NUOVI MARKERS NELLA DIAGNOSI DELLA DEMENZA DI ALZHEIMER

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Reference context for the research on Alzheimer's disease biomarkers

Literature context

ROUGH MEDLINE ANALYSIS

Medline AD biomarkers (limits: 10 yrs, human)	**2756**
+CSF	677
+PLASMA	332 (234 in 5 yrs)
+Genetic	532

CONCEPTUALIZING THE MARKERS (IMAGING EXCLUDED)

- **Pathogenesis related markers (mostly, not exclusively, CSF)**
- **Plasma biomarkers**

Conceptualizing the markers...further

Mol Diagn Ther 2011; 15 (2): 83-102 1177-1062/

Table I. Different types and uses of biomarkers[2]

Biomarker type	Use
Antecedent markers	Evaluation of the risk of a disorder
Screening markers	Early detection of diseases
Diagnostic markers	Identification of a disorder
Biomarker signatures	Indication of the disease state[a]
Prognostic markers	Prediction of the likely disease course or monitoring of the rate of disease progression
Stratification markers	Prediction of the likelihood of drug response or toxicity

a Usually linked to ongoing pathophysiology; may also provide information and insights into the underlying molecular mechanisms of a given disease.

Mol Diagn Ther 2011; 15 (2): 83-102 1177-1062/

Table I. Different types and uses of biomarkers[2]

Biomarker type	Use
Antecedent markers	Genetic risk markers
Screening markers	Genetic + CSF markers
Diagnostic markers	Genetic + CSF markers (+plasma mkrs)
Biomarker signatures	NMR, PET and SPECT studies (+plasma mkrs)
Prognostic markers	All the mentioned markers
Stratification markers	Endophenotype genetic markers + polimorphisms in drug metabolizing enzymes + target polimorphisms

ADAPTED TO ALZHEIMER'S DISEASE

PATHOGENESIS-RELATED BIOMARKERS (mostly, even if not exclusively, CSF markers)

WHICH PATHOGENESIS?

and consequently

WHICH MARKERS?

