefficient was determined by assaying the standard dilution with lowest concentration in 12 plates, and within-batch variation coefficient was determined by assaying the standard dilution with lowest concentration 20 times in a single plate. In our study, the PCR efficiencies of target genes ranged between 1.8 and 2.2. The between-batch and within-batch CV are 2.5-5.6% and 0.8-2.9% respectively.

5.2.7 Statistical analysis

5.2.7.1 Sample size estimation

Sample size was estimated by taking the potential difference of relative expression in CTSB between AD and NC. Among the candidate genes selected in the present study, CTSB was reported to be differentially expressed in peripheral blood with the minimum difference of 1.2 fold between AD and normal controls (Maes et al., 2007). The estimated difference suggested that total 200 subjects will have 90% power in detecting difference of 1.2 fold at a two-sided 0.05 significance level. Post-hoc power analysis was also reported in the Results section to illustrate the study power of subjects recruited.

5.2.7.2 Statistical analysis

The data was analyzed with the Statistical Package for Social Science for

windows, version 16.0. Continuous variables were compared with ANOVA or t-test, categorical variables were compared with chi-square test. Data with skewed distribution were analyzed with non-parametric tests. Logistic regression was adopted to analyze the predictive effect of gene expressions in the development of disease after adjustment for age, sex, education level, total CNPI score and ApoE4 status. Receiver operating characteristics (ROC) curves was constructed to evaluate the discriminative power for each gene. Linear regression analysis was performed to investigate the influence of each gene expression on cognitive and NP profiles after adjustment for age, education level, sex, total CIRS score and ApoE4 status. Bonferroni correction was adopted to adjust for multiple testing. A p value of <0.05 was considered significant.

5.2.7.3 Classification and gene biomarker selection

In order to optimize the combination of gene biomarker panel in producing the better predictive model, genes were selected based on two steps. Firstly, differentially expressed genes were selected based on Kruskal-Wallis test result ($p<0.05$). To get more conserved (small) gene feature sets, genes identified in the first step were further selected by backward conditional logistic regression in discriminating between AD and NC subjects. The final selected genes were set up as a gene biomarker panel. The binary diagnostic variable Y (control, 0; AD, 1 or control, 0; MCI, 1 or MCI, 0; AD, 1) was considered as a dependent variable, expression ratios of each selected gene of the biomarker panel were identified as covariables. When P is defined as the probability of a sample diagnosed as "AD" or "MCI", the function of Logit (P) is defined as following: $\text{Logit}P=\ln(P/(1-P))= b_0+b_1\text{Ratio}_1+b_2\text{Ratio}_2+b_3\text{Ratio}_3+\cdots\cdots+b_n\text{Ratio}_n$. Regression coefficients $\{b_n\}$ were

determined by Maximum-likelihood fitting method. ROC curve analysis was performed to evaluate the discriminatory power of gene combination in discriminating AD versus NC, MCI versus NC or AD versus MCI respectively. Sensitivity, specificity, positive and negative predictive values were calculated at different cut-off thresholds of Logit (*P*) value.

5.2.7.4 Cross validation

10-fold cross validation was adopted to assess the prediction accuracy of 5-gene biomarkers panel in our data. The samples were randomly divided into ten equal subsets. One from each of the ten subsets was left out in turn for evaluation of classification accuracy as a testing set, while the other nine training subsets were used as inputs to the classification model. This process was repeated 1000 times. The median and 95% confidence interval of AUC were calculated.

Chapter 6 Results

The results are summarized in three parts. In the first part, the demographic and clinical profiles were analyzed and compared among three groups. In the second part, gene expression levels were compared among AD, MCI and NC groups and genes were selected to optimize the combination of gene biomarkers panels in discriminating three groups. The third part, the association between each gene expression, cognitive and NP profiles was investigated in AD group.

6.1 Clinical profiles

6.1.1 Demographic characteristics (Table 6.1)

96 subjects with AD (87.5% women; mean age in years = 82.9, SD=6.0; range = 66-96; Education in year =1.1, SD=2.4; range = 0-11), 145 subjects with MCI (64.1% women; mean age in years =75.1, SD=8.2; range = 59-97; Education in year =3.5, SD=4.0; range = 0-16) were recruited in the study. Among the AD patients, 78 (81.2%) were suffering from mild dementia (CDR=1) and 18 (18.8%) were moderate dementia (CDR=2). 167 NC subjects (55.1% women; mean age in years =70.1, SD=7.1; range = 60-97; Education in year =6.6, SD=4.7; range = 0-18) were recruited. There are significant differences in gender, age, and education level among the three groups (Gender, Pearson χ^2 =28.8, df 2, p <0.001; Age, ANOVA, F=93.5, df 2, p<0.001; Education level, Kruskal-Wallis test, χ^2 =106.7, df 2, p<0.001). Further comparative studies in gender, age, and education level between any two of three groups showed there was significant differences (Gender, Pearson χ^2 test, P<0.05; Age, t-test, P<0.05; Education level, Mann-Whitney U test, p<0.05).

Table 6.1 Demographic characteristics of the AD, MCI and NC groups

	AD	MCI	NC	Differences	p-value
Sex (F/M)	84/12	93/52	92/75	$\chi 2$ =28.8	<0.001
Age in years (SD)	82.9 (6.0)	75.1 (8.2)	70.1 (7.1)	F=93.5	<0.001
Education (years)	1.1 (2.4)	3.5 (4.0)	6.6 (4.7)	$\chi 2$ =106.7	<0.001

6.1.2 Cognitive profiles (Table 6.2)

The mean (SD) MMSE score for AD, MCI and NC groups were 16.52 (3.56), 24.13 (2.80) and 27.39 (2.17) respectively. For the total ADAS-Cog score, the mean (SD) were 27.83 (7.29), 15.57 (5.57) and 8.56 (3.80) in three groups. The mean (SD) delay recall of list learning test were 0.76 (1.30) in AD subjects, 3.30 (2.37) in MCI subjects and 6.29 (2.04) in NC group. The mean (SD) combined CVFT score was 18.40 (7.20) in subjects with AD, 29.37 (7.20) in MCI and 36.36 (7.79) in NC group. There are significant differences in cognitive profiles among the three groups (Kruskal-Wallis test, $p<0.001$).

Table 6.2 Cognitive profiles of AD, MCI and NC groups

	AD	MCI	NC	χ^2	p-value
MMSE*	16.52 (3.56)	24.13 (2.80)	27.39 (2.17)	249.31	<0.001
ADAS-Cog Total score*	27.83 (7.29)	15.57 (5.57)	8.56 (3.80)	242.05	<0.001
Delayed recall*	0.76 (1.30)	3.30 (2.37)	6.29 (2.04)	207.47	<0.001
CVFT*	18.40 (7.20)	29.37 (7.20)	36.36 (7.79)	173.92	<0.001

MMSE, Mini-Mental State Examination; ADAS-Cog, Alzheimer's disease Assessment scale-Cognitive subscale; Delay recall, Delay recall of list learning test; CVFT, Category verbal fluency test.
*Kruskal-Wallis test, $p<0.001$

6.1.3 Profile of Neuropsychiatric symptoms (Table 6.3)

NP symptoms were evaluated by using the Chinese version of the NPI. The score of "Delusions" (Kruskal-Wallis test, $\chi 2$=13.11, p=0.001), "Apathy" (Kruskal-Wallis test, $\chi 2$=26.77, p<0.001), "Aberrant motor behavior" (Kruskal-Wallis test, $\chi 2$=6.97, p=0.031) and total CNPI score (Kruskal-Wallis test, $\chi 2$=16.22, p<0.001) were significantly different among the three groups.

Table 6.3 Profile of NP symptoms in AD, MCI and NC groups

NPI scores	AD	MCI	NC	χ^2	P-value
Delusions*	0.13 (0.47)	0.01 (0.08)	0.05 (0.70)	13.11	**0.001**
Hallucinations	0 (0)	0.01 (0.08)	0.02 (0.23)	0.58	0.747
Aggressivity	0.09 (0.45)	0.04 (0.35)	0.05 (0.48)	1.97	0.374
Depression	0.45 (1.22)	0.38 (1.38)	0.23 (1.01)	3.53	0.171
Anxiety	0.29 (1.11)	0.22 (0.72)	0.26 (0.88)	1.45	0.484
Euphoria	0.02 (0.21)	0.03 (0.25)	0 (0)	3.33	0.189
Apathy*	1.01 (1.97)	0.33 (0.86)	0.18 (0.87)	26.77	**<0.001**
Disinhibition	0.05 (0.26)	0.01 (0.08)	0.02 (0.19)	2.33	0.312
Irritability	0.13 (0.70)	0.09 (0.47)	0.36 (0.03)	1.77	0.413
Aberrant motor behavior*	0.23 (1.11)	0.06 (0.53)	0 (0)	6.97	**0.031**
Abnormal night-time behavior	0.33 (1.01)	0.32 (1.16)	0.25 (0.73)	0.14	0.930
Appetite disturbances	0.11 (0.88)	0.02 (0.19)	0.04 (0.35)	0.46	0.795
Total CNPI score*	2.86 (4.80)	1.51 (3.78)	1.12 (3.61)	16.22	**<0.001**

*Kruskal-Wallis test, $p<0.05$

6.1.4 Medical comorbidity

A cumulative illness rating scale (CIRS) with a physical illness checklist was used to document the number and severity of medical conditions in all subjects. The mean (SD) of CIRS total score in AD, MCI and NC groups are 2.56 (2.19), 2.34 (2.16) and 1.93 (1.86) respectively. The total CIRS score of AD subjects were higher than that in NC subjects (Mann-Whitney U test, z= -2.16, p=0.029).

6.1.5 ApoE gene polymorphisms (Table 6.4)

90 AD, 135 MCI subjects and 153 NC were genotyped with ApoE polymorphisms. The frequency of E4 allele (2/4, 3/4 and 4/4 genotype) in AD group was 10.0%, higher than that in NC group (7.1%), but the difference did not reach statistical significance (Pearson $\chi 2$ = 1.30, df 2, p=0.522). Neither was found between MCI group and NC group (Pearson $\chi 2$ = 4.47, df 2, p=0.107).

Table 6.4 ApoE allele distribution in AD, MCI and NC groups

	AD	MCI	NC	$\chi 2$	p-value
E2 allele	21 (11.7)	28 (10.4)	36 (11.6)		
E3 allele	141 (78.3)	209 (77.4)	252 (81.3)	4.53	0.339
E4 allele	18 (10.0)	33 (12.2)	22 (7.1)		

6.2 Validation of gene expression diagnostic potential in AD and MCI

6.2.1 Comparative analysis between gene expression levels of AD, MCI and NC groups (Table 6.5 and Figure 6.1-6.3)

Seven genes were found differently expressed between AD and NC group, with upregulation of CTSB (Mann-Whitney U test, z= -4.48, p<0.001), CTSD (Mann-Whitney U test, z= -4.77, p<0.001), DDT (Mann-Whitney U test, z= -2.63, p=0.009), TSC1 (Mann-Whitney U test, z= -2.64, p=0.008) and UQCRC1 (Mann-Whitney U test, z= -5.94, p<0.001), and downregulation of ITPKB (Mann-Whitney U test, z= -4.14, p<0.001) and PIN1(Mann-Whitney U test, z= -2.64, p=0.008) in AD patients. Two genes expression levels were found increased in MCI group compared with NC group, including CTSB (Mann-Whitney U test, z= -3.31, p=0.001), CTSD (Mann-Whitney U test, z= -2.04, p=0.041). In addition, an upregulation of CTSD (Mann-Whitney U test, z= -2.67, p=0.008), UQCRC1 (Mann-Whitney U test, z= -3.90, p<0.001), NRD1 (Mann-Whitney U test, z= -2.00, p=0.046) and downregulation of ITPKB (Mann-Whitney U test, z= -3.14, p=0.002) were observed in AD subjects in comparison with MCI group.

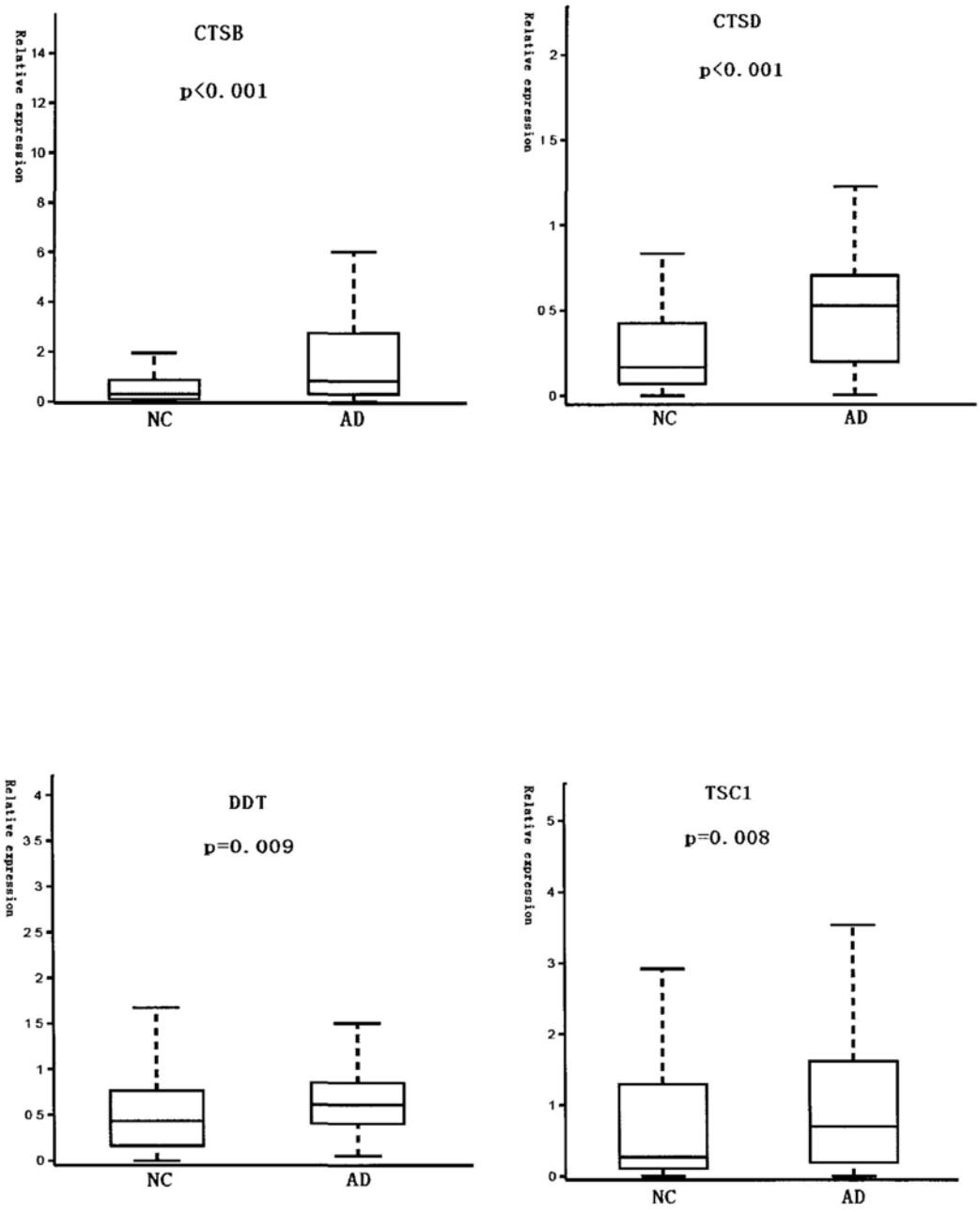
The expression levels of NDUFA6, SNX2, CNR2 and GSTM3 were not significantly different between AD, MCI and NC groups. Post-hoc power analyses showed that the present sample, with 89 AD and 145 NC, was able to detect the smallest difference of 1.11 fold change in SNX2 gene at a significance level of 0.05 with a satisfactory power of 95%.

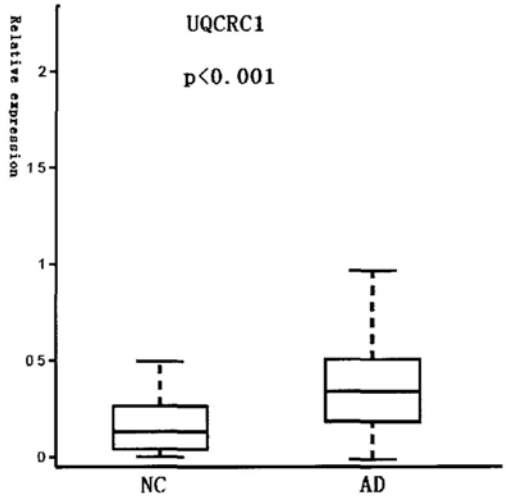
Table 6.5 Relative gene expression levels in AD, MCI and NC groups

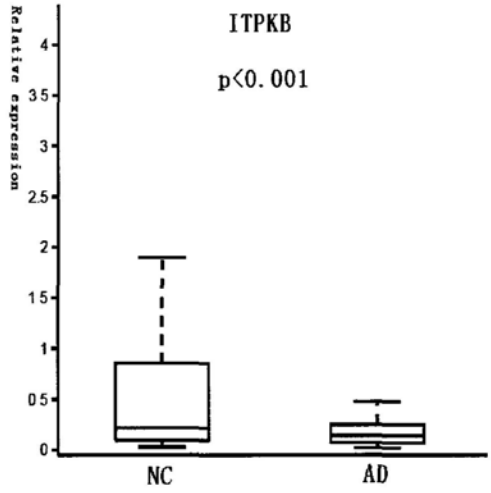
Relative expression (SD)	**AD**	**MCI**	**NC**	**χ^2**	**p-value**	**Up/down regulation**
CTSB*#Δ	2.86 (6.53)	1.84 (3.32)	1.32 (4.45)	22.78	**<0.001**	up
CTSD*#Δ&	0.50 (0.32)	0.41 (0.43)	0.32 (0.39)	22.41	**<0.001**	up
DDT*#	0.71 (0.70)	0.86 (1.22)	0.74 (1.23)	6.55	**0.038**	up
ITPKB*#&	0.36 (1.73)	0.73 (2.21)	0.88 (1.79)	17.97	**<0.001**	down
PIN1*#	0.45 (0.95)	0.66 (1.36)	0.69 (0.97)	7.12	**0.029**	down
TSC1*#	1.42 (2.25)	1.10 (1.60)	1.25 (3.05)	7.04	**0.030**	up
UQCRC1*#&	0.44 (0.38)	0.33 (0.51)	0.21(0.30)	34.74	**<0.001**	up
NRD1&	0.62 (0.51)	0.57 (0.69)	0.53 (0.46)	4.26	0.119	up
NDUFA6	0.39 (0.59)	0.55 (0.74)	0.78 (1.13)	0.07	0.968	
SNX2	0.34 (0.87)	0.34 (0.37)	0.38 (0.49)	3.22	0.322	
CNR2	0.35 (0.66)	0.28 (0.33)	0.27 (0.53)	3.41	3.414	
GSTM3	0.63 (0.97)	1.21 (3.91)	0.56 (1.03)	3.26	3.260	

*Kruskal-Wallis test, $p<0.001$
#Mann-Whitney U test, between AD and NC group, $p<0.05$
ΔMann-Whitney U test, between MCI and NC group, $p<0.05$
&Mann-Whitney U test, between MCI and AD group, $p<0.05$

Fig 6.1 Differentially expressed genes levels between AD and NC groups




UQCRC1
p<0.001
Relative expression
2
1.5
1
0.5
0
NC
AD


ITPKB
p<0.001
Relative expression
4
3.5
3
2.5
2
1.5
1
0.5
0
NC
AD

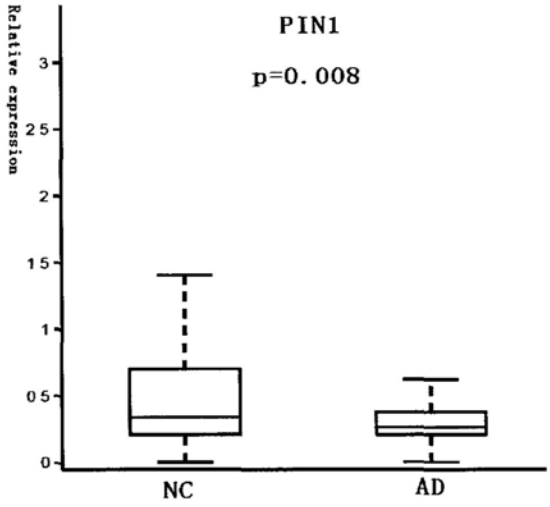

PIN1
p=0.008
Relative expression
3
2.5
2
1.5
1
0.5
0
NC
AD

Fig 6.2 Differentially expressed genes levels between MCI and NC groups

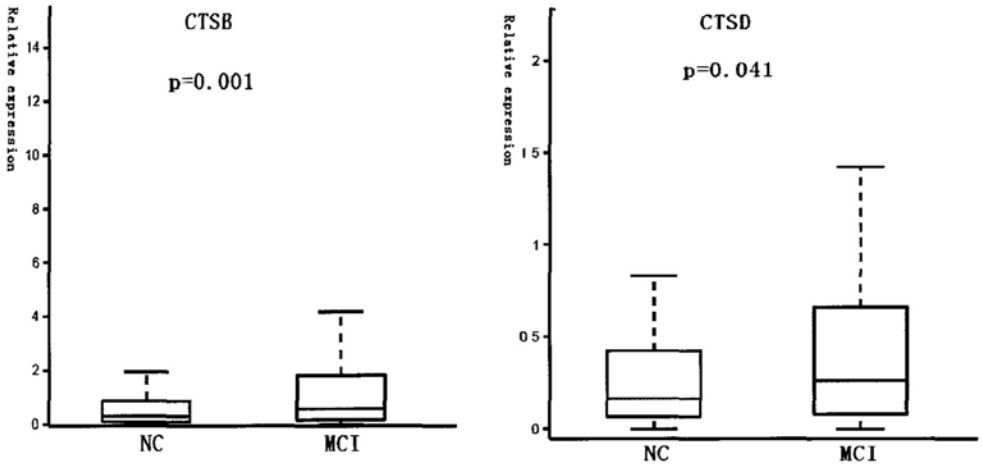
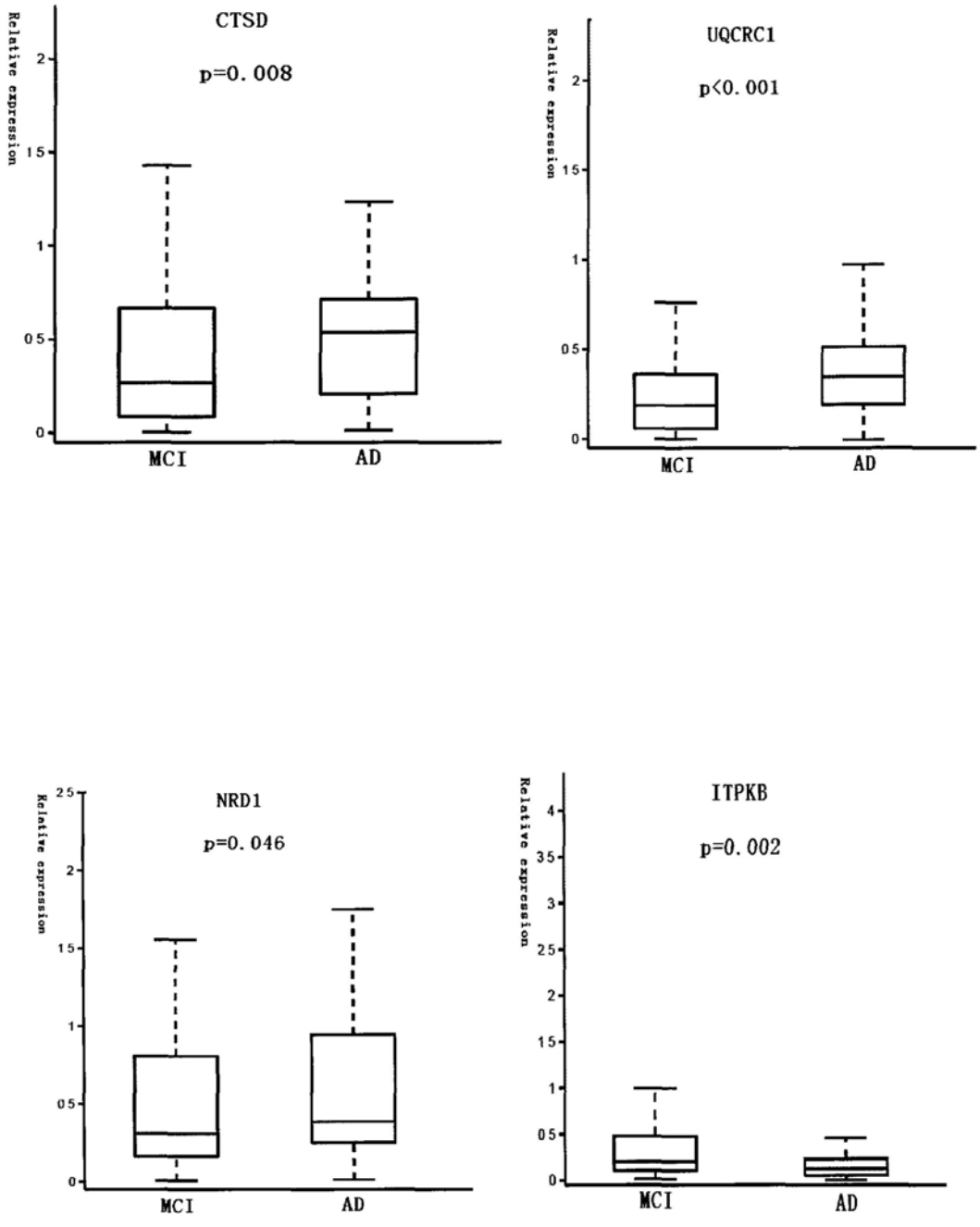


Fig 6.3 Differentially expressed genes levels between AD and MCI groups



6.2.2 Logistic-regression analysis of gene expression with clinical variables

Logistic regression analysis showed that expression level of any single gene was not significant contributor to AD or MCI after adjusting for confounding factors of age, gender, education level, ApoE4 status and the total CIRS score ($p>0.05$). Age (Odds ratio>1, $p<0.001$) and education level (Odds ratio<1, $p<0.001$) were associated with AD or MCI.

