Profiling of specific gene expression pathways in pheripheral cells from Alzheimer′s disease patients

M. Serpente, C. Fenoglio, SMG Cioffi, E. Oldoni, M. Arcaro, A. Arighi, G. Fumagalli, L. Ghezzi, M. Mercurio, E. Scarpini., D. Galimberti

Neurology Unit, Dept. of Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy.

BACKGROUND

❖ Alzheimer's disease (AD) is a neurodegenerative disorder charcterized by progressive loss of memory and decline of cognitive functions. Worldwide research has led to a growing knowledge of the genetics and molecular pathogenesis of AD, indicating that pathophysiological brain alterations occur decades before clinical signs and symptoms of cognitive decline appearance.

❖Currently, AD diagnosis is correctly performed by use of several biomarkers, such as structural and/or functional imaging (MRI, PET), cerebrospinal fluid (CSF) protein detection (β-amyloid, tau and p-tau) in accordance with Dubois criteria [1]. However, these biomarkers are invasive and expensive and in this framework, the identification of new peripheral biomarkers would be of critical importance in order to improve AD diagnosis.

❖To date, there is increasing evidence supporting a link between AD and insulin dysfunction [2,3].

To perform a whole gene expression profiling in peripheral cells from patients with MCI, Prodromal AD (MCI with AD CSF profile) and AD compared with controls

MATERIALS & METHODS

- Wide analysis with Sabioscience arrays in:
- 10 MCI (of which 5 prodromal AD)
- 7 AD patients (3 *PSEN1 Met146Leu* mutation carriers)
- 4 healthy controls

	MCI	Prodromal AD	AD	CTRLs
Number of subjects (screening)	5	5	7	4
Number of subjects (validation)			30	30
Gender (M:F)	1:4	1:4	17:20	16:18
Mean Aβ-42	1064.6	401.3	313.5	
Mean h-tau	223.25	614	820.5	
Mean P-tau	41.1	85.15	93.5	
Mean age, years ± SEM	69.41 ± 0.64	70.67±0.54	72.77 ± 0.65	68.82 ± 0.71
Mean age at onset, years ± SEM			70.84 ±0.71	
Mean disease duration, years ± SEM			2.87 ± 0.14	

Human Alzheimer's Disease PCR Array

SABiosciences
QIAGEN
A QIAGEN Company

84 genes important in the **onset, development**, and **progression** of Alzheimer's disease.

Array Layout

Housekeeping genes
Reverse transcription controls
Genomic DNA control
Positive PCR controls

- **Validation in a larger cohort of 30 AD patients and 30 ctrls by TaqMan assay**

CONCLUSIONS

❖ **This is the first attempt to test gene expression profile in a cohort of very mild AD (MCI with AD CSF signature)**

❖ **We observed a generalized up-regulation in patients as compared with controls and this data seem to be much evident in prodromal AD patients.**

❖ **Particularly, we observed a dysregulation of Insulin and Insulin receptor gene expression also in the validation cohort.**

❖ **Recently, an active role of insulin signal pathway was shown in AD pathogenesis**

❖ **In this context, our results suggest a possible future use of these molecules as peripheral biomarkers for early diagnosis**

FUTURE PLAN

❖ To enlarge validation cohort (MCI and Prodromal AD)

❖To replicate validation analysis for INS and INSr gene expression with RNA extracted from CSF cells

❖To Investigate a possible brain insulin resistance in AD patients by testing the role of insulin signaling (i.e. to evaluate CSF soluble phosphorylated IRS1 levels)

References
[1] Dubois B. et al., Lancet Neurol. 2014 Jun;13(6):614-29
[2] Dineley KT et al. Neurobiol Dis. 2014 Dec
[3] De Felice FG, Ferreira ST. Diabetes. 2014 Jul;63(7):2262-72

RESULTS

Screening:

Generalized up-regulation in patients compared with controls.

AD patients vs CTRLS

Magnitude of log2(Fold Change)
-6.1   6.1

AD *PSEN1 Met146Leu* patients vs CTRLS

Magnitude of log2(Fold Change)
-5.174   5.174

10 MCI

Normal CSF biomarkers
-High Abeta
-Low tau

MCI vs CTRLS

Magnitude of log2(Fold Change)
-2.828   0   2.828

AD-like CSF biomarkers
-Low Abeta
-High tau

Prodromal AD vs CTRLS

Alterations described in AD occur in very mild phase of the disease (prodromal AD)

Gene	MCI	AD prodromal	AD
ACHE	5.57	12.09	18.27
APLP1	4.29	6.60	13.26
IL1A	1.78	3.68	8.99
INS	5.38	8.44	39.20
INSR	-1.68	1.49	3.47

P>0.05   P<0.05

Fold regulation over controls

INS : encodes for insulin and it is located on chromosome 11p15.5

INSR: encodes for insulin receptor (chromosome 19p13.3). After removal of the precursor signal peptide, the insulin receptor precursor is post-translationally cleaved into two chains (alpha and beta) that are covalently linked.

Validation:

INS and INSr expression levels in PBMC

30 AD patients
30 healthy controls

Up-regulation was confirmed for both genes in the validation population (data represented as mean ± SEM)

* **INS**: AD 1.556 ± 0.26 versus ctrls 0.4256 ± 0.12 *P*=0.0009

** **INSr**: AD 3.545 ± 0.5 versus ctrls 2.089 ± 0.35 *P*=0.0372