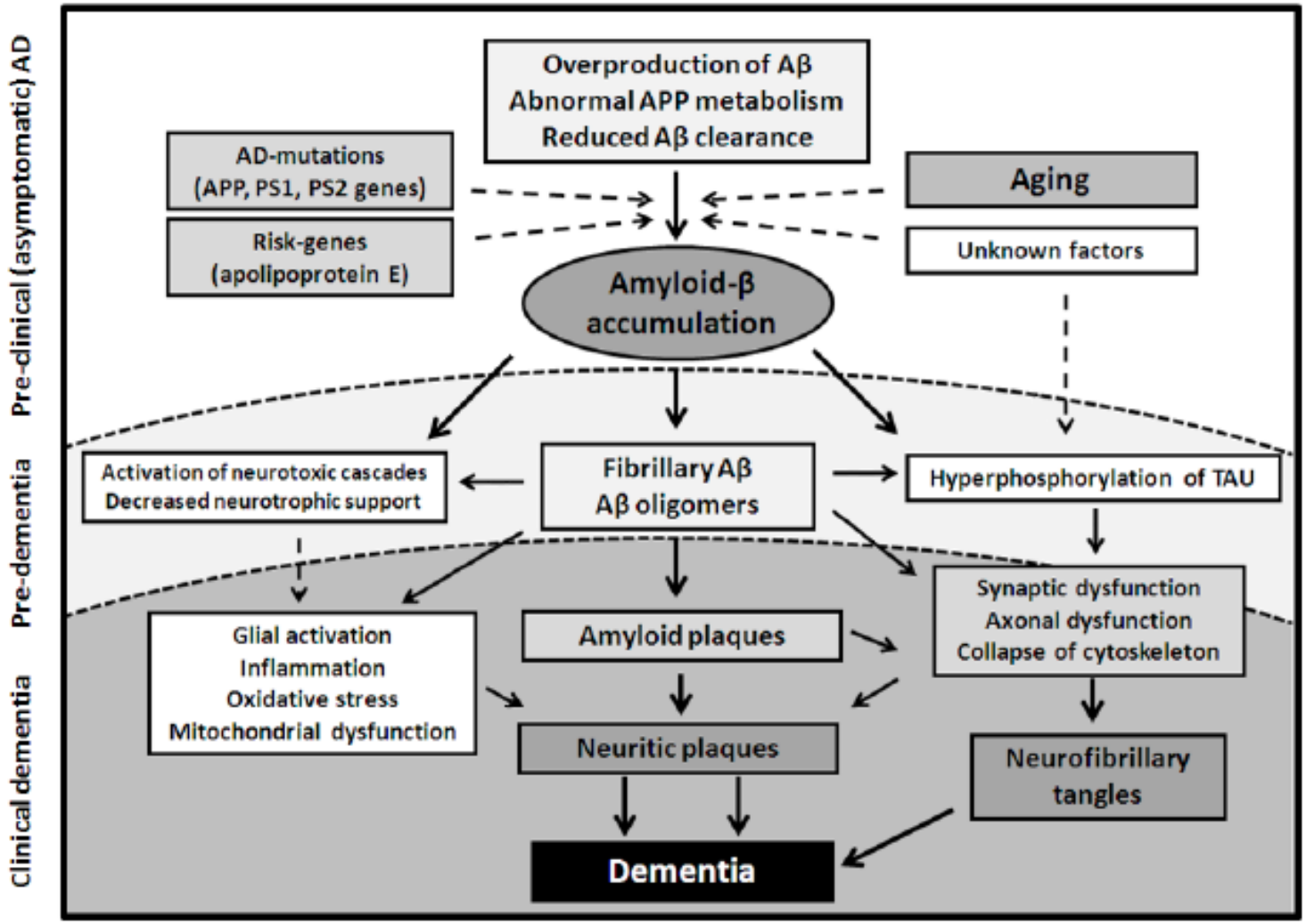
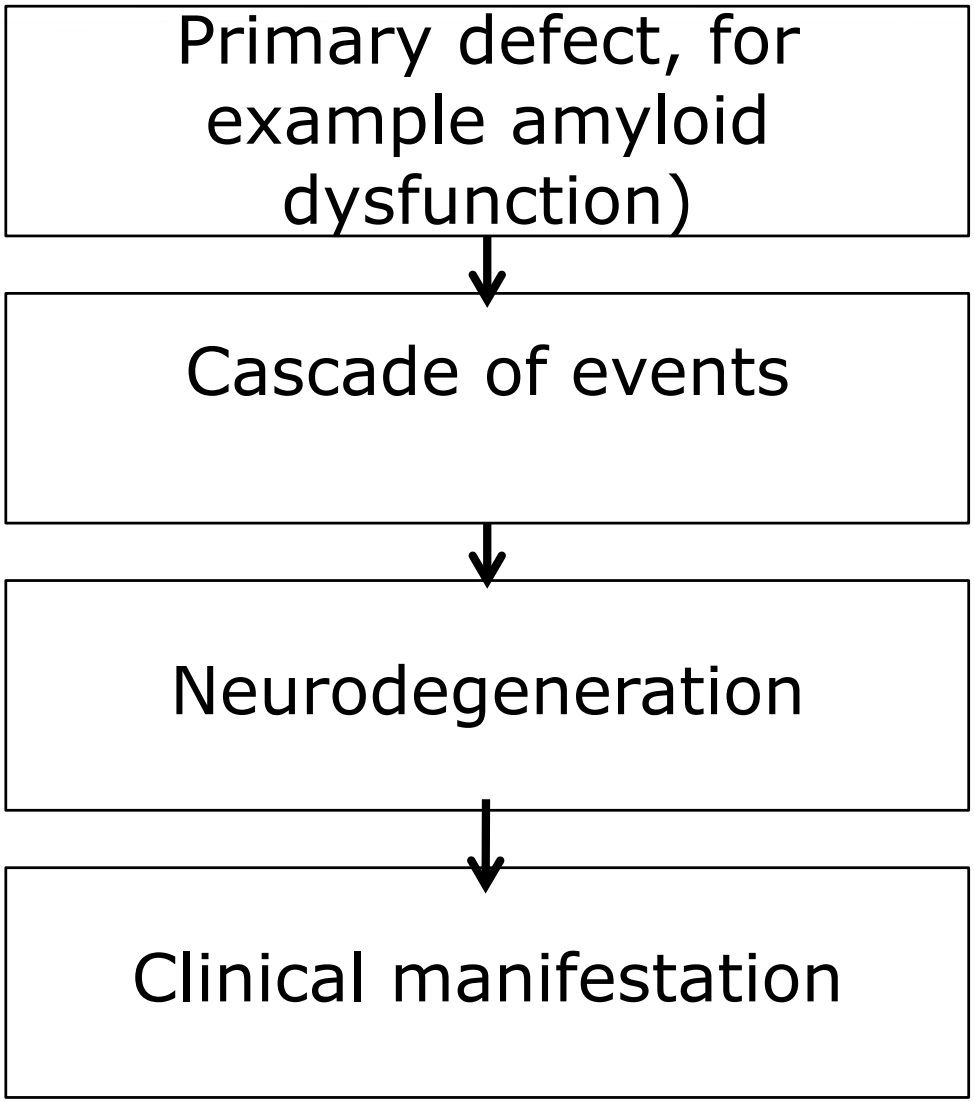