6.2.3 Classification using gene biomarkers (Table 6.6, 6.7 and 6.8)

The expression levels of 12 genes were compared among the NC, MCI and AD groups (Table 6.5). Significant differences were found in seven genes including CTSB (Kruskal-Wallis test, $\chi2=22.77$, $p<0.001$), CTSD (Kruskal-Wallis test, $\chi2=22.41$, $p<0.001$), DDT (Kruskal-Wallis test, $\chi2=6,55$, $p<0.038$), ITPKB (Kruskal-Wallis test, $\chi2=17.97$, $p<0.001$), PIN1 (Kruskal-Wallis test, $\chi2=7,12$, $p=0.029$), TSC1 (Kruskal-Wallis test, $\chi2=7.04$, $p=0.030$), and UQCRC1 (Kruskal-Wallis test, $\chi2=34.74$, $p=0.029$). The expression levels of these seven genes were further analyzed by backward conditional logistic regression and five genes including, DDT, ITPKB, PIN1, TSC1 and UQCRC1 were selected as the optimal gene features in discriminating AD and controls (Table 6.6). Then the five genes were set up as a 5-gene biomarker panel.

ROC analysis of expression data for each of five genes resulted in AUC value between 0.36-0.72 to classify AD, MCI and NC (Table 6.7). To evaluate the discriminatory power of the biomarker combination, logistic regression analysis

was analyzed for the relative expression levels of these five genes. The best combination equation can be formulated in discriminating between AD and NC as following:logit(P)=ln(P/(1-P))=-0.865+1.264R_{DDT}-1.145R_{ITPKB}-1.465R_{PIN1}+0.201 R_{TSC1} +1.643R_{UQCRC1}. Between MCI and NC, the equation was formulated as following:logit(P)=ln(P/(1-P))= -0.433+0.258R_{DDT}-0.258R_{ITPKB}-0.189R_{PIN1}+0.076 R_{TSC1}+0.734R_{UQCRC1}. Between AD and MCI, the combination equation was shown as following: logit(P) = ln(P/(1-P))= -0.502+0.458R_{DDT}-1.361R_{ITPKB}-0.259R_{PIN1}+ 0.161R_{TSC1}+0.751R_{UQCRC1}. The AUC for the 5-gene biomarkers panel in differentiating between AD and NC, between MCI and NC, between AD and MCI were 0.79 (95% CI, 0.72-0.86; p<0.001), 0.61 (95% CI, 0.53-0.69; p=0.007) and 0.68 (95% CI, 0.60-0.76; p<0.001) respectively (Table 6.7, Fig 6.4-6.6). The sensitivity, specificity, positive and negative predictive values were calculated in classifying two conditions of AD, MCI and NC subjects at different cut-off of Logit(P) value (Table 6.8).

Table 6.6 Variables selected in the final step of backward logistic regression

Step3	B	S.E	Wald	Sig.	Exp(B)	95% CI
DDT	1.28	0.42	9.47	0.002	3.60	1.59-8.14
ITPKB	-1.08	0.61	3.16	0.076	0.34	0.10-1.12
PIN1	-1.60	0.78	4.17	0.041	0.20	0.04-0.94
TSC1	0.19	0.11	2.89	0.089	1.21	0.97-1.51
UQCRC1	1.60	0.50	10.13	0.001	4.96	1.85-13.30

Table 6.7 AUC of each gene and 5-gene biomarker panels used for logistic regression

	AD vs NC	MCI vs NC	AD vs MCI
	AUC (95% CI)	**AUC (95% CI)**	**AUC (95% CI)**
DDT	0.62 (0.54-0.70)	0.55 (0.47-0.63)	0.56 (0.48-0.64)
ITPKB	0.36 (0.29-0.44)	0.48 (0.40-0.56)	0.38 (0.30-0.46)
PIN1	0.40 (0.32-0.48)	0.45 (0.37-0.53)	0.45 (0.36-0.53)
TSC1	0.62 (0.51-0.70)	0.54 (0.46-0.62)	0.58 (0.50-0.66)
UQCRC1	0.72 (0.65-0.80)	0.56 (0.48-0.64)	0.65 (0.57-0.73)
5-gene panel	0.79 (0.72-0.86)	0.61 (0.53-0.69)	0.68 (0.60-0.76)

Table 6.8 Sensitivity, specificity, positive and negative predictive values at different logit (P) cut-off value in classify AD, MCI and NC subjects by 5-gene panel

	Cut-off	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
AD vs NC	0.457	76.8	78.1	73.3	81.1
	0.464	74.4	81.9	76.3	80.4
	0.486	**70.7**	**86.7**	**80.6**	**79.1**
	0.516	67.1	86.7	79.7	77.1
	0.576	58.5	87.6	78.7	73.0
MCI vs NC	0.486	51.5	67.6	60.0	59.7
	0.491	50.5	72.4	63.3	60.8
	0.505	**42.4**	**76.2**	**62.7**	**58.4**
	0.510	39.4	78.3	62.9	57.7
	0.518	36.4	81.9	65.5	57.7
AD vs MCI	0.439	73.2	57.6	58.8	72.2
	0.468	72.0	65.7	63.4	73.9
	0.496	**61.0**	**73.7**	**65.8**	**69.5**
	0.531	50.0	76.8	64.0	65.0
	0.550	39.0	78.8	60.4	60.9

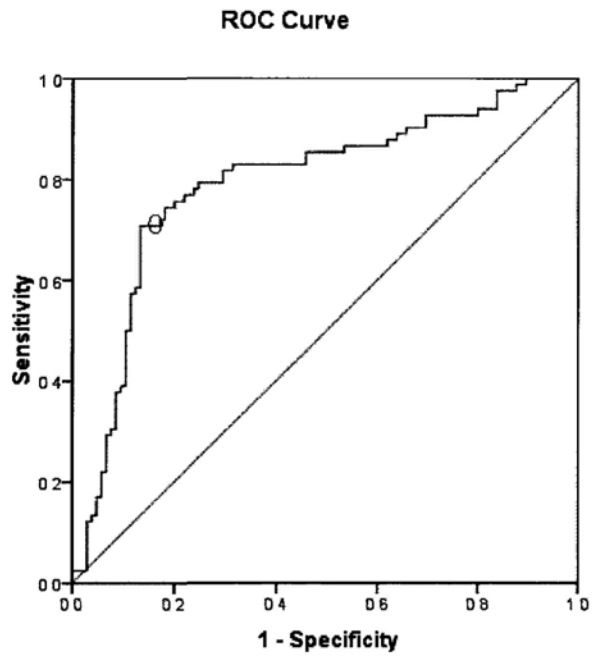


Fig 6.4 Discrimination power of 5-gene biomarkers panel between AD and NC

(Threshod: Logit(P)=0.486, sensitivity: 70.7%, 1-specificity: 13.3%)

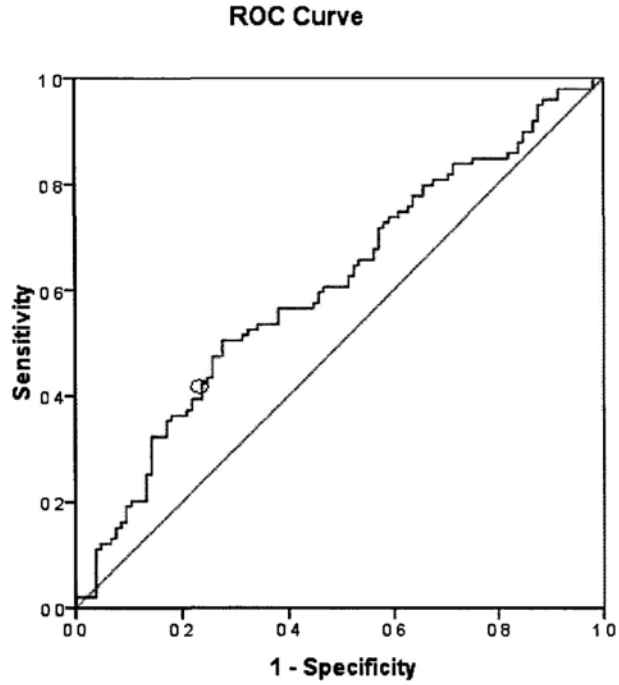


Fig 6.5 Discrimination power of 5-gene biomarkers panel between MCI and NC

(Threshod: Logit(P)=0.505, sensitivity: 42.4%, 1-specificity: 23.8%)

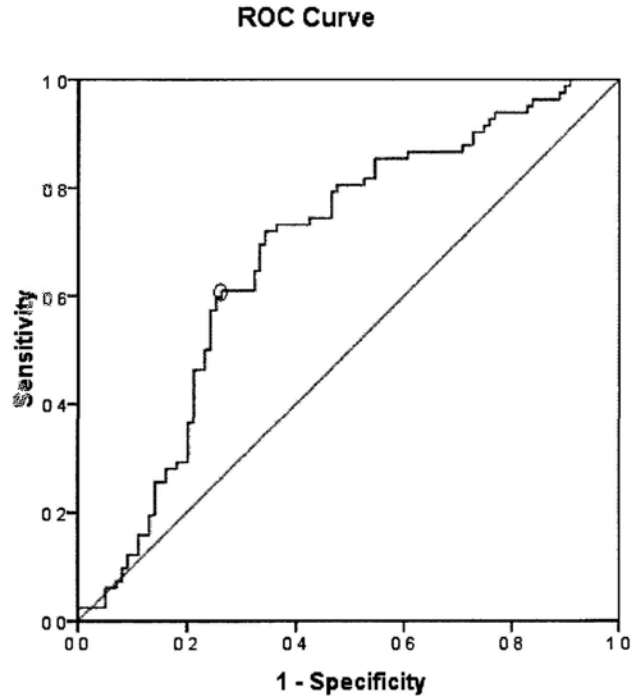


Fig 6.6 Discrimination power of 5-gene biomarkers panel between AD and MCI

(Threshold: Logit(P)=0.496, sensitivity: 61.0%, 1-specificity: 26.3%)

6.2.4 Cross validation

10-fold cross validation of our data in 1000 times showed that the medians of 5-gene biomarkers panel in discriminating AD from NC, MCI from NC and AD from MCI were 0.77 (95%CI: 0.69-0.84), 0.59 (95%CI: 0.52-0.65) and 0.65 (95%CI: 0.57-0.73) respectively.

6.3 Association between gene expression and cognitive and NP profiles in AD

6.3.1 Gene expression levels and cognitive profiles in AD group (Table 6.9)

Linear regression analysis for all 12 genes on each cognitive score were conducted after adjustment for age, sex, education levels, total CRIS score and ApoE4 status. Two genes including ITPKB (Linear regression, p=0.021) and NDUFA6 (Linear regression, p=0.011) showed a tendency to correlate with MMSE score. CTSD showed a tendency to correlate with ADAS-Cog score (Linear regression, p=0.049). After making Bonferroni correction, only expression level of NDUFA6 were still significantly associated with MMSE score.

Table 6.9 Regression coefficients and p-value of linear regression analysis between cognitive profiles and each gene expression in AD group

	MMSE		ADAS-Cog		Delayed recall		CVFT	
	coefficient	p	coefficient	p	coefficient	p	coefficient	p
CTSB	-0.01	0.945	-0.19	0.247	-0.04	0.825	-0.03	0.847
CTSD	-0.12	0.532	0.43	**0.049**	-0.33	0.150	-0.24	0.226
DDT	0.05	0.816	-0.10	0.646	0.26	0.275	0.29	0.150
ITPKB	-0.55	**0.021**	0.16	0.515	-0.18	0.512	-0.07	0.752
PIN1	0.19	0.361	0.17	0.446	0.09	0.704	-0.17	0.416
TSC1	-0.08	0.644	0.04	0.839	-0.13	0.487	0.01	0.977
UQCRC1	0.18	0.490	-0.26	0.367	0.29	0.356	0.21	0.449
NRD1	-0.02	0.895	0.22	0.274	-0.07	0.730	0.02	0.933
NDUFA6	0.51	**0.011***	-0.20	0.360	0.21	0.373	0.13	0.529
SNX2	-0.49	0.070	0.24	0.415	-0.14	0.649	-0.10	0.724
CNR2	0.09	0.547	-0.03	0.835	-0.02	0.897	-0.07	0.655
GSTM3	0.01	0.966	-0.20	0.427	-0.16	0.555	-0.33	0.159

Adjusted for age, sex, education year, CIRS total score, ApoE4 status in multiple linear regression model, *$p<0.02$

6.3.2 Gene expression levels and NP profiles in AD group (Table 6.10)

Linear regression analysis for all 12 genes on each subsymptom score and total CNPI score were conducted after adjustment for age, sex, education levels, total CRIS score and ApoE4 status in AD group. Since none of the AD subjects have reported the symptom of "Hallucination", the score of "Hallucination" could not be performed with linear regression. Several genes expression levels showed a tendency to correlate with NP subsymptom score and total CNPI score, however, after adjusting for multiple testing, the expression levels of TSC1 were associated with the score of "Delusion". DDT expression levels were significantly associated with the score of "Depression". CTSD, DDT and UQCRC1 expression were associated with the score of "Aberrant motor behavior". For total CIRS score, the significant correlation was found in DDT gene (Linear regression, $P<0.005$).

Table 6.10 Regression coefficients and p-value of linear regression analysis between NP profiles and each gene expression in AD group

	Delusion		Aggressivity		Depression		Anxiety		Euphoria		Apathy	
	coefficient	p	coefficient	p	coefficient	p	coefficient	p	coefficient	p	coefficient	p
CTSB	-0.28	0.052	0.001	0.994	-0.09	0.521	-0.03	0.850	-0.04	0.794	-0.12	0.486
CTSD	-0.22	0.291	-0.25	0.258	-0.53	**0.008**	-0.24	0.331	-0.07	0.759	-0.13	0.589
DDT	0.57	**0.007**	0.42	0.055	0.71	**<0.001***	0.20	0.400	0.32	0.136	-0.10	0.672
ITPKB	-0.44	0.071	0.53	**0.042**	0.02	0.934	0.06	0.834	0.52	**0.046**	-0.17	0.569
PIN1	0.16	0.129	-0.29	0.219	0.13	0.531	-0.08	0.744	-0.46	0.054	0.21	0.426
TSC1	0.13	**0.001***	-0.15	0.404	0.004	0.978	-0.06	0.746	-0.20	0.256	-0.02	0.939
UQCRC1	0.33	0.596	0.67	**0.031**	0.35	0.197	0.33	0.333	0.26	0.397	-0.17	0.621
NRD1	0.14	0.489	0.28	0.182	0.44	**0.018**	0.23	0.322	0.06	0.766	-0.03	0.914
NDUFA6	0.59	0.431	-0.09	0.699	-0.07	0.734	0.40	0.103	-0.06	0.785	-0.07	0.793
SNX2	-0.15	0.603	-0.05	0.863	-0.01	0.969	-0.26	0.421	-0.08	0.783	-0.10	0.774
CNR2	-0.08	0.585	0.07	0.658	0.05	0.730	0.01	0.950	0.13	0.411	-0.12	0.502
GSTM3	0.14	0.569	-0.58	**0.028**	-0.50	**0.032**	-0.08	0.774	-0.08	0.756	0.05	0.863

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	Disinhibition		Irritability		Aberrant motor behavior		Abnormal night-time behavior		Appetite disturbances		Total CNPI score	
	coefficient	p	coefficient	p	coefficient	p	coefficient	p	coefficient	p	coefficient	p
CTSB	-0.04	0.770	0.13	0.936	-0.14	0.237	-0.17	0.300	-0.04	0.794	-0.17	0.269
CTSD	0.02	0.935	-0.23	0.307	-0.60	**0.001***	-0.05	0.814	-0.07	0.759	-0.43	**0.045**
DDT	0.31	0.146	0.34	0.131	0.71	**<0.001***	0.29	0.201	0.32	0.136	0.64	**0.004***
ITPKB	0.12	0.632	0.37	0.165	-0.49	**0.015**	-0.61	0.028	0.52	**0.046**	-0.03	0.923
PIN1	-0.07	0.763	-0.05	0.832	0.16	0.334	0.09	0.701	-0.06	0.785	0.16	0.491
TSC1	-0.11	0.591	0.25	0.238	0.30	0.055	0.18	0.399	0.06	0.766	0.36	0.087
UQCRC1	-0.01	0.955	-0.18	0.451	0.57	**0.002***	0.60	**0.016**	-0.46	0.054	0.14	0.533
NRD1	0.10	0.734	-0.01	0.973	-0.30	0.173	-0.05	0.883	-0.08	0.783	-0.27	0.375
NDUFA6	0.26	0.136	-0.08	0.663	-0.02	0.882	-0.05	0.808	-0.20	0.256	-0.03	0.874
SNX2	0.07	0.813	0.53	0.092	-0.04	0.879	-0.25	0.437	0.26	0.397	0.32	0.297
CNR2	-0.04	0.782	0.04	0.808	0.05	0.709	0.05	0.746	0.13	0.411	0.05	0.761
GSTM3	0.20	0.439	-0.43	0.113	-0.10	0.623	-0.16	0.560	-0.08	0.756	-0.29	0.266

Adjusted for age, sex, education year, CIRS total score, ApoE4 status in multiple linear regression model, *p<0.005

Chapter 7 Discussion

AD is a complex disorder resulting from the interplay of genetic and environmental factors. Multiple genes with small effects are likely to contribute to the onset and progression of the disease. The heterogeneity nature in etiological factors and pathological mechanisms presented a diagnostic and therapeutic challenge of AD. Current diagnosis of AD depends on the combination of clinical, neuropsychological, and imaging assessments, being identified until the patients have apparent clinical symptoms. However, early intervention is a key to successful management of AD. From this perspective, additional disease markers would be of great value as diagnostic tools aiding in early detection of AD, as well as prediction of incipient Alzheimer's disease.

This thesis presented the findings of a cross-sectional study of the gene expression profiling in AD, MCI and controls and explored the potential of using gene expression as peripheral biomarkers for early diagnosis of AD. Combined gene expression biomarkers were shown to improve the diagnostic capability than only single gene. Association between several gene expression and quantitative phenotypes of cognitive and NP profiles further indicated the underlying biological mechanisms involved in the cognitive and NP manifestations of AD.

7.1 Clinical profiles of AD and MCI subjects

In the present study, the significant differences in age, education levels, female proportion, and total CIRS score between AD subjects and healthy controls substantiated the findings that older age, less education, female gender and medical comorbidity significantly increase the risk of AD. Logistic regression showed after adjustment for the confounding effects of age, gender, education level, ApoE4 status and CIRS total score, expression level of any single gene studied did not contribute significantly to AD or MCI. This suggested a minor effect of individual genetic factor in the development of disease. It also indicated that single gene expression biomarker may not be a determinant factor in the diagnosis of Alzheimer's disease. A marker panel may be more informative. On the other hand, it is possible that gene expression may interact with confounding factors such as age and gender to influence the risk of Alzheimer's disease.

Age and gender were previously reported to be associated with gene expression variation (Kim et al., 2007). Age is the most important determinant for Alzheimer's disease. Gene expression variation was assumed to affect the susceptibility of disease or represent the consequence or response to disease pathophysiology. Gene expression alteration interacts with age, also indicating it is a risk factor for Alzheimer's disease. But whether gene expression alteration follow or drive the process of aging still remain uncertain. Moreover, the current sample comprises subjects with geriatric age group. Although the age difference between

case and control was statistically significant, we would like to highlight that they still represent a clinically matched group. In addition, through searching a recently published meta analysis of age-related gene expression profiles (de Magalhaes et al., 2009), only expression level of one gene was reported to be associated with aging. This also lends support for our speculation that gene expression alteration may be an independent determinant for disease pathophysiology.

Gender may confound the association between peripheral gene expression and disease pathophysiology. We did not confine the comparative analysis by stratification of gender, because the sample size of male in AD group was so small that it may not have enough power to detect the significant differences. On the other hand, these genes were not linked to sex chromosome and it was thought that the gene expression variation between case and controls was larger than that between female and male. Logistic regression also indicated gender was not a significant contributor to AD or MCI in the present data.

ApoE is the only confirmed risk gene in late-onset AD. E4 allele is a risk factor and E2 allele is protective. In the present study, the prevalence of ApoE4 allele in healthy controls is 7.1% , which is comparable with other similar results in Chinese (Wong et al., 2005, Mak et al., 1996). However, the difference in the frequency of ApoE4 allele did not reach statistical significance between AD and healthy controls. This may be due to relative small sample size in the present study.

Post-hoc analyses showed a lack of statistical power for detecting significant differences. On the other hand, southern Chinese population has a lower genetic risk of AD attributable to Apo E4 allele.

7.2 Gene expression as peripheral biomarkers for AD

Numerous efforts have been made to look for potential biomarkers for AD, which may allow for more accurate and earlier diagnosis. Biomarkers also aid in tracking disease progression and evaluating therapeutic response. Although a wealth of biomarker candidates for AD has been identified, there has been no single biomarker that is reliable enough to be used in clinical practice. The identification of reliable biomarker is hindered by various limitations including methodological variability, inaccurate diagnosis protocol, and heterogeneity nature of the disease (Craig-Schapiro et al., 2009). Standard protocols for clinical assessment, assay methods and statistical analysis are required to overcome some limitations. Additionally, combination of biomarker panel is more informative for comprehensive evaluation of disease process as for AD, a complex disorder with multifactorial nature.

Peripheral gene expression has received special attention as potential biomarkers for AD. There is evidence to support that patterns of gene expression profiling in peripheral blood may reflect transcriptional changes in AD brain (Liew et al., 2006, Sullivan et al., 2006). Moreover, gene expression profiling allows for

reflecting body response to both inherited and environmental changes in a summative manner. In this study, we validated five differentially expressed genes between AD and healthy controls. The 5-gene combination was found to classify AD subjects from controls with higher discriminatory power, with the AUC ranging from 0.36-0.72 for each of the five genes to 0.78 for the biomarker combination. The sensitivity and specificity of 5-gene panel were influenced by chosen cut-off thresholds, with lower cut-off improving sensitivity but at the price of lower specificity. To identify the optimal cut-off, we chose decision criteria of excluding patients without disease as the primary concern, indicating lower type II error and higher specificity. The decision was based on several concerns. The gold standard for definitive diagnosis of AD is autopsy. Current clinical diagnosis, focusing on the exclusion of other causes of dementia refers to possible or probable AD. Although cognitive assessment such as MMSE is a widely used screening tool for dementia, it may not be used as a diagnostic test. From this perspective, the value of gene expression testing is that it showed satisfactory representative of AD process by demonstrating good sensitivity and specificity. On the other hand, MCI, as a more heterogeneous entity is not an absolute intermediate point between AD and normal aging. The subtype of aMCI with early AD pathology have a higher chance to progress to AD while non-amnestic subtypes are more likely to convert to non-AD dementia (Petersen and Negash, 2008). Therefore, it is important to identify those MCI patients without underlying pathological AD, as they are likely

to be stable and will not progress to AD. In this regard, high specificity will be an important issue for case identification.

The 5-gene combination was found to discriminate AD subjects from normal controls with good sensitivity and specificity of 70.7% and 86.7% respectively at an optimal cut-off of 0.486. Cross validation showed good predictive accuracy with the median of AUC of 0.77, suggesting this 5-gene biomarker set represents a potential diagnostic test for AD. However, the method of gene expression profiling is not better than any cognitive measures (Chu et al., 2000, Chiu HFK et al., 1994, Chiu HF et al., 1997), therefore it may serve as an adjuvant marker independent of cognitive assessment.

The low level of discriminatory power with unsatisfactory sensitivity (42.4%) in classifying MCI subjects and normal controls suggested that candidate gene combination may not replace the cognitive function as a disease marker. However, the acceptable specificity (76.2%) allows for facilitate identifying MCI subjects, who are more likely to develop AD than normal controls. If a subject is identified positively, by both cognitive and gene expression biomarker, they are more likely to having underlying AD pathology and the chances to progress to AD will be high. Further studies are required to follow up those with positive results by gene testing and see whether the gene biomarker panel is useful to predict the trajectory of disease.

Between AD and MCI subjects, gene combination showed better predictive value than each of five genes, but still did not satisfy the criteria of ideal biomarkers with the sensitivity of 61%, indicating that the 5-gene panel is not sensitive enough to discriminate the different severity of cognitive dysfunction. The higher specificity of 73.7% is clinically relevant, because we need to differentiate MCI patients without underlying AD pathology from those with AD pathology but still preclinical. It is known that the disease courses are different between the two subtypes. The negative predicative value of 69.5% suggested that further study should be carried out to see if gene biomarker panel could accurately predict those who will not progress to AD with time.

Compared with existing biomarkers such as CSF Aβ, tau proteins, neuroimaging biomarkers and genetic markers, gene expression biomarkers are worthy of further research. CSF markers are limited by the invasive nature of the procedure and difficult to be implemented in clinical practice in this region. Neuroimaging markers are expensive, time consuming and not sensitive enough to track disease progression. Genetic markers such as mutations in APP, PS-1, PS-2 and ApoE are only applicable for a small proportion of familial AD, but not strong enough as a determent factor for sporadic AD. Moreover, genetic predisposition is a trait marker which gives no implication on the severity of impairments. The dynamic gene expression profiling may provide additional insights into how constitutional predisposition interacts with complex health/physical factors to cause

neurodegeneration in AD. It may also offer information on evolution of disease process.

In the present study, the specificity of candidate gene expression panel met the consensus criteria of biomarkers for AD proposed by NIA. However, sensitivity was fairly low and unsatisfactory. At the prevalence of AD being 39% in the studied sample, the positive and negative predictive values were 80.6% and 79.1%, which is comparable to that of CSF biomarker such as P-tau (Luck et al., 2007), suggesting gene biomarker panel represented a potential diagnostic test for AD. However, the low predictive values when differentiating MCI from NC and MCI from AD suggested its limitation of using gene expression panel alone in the diagnostic or screening test of MCI. Future study that incorporating the gene panel along with other more accepted biomarkers such as CSF, MRI markers as predictors for incident AD or MCI are required to improve the discriminatory power of expression biomarker panel.

The gene biomarker panel could not be perfect because peripheral blood gene expression levels are susceptible to be affected by different physical and pathological factors. This led to systemic noises which interfere with the performance of biomarker. The heterogeneity nature of MCI also represented the difficulty of using gene biomarker panel alone to classify MCI and AD patients. Moreover, AD is a complex disorder with inconsistent correlation between clinical

and histological markers. The application is also limited because the present identified limited number of risk genes only accounted for a very small part of genetic factors of disease. A recent study has characterized a blood RNA signature composed of 133 genes that can correctly distinguish AD patients from normal controls with a sensitivity of 100% and specificity of 96% (Fehlbaum-Beurdeley et al., 2010).

The differentially expressed genes identified in our study rather reflected subtle alteration in blood gene expression serving as the systemic response to disease pathology. Cathepsin B (CTSB) is a lysosomal cysteine proteases presented in lysosomes. The protease is involved in diverse biological processes and pathological conditions, such as tumorigenesis, bone disorder, pancreatitis and AD (Watson and Kreuzaler, 2009, Halangk et al., 2000, Hopwood et al., 2009, Hook et al., 2008). Microarray studies found that CTSB transcript was downregulated in cerebral cortex of APP transgenic mice and PBMC of AD subjects but upregulated in hippocampus (Blalock et al., 2004, Reddy et al., 2004, Maes et al., 2007). In the present study, an upregulation of CTSB expression was observed in peripheral leukocytes of AD and MCI patients compared to healthy controls, suggested that CTSB related amyloid-beta production and apoptosis is probably the early response in disease pathophysiology process.

CTSD is a soluble lysosomal aspartic endopeptidase involved in multiple physical and pathological functions. One of the important functions of CTSD is to trigger apoptosis directly or act as a mediator of apoptosis (Benes et al., 2008). Recent studies demonstrated that CTSD has been implicated in the processing of APP, ApoE and tau protein (Kenessey et al., 1997, Zhou et al., 2006, Hook et al., 2008), which are important factors of AD pathogenesis. In our study, CTSD is the only gene which expression levels were significant different between any two of three groups, with highest in AD subjects, moderate in MCI subjects and lowest in healthy controls, indicating the essential role of CTSD related biological pathway in the whole process of AD pathology. It also substantiated the findings about the increased apoptotic vulnerability of AD lymphocytes and disturbed lysosomal functions in AD (Eckert et al., 1998, Nixon et al., 1992).

Another two upregulated genes in AD subjects compared to healthy controls, DDT and TSC1, are not differentially expressed between MCI subjects and controls or between AD and MCI subjects, suggesting the possibility of these two genes involved in the late pathological activities in the disease progression. DDT is an enzyme that converts D-dopachrome into 5,6-dihydroxyindole. This protein is shown to be related to the migration inhibitory factor (MIF) in terms of sequence, enzyme activity and structure. MIF was found to play a role in the pathogenesis of AD (Popp et al., 2009). The underlying mechanisms involved in inflammation and Aβ induced cytotoxicity (Bacher et al., 2010). DDT is speculated to function close

to MIF. Our data could not confirm the decreased expression of DDT in PBMC of AD subjects (Maes et al., 2007), but supported the observation that major upregulated function in AD was inflammatory response (Loring JF et al., 2002, Colangelo et al., 2002). Further studies are required to clarify the functions of DDT in the brain.

TSC1 encoded protein, hamartin binds TSC2-encoded protein tuberin to form a protein complex and function in protein translational regulation and cell growth (van Slegtenhorst et al., 1998). It modulated cell function via the mTOR signaling cascade (Rosner et al., 2008). Tuberin levels were found to be decreased in the brain tissues of AD patients (Ferrando-Miguel et al., 2005). The alteration of mTOR signaling activities were correlated with MMSE scores in AD subjects (Lafay-Chebassier et al., 2005). Our study also showed that abnormal TSC1 related pathway occur in AD in terms of gene transcription level.

NRD1 is another gene of special interests. It encodes a metalloendopeptidase that functions cleave peptides and also enhance the ectodomain shedding of a wide range of membrane proteins (Hiraoka et al., 2007, Hiraoka et al., 2008, Nishi et al., 2006). As the process of ectodomain shedding is essential for the activation of specific receptors, cytokines and growth factors, NRD1 was considered to play specific roles in cellular migration, proliferation and neurogenesis. In our study, the overexpression of NRD1 was observed in MCI subjects but not in AD patients,

suggesting that NRD1 regulated neurogeneration may be one of acute compensatory responses to the pathological damages at the onset of disease.

UQCRC1, was shown upregulated in AD subjects compared with NC and MCI subjects, indicating this gene plays an important role in the progression of the disease. UQCRC1 is a component of the ubiquinol-cytochrome c reductase complex (complex III), being a part of the mitochondrial respiratory chain. Overexpression of UQCRC1 was documented to affect mitochondrial morphology and physiology, leading to mitochondrial dysfunction (Johnston-Wilson et al., 2000). .

Two down-regulated genes, PIN1 and ITPKB, have been reported to regulate the initiation and progression of AD pathogenesis (Emilsson et al., 2006, Liou et al., 2003). Growing evidence showed that PIN1 is overexpressed in various tumors wherever downregulated in AD (Reddy et al., 2004, Maes et al., 2007, Bao et al., 2004). In this regard, it is interesting to find that several AD candidate genes such as CTSD and GAPD were associated with neurodegeneration and cancer (Li et al., 2004, Benes et al., 2008). In our study, mRNA level of PIN1 decreased in AD subjects, which is correspondence to the previous results and substantiated the observation that downregulation of PIN1 is associated with the increased risk of AD (Liou et al., 2003, Maes et al., 2007, Reddy et al., 2004).

ITPKB is a calcium-dependent inositol kinase and functions in converting IP3 to IP4. Alteration of ITPKB may lead to increased conversion of IP3 to IP4 affecting calcium homeostasis in the cells and severe T cell deficiency in mice (Pouillon et al., 2003). Our results not only substantiated the previous finding that mRNA expression of ITPKB was reduced in AD subjects (Emilsson et al., 2006, Maes et al., 2007), but also demonstrated that it is down-regulated in MCI subjects compared to healthy controls, suggesting the role of underlying processes regulated by ITPKB in initiating calcium deregulation in the early onset of cognitive impairment.

In summary, we demonstrated that AD leukocytes differ in the expression of seven genes compared with healthy controls. The major regulations are involved in diverse biological functions, including apoptosis, inflammation, neurogenesis, calcium disequilibrium and mitochondria insufficiency. In comparison with previous studies, our findings are consistent to some results but contradictory to others. For an instance, we have reaffirmed the downregulation of ITPKB and PIN1 in blood of AD subjects but did not replicate the downregulation of DDT and CTSD reported by Maes et al (Maes et al., 2007). Different technique platform (Microarray versus real-time RT-PCR), different ethnicity origin (Caucasian versus Chinese) and inconsistent target cell population (whole blood versus leukocytes) and patient cohort with larger sample size may account for some of these

discrepancies, but these discrepancies also highlight the importance of each laboratory establishing its own reference range for expression biomarker.

The peripheral gene expression alteration may result from the highly developed dialogue between brain and blood or due to the generalized systemic changes to disease pathophysiology. It is still not clear whether these peripheral abnormalities are related to the alteration in AD brain and how these genes are involved in pathophysiology of disease.

7.3 Genes link to cognitive and NP manifestations in AD

In our study, quantitative phenotypes of cognitive performance and neuropsychiatric assessment were also associated with gene expression data, because it may increase the statistical power to detect small polygenic effects and provide new insights into genetic factors involved in the development of cognitive and NP manifestations in dementia.

Numerous researches have attempted to explore the determinants of cognitive decline in AD. Molecular genetic studies have tried to provide us with important clues about constitutional differences. The most consistent findings are that ApoE4 allele was associated with poorer cognitive performance and faster cognitive deterioration (Lam et al., 2006, Cosentino et al., 2008, Wehling et al., 2007). Our previous finding also demonstrated the involvement of CYP46A1 gene in the

cognitive deficits (Fu et al., 2009). In the present study, we reported for the first time that gene expression of ITPKB, NDUFA6 and CTSD showed a tendency to correlate with cognitive performance of AD subjects, suggesting that multiple mechanisms are implicated in the development of cognitive impairment in AD, including calcium dysregulation, oxidative stress and lysosome disturbances. The functions of ITPKB and NDUFA6 in brain are not exactly known. One SNP in NDUFA6 (rs1801311) was found to be associated with cognitive ability in normal aging (Harris et al., 2007). CTSD, as a susceptible gene for AD, was shown to interact with ApoE4 in affecting cognitive ability in both demented and non-demented individuals (Payton et al., 2006). A functional C to T transition within exon 2 of CTSD that increased the secretion of the pro-CTSD was associated with decrease in several cognitive domains such as memory, executive and fluid intelligence (Payton et al., 2003). The important role of CTSD in early brain development has been highlighted recently.

Our study found a positive association between DDT gene expression and the score of "Depression", "Aberrant motor behavior" and total CNPI score of AD subjects, suggesting that DDT related biological processes may be involved in the pathogenesis of NP manifestation with the process of neurodegeneration. As the homology of DDT, MIF was found to participate in the detoxification of catecholamine metabolites and play a pivotal role in the synthesis of neuromelanin (Matsunaga et al., 1999). Hypothalamic MIF may modulate the nigro-striatal

dopaminergic activity and increase the secretion of melatonin in subjects with Parkinson's disease (Matsunaga et al., 1999). Changes in neurotransmitters were reported to be link to mood disturbance and aberrant motor behavior wherever decreased melatonin secretion was associated with disturbed circadian rhythms in AD patients (Wu and Swaab, 2007).

TSC1 gene was found in relation to the score of "Delusion" in AD subjects, indicating TSC1 encoding protein or its related mTOR signaling cascade may be involved in the development of delusion in AD. Additionally, a novel mTOR activating protein was reported to protect dopamine neurons against oxidative stress (Choi et al., 2010).

Another two genes of special interests, CTSD and UQCRC1, was associated with the score of "Aberrant motor behavior" of AD subjects. Aberrant motor behavior is closely related with disturbance in neurotransmitters (Scharre et al., 2003). Cognitive deficits and visuospatical impairments might lead to motor behavior abnormalities as well (Mega et al., 1996). In our study, we observed a tendency of association between CTSD and cognitive impairment as well as an inverse relation to psychiatric symptom, indicating the possibility of cognitive impairment regulated by CTSD contributing to the manifestation of NP symptom in dementia. More studies are required to clarify the functions of CTSD in the brain.

UQCRC1, as a component of the mitochondria respiratory chain, plays an important role in the maintenance of mitochondria function. Overexpression of UQCRC1 contributes to the mitochondrial dysfunction (Johnston-Wilson et al., 2000). Underlying mitochondria deficit influenced the synthesis and transport of neurotransmitters and was closely associated with psychiatric disorders (Jou et al., 2009). Studies showed an alteration of UQCRC1 transcript in mood disorders and schizophrenia (Prabakaran et al., 2004, Johnston-Wilson et al., 2000). In the present study, UQCRC1 was associated with diagnosis of AD, but also was positive correlated with the score of NP profile, lending support for the role of mitochondria dysfunction in the pathogenesis of this disorder.

7.4 Limitations of study

Confounding factors such as age, gender, ethnic group and the methodological procedure of RNA isolation were general concerns in producing false positive in gene expression studies (Grunblatt et al., 2009). Age and gender were previously reported to be associated with some variation, but the effect seems to be modest (Kim et al., 2007). Ethnic attributed most to the gene expression variation and the minor variance was observed within ethnic population (Storey et al., 2007). Variation in RNA extraction and storage procedure might influence the gene expression profile (Kim et al., 2007). In the present study, only subjects of Chinese ethnicity were recruited in order to control the major confounding factor of ethnic background. However, age and sex are not

matched between normal controls and AD or MCI group. This is one limitation of the present study, which is a component of prospective study but not a matched case-control study. Significant variables in univariate analysis may be confounding factors in gene expression variation. Statistical methods were adopted to adjust for the confounding variables such as age, gender, education levels and medical comorbidity. The variance from blood withdrawal and RNA isolation techniques was taken into account and minimized by strictly complying with the standard operating procedure of experiment protocol. It has to be addressed that we could not exclude the effects of medical treatments in the gene expression variation. The use of anti-psychotic medication may also confound the association between gene expression and the psychiatric symptoms in dementia. Such medications should be taken into account in the future validation study.

We used quantitative real-time RT-PCR in the present study due to its high accuracy and sensitivity. However, the limitation of SYBR green real-time PCR method is the possibility of false positive signal because SYBR green dye binds any double-strand DNA including specific and non-specific PCR products. To overcome the problems, all the RNA samples were treated with DNase and PCR products were electrophresed on 4% agarose gel to confirm only one gene-specific amplicon is amplified.

Gene biomarker combination was demonstrated with good sensitivity and

specificity in discriminating AD from healthy control in our sample, but it is still required to be validated in an independent cohort with large sample size. Moreover, we selected gene biomarkers based on the comparative results between AD subjects and healthy controls in our sample, and it is possible that more different genes may reach the better discriminatory power in other larger scale population. On the other hand, cross-sectional comparison in gene expression levels alone may limit our results in identifying the genes that represent the consequence or response rather than the cause to the disease pathophysiology. Therefore, more studies in clarifying the genetic pathway as well as integrating with genomic and proteomic information will help to comprehensively understand the biological complexity of AD. Patient classification in our study is dependent on clinical diagnosis which is not always accurate as AD is complex disorder with inconsistent correlation between clinical and histological markers and MCI is a more heterogeneous state than pre-symptomatic AD.

7.5 Direction for future research

Researches into biomarkers aim to facilitate accurate and early diagnosis of disease. In this project, a 5-gene combination set was demonstrated to be capable of discriminate AD from healthy controls with satisfactory sensitivity and specificity. As expression studies are well known to be difficult to replicate, we need to re-examine the findings in an independent sample for replication in order to show our findings are reliable and consistent. Besides, repeated samples could

be obtained in a prospective study. Stability of gene expression panel marker could be examined. In addition to cross-sectional analysis, a longitude study in MCI patients will be required to investigate the prognostic use of gene expression biomarkers. If further validated, it will allow identification the "high-risk" patients before the onset of clinical symptoms. Moreover, the potential of gene expression as biomarkers of therapeutic response in clinical trials could be explored.

Both transcriptomics and proteomics are high throughout approaches to search for novel biomarkers in neurodegenerative disorders. Transcriptome allows for simultaneously studying mRNA expression profiling in whole genome and identify the changes in acute/current cellular activities. Proteomics aims to study the whole protein profiling as well as the proteins functional modification and activities (Hochstrasser, 1998). Although mRNA expression alteration could provide some indication of protein levels, it may not substitute for detecting the protein itself. After all, protein is the mediator of the biological processes rather than mRNA (Devlin., 2010). In this regard, further analysis in protein expression related to the differently expressed genes pathway may be required to address the complex biological expression pattern and identify novel protein disease biomarkers.

Gene expression portrait could be regulated by genetic and epigenetic mechanisms. It has been widely accepted that genetic factors played an important role in the initiation of Alzheimer's disease. Studies incorporating expression and genotypic data will help to identify the genetic basis for expression regulation and uncover the underlying mechanisms of observed transcript variation. Epigenetic control of gene expression pattern by DNA methylation, alternative splicing or histone modification also contributes to the pathogenesis of Alzheimer's disease (Chouliaras et al., 2010). Research into gene-specific methylation and different transcript isoforms will be of value in analyzing the role of epigenetic regulation machinery apart from genetic sequence alteration in disease pathophysiology. Post-transcriptional regulation by non-coding microRNA also received special attention. It would be worthwhile to study the specific effects of microRNA in transcript regulation and further develop novel therapeutic strategy.

Chapter 8 Conclusion

AD is a major health challenge with the continuous expansion of the ageing population in the world. To date, more than 35 million people worldwide were estimated to suffer from AD and it may be 115 million in 2050. The rapid increase created a significant social and economic burden, yet there is no effective treatment that could prevent progression of the disease. AD is a complex disease caused by a combination of genetic and environmental factors. The nature of heterogeneity and complexity results in a diagnostic and therapeutic challenge of AD.

Current diagnosis of clinical probable AD is performed by clinical examination and neuroimaging with good reliability and validity (Blacker et al., 1994), but it could not be identified until the patients had apparent functional deficits. A wealth of studies attempted to identify potential biomarkers for AD and its risk state of MCI (Blennow et al., 2001). Peripheral gene expression has received special attention as potential biomarkers for AD in recent years. In this project, genes were selected based on the bioinformatics approach for studying the expression levels in leukocytes of AD, MCI subjects and healthy controls. Seven genes were identified differentially expressed between AD subjects and healthy controls with an upregulation of CTSB, CTSD, DDT, TSC1, UQCRC1 and downregulation of ITPKB and PIN1. NRD1 transcript was increased in MCI subjects compared with controls. The 5-gene biomarker set represented a

biomarker panel for the diagnosis of AD with good sensitivity and specificity. Further validation of the gene biomarker panel is required in a larger independent cohort.

Among the differentially expressed genes, PIN1 was hypothesized to be a major driving force for the initiation or progression of AD pathogenesis because of its diverse functions and the evidence that this protein is one of oxidized proteins common in MCI and AD brain. Downregulation of PIN1 probably leads to downstream pathway alteration including calcium disequilibrium and mitochondria insufficiency (ITPKB and UQCRC1), increased inflammatory response (DDT), neuronal apoptosis (CTSB and CTSD) and neurogeneration as compensation to the loss of synapses and neurons (NRD1 and TSC1).

Multiple genes with small effects each not only contributes to the development of AD, but also influence the manifestation of cognitive and NP symptoms. NDUFA6 gene expression was significantly correlated with cognitive ability. DDT, CTSD, TSC1, UQCRC1 were found in association with the behavior and psychiatric symptoms, indicating the role of genetic factors in moderating the presence of cognitive and NP profiles in demented individuals. The possible mechanisms are implicated in mediating the synthesis and metabolism of neurotransmitters and neurohormones, neural apoptosis and mitochondria deficiency. Further studies on gene signaling pathway, gene protein

interaction and transgenic animal may help to understand the function of genes in the brain.

The present project provided evidence that gene expression in blood can be used to develop molecular signature of AD, reflecting systemic response to disease pathophysiology. It is hoped that further validation in prospective studies will substantiate its clinical application and enhance our understanding of complex pathological mechanisms of AD.

References

ABDI, F., QUINN, J. F., JANKOVIC, J., MCINTOSH, M., LEVERENZ, J. B., PESKIND, E., NIXON, R., NUTT, J., CHUNG, K., ZABETIAN, C., SAMII, A., LIN, M., HATTAN, S., PAN, C., WANG, Y., JIN, J., ZHU, D., LI, G. J., LIU, Y., WAICHUNAS, D., MONTINE, T. J. & ZHANG, J. (2006) Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders. *J Alzheimers Dis,* 9, 293-348.

ABRAHAM, C. R., SELKOE, D. J. & POTTER, H. (1988) Immunochemical identification of the serine protease inhibitor alpha 1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell,* 52, 487-501.

AKIYAMA, H., SHIN, R. W., UCHIDA, C., KITAMOTO, T. & UCHIDA, T. (2005) Pin1 promotes production of Alzheimer's amyloid beta from beta-cleaved amyloid precursor protein. *Biochem Biophys Res Commun,* 336, 521-9.

AKUFFO, E. L., DAVIS, J. B., FOX, S. M., GLOGER, I. S., HOSFORD, D., KINSEY, E. E., JONES, N. A., NOCK, C. M., ROSES, A. D., SAUNDERS, A. M., SKEHEL, J. M., SMITH, M. A. & CUTLER, P. (2008) The discovery and early validation of novel plasma biomarkers in mild-to-moderate Alzheimer's disease patients responding to treatment with rosiglitazone. *Biomarkers,* 13, 618-36.

ANDREASEN, N., HESSE, C., DAVIDSSON, P., MINTHON, L., WALLIN, A., WINBLAD, B., VANDERSTICHELE, H., VANMECHELEN, E. & BLENNOW, K. (1999) Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch Neurol,* 56, 673-80.

ANDREASEN, N., MINTHON, L., DAVIDSSON, P., VANMECHELEN, E., VANDERSTICHELE, H., WINBLAD, B. & BLENNOW, K. (2001) Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. *Arch Neurol,* 58, 373-9.

ANDREASEN, N., VANMECHELEN, E., VANDERSTICHELE, H., DAVIDSSON, P. & BLENNOW, K. (2003) Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. *Acta Neurol Scand Suppl,* 179, 47-51.

APA (1994) *Diagnostic and statistical manual of mental disorders,* Washington,DC.

ARAI, H., MORIKAWA, Y., HIGUCHI, M., MATSUI, T., CLARK, C. M., MIURA, M., MACHIDA, N., LEE, V. M., TROJANOWSKI, J. Q. & SASAKI, H. (1997) Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. *Biochem Biophys Res Commun,* 236, 262-4.

ARRIAGADA, P. V., GROWDON, J. H., HEDLEY-WHYTE, E. T. & HYMAN, B. T. (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology,* 42, 631-9.

ASADA, T., KARIYA, T., YAMAGATA, Z., KINOSHITA, T. & ASAKA, A. (1996) ApoE epsilon 4 allele and cognitive decline in patients with Alzheimer's disease. *Neurology,* 47, 603.

ASTRINIDIS, A. & HENSKE, E. P. (2005) Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. *Oncogene,* 24, 7475-81.

AVILA, J., LUCAS, J. J., PEREZ, M. & HERNANDEZ, F. (2004) Role of tau protein in both physiological and pathological conditions. *Physiol Rev,* 84, 361-84.

AWANO, T., KATZ, M. L., O'BRIEN, D. P., TAYLOR, J. F., EVANS, J., KHAN, S., SOHAR, I., LOBEL, P. & JOHNSON, G. S. (2006) A mutation in the cathepsin D gene (CTSD) in American Bulldogs with neuronal ceroid lipofuscinosis. *Mol Genet Metab,* 87, 341-8.

AYALA, G., WANG, D., WULF, G., FROLOV, A., LI, R., SOWADSKI, J., WHEELER, T. M., LU, K. P. & BAO, L. (2003) The prolyl isomerase Pin1 is a novel prognostic marker in human prostate cancer. *Cancer Res,* 63, 6244-51.

BACHER, M., DEUSTER, O., ALJABARI, B., EGENSPERGER, R., NEFF, F., JESSEN, F., POPP, J., NOELKER, C., REESE, J. P., AL-ABED, Y. & DODEL, R. (2010) The role of macrophage migration inhibitory factor in Alzheimer's disease. *Mol Med,* 16, 116-21.

BAL-PRICE, A. & BROWN, G. C. (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci,* 21, 6480-91.

BALASTIK, M., LIM, J., PASTORINO, L. & LU, K. P. (2007) Pin1 in Alzheimer's disease: multiple substrates, one regulatory mechanism? *Biochim Biophys Acta,* 1772, 422-9.

BAO, L., KIMZEY, A., SAUTER, G., SOWADSKI, J. M., LU, K. P. & WANG, D. G. (2004) Prevalent overexpression of prolyl isomerase Pin1 in human cancers. *Am J Pathol,* 164, 1727-37.

BARNES, D. E., WHITMER, R. A. & YAFFE, K. (2007) Physical activity and dementia: The need for prevention trials. *Exerc Sport Sci Rev,* 35, 24-9.

BAUER, J., GANTER, U., STRAUSS, S., STADTMULLER, G., FROMMBERGER, U., BAUER, H., VOLK, B. & BERGER, M. (1992) The participation of interleukin-6 in the pathogenesis of Alzheimer's disease. *Res Immunol,* 143, 650-7.

BEHL, C., DAVIS, J. B., LESLEY, R. & SCHUBERT, D. (1994) Hydrogen peroxide mediates amyloid beta protein toxicity. *Cell,* 77, 817-27.

BENES, P., VETVICKA, V. & FUSEK, M. (2008) Cathepsin D--many functions of one aspartic protease. *Crit Rev Oncol Hematol,* 68, 12-28.

BENITO, C., NUNEZ, E., TOLON, R. M., CARRIER, E. J., RABANO, A., HILLARD, C. J. & ROMERO, J. (2003) Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci,* 23, 11136-41.

BENNETT, D. A., SCHNEIDER, J. A., BIENIAS, J. L., EVANS, D. A. & WILSON, R. S. (2005) Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. *Neurology,* 64, 834-41.

BERCHTOLD, N. C. & COTMAN, C. W. (1998) Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960s. *Neurobiol Aging,* 19, 173-89.