Figure 1 Hypothetical model of the pathological processes in Alzheimer's disease (AD), focusing on the amyloid β peptide

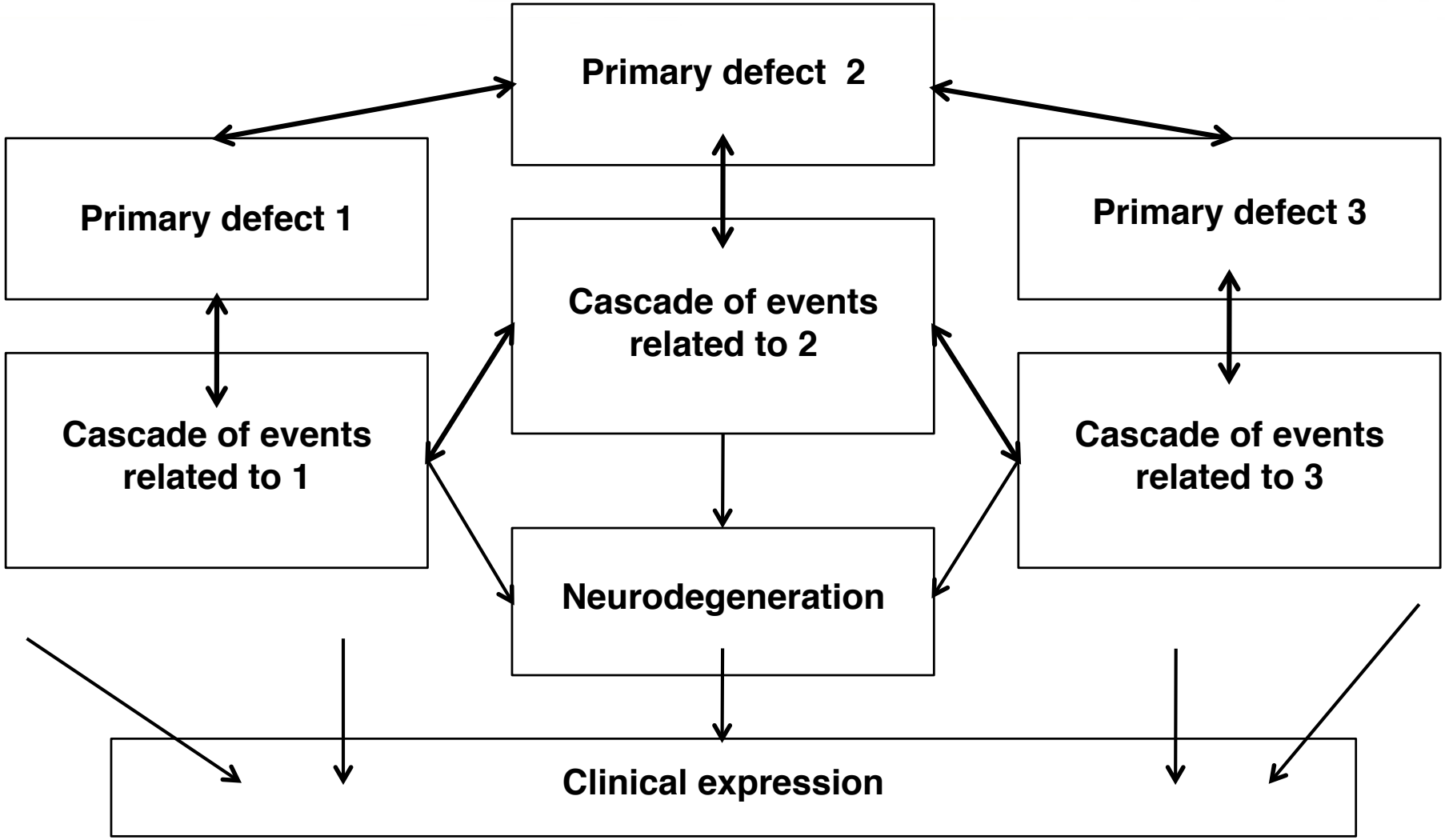
Govoni S et al. Psicogeriatria, Suppl. 1, gen-apr, 2012, pp 34-54