BERNSTEIN, H. G., STRICKER, R., DOBROWOLNY, H., TRUBNER, K., BOGERTS, B. & REISER, G. (2007) Histochemical evidence for wide expression of the metalloendopeptidase nardilysin in human brain neurons. *Neuroscience,* 146, 1513-23.

BIRD, T. D., LAMPE, T. H., NEMENS, E. J., MINER, G. W., SUMI, S. M. & SCHELLENBERG, G. D. (1988) Familial Alzheimer's disease in American descendants of the Volga Germans: probable genetic founder effect. *Ann Neurol,* 23, 25-31.

BLACKER, D., ALBERT, M. S., BASSETT, S. S., GO, R. C., HARRELL, L. E. & FOLSTEIN, M. F. (1994) Reliability and validity of NINCDS-ADRDA criteria for Alzheimer's disease. The National Institute of Mental Health Genetics Initiative. *Arch Neurol,* 51, 1198-204.

BLALOCK, E. M., GEDDES, J. W., CHEN, K. C., PORTER, N. M., MARKESBERY, W. R. & LANDFIELD, P. W. (2004) Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A,* 101, 2173-8.

BLASKO, I., JELLINGER, K., KEMMLER, G., KRAMPLA, W., JUNGWIRTH, S., WICHART, I., TRAGL, K. H. & FISCHER, P. (2008) Conversion from cognitive health to mild cognitive impairment and Alzheimer's disease: prediction by plasma amyloid beta 42, medial temporal lobe atrophy and homocysteine. *Neurobiol Aging,* 29, 1-11.

BLENNOW, K. (2004) Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx,* 1, 213-25.

BLENNOW, K., DAVIDSSON, P., WALLIN, A. & EKMAN, R. (1995) Chromogranin A in cerebrospinal fluid: a biochemical marker for synaptic degeneration in Alzheimer's disease? *Dementia,* 6, 306-11.

BLENNOW, K. & HAMPEL, H. (2003) CSF markers for incipient Alzheimer's disease. *Lancet Neurol,* 2, 605-13.

BLENNOW, K., VANMECHELEN, E. & HAMPEL, H. (2001) CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. *Mol Neurobiol,* 24, 87-97.

BLUM-DEGEN, D., MULLER, T., KUHN, W., GERLACH, M., PRZUNTEK, H. & RIEDERER, P. (1995) Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neurosci Lett,* 202, 17-20.

BOHNSTEDT, K. C., KARLBERG, B., WAHLUND, L. O., JONHAGEN, M. E., BASUN, H. & SCHMIDT, S. (2003) Determination of isoprostanes in urine samples from Alzheimer patients using porous graphitic carbon liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci,* 796, 11-9.

BOKDE, A. L., LOPEZ-BAYO, P., MEINDL, T., PECHLER, S., BORN, C., FALTRACO, F., TEIPEL, S. J., MOLLER, H. J. & HAMPEL, H. (2006) Functional connectivity of the fusiform gyrus during a face-matching task in subjects with mild cognitive impairment. *Brain,* 129, 1113-24.

BORCHELT, D. R., THINAKARAN, G., ECKMAN, C. B., LEE, M. K., DAVENPORT, F., RATOVITSKY, T., PRADA, C. M., KIM, G., SEEKINS, S., YAGER, D., SLUNT, H. H., WANG, R., SEEGER, M., LEVEY, A. I., GANDY, S. E., COPELAND, N. G., JENKINS, N. A., PRICE, D. L., YOUNKIN, S. G. & SISODIA, S. S. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron,* 17, 1005-13.

BOROVECKI, F., LOVRECIC, L., ZHOU, J., JEONG, H., THEN, F., ROSAS, H. D., HERSCH, S. M., HOGARTH, P., BOUZOU, B., JENSEN, R. V. & KRAINC, D. (2005) Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. *Proc Natl Acad Sci U S A,* 102, 11023-8.

BORRONI, B., ANCHISI, D., PAGHERA, B., VICINI, B., KERROUCHE, N., GARIBOTTO, V., TERZI, A., VIGNOLO, L. A., DI LUCA, M., GIUBBINI, R., PADOVANI, A. & PERANI,

D. (2006) Combined 99mTc-ECD SPECT and neuropsychological studies in MCI for the assessment of conversion to AD. *Neurobiol Aging,* 27, 24-31.

BRAAK, E., BRAAK, H. & MANDELKOW, E. M. (1994) A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. *Acta Neuropathol,* 87, 554-67.

BRAAK, H. & BRAAK, E. (1998) Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria. *J Neural Transm,* 105, 801-19.

BREEN, K. C., BRUCE, M. & ANDERTON, B. H. (1991) Beta amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. *J Neurosci Res,* 28, 90-100.

BROWN, G. C. & BAL-PRICE, A. (2003) Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. *Mol Neurobiol,* 27, 325-55.

BRUGGE, K., KATZMAN, R., HILL, L. R., HANSEN, L. A. & SAITOH, T. (1992) Serological alpha 1-antichymotrypsin in Down's syndrome and Alzheimer's disease. *Ann Neurol,* 32, 193-7.

BUCALA, R. (1994) Identification of MIF as a new pituitary hormone and macrophage cytokine and its role in endotoxic shock. *Immunol Lett,* 43, 23-6.

BUERGER, K., TEIPEL, S. J., ZINKOWSKI, R., BLENNOW, K., ARAI, H., ENGEL, R., HOFMANN-KIEFER, K., MCCULLOCH, C., PTOK, U., HEUN, R., ANDREASEN, N., DEBERNARDIS, J., KERKMAN, D., MOELLER, H., DAVIES, P. & HAMPEL, H. (2002a) CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. *Neurology,* 59, 627-9.

BUERGER, K., ZINKOWSKI, R., TEIPEL, S. J., ARAI, H., DEBERNARDIS, J., KERKMAN, D., MCCULLOCH, C., PADBERG, F., FALTRACO, F., GOERNITZ, A., TAPIOLA, T., RAPOPORT, S. I., PIRTTILA, T., MOLLER, H. J. & HAMPEL, H. (2003) Differentiation of geriatric major depression from Alzheimer's disease with CSF tau protein phosphorylated at threonine 231. *Am J Psychiatry,* 160, 376-9.

BUERGER, K., ZINKOWSKI, R., TEIPEL, S. J., TAPIOLA, T., ARAI, H., BLENNOW, K., ANDREASEN, N., HOFMANN-KIEFER, K., DEBERNARDIS, J., KERKMAN, D., MCCULLOCH, C., KOHNKEN, R., PADBERG, F., PIRTTILA, T., SCHAPIRO, M. B., RAPOPORT, S. I., MOLLER, H. J., DAVIES, P. & HAMPEL, H. (2002b) Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. *Arch Neurol,* 59, 1267-72.

BURNS, A., BYRNE, E. J. & MAURER, K. (2002) Alzheimer's disease. *Lancet,* 360, 163-5.

BURNS, A., PHILPOT, M. P., COSTA, D. C., ELL, P. J. & LEVY, R. (1989) The investigation of Alzheimer's disease with single photon emission tomography. *J Neurol Neurosurg Psychiatry,* 52, 248-53.

BUTTERFIELD, D. A., POON, H. F., ST CLAIR, D., KELLER, J. N., PIERCE, W. M., KLEIN, J. B. & MARKESBERY, W. R. (2006) Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol Dis,* 22, 223-32.

CABRAL, G. A., RABORN, E. S., GRIFFIN, L., DENNIS, J. & MARCIANO-CABRAL, F. (2008) CB2 receptors in the brain: role in central immune function. *Br J Pharmacol,* 153, 240-51.

CACABELOS, R. (2007) Molecular pathology and pharmacogenomics in Alzheimer's disease: polygenic-related effects of multifactorial treatments on cognition, anxiety and depression. *Methods Find Exp Clin Pharmacol,* 29 Suppl A, 1-91.

CARRETTE, O., DEMALTE, I., SCHERL, A., YALKINOGLU, O., CORTHALS, G., BURKHARD, P., HOCHSTRASSER, D. F. & SANCHEZ, J. C. (2003) A panel of cerebrospinal fluid potential biomarkers for the diagnosis of Alzheimer's disease. *Proteomics,* 3, 1486-94.

CASTELLANI, R., HIRAI, K., ALIEV, G., DREW, K. L., NUNOMURA, A., TAKEDA, A., CASH, A. D., OBRENOVICH, M. E., PERRY, G. & SMITH, M. A. (2002) Role of mitochondrial dysfunction in Alzheimer's disease. *J Neurosci Res,* 70, 357-60.

CASTELLANI, R. J., NUNOMURA, A., LEE, H. G., PERRY, G. & SMITH, M. A. (2008) Phosphorylated tau: toxic, protective, or none of the above. *J Alzheimers Dis,* 14, 377-83.

CATALDO, A. M., BARNETT, J. L., BERMAN, S. A., LI, J., QUARLESS, S., BURSZTAJN, S., LIPPA, C. & NIXON, R. A. (1995) Gene expression and cellular content of cathepsin D in Alzheimer's disease brain: evidence for early up-regulation of the endosomal-lysosomal system. *Neuron,* 14, 671-80.

CELONE, K. A., CALHOUN, V. D., DICKERSON, B. C., ATRI, A., CHUA, E. F., MILLER, S. L., DEPEAU, K., RENTZ, D. M., SELKOE, D. J., BLACKER, D., ALBERT, M. S. & SPERLING, R. A. (2006) Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. *J Neurosci,* 26, 10222-31.

CHANDRASEKARAN, K., GIORDANO, T., BRADY, D. R., STOLL, J., MARTIN, L. J. & RAPOPORT, S. I. (1994) Impairment in mitochondrial cytochrome oxidase gene expression in Alzheimer disease. *Brain Res Mol Brain Res,* 24, 336-40.

CHANG, S., RAN MA, T., MIRANDA, R. D., BALESTRA, M. E., MAHLEY, R. W. & HUANG, Y. (2005) Lipid- and receptor-binding regions of apolipoprotein E4 fragments act in concert to cause mitochondrial dysfunction and neurotoxicity. *Proc Natl Acad Sci U S A,* 102, 18694-9.

CHANO, T., OKABE, H. & HULETTE, C. M. (2007) RB1CC1 insufficiency causes neuronal atrophy through mTOR signaling alteration and involved in the pathology of Alzheimer's diseases. *Brain Res,* 1168, 97-105.

CHARTIER-HARLIN, M. C., CRAWFORD, F., HOULDEN, H., WARREN, A., HUGHES, D., FIDANI, L., GOATE, A., ROSSOR, M., ROQUES, P., HARDY, J. & ET AL. (1991) Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature,* 353, 844-6.

CHEE, F., MUDHER, A., NEWMAN, T. A., CUTTLE, M., LOVESTONE, S. & SHEPHERD, D. (2006) Overexpression of tau results in defective synaptic transmission in Drosophila neuromuscular junctions. *Biochem Soc Trans,* 34, 88-90.

CHEN LM, B ‧ , X. & JC., Y. (2009) Value of cerebrospinal fliud beta-amyloid protein/phosphorylated tau protein ratio on differential diagnosis of Alzheimer's disease and vascular dementia. *Progress in Modern Biomedicine,* 9, 922-4.

CHEN, M., INESTROSA, N. C., ROSS, G. S. & FERNANDEZ, H. L. (1995) Platelets are the primary source of amyloid beta-peptide in human blood. *Biochem Biophys Res Commun,* 213, 96-103.

CHENG, Y. & HITCHCOCK, S. A. (2007) Targeting cannabinoid agonists for inflammatory and neuropathic pain. *Expert Opin Investig Drugs,* 16, 951-65.

CHESNEAU, V., PIEROTTI, A. R., PRAT, A., GAUDOUX, F., FOULON, T. & COHEN, P. (1994) N-arginine dibasic convertase (NRD convertase): a newcomer to the family of processing endopeptidases. An overview. *Biochimie,* 76, 234-40.

CHETELAT, G., LANDEAU, B., EUSTACHE, F., MEZENGE, F., VIADER, F., DE LA SAYETTE, V., DESGRANGES, B. & BARON, J. C. (2005) Using voxel-based morphometry to map the structural changes associated with rapid conversion in MCI: a longitudinal MRI study. *Neuroimage,* 27, 934-46.

CHIBNALL, J. T. & EASTWOOD, R. (1998) Postsecondary education and dementia risk in older Jesuit priests. *Int Psychogeriatr,* 10, 359-68.

CHIU HF, CHAN CK, LAM LC, NG KO, LI SW, WONG M & WF, C. (1997) The modified Fuid Verbal Fluency Test: a validation study in Hong Kong. *J Gerontol B Psychol Sci Soc Sci.,* 52, 247-50.

CHIU, H. F., LAM, L. C., CHI, I., LEUNG, T., LI, S. W., LAW, W. T., CHUNG, D. W., FUNG, H. H., KAN, P. S., LUM, C. M., NG, J. & LAU, J. (1998) Prevalence of dementia in Chinese elderly in Hong Kong. *Neurology,* 50, 1002-9.

CHIU HFK, LEE HC, CHUNG WS & PK., K. (1994) Reliability and validity of the Cantonese version of the Min-Mental State Examination-A preliminary study. *Journal of Hong Kong College of Psychiatrists,* 4(suppl 2), 25-8.

CHOI, K. C., KIM, S. H., HA, J. Y., KIM, S. T. & SON, J. H. (2010) A novel mTOR activating protein protects dopamine neurons against oxidative stress by repressing autophagy related cell death. *J Neurochem,* 112, 366-76.

CHONG, J. K., MILLER, B. E. & GHANBARI, H. A. (1990) Detection of amyloid beta protein precursor immunoreactivity in normal and Alzheimer's disease cerebrospinal fluid. *Life Sci,* 47, 1163-71.

CHOULIARAS, L., RUTTEN, B. P., KENIS, G., PEERBOOMS, O., VISSER, P. J., VERHEY, F., VAN OS, J., STEINBUSCH, H. W. & VAN DEN HOVE, D. L. (2010) Epigenetic regulation in the pathophysiology of Alzheimer's disease. *Prog Neurobiol,* 90, 498-510.

CHU, L. W., CHIU, K. C., HUI, S. L., YU, G. K., TSUI, W. J. & LEE, P. W. (2000) The reliability and validity of the Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog) among the elderly Chinese in Hong Kong. *Ann Acad Med Singapore,* 29, 474-85.

CITRON, M. (2004) Beta-secretase inhibition for the treatment of Alzheimer's disease--promise and challenge. *Trends Pharmacol Sci,* 25, 92-7.

CLARK, C. M., XIE, S., CHITTAMS, J., EWBANK, D., PESKIND, E., GALASKO, D., MORRIS, J. C., MCKEEL, D. W., JR., FARLOW, M., WEITLAUF, S. L., QUINN, J., KAYE, J., KNOPMAN, D., ARAI, H., DOODY, R. S., DECARLI, C., LEIGHT, S., LEE, V. M. & TROJANOWSKI, J. Q. (2003) Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol,* 60, 1696-702.

COHEN-MANSFIELD, J. (1996) Conceptualization of agitation: results based on the Cohen-Mansfield Agitation Inventory and the Agitation Behavior Mapping Instrument. *Int Psychogeriatr,* 8 Suppl 3, 309-15; discussion 351-4.

COLANGELO, V., SCHURR, J., BALL, M. J., PELAEZ, R. P., BAZAN, N. G. & LUKIW, W. J. (2002) 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. *J Neurosci Res,* 70, 462-73.

COPELAND, M. P., DALY, E., HINES, V., MASTROMAURO, C., ZAITCHIK, D., GUNTHER, J. & ALBERT, M. (2003) Psychiatric symptomatology and prodromal Alzheimer's disease. *Alzheimer Dis Assoc Disord,* 17, 1-8.

COSENTINO, S., SCARMEAS, N., HELZNER, E., GLYMOUR, M. M., BRANDT, J., ALBERT, M., BLACKER, D. & STERN, Y. (2008) APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease. *Neurology,* 70, 1842-9.

COSKUN, P. E., BEAL, M. F. & WALLACE, D. C. (2004) Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc Natl Acad Sci U S A,* 101, 10726-31.

CRAFT, S. (2006) Insulin resistance syndrome and Alzheimer disease: pathophysiologic mechanisms and therapeutic implications. *Alzheimer Dis Assoc Disord,* 20, 298-301.

CRAFT, S., TERI, L., EDLAND, S. D., KUKULL, W. A., SCHELLENBERG, G., MCCORMICK, W. C., BOWEN, J. D. & LARSON, E. B. (1998) Accelerated decline in apolipoprotein E-epsilon4 homozygotes with Alzheimer's disease. *Neurology,* 51, 149-53.

CRAIG-SCHAPIRO, R., FAGAN, A. M. & HOLTZMAN, D. M. (2009) Biomarkers of Alzheimer's disease. *Neurobiol Dis,* 35, 128-40.

CSERNANSKY, J. G., MILLER, J. P., MCKEEL, D. & MORRIS, J. C. (2002) Relationships among cerebrospinal fluid biomarkers in dementia of the Alzheimer type. *Alzheimer Dis Assoc Disord,* 16, 144-9.

CSERNANSKY, J. G., WANG, L., SWANK, J., MILLER, J. P., GADO, M., MCKEEL, D., MILLER, M. I. & MORRIS, J. C. (2005) Preclinical detection of Alzheimer's disease: hippocampal shape and volume predict dementia onset in the elderly. *Neuroimage,* 25, 783-92.

D'SOUZA, I. & SCHELLENBERG, G. D. (2005) Regulation of tau isoform expression and dementia. *Biochim Biophys Acta,* 1739, 104-15.

DANYSZ, W., PARSONS, C. G., MOBIUS, H. J., STOFFLER, A. & QUACK, G. (2000) Neuroprotective and symptomatological action of memantine relevant for Alzheimer's disease--a unified glutamatergic hypothesis on the mechanism of action. *Neurotox Res,* 2, 85-97.

DAVIDSON, Y., GIBBONS, L., PRITCHARD, A., HARDICRE, J., WREN, J., TIAN, J., SHI, J., STOPFORD, C., JULIEN, C., THOMPSON, J., PAYTON, A., THAKER, U., HAYES, A. J., IWATSUBO, T., PICKERING-BROWN, S. M., PENDLETON, N., HORAN, M. A., BURNS, A., PURANDARE, N., LENDON, C. L., NEARY, D., SNOWDEN, J. S. & MANN, D. M. (2006) Genetic associations between cathepsin D exon 2 C-->T polymorphism and Alzheimer's disease, and pathological correlations with genotype. *J Neurol Neurosurg Psychiatry,* 77, 515-7.

DE CRAEN, A. J., GUSSEKLOO, J., VRIJSEN, B. & WESTENDORP, R. G. (2005) Meta-analysis of nonsteroidal antiinflammatory drug use and risk of dementia. *Am J Epidemiol,* 161, 114-20.

DE KLOET, E. R. (2004) Hormones and the stressed brain. *Ann N Y Acad Sci,* 1018, 1-15.

DE LEON, M. J., GEORGE, A. E., GOLOMB, J., TARSHISH, C., CONVIT, A., KLUGER, A., DE SANTI, S., MCRAE, T., FERRIS, S. H., REISBERG, B., INCE, C., RUSINEK, H.,

BOBINSKI, M., QUINN, B., MILLER, D. C. & WISNIEWSKI, H. M. (1997) Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease. *Neurobiol Aging,* 18, 1-11.

DE MAGALHAES, J. P., CURADO, J. & CHURCH, G. M. (2009) Meta-analysis of age-related gene expression profiles identifies common signatures of aging. *Bioinformatics,* 25, 875-81.

DEBEY, S., SCHOENBECK, U., HELLMICH, M., GATHOF, B. S., PILLAI, R., ZANDER, T. & SCHULTZE, J. L. (2004) Comparison of different isolation techniques prior gene expression profiling of blood derived cells: impact on physiological responses, on overall expression and the role of different cell types. *Pharmacogenomics J,* 4, 193-207.

DEKOSKY, S. T., IKONOMOVIC, M. D., WANG, X., FARLOW, M., WISNIEWSKI, S., LOPEZ, O. L., BECKER, J. T., SAXTON, J., KLUNK, W. E., SWEET, R., KAUFER, D. I. & KAMBOH, M. I. (2003) Plasma and cerebrospinal fluid alpha1-antichymotrypsin levels in Alzheimer's disease: correlation with cognitive impairment. *Ann Neurol,* 53, 81-90.

DELACOURTE, A., DAVID, J. P., SERGEANT, N., BUEE, L., WATTEZ, A., VERMERSCH, P., GHOZALI, F., FALLET-BIANCO, C., PASQUIER, F., LEBERT, F., PETIT, H. & DI MENZA, C. (1999) The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology,* 52, 1158-65.

DELLES, C., PADMANABHAN, S., LEE, W. K., MILLER, W. H., MCBRIDE, M. W., MCCLURE, J. D., BRAIN, N. J., WALLACE, C., MARCANO, A. C., SCHMIEDER, R. E., BROWN, M. J., CAULFIELD, M. J., MUNROE, P. B., FARRALL, M., WEBSTER, J., CONNELL, J. M. & DOMINICZAK, A. F. (2008) Glutathione S-transferase variants and hypertension. *J Hypertens,* 26, 1343-52.

DEMATTOS, R. B., BALES, K. R., PARSADANIAN, M., O'DELL, M. A., FOSS, E. M., PAUL, S. M. & HOLTZMAN, D. M. (2002) Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. *J Neurochem,* 81, 229-36.

DENIZ-NARANJO, M. C., MUNOZ-FERNANDEZ, C., ALEMANY-RODRIGUEZ, M. J., PEREZ-VIEITEZ, M. C., ALADRO-BENITO, Y., IRURITA-LATASA, J. & SANCHEZ-GARCIA, F. (2008) Cytokine IL-1 beta but not IL-1 alpha promoter polymorphism is associated with Alzheimer disease in a population from the Canary Islands, Spain. *Eur J Neurol,* 15, 1080-4.

DEVLIN., T. M. (2010) *Textbook of biochemistry : with clinical correlations*, John Wiley & Sons.

DHANDAPANI, K. M., HADMAN, M., DE SEVILLA, L., WADE, M. F., MAHESH, V. B. & BRANN, D. W. (2003) Astrocyte protection of neurons: role of transforming growth factor-beta signaling via a c-Jun-AP-1 protective pathway. *J Biol Chem,* 278, 43329-39.

DI BONA, D., PLAIA, A., VASTO, S., CAVALLONE, L., LESCAI, F., FRANCESCHI, C., LICASTRO, F., COLONNA-ROMANO, G., LIO, D., CANDORE, G. & CARUSO, C. (2008) Association between the interleukin-1beta polymorphisms and Alzheimer's disease: a systematic review and meta-analysis. *Brain Res Rev,* 59, 155-63.

DICKERSON, B. C., SALAT, D. H., GREVE, D. N., CHUA, E. F., RAND-GIOVANNETTI, E., RENTZ, D. M., BERTRAM, L., MULLIN, K., TANZI, R. E., BLACKER, D., ALBERT, M. S. & SPERLING, R. A. (2005) Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. *Neurology,* 65, 404-11.

DICKEY, C. A., LORING, J. F., MONTGOMERY, J., GORDON, M. N., EASTMAN, P. S. & MORGAN, D. (2003) Selectively reduced expression of synaptic plasticity-related genes in amyloid precursor protein + presenilin-1 transgenic mice. *J Neurosci,* 23, 5219-26.

DOODY, R. S., FERRIS, S. H., SALLOWAY, S., SUN, Y., GOLDMAN, R., WATKINS, W. E., XU, Y. & MURTHY, A. K. (2009) Donepezil treatment of patients with MCI: a 48-week randomized, placebo-controlled trial. *Neurology,* 72, 1555-61.

DOODY, R. S., GELDMACHER, D. S., GORDON, B., PERDOMO, C. A. & PRATT, R. D. (2001) Open-label, multicenter, phase 3 extension study of the safety and efficacy of donepezil in patients with Alzheimer disease. *Arch Neurol,* 58, 427-33.

DORAISWAMY, P. M., LEON, J., CUMMINGS, J. L., MARIN, D. & NEUMANN, P. J. (2002) Prevalence and impact of medical comorbidity in Alzheimer's disease. *J Gerontol A Biol Sci Med Sci,* 57, M173-7.

DRZEZGA, A., GRIMMER, T., RIEMENSCHNEIDER, M., LAUTENSCHLAGER, N., SIEBNER, H., ALEXOPOULUS, P., MINOSHIMA, S., SCHWAIGER, M. & KURZ, A. (2005) Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. *J Nucl Med,* 46, 1625-32.

DU, A. T., SCHUFF, N., AMEND, D., LAAKSO, M. P., HSU, Y. Y., JAGUST, W. J., YAFFE, K., KRAMER, J. H., REED, B., NORMAN, D., CHUI, H. C. & WEINER, M. W. (2001) Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 71, 441-7.

DUNN, N., MULLEE, M., PERRY, V. H. & HOLMES, C. (2005) Association between dementia and infectious disease: evidence from a case-control study. *Alzheimer Dis Assoc Disord,* 19, 91-4.

DURAN, M. C., MARTIN-VENTURA, J. L., MOHAMMED, S., BARDERAS, M. G., BLANCO-COLIO, L. M., MAS, S., MORAL, V., ORTEGA, L., TUNON, J., JENSEN, O. N., VIVANCO, F. & EGIDO, J. (2007) Atorvastatin modulates the profile of proteins released by human atherosclerotic plaques. *Eur J Pharmacol,* 562, 119-29.

ECKERT, A., COTMAN, C. W., ZERFASS, R., HENNERICI, M. & MULLER, W. E. (1998) Lymphocytes as cell model to study apoptosis in Alzheimer's disease: vulnerability to programmed cell death appears to be altered. *J Neural Transm Suppl,* 54, 259-67.

ECKMAN, C. B., MEHTA, N. D., CROOK, R., PEREZ-TUR, J., PRIHAR, G., PFEIFFER, E., GRAFF-RADFORD, N., HINDER, P., YAGER, D., ZENK, B., REFOLO, L. M., PRADA, C. M., YOUNKIN, S. G., HUTTON, M. & HARDY, J. (1997) A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). *Hum Mol Genet,* 6, 2087-9.

EMILSSON, L., SAETRE, P. & JAZIN, E. (2006) Alzheimer's disease: mRNA expression profiles of multiple patients show alterations of genes involved with calcium signaling. *Neurobiol Dis,* 21, 618-25.

ENGELBORGHS, S., DE VREESE, K., VAN DE CASTEELE, T., VANDERSTICHELE, H., VAN EVERBROECK, B., CRAS, P., MARTIN, J. J., VANMECHELEN, E. & DE DEYN, P. P. (2008) Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. *Neurobiol Aging,* 29, 1143-59.

ENGELHART, M. J., GEERLINGS, M. I., MEIJER, J., KILIAAN, A., RUITENBERG, A., VAN SWIETEN, J. C., STIJNEN, T., HOFMAN, A., WITTEMAN, J. C. & BRETELER, M. M.

(2004) Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. *Arch Neurol,* 61, 668-72.

FAGAN, A. M., MINTUN, M. A., MACH, R. H., LEE, S. Y., DENCE, C. S., SHAH, A. R., LAROSSA, G. N., SPINNER, M. L., KLUNK, W. E., MATHIS, C. A., DEKOSKY, S. T., MORRIS, J. C. & HOLTZMAN, D. M. (2006) Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Ann Neurol,* 59, 512-9.

FAGAN, A. M., ROE, C. M., XIONG, C., MINTUN, M. A., MORRIS, J. C. & HOLTZMAN, D. M. (2007) Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol,* 64, 343-9.

FEEZOR, R. J., BAKER, H. V., MINDRINOS, M., HAYDEN, D., TANNAHILL, C. L., BROWNSTEIN, B. H., FAY, A., MACMILLAN, S., LARAMIE, J., XIAO, W., MOLDAWER, L. L., COBB, J. P., LAUDANSKI, K., MILLER-GRAZIANO, C. L., MAIER, R. V., SCHOENFELD, D., DAVIS, R. W. & TOMPKINS, R. G. (2004) Whole blood and leukocyte RNA isolation for gene expression analyses. *Physiol Genomics,* 19, 247-54.

FEHLBAUM-BEURDELEY, P., JARRIGE-LE PRADO, A. C., PALLARES, D., CARRIERE, J., GUIHAL, C., SOUCAILLE, C., ROUET, F., DROUIN, D., SOL, O., JORDAN, H., WU, D., LEI, L., EINSTEIN, R., SCHWEIGHOFFER, F. & BRACCO, L. (2010) Toward an Alzheimer's disease diagnosis via high-resolution blood gene expression. *Alzheimers Dement,* 6, 25-38.

FEILLET-COUDRAY, C., TOURTAUCHAUX, R., NICULESCU, M., ROCK, E., TAUVERON, I., ALEXANDRE-GOUABAU, M. C., RAYSSIGUIER, Y., JALENQUES, I. & MAZUR, A. (1999) Plasma levels of 8-epiPGF2alpha, an in vivo marker of oxidative stress, are not affected by aging or Alzheimer's disease. *Free Radic Biol Med,* 27, 463-9.

FELDMAN, H. H., FERRIS, S., WINBLAD, B., SFIKAS, N., MANCIONE, L., HE, Y., TEKIN, S., BURNS, A., CUMMINGS, J., DEL SER, T., INZITARI, D., ORGOGOZO, J. M., SAUER, H., SCHELTENS, P., SCARPINI, E., HERRMANN, N., FARLOW, M., POTKIN, S., CHARLES, H. C., FOX, N. C. & LANE, R. (2007) Effect of rivastigmine on delay to diagnosis of Alzheimer's disease from mild cognitive impairment: the InDDEx study. *Lancet Neurol,* 6, 501-12.

FERRANDO-MIGUEL, R., ROSNER, M., FREILINGER, A., LUBEC, G. & HENGSTSCHLAGER, M. (2005) Tuberin--a new molecular target in Alzheimer's disease? *Neurochem Res,* 30, 1413-9.

FINEHOUT, E. J., FRANCK, Z., CHOE, L. H., RELKIN, N. & LEE, K. H. (2007) Cerebrospinal fluid proteomic biomarkers for Alzheimer's disease. *Ann Neurol,* 61, 120-9.

FITZPATRICK, J. L., MIZE, A. L., WADE, C. B., HARRIS, J. A., SHAPIRO, R. A. & DORSA, D. M. (2002) Estrogen-mediated neuroprotection against beta-amyloid toxicity requires expression of estrogen receptor alpha or beta and activation of the MAPK pathway. *J Neurochem,* 82, 674-82.

FOLSTEIN, M. F., FOLSTEIN, S. E. & MCHUGH, P. R. (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res,* 12, 189-98.

FONG, D., CHAN, M. M., HSIEH, W. T., MENNINGER, J. C. & WARD, D. C. (1992) Confirmation of the human cathepsin B gene (CTSB) assignment to chromosome 8. *Hum Genet,* 89, 10-2.

FORSBERG, A., ALMKVIST, O., ENGLER, H., WALL, A., LANGSTROM, B. & NORDBERG, A. High PIB retention in Alzheimer's disease is an early event with complex relationship with CSF biomarkers and functional parameters. *Curr Alzheimer Res,* 7, 56-66.

FRANK, R. A., GALASKO, D., HAMPEL, H., HARDY, J., DE LEON, M. J., MEHTA, P. D., ROGERS, J., SIEMERS, E. & TROJANOWSKI, J. Q. (2003) Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. *Neurobiol Aging,* 24, 521-36.

FRAUTSCHY, S. A., HORN, D. L., SIGEL, J. J., HARRIS-WHITE, M. E., MENDOZA, J. J., YANG, F., SAIDO, T. C. & COLE, G. M. (1998) Protease inhibitor coinfusion with amyloid beta-protein results in enhanced deposition and toxicity in rat brain. *J Neurosci,* 18, 8311-21.

FU, B. Y., MA, S. L., TANG, N. L., TAM, C. W., LUI, V. W., CHIU, H. F. & LAM, L. C. (2009) Cholesterol 24-hydroxylase (CYP46A1) polymorphisms are associated with faster cognitive deterioration in Chinese older persons: a two-year follow up study. *Int J Geriatr Psychiatry,* 24, 921-6.

FUKUMOTO, H., TENNIS, M., LOCASCIO, J. J., HYMAN, B. T., GROWDON, J. H. & IRIZARRY, M. C. (2003) Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. *Arch Neurol,* 60, 958-64.

FUKUYAMA, R., MIZUNO, T., MORI, S., NAKAJIMA, K., FUSHIKI, S. & YANAGISAWA, K. (2000) Age-dependent change in the levels of Abeta40 and Abeta42 in cerebrospinal fluid from control subjects, and a decrease in the ratio of Abeta42 to Abeta40 level in cerebrospinal fluid from Alzheimer's disease patients. *Eur Neurol,* 43, 155-60.

FUMAGALLI, P., ACCARINO, M., EGEO, A., SCARTEZZINI, P., RAPPAZZO, G., PIZZUTI, A., AVVANTAGGIATO, V., SIMEONE, A., ARRIGO, G., ZUFFARDI, O., OTTOLENGHI, S. & TARAMELLI, R. (1998) Human NRD convertase: a highly conserved metalloendopeptidase expressed at specific sites during development and in adult tissues. *Genomics,* 47, 238-45.

FURBY, A., LEYS, D., DELACOURTE, A., BUEE, L., SOETAERT, G. & PETIT, H. (1991) Are alpha-1-antichymotrypsin and inter-alpha-trypsin inhibitor peripheral markers of Alzheimer's disease? *J Neurol Neurosurg Psychiatry,* 54, 469.

GABRYELEWICZ, T., STYCZYNSKA, M., LUCZYWEK, E., BARCZAK, A., PFEFFER, A., ANDROSIUK, W., CHODAKOWSKA-ZEBROWSKA, M., WASIAK, B., PEPLONSKA, B. & BARCIKOWSKA, M. (2007) The rate of conversion of mild cognitive impairment to dementia: predictive role of depression. *Int J Geriatr Psychiatry,* 22, 563-7.

GALASKO, D. (1998) Cerebrospinal fluid levels of A beta 42 and tau: potential markers of Alzheimer's disease. *J Neural Transm Suppl,* 53, 209-21.

GAN, L., YE, S., CHU, A., ANTON, K., YI, S., VINCENT, V. A., VON SCHACK, D., CHIN, D., MURRAY, J., LOHR, S., PATTHY, L., GONZALEZ-ZULUETA, M., NIKOLICH, K. & URFER, R. (2004) Identification of cathepsin B as a mediator of neuronal death induced by

Abeta-activated microglial cells using a functional genomics approach. *J Biol Chem,* 279, 5565-72.

GANGULI, M., DODGE, H. H., SHEN, C. & DEKOSKY, S. T. (2004) Mild cognitive impairment, amnestic type: an epidemiologic study. *Neurology,* 63, 115-21.

GAO, S., HENDRIE, H. C., HALL, K. S. & HUI, S. (1998) The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. *Arch Gen Psychiatry,* 55, 809-15.

GAUTHIER, S., REISBERG, B., ZAUDIG, M., PETERSEN, R. C., RITCHIE, K., BROICH, K., BELLEVILLE, S., BRODATY, H., BENNETT, D., CHERTKOW, H., CUMMINGS, J. L., DE LEON, M., FELDMAN, H., GANGULI, M., HAMPEL, H., SCHELTENS, P., TIERNEY, M. C., WHITEHOUSE, P. & WINBLAD, B. (2006) Mild cognitive impairment. *Lancet,* 367, 1262-70.

GEDA, Y. E., SMITH, G. E., KNOPMAN, D. S., BOEVE, B. F., TANGALOS, E. G., IVNIK, R. J., MRAZEK, D. A., EDLAND, S. D. & PETERSEN, R. C. (2004) De novo genesis of neuropsychiatric symptoms in mild cognitive impairment (MCI). *Int Psychogeriatr,* 16, 51-60.

GEORGE PERRY, R. B. M. (2009) *Current Hypotheses and Research Milestones in Alzheimer's disease*, Springer US.

GERMAN, D. C., GURNANI, P., NANDI, A., GARNER, H. R., FISHER, W., DIAZ-ARRASTIA, R., O'SUILLEABHAIN, P. & ROSENBLATT, K. P. (2007) Serum biomarkers for Alzheimer's disease: proteomic discovery. *Biomed Pharmacother,* 61, 383-9.

GHISO, J., TAGLIAVINI, F., TIMMERS, W. F. & FRANGIONE, B. (1989) Alzheimer's disease amyloid precursor protein is present in senile plaques and cerebrospinal fluid: immunohistochemical and biochemical characterization. *Biochem Biophys Res Commun,* 163, 430-7.

GIBSON, G. E., SHEU, K. F. & BLASS, J. P. (1998) Abnormalities of mitochondrial enzymes in Alzheimer disease. *J Neural Transm,* 105, 855-70.

GINSBERG, S. D., CRINO, P. B., HEMBY, S. E., WEINGARTEN, J. A., LEE, V. M., EBERWINE, J. H. & TROJANOWSKI, J. Q. (1999) Predominance of neuronal mRNAs in individual Alzheimer's disease senile plaques. *Ann Neurol,* 45, 174-81.

GINSBERG, S. D., ELAROVA, I., RUBEN, M., TAN, F., COUNTS, S. E., EBERWINE, J. H., TROJANOWSKI, J. Q., HEMBY, S. E., MUFSON, E. J. & CHE, S. (2004) Single-cell gene expression analysis: implications for neurodegenerative and neuropsychiatric disorders. *Neurochem Res,* 29, 1053-64.

GINSBERG, S. D., HEMBY, S. E., LEE, V. M., EBERWINE, J. H. & TROJANOWSKI, J. Q. (2000) Expression profile of transcripts in Alzheimer's disease tangle-bearing CA1 neurons. *Ann Neurol,* 48, 77-87.

GLADKEVICH, A., KAUFFMAN, H. F. & KORF, J. (2004) Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry,* 28, 559-76.

GOATE, A., CHARTIER-HARLIN, M. C., MULLAN, M., BROWN, J., CRAWFORD, F., FIDANI, L., GIUFFRA, L., HAYNES, A., IRVING, N., JAMES, L. & ET AL. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature,* 349, 704-6.

GOLKA, K., SCHMIDT, T., SEIDEL, T., DIETRICH, H., ROEMER, H. C., LOHLEIN, D., RECKWITZ, T., SOKELAND, J., WEISTENHOFER, W., BLASZKEWICZ, M. & SELINSKI, S. (2008) The influence of polymorphisms of glutathione S-transferases M1 and M3 on the development of human urothelial cancer. *J Toxicol Environ Health A,* 71, 881-6.

GRAY, E. G., PAULA-BARBOSA, M. & ROHER, A. (1987) Alzheimer's disease: paired helical filaments and cytomembranes. *Neuropathol Appl Neurobiol,* 13, 91-110.

GROSSMAN, M., FARMER, J., LEIGHT, S., WORK, M., MOORE, P., VAN DEERLIN, V., PRATICO, D., CLARK, C. M., COSLETT, H. B., CHATTERJEE, A., GEE, J., TROJANOWSKI, J. Q. & LEE, V. M. (2005) Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease. *Ann Neurol,* 57, 721-9.

GROUP, A. R., LYKETSOS, C. G., BREITNER, J. C., GREEN, R. C., MARTIN, B. K., MEINERT, C., PIANTADOSI, S. & SABBAGH, M. (2007) Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. *Neurology,* 68, 1800-8.

GRUNBLATT, E., BARTL, J., ZEHETMAYER, S., RINGEL, T. M., BAUER, P., RIEDERER, P. & JACOB, C. P. (2009) Gene expression as peripheral biomarkers for sporadic Alzheimer's disease. *J Alzheimers Dis,* 16, 627-34.

GRUNBLATT, E., ZANDER, N., BARTL, J., JIE, L., MONORANU, C. M., ARZBERGER, T., RAVID, R., ROGGENDORF, W., GERLACH, M. & RIEDERER, P. (2007) Comparison analysis of gene expression patterns between sporadic Alzheimer's and Parkinson's disease. *J Alzheimers Dis,* 12, 291-311.

GUERREIRO, R. J., BECK, J., GIBBS, J. R., SANTANA, I., ROSSOR, M. N., SCHOTT, J. M., NALLS, M. A., RIBEIRO, H., SANTIAGO, B., FOX, N. C., OLIVEIRA, C., COLLINGE, J., MEAD, S., SINGLETON, A. & HARDY, J. (2010) Genetic variability in CLU and its association with Alzheimer's disease. *PLoS One,* 5, e9510.

GUO, L., LADU, M. J. & VAN ELDIK, L. J. (2004) A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci,* 23, 205-12.

HADDAD, L. A., SMITH, N., BOWSER, M., NIIDA, Y., MURTHY, V., GONZALEZ-AGOSTI, C. & RAMESH, V. (2002) The TSC1 tumor suppressor hamartin interacts with neurofilament-L and possibly functions as a novel integrator of the neuronal cytoskeleton. *J Biol Chem,* 277, 44180-6.

HALANGK, W., LERCH, M. M., BRANDT-NEDELEV, B., ROTH, W., RUTHENBUERGER, M., REINHECKEL, T., DOMSCHKE, W., LIPPERT, H., PETERS, C. & DEUSSING, J. (2000) Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest,* 106, 773-81.

HAMPEL, H., BURGER, K., TEIPEL, S. J., BOKDE, A. L., ZETTERBERG, H. & BLENNOW, K. (2008) Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimers Dement,* 4, 38-48.

HAMPEL, H., TEIPEL, S. J., FUCHSBERGER, T., ANDREASEN, N., WILTFANG, J., OTTO, M., SHEN, Y., DODEL, R., DU, Y., FARLOW, M., MOLLER, H. J., BLENNOW, K. & BUERGER, K. (2004) Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry,* 9, 705-10.

HANSSON, O., ZETTERBERG, H., BUCHHAVE, P., ANDREASSON, U., LONDOS, E., MINTHON, L. & BLENNOW, K. (2007) Prediction of Alzheimer's disease using the CSF

Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord,* 23, 316-20.

HANSSON, O., ZETTERBERG, H., BUCHHAVE, P., LONDOS, E., BLENNOW, K. & MINTHON, L. (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol,* 5, 228-34.

HANSSON, O., ZETTERBERG, H., VANMECHELEN, E., VANDERSTICHELE, H., ANDREASSON, U., LONDOS, E., WALLIN, A., MINTHON, L. & BLENNOW, K. (2008) Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. *Neurobiol Aging,* 31, 357-67.

HARDY, J. & ALLSOP, D. (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci,* 12, 383-8.

HARIGAYA, Y., SHOJI, M., NAKAMURA, T., MATSUBARA, E., HOSODA, K. & HIRAI, S. (1995) Alpha 1-antichymotrypsin level in cerebrospinal fluid is closely associated with late onset Alzheimer's disease. *Intern Med,* 34, 481-4.

HARRIS, F. M., BRECHT, W. J., XU, Q., TESSEUR, I., KEKONIUS, L., WYSS-CORAY, T., FISH, J. D., MASLIAH, E., HOPKINS, P. C., SCEARCE-LEVIE, K., WEISGRABER, K. H., MUCKE, L., MAHLEY, R. W. & HUANG, Y. (2003) Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci U S A,* 100, 10966-71.

HARRIS, G. J., LEWIS, R. F., SATLIN, A., ENGLISH, C. D., SCOTT, T. M., YURGELUN-TODD, D. A. & RENSHAW, P. F. (1998) Dynamic susceptibility contrast MR imaging of regional cerebral blood volume in Alzheimer disease: a promising alternative to nuclear medicine. *AJNR Am J Neuroradiol,* 19, 1727-32.

HARRIS, S. E., FOX, H., WRIGHT, A. F., HAYWARD, C., STARR, J. M., WHALLEY, L. J. & DEARY, I. J. (2007) A genetic association analysis of cognitive ability and cognitive ageing using 325 markers for 109 genes associated with oxidative stress or cognition. *BMC Genet,* 8, 43.

HARVEY, R. J., SKELTON-ROBINSON, M. & ROSSOR, M. N. (2003) The prevalence and causes of dementia in people under the age of 65 years. *J Neurol Neurosurg Psychiatry,* 74, 1206-9.

HASILIK, A. & NEUFELD, E. F. (1980) Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. *J Biol Chem,* 255, 4937-45.

HATA, R., MASUMURA, M., AKATSU, H., LI, F., FUJITA, H., NAGAI, Y., YAMAMOTO, T., OKADA, H., KOSAKA, K., SAKANAKA, M. & SAWADA, T. (2001) Up-regulation of calcineurin Abeta mRNA in the Alzheimer's disease brain: assessment by cDNA microarray. *Biochem Biophys Res Commun,* 284, 310-6.

HAYES, J. D., CHANAS, S. A., HENDERSON, C. J., MCMAHON, M., SUN, C., MOFFAT, G. J., WOLF, C. R. & YAMAMOTO, M. (2000) The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. *Biochem Soc Trans,* 28, 33-41.

HAYES, J. D. & PULFORD, D. J. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol,* 30, 445-600.

HEIDUSCHKA, P., BLITGEN-HEINECKE, P., TURA, A., KOKKINOU, D., JULIEN, S., HOFMEISTER, S., BARTZ-SCHMIDT, K. U. & SCHRAERMEYER, U. (2007) Melanin precursor 5,6-dihydroxyindol: protective effects and cytotoxicity on retinal cells in vitro and in vivo. *Toxicol Pathol,* 35, 1030-8.

HENDRIKS, L., VAN DUIJN, C. M., CRAS, P., CRUTS, M., VAN HUL, W., VAN HARSKAMP, F., WARREN, A., MCINNIS, M. G., ANTONARAKIS, S. E., MARTIN, J. J. & ET AL. (1992) Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nat Genet,* 1, 218-21.

HENRIKSSON, T., BARBOUR, R. M., BRAA, S., WARD, P., FRITZ, L. C., JOHNSON-WOOD, K., CHUNG, H. D., BURKE, W., REINIKAINEN, K. J., RIEKKINEN, P. & ET AL. (1991) Analysis and quantitation of the beta-amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients with a monoclonal antibody-based immunoassay. *J Neurochem,* 56, 1037-42.

HESSE, C., LARSSON, H., FREDMAN, P., MINTHON, L., ANDREASEN, N., DAVIDSSON, P. & BLENNOW, K. (2000) Measurement of apolipoprotein E (apoE) in cerebrospinal fluid. *Neurochem Res,* 25, 511-7.

HEYN, P., ABREU, B. C. & OTTENBACHER, K. J. (2004) The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis. *Arch Phys Med Rehabil,* 85, 1694-704.

HILL, M. N. & GORZALKA, B. B. (2009) The endocannabinoid system and the treatment of mood and anxiety disorders. *CNS Neurol Disord Drug Targets,* 8, 451-8.

HINDS, T. R., KUKULL, W. A., VAN BELLE, G., SCHELLENBERG, G. D., VILLACRES, E. C. & LARSON, E. B. (1994) Relationship between serum alpha 1-antichymotrypsin and Alzheimer's disease. *Neurobiol Aging,* 15, 21-7.

HIRAI, K., ALIEV, G., NUNOMURA, A., FUJIOKA, H., RUSSELL, R. L., ATWOOD, C. S., JOHNSON, A. B., KRESS, Y., VINTERS, H. V., TABATON, M., SHIMOHAMA, S., CASH, A. D., SIEDLAK, S. L., HARRIS, P. L., JONES, P. K., PETERSEN, R. B., PERRY, G. & SMITH, M. A. (2001) Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci,* 21, 3017-23.

HIRAOKA, Y., OHNO, M., YOSHIDA, K., OKAWA, K., TOMIMOTO, H., KITA, T. & NISHI, E. (2007) Enhancement of alpha-secretase cleavage of amyloid precursor protein by a metalloendopeptidase nardilysin. *J Neurochem,* 102, 1595-605.

HIRAOKA, Y., YOSHIDA, K., OHNO, M., MATSUOKA, T., KITA, T. & NISHI, E. (2008) Ectodomain shedding of TNF-alpha is enhanced by nardilysin via activation of ADAM proteases. *Biochem Biophys Res Commun,* 370, 154-8.

HOCHSTRASSER, D. F. (1998) Proteome in perspective. *Clin Chem Lab Med,* 36, 825-36.

HOFFMAN, J. M., WELSH-BOHMER, K. A., HANSON, M., CRAIN, B., HULETTE, C., EARL, N. & COLEMAN, R. E. (2000) FDG PET imaging in patients with pathologically verified dementia. *J Nucl Med,* 41, 1920-8.

HOFMAN, A., ROCCA, W. A., BRAYNE, C., BRETELER, M. M., CLARKE, M., COOPER, B., COPELAND, J. R., DARTIGUES, J. F., DA SILVA DROUX, A., HAGNELL, O. & ET

AL. (1991) The prevalence of dementia in Europe: a collaborative study of 1980-1990 findings. Eurodem Prevalence Research Group. *Int J Epidemiol,* 20, 736-48.

HOLMES, C., BOCHE, D., WILKINSON, D., YADEGARFAR, G., HOPKINS, V., BAYER, A., JONES, R. W., BULLOCK, R., LOVE, S., NEAL, J. W., ZOTOVA, E. & NICOLL, J. A. (2008) Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet,* 372, 216-23.

HONG, G. S., HEUN, R., JESSEN, F., POPP, J., HENTSCHEL, F., KELEMEN, P., SCHULZ, A., MAIER, W. & KOLSCH, H. (2009) Gene variations in GSTM3 are a risk factor for Alzheimer's disease. *Neurobiol Aging,* 30, 691-6.

HOOK, V., SCHECHTER, I., DEMUTH, H. U. & HOOK, G. (2008) Alternative pathways for production of beta-amyloid peptides of Alzheimer's disease. *Biol Chem,* 389, 993-1006.

HOOZEMANS, J. J., VEERHUIS, R., ROZEMULLER, J. M. & EIKELENBOOM, P. (2006) Neuroinflammation and regeneration in the early stages of Alzheimer's disease pathology. *Int J Dev Neurosci,* 24, 157-65.

HOPWOOD, B., TSYKIN, A., FINDLAY, D. M. & FAZZALARI, N. L. (2009) Gene expression profile of the bone microenvironment in human fragility fracture bone. *Bone,* 44, 87-101.

HORI, T., NAKASHIMA, T., TAKE, S., KAIZUKA, Y., MORI, T. & KATAFUCHI, T. (1991) Immune cytokines and regulation of body temperature, food intake and cellular immunity. *Brain Res Bull,* 27, 309-13.

HSIAO, K. K., BORCHELT, D. R., OLSON, K., JOHANNSDOTTIR, R., KITT, C., YUNIS, W., XU, S., ECKMAN, C., YOUNKIN, S., PRICE, D. & ET AL. (1995) Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron,* 15, 1203-18.

HSU, Y. Y., SCHUFF, N., DU, A. T., MARK, K., ZHU, X., HARDIN, D. & WEINER, M. W. (2002) Comparison of automated and manual MRI volumetry of hippocampus in normal aging and dementia. *J Magn Reson Imaging,* 16, 305-10.

HUANG, C., EIDELBERG, D., HABECK, C., MOELLER, J., SVENSSON, L., TARABULA, T. & JULIN, P. (2007) Imaging markers of mild cognitive impairment: multivariate analysis of CBF SPECT. *Neurobiol Aging,* 28, 1062-9.