HYERARCHICAL MODEL



Govoni S et al. Psicogeriatria, Suppl. 1, gen-apr, 2012, pp 34-54

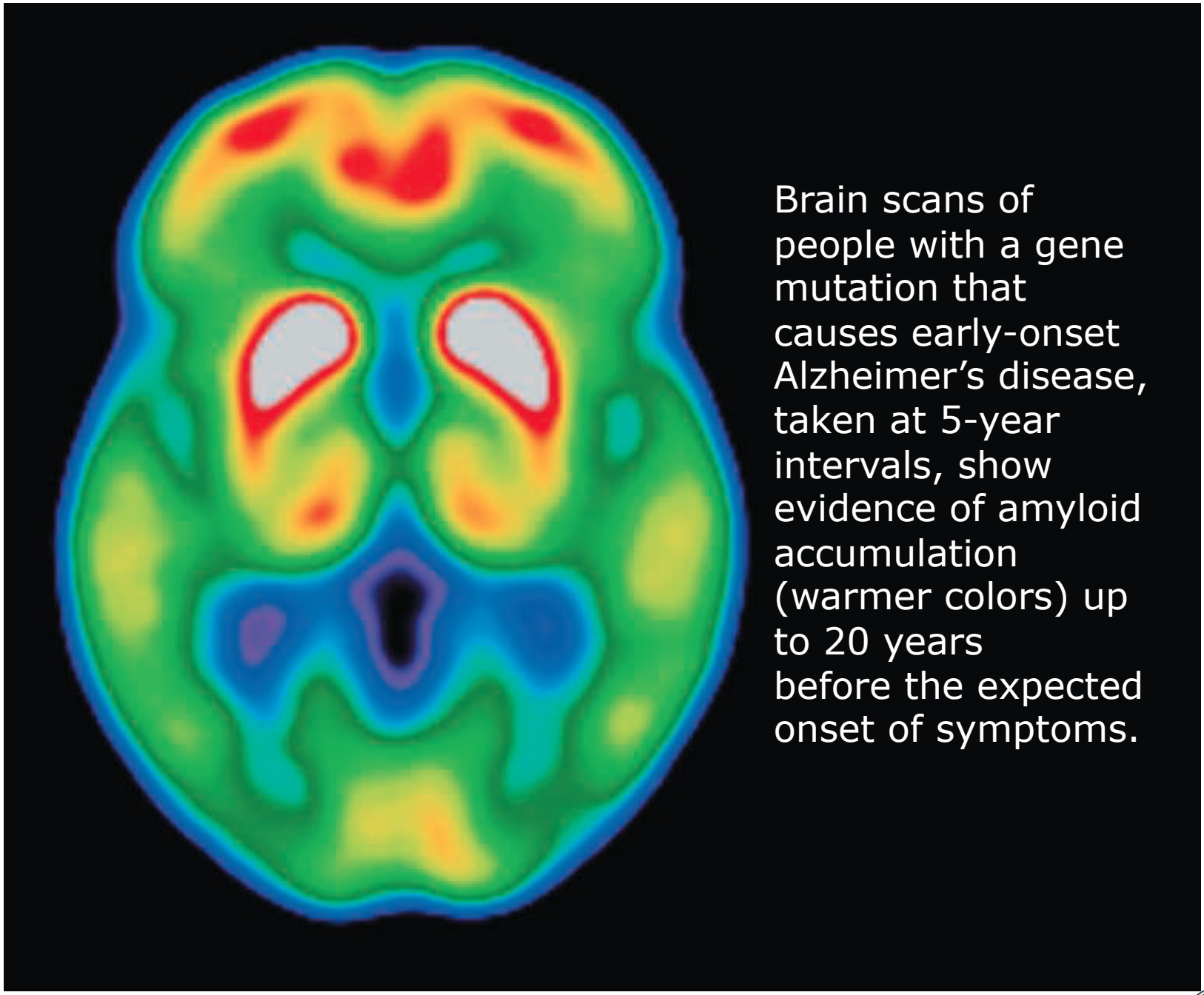
NETWORK MODEL



BEST EXAMPLE

PATHOGENESIS RELATED MARKERS IN FAMILIAL AD

SCIENCE VOL 337 17 AUGUST 2012



Brain scans of people with a gene mutation that causes early-onset Alzheimer's disease, taken at 5-year intervals, show evidence of amyloid accumulation (warmer colors) up to 20 years before the expected onset of symptoms.