HUGHES, C. P., BERG, L., DANZIGER, W. L., COBEN, L. A. & MARTIN, R. L. (1982) A new clinical scale for the staging of dementia. *Br J Psychiatry,* 140, 566-72.

HULSTAERT, F., BLENNOW, K., IVANOIU, A., SCHOONDERWALDT, H. C., RIEMENSCHNEIDER, M., DE DEYN, P. P., BANCHER, C., CRAS, P., WILTFANG, J., MEHTA, P. D., IQBAL, K., POTTEL, H., VANMECHELEN, E. & VANDERSTICHELE, H. (1999) Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. *Neurology,* 52, 1555-62.

HUNG, Y. C., HUANG, G. S., SAVA, V. M., BLAGODARSKY, V. A. & HONG, M. Y. (2006) Protective effects of tea melanin against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity: antioxidant activity and aryl hydrocarbon receptor suppressive effect. *Biol Pharm Bull,* 29, 2284-91.

HUTTON, M., LENDON, C. L., RIZZU, P., BAKER, M., FROELICH, S., HOULDEN, H., PICKERING-BROWN, S., CHAKRAVERTY, S., ISAACS, A., GROVER, A., HACKETT, J., ADAMSON, J., LINCOLN, S., DICKSON, D., DAVIES, P., PETERSEN, R. C., STEVENS, M., DE GRAAFF, E., WAUTERS, E., VAN BAREN, J., HILLEBRAND, M.,

JOOSSE, M., KWON, J. M., NOWOTNY, P., CHE, L. K., NORTON, J., MORRIS, J. C., REED, L. A., TROJANOWSKI, J., BASUN, H., LANNFELT, L., NEYSTAT, M., FAHN, S., DARK, F., TANNENBERG, T., DODD, P. R., HAYWARD, N., KWOK, J. B., SCHOFIELD, P. R., ANDREADIS, A., SNOWDEN, J., CRAUFURD, D., NEARY, D., OWEN, F., OOSTRA, B. A., HARDY, J., GOATE, A., VAN SWIETEN, J., MANN, D., LYNCH, T. & HEUTINK, P. (1998) Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature,* 393, 702-5.

HWANG, S. Y., YOO, B. C., JUNG, J. W., OH, E. S., HWANG, J. S., SHIN, J. A., KIM, S. Y., CHA, S. H. & HAN, I. O. (2009) Induction of glioma apoptosis by microglia-secreted molecules: The role of nitric oxide and cathepsin B. *Biochim Biophys Acta,* 1793, 1656-68.

HWANG, T. J., MASTERMAN, D. L., ORTIZ, F., FAIRBANKS, L. A. & CUMMINGS, J. L. (2004) Mild cognitive impairment is associated with characteristic neuropsychiatric symptoms. *Alzheimer Dis Assoc Disord,* 18, 17-21.

IDA, N., HARTMANN, T., PANTEL, J., SCHRODER, J., ZERFASS, R., FORSTL, H., SANDBRINK, R., MASTERS, C. L. & BEYREUTHER, K. (1996) Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem,* 271, 22908-14.

IN T' VELD, B. A., RUITENBERG, A., HOFMAN, A., LAUNER, L. J., VAN DUIJN, C. M., STIJNEN, T., BRETELER, M. M. & STRICKER, B. H. (2001) Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N Engl J Med,* 345, 1515-21.

IPA (1998) Behavioral and Psychological Symptoms of Dementia (BPSD) Educational Pack

IRIZARRY, M. C., MCNAMARA, M., FEDORCHAK, K., HSIAO, K. & HYMAN, B. T. (1997) APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol,* 56, 965-73.

IRIZARRY, M. C., YAO, Y., HYMAN, B. T., GROWDON, J. H. & PRATICO, D. (2007) Plasma F2A isoprostane levels in Alzheimer's and Parkinson's disease. *Neurodegener Dis,* 4, 403-5.

ISHII, K., II, K., HASEGAWA, T., SHOJI, S., DOI, A. & MORI, H. (1997) Increased A beta 42(43)-plaque deposition in early-onset familial Alzheimer's disease brains with the deletion of exon 9 and the missense point mutation (H163R) in the PS-1 gene. *Neurosci Lett,* 228, 17-20.

JACK, C. R., JR., LOWE, V. J., SENJEM, M. L., WEIGAND, S. D., KEMP, B. J., SHIUNG, M. M., KNOPMAN, D. S., BOEVE, B. F., KLUNK, W. E., MATHIS, C. A. & PETERSEN, R. C. (2008) 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain,* 131, 665-80.

JACK, C. R., JR., PETERSEN, R. C., XU, Y. C., O'BRIEN, P. C., SMITH, G. E., IVNIK, R. J., BOEVE, B. F., WARING, S. C., TANGALOS, E. G. & KOKMEN, E. (1999) Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology,* 52, 1397-403.

JENSEN, M., SCHRODER, J., BLOMBERG, M., ENGVALL, B., PANTEL, J., IDA, N., BASUN, H., WAHLUND, L. O., WERLE, E., JAUSS, M., BEYREUTHER, K., LANNFELT, L. & HARTMANN, T. (1999) Cerebrospinal fluid A beta42 is increased early in sporadic Alzheimer's disease and declines with disease progression. *Ann Neurol,* 45, 504-11.

JIA, J. P., MENG, R., SUN, Y. X., SUN, W. J., JI, X. M. & JIA, L. F. (2005) Cerebrospinal fluid tau, Abeta1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia. *Neurosci Lett,* 383, 12-6.

JICHA, G. A., PARISI, J. E., DICKSON, D. W., JOHNSON, K., CHA, R., IVNIK, R. J., TANGALOS, E. G., BOEVE, B. F., KNOPMAN, D. S., BRAAK, H. & PETERSEN, R. C. (2006) Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch Neurol,* 63, 674-81.

JIN, K., MAO, X. O., SUN, Y., XIE, L., JIN, L., NISHI, E., KLAGSBRUN, M. & GREENBERG, D. A. (2002) Heparin-binding epidermal growth factor-like growth factor: hypoxia-inducible expression in vitro and stimulation of neurogenesis in vitro and in vivo. *J Neurosci,* 22, 5365-73.

JOHNSTON-WILSON, N. L., SIMS, C. D., HOFMANN, J. P., ANDERSON, L., SHORE, A. D., TORREY, E. F. & YOLKEN, R. H. (2000) Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium. *Mol Psychiatry,* 5, 142-9.

JORM, A. F. (2001) History of depression as a risk factor for dementia: an updated review. *Aust N Z J Psychiatry,* 35, 776-81.

JORM, A. F., KORTEN, A. E. & HENDERSON, A. S. (1987) The prevalence of dementia: a quantitative integration of the literature. *Acta Psychiatr Scand,* 76, 465-79.

JOSEPH, S. K., THOMAS, A. P., WILLIAMS, R. J., IRVINE, R. F. & WILLIAMSON, J. R. (1984) myo-Inositol 1,4,5-trisphosphate. A second messenger for the hormonal mobilization of intracellular Ca2+ in liver. *J Biol Chem,* 259, 3077-81.

JOU, S. H., CHIU, N. Y. & LIU, C. S. (2009) Mitochondrial dysfunction and psychiatric disorders. *Chang Gung Med J,* 32, 370-9.

KABIR, I., MALEK, M. A., RAHMAN, M. M., KHALED, M. A. & MAHALANABIS, D. (1994) Changes in body composition of malnourished children after dietary supplementation as measured by bioelectrical impedance. *Am J Clin Nutr,* 59, 5-9.

KALMAN, J., KITAJKA, K., PAKASKI, M., ZVARA, A., JUHASZ, A., VINCZE, G., JANKA, Z. & PUSKAS, L. G. (2005) Gene expression profile analysis of lymphocytes from Alzheimer's patients. *Psychiatr Genet,* 15, 1-6.

KALMIJN, S., LAUNER, L. J., LINDEMANS, J., BOTS, M. L., HOFMAN, A. & BRETELER, M. M. (1999) Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. *Am J Epidemiol,* 150, 283-9.

KANEMARU, K., KAMEDA, N. & YAMANOUCHI, H. (2000) Decreased CSF amyloid beta42 and normal tau levels in dementia with Lewy bodies. *Neurology,* 54, 1875-6.

KARAS, G. B., SCHELTENS, P., ROMBOUTS, S. A., VISSER, P. J., VAN SCHIJNDEL, R. A., FOX, N. C. & BARKHOF, F. (2004) Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. *Neuroimage,* 23, 708-16.

KATAYAMA, T., IMAIZUMI, K., SATO, N., MIYOSHI, K., KUDO, T., HITOMI, J., MORIHARA, T., YONEDA, T., GOMI, F., MORI, Y., NAKANO, Y., TAKEDA, J., TSUDA, T., ITOYAMA, Y., MURAYAMA, O., TAKASHIMA, A., ST GEORGE-HYSLOP, P., TAKEDA, M. & TOHYAMA, M. (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat Cell Biol,* 1, 479-85.

KAWAMATA, J. & SHIMOHAMA, S. (2002) Association of novel and established polymorphisms in neuronal nicotinic acetylcholine receptors with sporadic Alzheimer's disease. *J Alzheimers Dis,* 4, 71-6.

KEMPERMANN, G. & NEUMANN, H. (2003) Neuroscience. Microglia: the enemy within? *Science,* 302, 1689-90.

KENESSEY, A., NACHARAJU, P., KO, L. W. & YEN, S. H. (1997) Degradation of tau by lysosomal enzyme cathepsin D: implication for Alzheimer neurofibrillary degeneration. *J Neurochem,* 69, 2026-38.

KIM, J., BASAK, J. M. & HOLTZMAN, D. M. (2009) The role of apolipoprotein E in Alzheimer's disease. *Neuron,* 63, 287-303.

KIM, S. J., DIX, D. J., THOMPSON, K. E., MURRELL, R. N., SCHMID, J. E., GALLAGHER, J. E. & ROCKETT, J. C. (2007) Effects of storage, RNA extraction, genechip type, and donor sex on gene expression profiling of human whole blood. *Clin Chem,* 53, 1038-45.

KLUNK, W. E., PRICE, J. C., MATHIS, C. A., TSOPELAS, N. D., LOPRESTI, B. J., ZIOLKO, S. K., BI, W., HOGE, J. A., COHEN, A. D., IKONOMOVIC, M. D., SAXTON, J. A., SNITZ, B. E., POLLEN, D. A., MOONIS, M., LIPPA, C. F., SWEARER, J. M., JOHNSON, K. A., RENTZ, D. M., FISCHMAN, A. J., AIZENSTEIN, H. J. & DEKOSKY, S. T. (2007) Amyloid deposition begins in the striatum of presenilin-1 mutation carriers from two unrelated pedigrees. *J Neurosci,* 27, 6174-84.

KOSAKA, T., IMAGAWA, M., SEKI, K., ARAI, H., SASAKI, H., TSUJI, S., ASAMI-ODAKA, A., FUKUSHIMA, T., IMAI, K. & IWATSUBO, T. (1997) The beta APP717 Alzheimer mutation increases the percentage of plasma amyloid-beta protein ending at A beta42(43). *Neurology,* 48, 741-5.

KRIAUCIONIS, S., PATERSON, A., CURTIS, J., GUY, J., MACLEOD, N. & BIRD, A. (2006) Gene expression analysis exposes mitochondrial abnormalities in a mouse model of Rett syndrome. *Mol Cell Biol,* 26, 5033-42.

KRISHNAN, K. R., CHARLES, H. C., DORAISWAMY, P. M., MINTZER, J., WEISLER, R., YU, X., PERDOMO, C., IENI, J. R. & ROGERS, S. (2003) Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. *Am J Psychiatry,* 160, 2003-11.

KULAWIEC, M., ARNOUK, H., DESOUKI, M. M., KAZIM, L., STILL, I. & SINGH, K. K. (2006) Proteomic analysis of mitochondria-to-nucleus retrograde response in human cancer. *Cancer Biol Ther,* 5, 967-75.

LADHA, J. S., TRIPATHY, M. K. & MITRA, D. (2005) Mitochondrial complex I activity is impaired during HIV-1-induced T-cell apoptosis. *Cell Death Differ,* 12, 1417-28.

LAFAY-CHEBASSIER, C., PACCALIN, M., PAGE, G., BARC-PAIN, S., PERAULT-POCHAT, M. C., GIL, R., PRADIER, L. & HUGON, J. (2005) mTOR/p70S6k signalling alteration by Abeta exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. *J Neurochem,* 94, 215-25.

LAFERLA, F. M., HALL, C. K., NGO, L. & JAY, G. (1996) Extracellular deposition of beta-amyloid upon p53-dependent neuronal cell death in transgenic mice. *J Clin Invest,* 98, 1626-32.

LAHIRI, D. K., GE, Y. W., MALONEY, B., WAVRANT-DE VRIEZE, F. & HARDY, J. (2005) Characterization of two APP gene promoter polymorphisms that appear to influence risk of late-onset Alzheimer's disease. *Neurobiol Aging,* 26, 1329-41.

LAM, L. C., TANG, N. L., MA, S. L., LUI, V. W., CHAN, A. S., LEUNG, P. Y. & CHIU, H. F. (2006) Apolipoprotein epsilon-4 allele and the two-year progression of cognitive function in Chinese subjects with late-onset Alzheimer's disease. *Am J Alzheimers Dis Other Demen,* 21, 92-9.

LAMBERT, D. M. & FOWLER, C. J. (2005) The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J Med Chem,* 48, 5059-87.

LANNFELT, L., BASUN, H., VIGO-PELFREY, C., WAHLUND, L. O., WINBLAD, B., LIEBERBURG, I. & SCHENK, D. (1995) Amyloid beta-peptide in cerebrospinal fluid in individuals with the Swedish Alzheimer amyloid precursor protein mutation. *Neurosci Lett,* 199, 203-6.

LANZREIN, A. S., JOHNSTON, C. M., PERRY, V. H., JOBST, K. A., KING, E. M. & SMITH, A. D. (1998) Longitudinal study of inflammatory factors in serum, cerebrospinal fluid, and brain tissue in Alzheimer disease: interleukin-1beta, interleukin-6, interleukin-1 receptor antagonist, tumor necrosis factor-alpha, the soluble tumor necrosis factor receptors I and II, and alpha1-antichymotrypsin. *Alzheimer Dis Assoc Disord,* 12, 215-27.

LAWS, S. M., PERNECZKY, R., WAGENPFEIL, S., MULLER, U., FORSTL, H., MARTINS, R. N., KURZ, A. & RIEMENSCHNEIDER, M. (2005) TNF polymorphisms in Alzheimer disease and functional implications on CSF beta-amyloid levels. *Hum Mutat,* 26, 29-35.

LE BASTARD, N., MARTIN, J. J., VANMECHELEN, E., VANDERSTICHELE, H., DE DEYN, P. P. & ENGELBORGHS, S. (2009) Added diagnostic value of CSF biomarkers in differential dementia diagnosis. *Neurobiol Aging.*

LEBOEUF, R. (2003) Homocysteine and Alzheimer's disease. *J Am Diet Assoc,* 103, 304-7.

LEE, M. K., BORCHELT, D. R., KIM, G., THINAKARAN, G., SLUNT, H. H., RATOVITSKI, T., MARTIN, L. J., KITTUR, A., GANDY, S., LEVEY, A. I., JENKINS, N., COPELAND, N., PRICE, D. L. & SISODIA, S. S. (1997) Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. *Nat Med,* 3, 756-60.

LERCH, J. P., PRUESSNER, J., ZIJDENBOS, A. P., COLLINS, D. L., TEIPEL, S. J., HAMPEL, H. & EVANS, A. C. (2008) Automated cortical thickness measurements from MRI can accurately separate Alzheimer's patients from normal elderly controls. *Neurobiol Aging,* 29, 23-30.

LEUNG, V. P., LAM, L. C., CHIU, H. F., CUMMINGS, J. L. & CHEN, Q. L. (2001) Validation study of the Chinese version of the neuropsychiatric inventory (CNPI). *Int J Geriatr Psychiatry,* 16, 789-93.

LEWCZUK, P., ESSELMANN, H., OTTO, M., MALER, J. M., HENKEL, A. W., HENKEL, M. K., EIKENBERG, O., ANTZ, C., KRAUSE, W. R., REULBACH, U., KORNHUBER, J. & WILTFANG, J. (2004) Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau. *Neurobiol Aging,* 25, 273-81.

LI, H. L., WANG, H. H., LIU, S. J., DENG, Y. Q., ZHANG, Y. J., TIAN, Q., WANG, X. C., CHEN, X. Q., YANG, Y., ZHANG, J. Y., WANG, Q., XU, H., LIAO, F. F. & WANG, J. Z. (2007) Phosphorylation of tau antagonizes apoptosis by stabilizing beta-catenin, a mechanism involved in Alzheimer's neurodegeneration. *Proc Natl Acad Sci U S A,* 104, 3591-6.

LI, Y., NOWOTNY, P., HOLMANS, P., SMEMO, S., KAUWE, J. S., HINRICHS, A. L., TACEY, K., DOIL, L., VAN LUCHENE, R., GARCIA, V., ROWLAND, C., SCHRODI, S., LEONG, D., GOGIC, G., CHAN, J., CRAVCHIK, A., ROSS, D., LAU, K., KWOK, S., CHANG, S. Y., CATANESE, J., SNINSKY, J., WHITE, T. J., HARDY, J., POWELL, J., LOVESTONE, S., MORRIS, J. C., THAL, L., OWEN, M., WILLIAMS, J., GOATE, A. & GRUPE, A. (2004) Association of late-onset Alzheimer's disease with genetic variation in multiple members of the GAPD gene family. *Proc Natl Acad Sci U S A,* 101, 15688-93.

LIAO, P. C., YU, L., KUO, C.-C., LIN, C., KUO, Y.-M. (2007) Proteomics analysis of plasma for potential biomarkers in the diagnosis of Alzheimer's disease. *Proteomics Clin Appl,* 1, 506-512.

LICASTRO, F., PEDRINI, S., CAPUTO, L., ANNONI, G., DAVIS, L. J., FERRI, C., CASADEI, V. & GRIMALDI, L. M. (2000) Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? *J Neuroimmunol,* 103, 97-102.

LIEW, C. C., MA, J., TANG, H. C., ZHENG, R. & DEMPSEY, A. A. (2006) The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med,* 147, 126-32.

LINDH, M., BLOMBERG, M., JENSEN, M., BASUN, H., LANNFELT, L., ENGVALL, B., SCHARNAGEL, H., MARZ, W., WAHLUND, L. O. & COWBURN, R. F. (1997) Cerebrospinal fluid apolipoprotein E (apoE) levels in Alzheimer's disease patients are increased at follow up and show a correlation with levels of tau protein. *Neurosci Lett,* 229, 85-8.

LIOU, Y. C., SUN, A., RYO, A., ZHOU, X. Z., YU, Z. X., HUANG, H. K., UCHIDA, T., BRONSON, R., BING, G., LI, X., HUNTER, T. & LU, K. P. (2003) Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature,* 424, 556-61.

LOCASCIO, J. J., FUKUMOTO, H., YAP, L., BOTTIGLIERI, T., GROWDON, J. H., HYMAN, B. T. & IRIZARRY, M. C. (2008) Plasma amyloid beta-protein and C-reactive protein in relation to the rate of progression of Alzheimer disease. *Arch Neurol,* 65, 776-85.

LOPEZ, O. L., KULLER, L. H., MEHTA, P. D., BECKER, J. T., GACH, H. M., SWEET, R. A., CHANG, Y. F., TRACY, R. & DEKOSKY, S. T. (2008) Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. *Neurology,* 70, 1664-71.

LORING JF, WEN X, LEE JM, SEILHAMER J & R., S. (2002) A gene expression profile of Alzheimer's disease. *DNA Cell Biol,* 21, 241.

LOVELL, M. A., XIE, C. & MARKESBERY, W. R. (1998) Decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease. *Neurology,* 51, 1562-6.

LU, K. P. (2003) Prolyl isomerase Pin1 as a molecular target for cancer diagnostics and therapeutics. *Cancer Cell,* 4, 175-80.

LU, K. P., HANES, S. D. & HUNTER, T. (1996) A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature,* 380, 544-7.

LU, K. P., SUIZU, F., ZHOU, X. Z., FINN, G., LAM, P. & WULF, G. (2006) Targeting carcinogenesis: a role for the prolyl isomerase Pin1? *Mol Carcinog,* 45, 397-402.

LU, P. J., ZHOU, X. Z., SHEN, M. & LU, K. P. (1999) Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science,* 283, 1325-8.

LUCK, T., RIEDEL-HELLER, S. G., KADUSZKIEWICZ, H., BICKEL, H., JESSEN, F., PENTZEK, M., WIESE, B., KOELSCH, H., VAN DEN BUSSCHE, H., ABHOLZ, H. H., MOESCH, E., GORFER, S., ANGERMEYER, M. C., MAIER, W. & WEYERER, S. (2007) Mild cognitive impairment in general practice: age-specific prevalence and correlate results from the German study on ageing, cognition and dementia in primary care patients (AgeCoDe). *Dement Geriatr Cogn Disord,* 24, 307-16.

LYE, T. C. & SHORES, E. A. (2000) Traumatic brain injury as a risk factor for Alzheimer's disease: a review. *Neuropsychol Rev,* 10, 115-29.

LYKETSOS, C. G., STEINBERG, M., TSCHANZ, J. T., NORTON, M. C., STEFFENS, D. C. & BREITNER, J. C. (2000) Mental and behavioral disturbances in dementia: findings from the Cache County Study on Memory in Aging. *Am J Psychiatry,* 157, 708-14.

MA, S. L., TANG, N. L., LAM, L. C. & CHIU, H. F. (2005) The association between promoter polymorphism of the interleukin-10 gene and Alzheimer's disease. *Neurobiol Aging,* 26, 1005-10.

MACHULDA, M. M., WARD, H. A., BOROWSKI, B., GUNTER, J. L., CHA, R. H., O'BRIEN, P. C., PETERSEN, R. C., BOEVE, B. F., KNOPMAN, D., TANG-WAI, D. F., IVNIK, R. J., SMITH, G. E., TANGALOS, E. G. & JACK, C. R., JR. (2003) Comparison of memory fMRI response among normal, MCI, and Alzheimer's patients. *Neurology,* 61, 500-6.

MADDALENA, A., PAPASSOTIROPOULOS, A., MULLER-TILLMANNS, B., JUNG, H. H., HEGI, T., NITSCH, R. M. & HOCK, C. (2003) Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to beta-amyloid peptide42. *Arch Neurol,* 60, 1202-6.

MAES, O. C., SCHIPPER, H. M., CHONG, G., CHERTKOW, H. M. & WANG, E. (2010) A GSTM3 polymorphism associated with an etiopathogenetic mechanism in Alzheimer disease. *Neurobiol Aging,* 31, 34-45.

MAES, O. C., XU, S., YU, B., CHERTKOW, H. M., WANG, E. & SCHIPPER, H. M. (2007) Transcriptional profiling of Alzheimer blood mononuclear cells by microarray. *Neurobiol Aging,* 28, 1795-809.

MAHLEY, R. W., HUANG, Y. & WEISGRABER, K. H. (2007) Detrimental effects of apolipoprotein E4: potential therapeutic targets in Alzheimer's disease. *Curr Alzheimer Res,* 4, 537-40.

MAHLEY, R. W., WEISGRABER, K. H. & HUANG, Y. (2006) Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A,* 103, 5644-51.

MAK, Y. T., CHIU, H., WOO, J., KAY, R., CHAN, Y. S., HUI, E., SZE, K. H., LUM, C., KWOK, T. & PANG, C. P. (1996) Apolipoprotein E genotype and Alzheimer's disease in Hong Kong elderly Chinese. *Neurology,* 46, 146-9.

MANLY, J. J., TANG, M. X., SCHUPF, N., STERN, Y., VONSATTEL, J. P. & MAYEUX, R. (2008) Frequency and course of mild cognitive impairment in a multiethnic community. *Ann Neurol,* 63, 494-506.

MARECHAL, Y., PESESSE, X., JIA, Y., POUILLON, V., PEREZ-MORGA, D., DANIEL, J., IZUI, S., CULLEN, P. J., LEO, O., LUO, H. R., ERNEUX, C. & SCHURMANS, S. (2007) Inositol 1,3,4,5-tetrakisphosphate controls proapoptotic Bim gene expression and survival in B cells. *Proc Natl Acad Sci U S A,* 104, 13978-83.

MARKESBERY, W. R., SCHMITT, F. A., KRYSCIO, R. J., DAVIS, D. G., SMITH, C. D. & WEKSTEIN, D. R. (2006) Neuropathologic substrate of mild cognitive impairment. *Arch Neurol,* 63, 38-46.

MATSUBARA, E., HIRAI, S., AMARI, M., SHOJI, M., YAMAGUCHI, H., OKAMOTO, K., ISHIGURO, K., HARIGAYA, Y. & WAKABAYASHI, K. (1990) Alpha 1-antichymotrypsin as a possible biochemical marker for Alzheimer-type dementia. *Ann Neurol,* 28, 561-7.

MATSUNAGA, J., SINHA, D., SOLANO, F., SANTIS, C., WISTOW, G. & HEARING, V. (1999) Macrophage migration inhibitory factor (MIF)--its role in catecholamine metabolism. *Cell Mol Biol (Noisy-le-grand),* 45, 1035-40.

MAYEUX, R., SAUNDERS, A. M., SHEA, S., MIRRA, S., EVANS, D., ROSES, A. D., HYMAN, B. T., CRAIN, B., TANG, M. X. & PHELPS, C. H. (1998) Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's Disease Centers Consortium on Apolipoprotein E and Alzheimer's Disease. *N Engl J Med,* 338, 506-11.

MAYEUX, R., TANG, M. X., JACOBS, D. M., MANLY, J., BELL, K., MERCHANT, C., SMALL, S. A., STERN, Y., WISNIEWSKI, H. M. & MEHTA, P. D. (1999) Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. *Ann Neurol,* 46, 412-6.

MCGEER, P. L., SCHULZER, M. & MCGEER, E. G. (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology,* 47, 425-32.

MCKHANN, G., DRACHMAN, D., FOLSTEIN, M., KATZMAN, R., PRICE, D. & STADLAN, E. M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology,* 34, 939-44.

MEGA, M. S., CUMMINGS, J. L., FIORELLO, T. & GORNBEIN, J. (1996) The spectrum of behavioral changes in Alzheimer's disease. *Neurology,* 46, 130-5.

MILLER, A. T., SANDBERG, M., HUANG, Y. H., YOUNG, M., SUTTON, S., SAUER, K. & COOKE, M. P. (2007) Production of Ins(1,3,4,5)P4 mediated by the kinase Itpkb inhibits store-operated calcium channels and regulates B cell selection and activation. *Nat Immunol,* 8, 514-21.

MILLER, J. W., GREEN, R., MUNGAS, D. M., REED, B. R. & JAGUST, W. J. (2002) Homocysteine, vitamin B6, and vascular disease in AD patients. *Neurology,* 58, 1471-5.

MINOSHIMA, S., GIORDANI, B., BERENT, S., FREY, K. A., FOSTER, N. L. & KUHL, D. E. (1997) Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol,* 42, 85-94.

MOHS, R. C., ROSEN, W. G. & DAVIS, K. L. (1983) The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. *Psychopharmacol Bull,* 19, 448-50.

MOK, E. H., LAM, L. C. & CHIU, H. F. (2004) Category verbal fluency test performance in chinese elderly with Alzheimer's disease. *Dement Geriatr Cogn Disord,* 18, 120-4.

MONTALDI, D., BROOKS, D. N., MCCOLL, J. H., WYPER, D., PATTERSON, J., BARRON, E. & MCCULLOCH, J. (1990) Measurements of regional cerebral blood flow and cognitive performance in Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 53, 33-8.

MONTINE, K. S., QUINN, J. F., ZHANG, J., FESSEL, J. P., ROBERTS, L. J., 2ND, MORROW, J. D. & MONTINE, T. J. (2004) Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chem Phys Lipids,* 128, 117-24.

MONTINE, T. J., BEAL, M. F., CUDKOWICZ, M. E., O'DONNELL, H., MARGOLIN, R. A., MCFARLAND, L., BACHRACH, A. F., ZACKERT, W. E., ROBERTS, L. J. & MORROW, J. D. (1999a) Increased CSF F2-isoprostane concentration in probable AD. *Neurology,* 52, 562-5.

MONTINE, T. J., BEAL, M. F., ROBERTSON, D., CUDKOWICZ, M. E., BIAGGIONI, I., O'DONNELL, H., ZACKERT, W. E., ROBERTS, L. J. & MORROW, J. D. (1999b) Cerebrospinal fluid F2-isoprostanes are elevated in Huntington's disease. *Neurology,* 52, 1104-5.

MONTINE, T. J., SHINOBU, L., MONTINE, K. S., ROBERTS, L. J., 2ND, KOWALL, N. W., BEAL, M. F. & MORROW, J. D. (2000) No difference in plasma or urinary F2-isoprostanes among patients with Huntington's disease or Alzheimer's disease and controls. *Ann Neurol,* 48, 950.

MORRIS, J. C. (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology,* 43, 2412-4.

MORRIS, J. C., ERNESTO, C., SCHAFER, K., COATS, M., LEON, S., SANO, M., THAL, L. J. & WOODBURY, P. (1997) Clinical dementia rating training and reliability in multicenter studies: the Alzheimer's Disease Cooperative Study experience. *Neurology,* 48, 1508-10.

MORRIS, J. C., MCKEEL, D. W., JR., FULLING, K., TORACK, R. M. & BERG, L. (1988) Validation of clinical diagnostic criteria for Alzheimer's disease. *Ann Neurol,* 24, 17-22.

MORRIS, M. S., BOSTOM, A. G., JACQUES, P. F., SELHUB, J. & ROSENBERG, I. H. (2001) Hyperhomocysteinemia and hypercholesterolemia associated with hypothyroidism in the third US National Health and Nutrition Examination Survey. *Atherosclerosis,* 155, 195-200.

MORROW, J. D., AWAD, J. A., BOSS, H. J., BLAIR, I. A. & ROBERTS, L. J., 2ND (1992) Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. *Proc Natl Acad Sci U S A,* 89, 10721-5.

MORROW, J. D. & ROBERTS, L. J. (1997) The isoprostanes: unique bioactive products of lipid peroxidation. *Prog Lipid Res,* 36, 1-21.

MORSCH, R., SIMON, W. & COLEMAN, P. D. (1999) Neurons may live for decades with neurofibrillary tangles. *J Neuropathol Exp Neurol,* 58, 188-97.

MOSCONI, L., PERANI, D., SORBI, S., HERHOLZ, K., NACMIAS, B., HOLTHOFF, V., SALMON, E., BARON, J. C., DE CRISTOFARO, M. T., PADOVANI, A., BORRONI, B., FRANCESCHI, M., BRACCO, L. & PUPI, A. (2004) MCI conversion to dementia and the APOE genotype: a prediction study with FDG-PET. *Neurology,* 63, 2332-40.

MOTTER, R., VIGO-PELFREY, C., KHOLODENKO, D., BARBOUR, R., JOHNSON-WOOD, K., GALASKO, D., CHANG, L., MILLER, B., CLARK, C., GREEN, R. & ET AL. (1995) Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol,* 38, 643-8.

MUDHER, A. & LOVESTONE, S. (2002) Alzheimer's disease-do tauists and baptists finally shake hands? *Trends Neurosci,* 25, 22-6.

MUFSON, E. J., COUNTS, S. E. & GINSBERG, S. D. (2002) Gene expression profiles of cholinergic nucleus basalis neurons in Alzheimer's disease. *Neurochem Res,* 27, 1035-48.

MUHAMMAD, A., FLORES, I., ZHANG, H., YU, R., STANISZEWSKI, A., PLANEL, E., HERMAN, M., HO, L., KREBER, R., HONIG, L. S., GANETZKY, B., DUFF, K., ARANCIO, O. & SMALL, S. A. (2008) Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. *Proc Natl Acad Sci U S A,* 105, 7327-32.

MULLAN, M., CRAWFORD, F., AXELMAN, K., HOULDEN, H., LILIUS, L., WINBLAD, B. & LANNFELT, L. (1992) A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat Genet,* 1, 345-7.

MUNRO, S., THOMAS, K. L. & ABU-SHAAR, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* 365, 61-5.

NATHAN, B. P., BELLOSTA, S., SANAN, D. A., WEISGRABER, K. H., MAHLEY, R. W. & PITAS, R. E. (1994) Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science,* 264, 850-2.

NESTOR, P. J., FRYER, T. D., SMIELEWSKI, P. & HODGES, J. R. (2003) Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. *Ann Neurol,* 54, 343-51.

NEVE, R. L., MCPHIE, D. L. & CHEN, Y. (2000) Alzheimer's disease: a dysfunction of the amyloid precursor protein(1). *Brain Res,* 886, 54-66.

NIELSEN, H. M., MINTHON, L., LONDOS, E., BLENNOW, K., MIRANDA, E., PEREZ, J., CROWTHER, D. C., LOMAS, D. A. & JANCIAUSKIENE, S. M. (2007) Plasma and CSF serpins in Alzheimer disease and dementia with Lewy bodies. *Neurology,* 69, 1569-79.

NISHI, E., HIRAOKA, Y., YOSHIDA, K., OKAWA, K. & KITA, T. (2006) Nardilysin enhances ectodomain shedding of heparin-binding epidermal growth factor-like growth factor through activation of tumor necrosis factor-alpha-converting enzyme. *J Biol Chem,* 281, 31164-72.

NISHI, E., PRAT, A., HOSPITAL, V., ELENIUS, K. & KLAGSBRUN, M. (2001) N-arginine dibasic convertase is a specific receptor for heparin-binding EGF-like growth factor that mediates cell migration. *Embo J,* 20, 3342-50.

NIXON, R. A., CATALDO, A. M., PASKEVICH, P. A., HAMILTON, D. J., WHEELOCK, T. R. & KANALEY-ANDREWS, L. (1992) The lysosomal system in neurons. Involvement at multiple stages of Alzheimer's disease pathogenesis. *Ann N Y Acad Sci,* 674, 65-88.

NOTKOLA, I. L., SULKAVA, R., PEKKANEN, J., ERKINJUNTTI, T., EHNHOLM, C., KIVINEN, P., TUOMILEHTO, J. & NISSINEN, A. (1998) Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology,* 17, 14-20.

NTAIS, C., POLYCARPOU, A. & IOANNIDIS, J. P. (2004) Meta-analysis of the association of the cathepsin D Ala224Val gene polymorphism with the risk of Alzheimer's disease: a HuGE gene-disease association review. *Am J Epidemiol,* 159, 527-36.

NUNOMURA, A., PERRY, G., HIRAI, K., ALIEV, G., TAKEDA, A., CHIBA, S. & SMITH, M. A. (1999a) Neuronal RNA oxidation in Alzheimer's disease and Down's syndrome. *Ann N Y Acad Sci,* 893, 362-4.

NUNOMURA, A., PERRY, G., PAPPOLLA, M. A., WADE, R., HIRAI, K., CHIBA, S. & SMITH, M. A. (1999b) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J Neurosci,* 19, 1959-64.

OHNO, M., HIRAOKA, Y., MATSUOKA, T., TOMIMOTO, H., TAKAO, K., MIYAKAWA, T., OSHIMA, N., KIYONARI, H., KIMURA, T., KITA, T. & NISHI, E. (2009) Nardilysin regulates axonal maturation and myelination in the central and peripheral nervous system. *Nat Neurosci,* 12, 1506-13.

OKAMOTO, T., TAKEDA, S., MURAYAMA, Y., OGATA, E. & NISHIMOTO, I. (1995) Ligand-dependent G protein coupling function of amyloid transmembrane precursor. *J Biol Chem,* 270, 4205-8.

OKELLO, A., EDISON, P., ARCHER, H. A., TURKHEIMER, F. E., KENNEDY, J., BULLOCK, R., WALKER, Z., KENNEDY, A., FOX, N., ROSSOR, M. & BROOKS, D. J. (2009) Microglial activation and amyloid deposition in mild cognitive impairment: a PET study. *Neurology,* 72, 56-62.

ORELLANA, D. I., QUINTANILLA, R. A., GONZALEZ-BILLAULT, C. & MACCIONI, R. B. (2005) Role of the JAKs/STATs pathway in the intracellular calcium changes induced by interleukin-6 in hippocampal neurons. *Neurotox Res,* 8, 295-304.

ORGOGOZO, J. M., RIGAUD, A. S., STOFFLER, A., MOBIUS, H. J. & FORETTE, F. (2002) Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). *Stroke,* 33, 1834-9.

OTSUKA, M., YAMAGUCHI, K. & UEKI, A. (2002) Similarities and differences between Alzheimer's disease and vascular dementia from the viewpoint of nutrition. *Ann N Y Acad Sci,* 977, 155-61.

OWNBY, R. L., CROCCO, E., ACEVEDO, A., JOHN, V. & LOEWENSTEIN, D. (2006) Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. *Arch Gen Psychiatry,* 63, 530-8.

PAPASSOTIROPOULOS, A., LEWIS, H. D., BAGLI, M., JESSEN, F., PTOK, U., SCHULTE, A., SHEARMAN, M. S. & HEUN, R. (2002a) Cerebrospinal fluid levels of beta-amyloid(42) in patients with Alzheimer's disease are related to the exon 2 polymorphism of the cathepsin D gene. *Neuroreport,* 13, 1291-4.

PAPASSOTIROPOULOS, A., LUTJOHANN, D., BAGLI, M., LOCATELLI, S., JESSEN, F., BUSCHFORT, R., PTOK, U., BJORKHEM, I., VON BERGMANN, K. & HEUN, R. (2002b) 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia. *J Psychiatr Res,* 36, 27-32.

PARADOWSKI, B., CELCZYNSKA, D., DOBOSZ, T. & NOGA, L. (2008) Polymorphism 174 G/C of interleukin 6 gene in Alzheimer's disease--preliminary report. *Neurol Neurochir Pol,* 42, 312-5.

PARNETTI, L., LANARI, A., AMICI, S., GALLAI, V., VANMECHELEN, E. & HULSTAERT, F. (2001) CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. Phospho-Tau International Study Group. *Neurol Sci,* 22, 77-8.

PARNETTI, L., REBOLDI, G. P. & GALLAI, V. (2000) Cerebrospinal fluid pyruvate levels in Alzheimer's disease and vascular dementia. *Neurology,* 54, 735-7.

PASINETTI, G. M. (2001) Use of cDNA microarray in the search for molecular markers involved in the onset of Alzheimer's disease dementia. *J Neurosci Res,* 65, 471-6.

PASTORINO, L., SUN, A., LU, P. J., ZHOU, X. Z., BALASTIK, M., FINN, G., WULF, G., LIM, J., LI, S. H., LI, X., XIA, W., NICHOLSON, L. K. & LU, K. P. (2006) The prolyl isomerase

Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature,* 440, 528-34.

PAYTON, A., HOLLAND, F., DIGGLE, P., RABBITT, P., HORAN, M., DAVIDSON, Y., GIBBONS, L., WORTHINGTON, J., OLLIER, W. E. & PENDLETON, N. (2003) Cathepsin D exon 2 polymorphism associated with general intelligence in a healthy older population. *Mol Psychiatry,* 8, 14-8.

PAYTON, A., VAN DEN BOOGERD, E., DAVIDSON, Y., GIBBONS, L., OLLIER, W., RABBITT, P., WORTHINGTON, J., HORAN, M. & PENDLETON, N. (2006) Influence and interactions of cathepsin D, HLA-DRB1 and APOE on cognitive abilities in an older non-demented population. *Genes Brain Behav,* 5 Suppl 1, 23-31.

PETERSEN, R. C. (2004) Mild cognitive impairment as a diagnostic entity. *J Intern Med,* 256, 183-94.

PETERSEN, R. C. & NEGASH, S. (2008) Mild cognitive impairment: an overview. *CNS Spectr,* 13, 45-53.

PETERSEN, R. C., SMITH, G. E., WARING, S. C., IVNIK, R. J., TANGALOS, E. G. & KOKMEN, E. (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol,* 56, 303-8.

PETERSEN, R. C., STEVENS, J. C., GANGULI, M., TANGALOS, E. G., CUMMINGS, J. L. & DEKOSKY, S. T. (2001) Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology,* 56, 1133-42.

PETERSEN, R. C., THOMAS, R. G., GRUNDMAN, M., BENNETT, D., DOODY, R., FERRIS, S., GALASKO, D., JIN, S., KAYE, J., LEVEY, A., PFEIFFER, E., SANO, M., VAN DYCK, C. H. & THAL, L. J. (2005) Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med,* 352, 2379-88.

PFAFFL, M. W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res,* 29, e45.

PIKE, K. E., SAVAGE, G., VILLEMAGNE, V. L., NG, S., MOSS, S. A., MARUFF, P., MATHIS, C. A., KLUNK, W. E., MASTERS, C. L. & ROWE, C. C. (2007) Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain,* 130, 2837-44.

PIRTTILA, T., MEHTA, P. D., FREY, H. & WISNIEWSKI, H. M. (1994) Alpha 1-antichymotrypsin and IL-1 beta are not increased in CSF or serum in Alzheimer's disease. *Neurobiol Aging,* 15, 313-7.

PLASSMAN, B. L., LANGA, K. M., FISHER, G. G., HEERINGA, S. G., WEIR, D. R., OFSTEDAL, M. B., BURKE, J. R., HURD, M. D., POTTER, G. G., RODGERS, W. L., STEFFENS, D. C., MCARDLE, J. J., WILLIS, R. J. & WALLACE, R. B. (2008) Prevalence of cognitive impairment without dementia in the United States. *Ann Intern Med,* 148, 427-34.

POMARA, N., WILLOUGHBY, L. M., SIDTIS, J. J. & MEHTA, P. D. (2005) Selective reductions in plasma Abeta 1-42 in healthy elderly subjects during longitudinal follow-up: a preliminary report. *Am J Geriatr Psychiatry,* 13, 914-7.

POORKAJ, P., BIRD, T. D., WIJSMAN, E., NEMENS, E., GARRUTO, R. M., ANDERSON, L., ANDREADIS, A., WIEDERHOLT, W. C., RASKIND, M. & SCHELLENBERG, G. D.

(1998) Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol,* 43, 815-25.

POPP, J., BACHER, M., KOLSCH, H., NOELKER, C., DEUSTER, O., DODEL, R. & JESSEN, F. (2009) Macrophage migration inhibitory factor in mild cognitive impairment and Alzheimer's disease. *J Psychiatr Res,* 43, 749-53.

POTKIN, S. G., ANAND, R., FLEMING, K., ALVA, G., KEATOR, D., CARREON, D., MESSINA, J., WU, J. C., HARTMAN, R. & FALLON, J. H. (2001) Brain metabolic and clinical effects of rivastigmine in Alzheimer's disease. *Int J Neuropsychopharmacol,* 4, 223-30.

POUILLON, V., HASCAKOVA-BARTOVA, R., PAJAK, B., ADAM, E., BEX, F., DEWASTE, V., VAN LINT, C., LEO, O., ERNEUX, C. & SCHURMANS, S. (2003) Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development. *Nat Immunol,* 4, 1136-43.

PRABAKARAN, S., SWATTON, J. E., RYAN, M. M., HUFFAKER, S. J., HUANG, J. T., GRIFFIN, J. L., WAYLAND, M., FREEMAN, T., DUDBRIDGE, F., LILLEY, K. S., KARP, N. A., HESTER, S., TKACHEV, D., MIMMACK, M. L., YOLKEN, R. H., WEBSTER, M. J., TORREY, E. F. & BAHN, S. (2004) Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry,* 9, 684-97, 643.

PRATICO, D., CLARK, C. M., LEE, V. M., TROJANOWSKI, J. Q., ROKACH, J. & FITZGERALD, G. A. (2000) Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann Neurol,* 48, 809-12.

PRATICO, D., V, M. Y. L., TROJANOWSKI, J. Q., ROKACH, J. & FITZGERALD, G. A. (1998) Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *Faseb J,* 12, 1777-83.

QUINN, J. F., MONTINE, K. S., MOORE, M., MORROW, J. D., KAYE, J. A. & MONTINE, T. J. (2004) Suppression of longitudinal increase in CSF F2-isoprostanes in Alzheimer's disease. *J Alzheimers Dis,* 6, 93-7.

RAFII, M. S. & AISEN, P. S. (2009) Recent developments in Alzheimer's disease therapeutics. *BMC Med,* 7, 7.

RAMIREZ, B. G., BLAZQUEZ, C., GOMEZ DEL PULGAR, T., GUZMAN, M. & DE CEBALLOS, M. L. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci,* 25, 1904-13.

RAUX, G., GUYANT-MARECHAL, L., MARTIN, C., BOU, J., PENET, C., BRICE, A., HANNEQUIN, D., FREBOURG, T. & CAMPION, D. (2005) Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. *J Med Genet,* 42, 793-5.

RAZ, N., RODRIGUE, K. M., HEAD, D., KENNEDY, K. M. & ACKER, J. D. (2004) Differential aging of the medial temporal lobe: a study of a five-year change. *Neurology,* 62, 433-8.

REBECK, G. W., REITER, J. S., STRICKLAND, D. K. & HYMAN, B. T. (1993) Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron,* 11, 575-80.

REDDY, P. H., MCWEENEY, S., PARK, B. S., MANCZAK, M., GUTALA, R. V., PARTOVI, D., JUNG, Y., YAU, V., SEARLES, R., MORI, M. & QUINN, J. (2004) Gene expression

profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Hum Mol Genet,* 13, 1225-40.

REIMAN, E. M., WEBSTER, J. A., MYERS, A. J., HARDY, J., DUNCKLEY, T., ZISMANN, V. L., JOSHIPURA, K. D., PEARSON, J. V., HU-LINCE, D., HUENTELMAN, M. J., CRAIG, D. W., COON, K. D., LIANG, W. S., HERBERT, R. H., BEACH, T., ROHRER, K. C., ZHAO, A. S., LEUNG, D., BRYDEN, L., MARLOWE, L., KALEEM, M., MASTROENI, D., GROVER, A., HEWARD, C. B., RAVID, R., ROGERS, J., HUTTON, M. L., MELQUIST, S., PETERSEN, R. C., ALEXANDER, G. E., CASELLI, R. J., KUKULL, W., PAPASSOTIROPOULOS, A. & STEPHAN, D. A. (2007) GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. *Neuron,* 54, 713-20.

REISER, G. & BERNSTEIN, H. G. (2002) Neurons and plaques of Alzheimer's disease patients highly express the neuronal membrane docking protein p42IP4/centaurin alpha. *Neuroreport,* 13, 2417-9.

REISER, G. & BERNSTEIN, H. G. (2004) Altered expression of protein p42IP4/centaurin-alpha 1 in Alzheimer's disease brains and possible interaction of p42IP4 with nucleolin. *Neuroreport,* 15, 147-8.

RIEMENSCHNEIDER, M., BLENNOW, K., WAGENPFEIL, S., ANDREASEN, N., PRINCE, J. A., LAWS, S. M., FORSTL, H. & KURZ, A. (2006) The cathepsin D rs17571 polymorphism: effects on CSF tau concentrations in Alzheimer disease. *Hum Mutat,* 27, 532-7.

RIEMENSCHNEIDER, M., LAUTENSCHLAGER, N., WAGENPFEIL, S., DIEHL, J., DRZEZGA, A. & KURZ, A. (2002) Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer disease in subjects with mild cognitive impairment. *Arch Neurol,* 59, 1729-34.

RINGMAN, J. M., YOUNKIN, S. G., PRATICO, D., SELTZER, W., COLE, G. M., GESCHWIND, D. H., RODRIGUEZ-AGUDELO, Y., SCHAFFER, B., FEIN, J., SOKOLOW, S., ROSARIO, E. R., GYLYS, K. H., VARPETIAN, A., MEDINA, L. D. & CUMMINGS, J. L. (2008) Biochemical markers in persons with preclinical familial Alzheimer disease. *Neurology,* 71, 85-92.

RITCHIE, K., KILDEA, D. & ROBINE, J. M. (1992) The relationship between age and the prevalence of senile dementia: a meta-analysis of recent data. *Int J Epidemiol,* 21, 763-9.

ROE, C. M., XIONG, C., MILLER, J. P. & MORRIS, J. C. (2007) Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. *Neurology,* 68, 223-8.

ROGAEV, E. I., SHERRINGTON, R., ROGAEVA, E. A., LEVESQUE, G., IKEDA, M., LIANG, Y., CHI, H., LIN, C., HOLMAN, K., TSUDA, T. & ET AL. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature,* 376, 775-8.

ROGERS, J. T., LEITER, L. M., MCPHEE, J., CAHILL, C. M., ZHAN, S. S., POTTER, H. & NILSSON, L. N. (1999) Translation of the alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5'-untranslated region sequences. *J Biol Chem,* 274, 6421-31.

ROHRBACHER, J., BIJAK, M. & MISGELD, U. (1994) Suppression by memantine and amantadine of synaptic excitation intrastriatally evoked in rat neostriatal slices. *Neurosci Lett,* 182, 95-8.

ROJAS, R., KAMETAKA, S., HAFT, C. R. & BONIFACINO, J. S. (2007) Interchangeable but essential functions of SNX1 and SNX2 in the association of retromer with endosomes and the trafficking of mannose 6-phosphate receptors. *Mol Cell Biol,* 27, 1112-24.

ROJO, L. E., FERNANDEZ, J. A., MACCIONI, A. A., JIMENEZ, J. M. & MACCIONI, R. B. (2008) Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. *Arch Med Res,* 39, 1-16.

ROLLINS, B., MARTIN, M. V., MORGAN, L. & VAWTER, M. P. (2010) Analysis of whole genome biomarker expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet.*

ROMAS, S. N., TANG, M. X., BERGLUND, L. & MAYEUX, R. (1999) APOE genotype, plasma lipids, lipoproteins, and AD in community elderly. *Neurology,* 53, 517-21.

ROSEN, W. G., MOHS, R. C. & DAVIS, K. L. (1984) A new rating scale for Alzheimer's disease. *Am J Psychiatry,* 141, 1356-64.

ROSES, A. D. (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med,* 47, 387-400.

ROSNER, M., HANNEDER, M., SIEGEL, N., VALLI, A., FUCHS, C. & HENGSTSCHLAGER, M. (2008) The mTOR pathway and its role in human genetic diseases. *Mutat Res,* 659, 284-92.

RYO, A., UEMURA, H., ISHIGURO, H., SAITOH, T., YAMAGUCHI, A., PERREM, K., KUBOTA, Y., LU, K. P. & AOKI, I. (2005) Stable suppression of tumorigenicity by Pin1-targeted RNA interference in prostate cancer. *Clin Cancer Res,* 11, 7523-31.

SACHDEV, P. S. (2005) Homocysteine and brain atrophy. *Prog Neuropsychopharmacol Biol Psychiatry,* 29, 1152-61.

SAKAMOTO, S., ISHII, K., SASAKI, M., HOSAKA, K., MORI, T., MATSUI, M., HIRONO, N. & MORI, E. (2002) Differences in cerebral metabolic impairment between early and late onset types of Alzheimer's disease. *J Neurol Sci,* 200, 27-32.

SALOMON, R. N., UNDERWOOD, R., DOYLE, M. V., WANG, A. & LIBBY, P. (1992) Increased apolipoprotein E and c-fms gene expression without elevated interleukin 1 or 6 mRNA levels indicates selective activation of macrophage functions in advanced human atheroma. *Proc Natl Acad Sci U S A,* 89, 2814-8.

SANAN, D. A., WEISGRABER, K. H., RUSSELL, S. J., MAHLEY, R. W., HUANG, D., SAUNDERS, A., SCHMECHEL, D., WISNIEWSKI, T., FRANGIONE, B., ROSES, A. D. & ET AL. (1994) Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J Clin Invest,* 94, 860-9.

SAUNDERS, A. M., STRITTMATTER, W. J., SCHMECHEL, D., GEORGE-HYSLOP, P. H., PERICAK-VANCE, M. A., JOO, S. H., ROSI, B. L., GUSELLA, J. F., CRAPPER-MACLACHLAN, D. R., ALBERTS, M. J. & ET AL. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology,* 43, 1467-72.

SCHARRE, D. W., DAVIS, R. A., WARNER, J. L., CHANG, S. I. & BEVERSDORF, D. Q. (2003) A pilot open-label trial of citalopram for restless activity and aberrant motor behaviors in Alzheimer disease. *Am J Geriatr Psychiatry,* 11, 687-91.

SCHEUNER, D., ECKMAN, C., JENSEN, M., SONG, X., CITRON, M., SUZUKI, N., BIRD, T. D., HARDY, J., HUTTON, M., KUKULL, W., LARSON, E., LEVY-LAHAD, E., VIITANEN, M., PESKIND, E., POORKAJ, P., SCHELLENBERG, G., TANZI, R., WASCO, W., LANNFELT, L., SELKOE, D. & YOUNKIN, S. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med,* 2, 864-70.

SCHMECHEL, D. E., SAUNDERS, A. M., STRITTMATTER, W. J., CRAIN, B. J., HULETTE, C. M., JOO, S. H., PERICAK-VANCE, M. A., GOLDGABER, D. & ROSES, A. D. (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A,* 90, 9649-53.

SEAMAN, M. N. (2005) Recycle your receptors with retromer. *Trends Cell Biol,* 15, 68-75.

SEGAT, L., PONTILLO, A., ANNONI, G., TRABATTONI, D., VERGANI, C., CLERICI, M., AROSIO, B. & CROVELLA, S. (2007) PIN1 promoter polymorphisms are associated with Alzheimer's disease. *Neurobiol Aging,* 28, 69-74.

SELWOOD, S. P., PARVATHY, S., CORDELL, B., RYAN, H. S., OSHIDARI, F., VINCENT, V., YESAVAGE, J., LAZZERONI, L. C. & MURPHY, G. M., JR. (2009) Gene expression profile of the PDAPP mouse model for Alzheimer's disease with and without Apolipoprotein E. *Neurobiol Aging,* 30, 574-90.

SESHADRI, S., BEISER, A., SELHUB, J., JACQUES, P. F., ROSENBERG, I. H., D'AGOSTINO, R. B., WILSON, P. W. & WOLF, P. A. (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med,* 346, 476-83.

SHADLEN, M. F., SISCOVICK, D., FITZPATRICK, A. L., DULBERG, C., KULLER, L. H. & JACKSON, S. (2006) Education, cognitive test scores, and black-white differences in dementia risk. *J Am Geriatr Soc,* 54, 898-905.

SHEN YC, L. G., LI SR, CHEN CH, ZHAO YW, LIU M. (1994) Three Years Follow-up of Dementia in Beijing district *Chinese Mental Health Journal,* 8, 165-6.

SHENG, J. G., BORA, S. H., XU, G., BORCHELT, D. R., PRICE, D. L. & KOLIATSOS, V. E. (2003) Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. *Neurobiol Dis,* 14, 133-45.

SIEMERS, E. R., DEAN, R. A., FRIEDRICH, S., FERGUSON-SELLS, L., GONZALES, C., FARLOW, M. R. & MAY, P. C. (2007) Safety, tolerability, and effects on plasma and cerebrospinal fluid amyloid-beta after inhibition of gamma-secretase. *Clin Neuropharmacol,* 30, 317-25.

SIMMONS, D. L., BOTTING, R. M. & HLA, T. (2004) Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol Rev,* 56, 387-437.

SIMONSEN, A. H., MCGUIRE, J., HANSSON, O., ZETTERBERG, H., PODUST, V. N., DAVIES, H. A., WALDEMAR, G., MINTHON, L. & BLENNOW, K. (2007) Novel panel of cerebrospinal fluid biomarkers for the prediction of progression to Alzheimer dementia in patients with mild cognitive impairment. *Arch Neurol,* 64, 366-70.

SINGH, H., SACHAN, R., DEVI, S., PANDEY, S. N. & MITTAL, B. (2008) Association of GSTM1, GSTT1, and GSTM3 gene polymorphisms and susceptibility to cervical cancer in a North Indian population. *Am J Obstet Gynecol,* 198, 303 e1-6.

SJOGREN, M., DAVIDSSON, P., TULLBERG, M., MINTHON, L., WALLIN, A., WIKKELSO, C., GRANERUS, A. K., VANDERSTICHELE, H., VANMECHELEN, E. & BLENNOW, K. (2001a) Both total and phosphorylated tau are increased in Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 70, 624-30.

SJOGREN, M., DAVIDSSON, P., WALLIN, A., GRANERUS, A. K., GRUNDSTROM, E., ASKMARK, H., VANMECHELEN, E. & BLENNOW, K. (2002) Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mismetabolism of beta-amyloid induced by disparate mechanisms. *Dement Geriatr Cogn Disord,* 13, 112-8.

SJOGREN, M., MINTHON, L., DAVIDSSON, P., GRANERUS, A. K., CLARBERG, A., VANDERSTICHELE, H., VANMECHELEN, E., WALLIN, A. & BLENNOW, K. (2000) CSF levels of tau, beta-amyloid(1-42) and GAP-43 in frontotemporal dementia, other types of dementia and normal aging. *J Neural Transm,* 107, 563-79.

SJOGREN, M., VANDERSTICHELE, H., AGREN, H., ZACHRISSON, O., EDSBAGGE, M., WIKKELSO, C., SKOOG, I., WALLIN, A., WAHLUND, L. O., MARCUSSON, J., NAGGA, K., ANDREASEN, N., DAVIDSSON, P., VANMECHELEN, E. & BLENNOW, K. (2001b) Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values. *Clin Chem,* 47, 1776-81.

SLEEGERS, K., LAMBERT, J. C., BERTRAM, L., CRUTS, M., AMOUYEL, P. & VAN BROECKHOVEN, C. (2010) The pursuit of susceptibility genes for Alzheimer's disease: progress and prospects. *Trends Genet,* 26, 84-93.

SMALL, S. A., KENT, K., PIERCE, A., LEUNG, C., KANG, M. S., OKADA, H., HONIG, L., VONSATTEL, J. P. & KIM, T. W. (2005) Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann Neurol,* 58, 909-19.

SMITS, H. A., RIJSMUS, A., VAN LOON, J. H., WAT, J. W., VERHOEF, J., BOVEN, L. A. & NOTTET, H. S. (2002) Amyloid-beta-induced chemokine production in primary human macrophages and astrocytes. *J Neuroimmunol,* 127, 160-8.

SMITS, H. A., VAN BEELEN, A. J., DE VOS, N. M., RIJSMUS, A., VAN DER BRUGGEN, T., VERHOEF, J., VAN MUISWINKEL, F. L. & NOTTET, H. S. (2001) Activation of human macrophages by amyloid-beta is attenuated by astrocytes. *J Immunol,* 166, 6869-76.

SOLFRIZZI, V., COLACICCO, A. M., D'INTRONO, A., CAPURSO, C., PARIGI, A. D., CAPURSO, S. A., TORRES, F., CAPURSO, A. & PANZA, F. (2006) Macronutrients, aluminium from drinking water and foods, and other metals in cognitive decline and dementia. *J Alzheimers Dis,* 10, 303-30.

SOLOMON, A., KAREHOLT, I., NGANDU, T., WINBLAD, B., NISSINEN, A., TUOMILEHTO, J., SOININEN, H. & KIVIPELTO, M. (2007) Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study. *Neurology,* 68, 751-6.

SONG, F., POLJAK, A., SMYTHE, G. A. & SACHDEV, P. (2009) Plasma biomarkers for mild cognitive impairment and Alzheimer's disease. *Brain Res Rev,* 61, 69-80.

SOUTHWICK, P. C., YAMAGATA, S. K., ECHOLS, C. L., JR., HIGSON, G. J., NEYNABER, S. A., PARSON, R. E. & MUNROE, W. A. (1996) Assessment of amyloid beta protein in

cerebrospinal fluid as an aid in the diagnosis of Alzheimer's disease. *J Neurochem,* 66, 259-65.

SPERLING, R. A., BATES, J. F., CHUA, E. F., COCCHIARELLA, A. J., RENTZ, D. M., ROSEN, B. R., SCHACTER, D. L. & ALBERT, M. S. (2003) fMRI studies of associative encoding in young and elderly controls and mild Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 74, 44-50.

STOREY, J. D., MADEOY, J., STROUT, J. L., WURFEL, M., RONALD, J. & AKEY, J. M. (2007) Gene-expression variation within and among human populations. *Am J Hum Genet,* 80, 502-9.

STRITTMATTER, W. J., SAUNDERS, A. M., SCHMECHEL, D., PERICAK-VANCE, M., ENGHILD, J., SALVESEN, G. S. & ROSES, A. D. (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A,* 90, 1977-81.

STROZYK, D., BLENNOW, K., WHITE, L. R. & LAUNER, L. J. (2003) CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology,* 60, 652-6.

SU, B., WANG, X., NUNOMURA, A., MOREIRA, P. I., LEE, H. G., PERRY, G., SMITH, M. A. & ZHU, X. (2008) Oxidative stress signaling in Alzheimer's disease. *Curr Alzheimer Res,* 5, 525-32.

SULLIVAN, P. F., FAN, C. & PEROU, C. M. (2006) Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet,* 141B, 261-8.

SULTANA, R., BOYD-KIMBALL, D., POON, H. F., CAI, J., PIERCE, W. M., KLEIN, J. B., MARKESBERY, W. R., ZHOU, X. Z., LU, K. P. & BUTTERFIELD, D. A. (2006) Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: A redox proteomics analysis. *Neurobiol Aging,* 27, 918-25.

SUN, M. K. & ALKON, D. L. (2006) Links between Alzheimer's disease and diabetes. *Drugs Today (Barc),* 42, 481-9.

TAKAHASHI, K., UCHIDA, C., SHIN, R. W., SHIMAZAKI, K. & UCHIDA, T. (2008) Prolyl isomerase, Pin1: new findings of post-translational modifications and physiological substrates in cancer, asthma and Alzheimer's disease. *Cell Mol Life Sci,* 65, 359-75.

TAMAOKA, A., FUKUSHIMA, T., SAWAMURA, N., ISHIKAWA, K., OGUNI, E., KOMATSUZAKI, Y. & SHOJI, S. (1996) Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease. *J Neurol Sci,* 141, 65-8.

TANG, Y., GILBERT, D. L., GLAUSER, T. A., HERSHEY, A. D. & SHARP, F. R. (2005) Blood gene expression profiling of neurologic diseases: a pilot microarray study. *Arch Neurol,* 62, 210-5.

TANG, Y., SCHAPIRO, M. B., FRANZ, D. N., PATTERSON, B. J., HICKEY, F. J., SCHORRY, E. K., HOPKIN, R. J., WYLIE, M., NARAYAN, T., GLAUSER, T. A., GILBERT, D. L., HERSHEY, A. D. & SHARP, F. R. (2004) Blood expression profiles for tuberous sclerosis complex 2, neurofibromatosis type 1, and Down's syndrome. *Ann Neurol,* 56, 808-14.

TANG Z, ZHANG XQ, WU XG, LIU HJ, DIAO LJ, GUAN SC & AL., E. (2007) Prevalence of mild cognitive impairment among elderly in Beijing *Chinese Mental Health Journal,* 21, 116-118.

TANG Z, M. S., CHEN B. (2003) Epidemiology study in dementia in Beijing district of China. *Chin J Epidemiol,* 24, 734-6.

TAPIOLA, T., PIRTTILA, T., MEHTA, P. D., ALAFUZOFFF, I., LEHTOVIRTA, M. & SOININEN, H. (2000) Relationship between apoE genotype and CSF beta-amyloid (1-42) and tau in patients with probable and definite Alzheimer's disease. *Neurobiol Aging,* 21, 735-40.

TCHAIKOVSKAYA, T., FRAIFELD, V., URPHANISHVILI, T., ANDORFER, J. H., DAVIES, P. & LISTOWSKY, I. (2005) Glutathione S-transferase hGSTM3 and ageing-associated neurodegeneration: relationship to Alzheimer's disease. *Mech Ageing Dev,* 126, 309-15.

TEIPEL, S. J., BORN, C., EWERS, M., BOKDE, A. L., REISER, M. F., MOLLER, H. J. & HAMPEL, H. (2007) Multivariate deformation-based analysis of brain atrophy to predict Alzheimer's disease in mild cognitive impairment. *Neuroimage,* 38, 13-24.

TEIPEL, S. J., PRUESSNER, J. C., FALTRACO, F., BORN, C., ROCHA-UNOLD, M., EVANS, A., MOLLER, H. J. & HAMPEL, H. (2006) Comprehensive dissection of the medial temporal lobe in AD: measurement of hippocampus, amygdala, entorhinal, perirhinal and parahippocampal cortices using MRI. *J Neurol,* 253, 794-800.

TETLOW, N., ROBINSON, A., MANTLE, T. & BOARD, P. (2004) Polymorphism of human mu class glutathione transferases. *Pharmacogenetics,* 14, 359-68.

THAL, L. J., FERRIS, S. H., KIRBY, L., BLOCK, G. A., LINES, C. R., YUEN, E., ASSAID, C., NESSLY, M. L., NORMAN, B. A., BARANAK, C. C. & REINES, S. A. (2005) A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. *Neuropsychopharmacology,* 30, 1204-15.

THORPE, S. M., ROCHEFORT, H., GARCIA, M., FREISS, G., CHRISTENSEN, I. J., KHALAF, S., PAOLUCCI, F., PAU, B., RASMUSSEN, B. B. & ROSE, C. (1989) Association between high concentrations of Mr 52,000 cathepsin D and poor prognosis in primary human breast cancer. *Cancer Res,* 49, 6008-14.

TIERNEY, M. C., SZALAI, J. P., SNOW, W. G., FISHER, R. H., NORES, A., NADON, G., DUNN, E. & ST GEORGE-HYSLOP, P. H. (1996) Prediction of probable Alzheimer's disease in memory-impaired patients: A prospective longitudinal study. *Neurology,* 46, 661-5.

TIRABOSCHI, P., HANSEN, L. A., THAL, L. J. & COREY-BLOOM, J. (2004) The importance of neuritic plaques and tangles to the development and evolution of AD. *Neurology,* 62, 1984-9.

TON, C., HWANG, D. M., DEMPSEY, A. A. & LIEW, C. C. (1997) Identification and primary structure of five human NADH-ubiquinone oxidoreductase subunits. *Biochem Biophys Res Commun,* 241, 589-94.

TSUANG, M. T., NOSSOVA, N., YAGER, T., TSUANG, M. M., GUO, S. C., SHYU, K. G., GLATT, S. J. & LIEW, C. C. (2005) Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. *Am J Med Genet B Neuropsychiatr Genet,* 133B, 1-5.

TURK, B., TURK, D. & TURK, V. (2000) Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta,* 1477, 98-111.

UNVERZAGT, F. W., GAO, S., BAIYEWU, O., OGUNNIYI, A. O., GUREJE, O., PERKINS, A., EMSLEY, C. L., DICKENS, J., EVANS, R., MUSICK, B., HALL, K. S., HUI, S. L. &

HENDRIE, H. C. (2001) Prevalence of cognitive impairment: data from the Indianapolis Study of Health and Aging. *Neurology,* 57, 1655-62.

VACA, K. & WENDT, E. (1992) Divergent effects of astroglial and microglial secretions on neuron growth and survival. *Exp Neurol,* 118, 62-72.

VAN NOSTRAND, W. E., WAGNER, S. L., SHANKLE, W. R., FARROW, J. S., DICK, M., ROZEMULLER, J. M., KUIPER, M. A., WOLTERS, E. C., ZIMMERMAN, J., COTMAN, C. W. & ET AL. (1992) Decreased levels of soluble amyloid beta-protein precursor in cerebrospinal fluid of live Alzheimer disease patients. *Proc Natl Acad Sci U S A,* 89, 2551-5.

VAN SLEGTENHORST, M., NELLIST, M., NAGELKERKEN, B., CHEADLE, J., SNELL, R., VAN DEN OUWELAND, A., REUSER, A., SAMPSON, J., HALLEY, D. & VAN DER SLUIJS, P. (1998) Interaction between hamartin and tuberin, the TSC1 and TSC2 gene products. *Hum Mol Genet,* 7, 1053-7.

VARADARAJAN, S., YATIN, S., AKSENOVA, M. & BUTTERFIELD, D. A. (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. *J Struct Biol,* 130, 184-208.

VASILJEVA, O., PAPAZOGLOU, A., KRUGER, A., BRODOEFEL, H., KOROVIN, M., DEUSSING, J., AUGUSTIN, N., NIELSEN, B. S., ALMHOLT, K., BOGYO, M., PETERS, C. & REINHECKEL, T. (2006) Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. *Cancer Res,* 66, 5242-50.

VISSER, P. J., SCHELTENS, P., VERHEY, F. R., SCHMAND, B., LAUNER, L. J., JOLLES, J. & JONKER, C. (1999) Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. *J Neurol,* 246, 477-85.

WANG, P. N., LIRNG, J. F., LIN, K. N., CHANG, F. C. & LIU, H. C. (2006) Prediction of Alzheimer's disease in mild cognitive impairment: a prospective study in Taiwan. *Neurobiol Aging,* 27, 1797-806.

WATSON, C. J. & KREUZALER, P. A. (2009) The role of cathepsins in involution and breast cancer. *J Mammary Gland Biol Neoplasia,* 14, 171-9.

WEBSTER, B., HANSEN, L., ADAME, A., CREWS, L., TORRANCE, M., THAL, L. & MASLIAH, E. (2006) Astroglial activation of extracellular-regulated kinase in early stages of Alzheimer disease. *J Neuropathol Exp Neurol,* 65, 142-51.

WEHLING, E., LUNDERVOLD, A. J., STANDNES, B., GJERSTAD, L. & REINVANG, I. (2007) APOE status and its association to learning and memory performance in middle aged and older Norwegians seeking assessment for memory deficits. *Behav Brain Funct,* 3, 57.

WEIDEMANN, A., KONIG, G., BUNKE, D., FISCHER, P., SALBAUM, J. M., MASTERS, C. L. & BEYREUTHER, K. (1989) Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell,* 57, 115-26.

WEISER, W. Y., TEMPLE, P. A., WITEK-GIANNOTTI, J. S., REMOLD, H. G., CLARK, S. C. & DAVID, J. R. (1989) Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. *Proc Natl Acad Sci U S A,* 86, 7522-6.

WHITNEY, A. R., DIEHN, M., POPPER, S. J., ALIZADEH, A. A., BOLDRICK, J. C., RELMAN, D. A. & BROWN, P. O. (2003) Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci U S A,* 100, 1896-901.

WHO (1992) International statistical classification of diseases and related health problems: tenth revision: ICD-10.1992. Geneva.

WIJSMAN, E. M., DAW, E. W., YU, C. E., PAYAMI, H., STEINBART, E. J., NOCHLIN, D., CONLON, E. M., BIRD, T. D. & SCHELLENBERG, G. D. (2004) Evidence for a novel late-onset Alzheimer disease locus on chromosome 19p13.2. *Am J Hum Genet,* 75, 398-409.

WONG, S. Y., LAU, E. M., LI, M., CHUNG, T., SHAM, A. & WOO, J. (2005) The prevalence of Apo E4 genotype and its relationship to bone mineral density in Hong Kong Chinese. *J Bone Miner Metab,* 23, 261-5.

WOOD, J. A., WOOD, P. L., RYAN, R., GRAFF-RADFORD, N. R., PILAPIL, C., ROBITAILLE, Y. & QUIRION, R. (1993) Cytokine indices in Alzheimer's temporal cortex: no changes in mature IL-1 beta or IL-1RA but increases in the associated acute phase proteins IL-6, alpha 2-macroglobulin and C-reactive protein. *Brain Res,* 629, 245-52.

WOOD, L. S., PICKERING, E. H., MCHALE, D. & DECHAIRO, B. M. (2007) Association between neprilysin polymorphisms and sporadic Alzheimer's disease. *Neurosci Lett,* 427, 103-6.

WRAGG, R. E. & JESTE, D. V. (1989) Overview of depression and psychosis in Alzheimer's disease. *Am J Psychiatry,* 146, 577-87.

WU, Y. H. & SWAAB, D. F. (2007) Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. *Sleep Med,* 8, 623-36.

WU, Z. L., CIALLELLA, J. R., FLOOD, D. G., O'KANE, T. M., BOZYCZKO-COYNE, D. & SAVAGE, M. J. (2006) Comparative analysis of cortical gene expression in mouse models of Alzheimer's disease. *Neurobiol Aging,* 27, 377-86.

WULF, G., GARG, P., LIOU, Y. C., IGLEHART, D. & LU, K. P. (2004) Modeling breast cancer in vivo and ex vivo reveals an essential role of Pin1 in tumorigenesis. *Embo J,* 23, 3397-407.

XU, Y., JACK, C. R., JR., O'BRIEN, P. C., KOKMEN, E., SMITH, G. E., IVNIK, R. J., BOEVE, B. F., TANGALOS, R. G. & PETERSEN, R. C. (2000) Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. *Neurology,* 54, 1760-7.

YAFFE, K., BARNES, D., NEVITT, M., LUI, L. Y. & COVINSKY, K. (2001) A prospective study of physical activity and cognitive decline in elderly women: women who walk. *Arch Intern Med,* 161, 1703-8.

YAMAUCHI K, T. M., NAKABAYASHI T, SUGANO M, HIDAKA H, KONDO Y, KATSUYAMA T.. (1999) Higher avidity binding of apolipoprotein (E-AII) complex than of apolipoprotein E monomer to beta-amyloid. *J Neurosci Res,* 58, 301-307.

YAMAZAKI, Y., KARAKIDA, T. & HOMMA, S. (1990) Conduction velocity of motor nerve and cervical sympathetic and vagus nerve in streptozotocin diabetic rats. *Neurosci Lett,* 113, 29-33.

YAN, B. Z., WANG, W., CHEN, L. Y., BI, M. R., LU, Y. J., LI, B. X. & YANG, B. S. (2009) Role of cathepsin B-mediated apoptosis in fulminant hepatic failure in mice. *World J Gastroenterol,* 15, 1231-6.

YAO, P. J., ZHU, M., PYUN, E. I., BROOKS, A. I., THERIANOS, S., MEYERS, V. E. & COLEMAN, P. D. (2003a) Defects in expression of genes related to synaptic vesicle trafficking in frontal cortex of Alzheimer's disease. *Neurobiol Dis,* 12, 97-109.

YAO, Y., ZHUKAREVA, V., SUNG, S., CLARK, C. M., ROKACH, J., LEE, V. M., TROJANOWSKI, J. Q. & PRATICO, D. (2003b) Enhanced brain levels of 8,12-iso-iPF2alpha-VI differentiate AD from frontotemporal dementia. *Neurology,* 61, 475-8.

YATES, S. L., BURGESS, L. H., KOCSIS-ANGLE, J., ANTAL, J. M., DORITY, M. D., EMBURY, P. B., PIOTRKOWSKI, A. M. & BRUNDEN, K. R. (2000) Amyloid beta and amylin fibrils induce increases in proinflammatory cytokine and chemokine production by THP-1 cells and murine microglia. *J Neurochem,* 74, 1017-25.

YOSHIYAMA, Y., HIGUCHI, M., ZHANG, B., HUANG, S. M., IWATA, N., SAIDO, T. C., MAEDA, J., SUHARA, T., TROJANOWSKI, J. Q. & LEE, V. M. (2007) Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron,* 53, 337-51.

YOUNKIN, S. G. (1998) The role of A beta 42 in Alzheimer's disease. *J Physiol Paris,* 92, 289-92.

YU, B. P. (1994) Cellular defenses against damage from reactive oxygen species. *Physiol Rev,* 74, 139-62.

ZHANG, J., SOKAL, I., PESKIND, E. R., QUINN, J. F., JANKOVIC, J., KENNEY, C., CHUNG, K. A., MILLARD, S. P., NUTT, J. G. & MONTINE, T. J. (2008) CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases. *Am J Clin Pathol,* 129, 526-9.

ZHOU, W., SCOTT, S. A., SHELTON, S. B. & CRUTCHER, K. A. (2006) Cathepsin D-mediated proteolysis of apolipoprotein E: possible role in Alzheimer's disease. *Neuroscience,* 143, 689-701.

ZHOU, X. Z., KOPS, O., WERNER, A., LU, P. J., SHEN, M., STOLLER, G., KULLERTZ, G., STARK, M., FISCHER, G. & LU, K. P. (2000) Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. *Mol Cell,* 6, 873-83.

ZHOU, X. Z., LU, P. J., WULF, G. & LU, K. P. (1999) Phosphorylation-dependent prolyl isomerization: a novel signaling regulatory mechanism. *Cell Mol Life Sci,* 56, 788-806.

ZLOKOVIC, B. V. (2004) Clearing amyloid through the blood-brain barrier. *J Neurochem,* 89, 807-11.