BEYOND PATHOGENESIS RELATED MARKERS IN FAMILIAL AD

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ORIGINAL ARTICLE

Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease

CONCLUSIONS:

We found that autosomal dominant Alzheimer's disease was associated with a series of pathophysiological changes over decades in CSF biochemical markers of Alzheimer's disease, brain amyloid deposition, and brain metabolism as well as progressive cognitive impairment. Our results require confirmation with the use of longitudinal data and may not apply to patients with sporadic Alzheimer's disease. (Funded by the National Institute on Aging and others; DIAN ClinicalTrials.gov number, NCT00869817.)

PLASMA BIOMARKERS

Requirements for Screening Tests

- Test must be quick, easy and inexpensive
- Test must be safe, acceptable to persons screened and physicians or health care workers screening
- Sensitivity, specificity and predictive values must be known and acceptable to medical community
- Adequate follow-up for screened positives with and without disease

PATHOGENESIS-UNRELATED BIOMARKERS (mostly, even if not exclusively, plasma markers)

2007

Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins

Sandip Ray[1,16], Markus Britschgi[2,16], Charles Herbert[1], Yoshiko Takeda-Uchimura[2], Adam Boxer[3], Kaj Blennow[4], Leah F Friedman[5], Douglas R Galasko[6], Marek Jutel[7], Anna Karydas[3], Jeffrey A Kaye[8], Jerzy Leszek[9], Bruce L Miller[3], Lennart Minthon[10], Joseph F Quinn[8], Gil D Rabinovici[3], William H Robinson[11], Marwan N Sabbagh[12], Yuen T So[2], D Larry Sparks[12], Massimo Tabaton[13], Jared Tinklenberg[5], Jerome A Yesavage[5], Robert Tibshirani[14] & Tony Wyss-Coray[2,15]

A molecular test for Alzheimer's disease could lead to better treatment and therapies. We found 18 signaling proteins in blood plasma that can be used to classify blinded samples from Alzheimer's and control subjects with close to 90% accuracy and to identify patients who had mild cognitive impairment that progressed to Alzheimer's disease 2–6 years later. Biological analysis of the 18 proteins points to systemic dysregulation of hematopoiesis, immune responses, apoptosis and neuronal support in presymptomatic Alzheimer's disease.

increasingly implicated in Alzheimer's[3] and related diseases[4], we hypothesized that the pathological processes leading to Alzheimer's would cause characteristic changes in the concentrations of signaling proteins in the blood, generating a detectable disease-specific molecular phenotype.

We collected a total of 259 archived plasma samples from individuals with presymptomatic to late-stage Alzheimer's disease and from various controls (**Supplementary Table 1** online) and measured the abundance of 120 known signaling proteins (**Supplementary Table 2** online) in these samples with filter-based, arrayed sandwich ELISAs[5]

Nat Med 2007;13:1359 –1362.

NATURE MEDICINE VOLUME 13 [NUMBER 11
[NOVEMBER 2007 1359

Table 1 Eighteen plasma signaling proteins that predict clinical Alzheimer's diagnosis

Predictors	
ANG-2	ANG-2, angiopoietin-2.
CCL5	CCL, chemokine that contains a C-C motif;
CCL7	
CCL15	CXCL, chemokine that contains a C-X-C motif;
CCL18	
CXCL8	G-CSF, granulocyte-colony stimulating factor;
EGF	
G-CSF	GDNF, glial-derived neurotrophic factor;
GDNF	ICAM-1, intercellular adhesion molecule-1;
ICAM-1	
IGFBP-6	IGFBP-1, insulin-like growth factor–binding protein-6;
IL-1α	
IL-3	IL, interleukin;
IL-11	PDGF-BB, platelet-derived growth factor BB;
M-CSF	
PDGF-BB	TRAIL-R4, TNF-related apoptosis-inducing ligand receptor-4.
TNF-α	
TRAIL-R4	

2011

Differences in Abundances of Cell-Signalling Proteins in Blood Reveal Novel Biomarkers for Early Detection Of Clinical Alzheimer's Disease

Mateus Rocha de Paula[1], Martín Gómez Ravetti[2], Regina Berretta[1], Pablo Moscato[1]*

1 Centre for Bioinformatics, Biomarker Discovery & Information-Based Medicine, The University of Newcastle, Callaghan, Australia, **2** Departamento de Engenharia de Produção, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

Abstract

Background: In November 2007 a study published in Nature Medicine proposed a simple test based on the abundance of 18 proteins in blood to predict the onset of clinical symptoms of Alzheimer's Disease (AD) two to six years before these symptoms manifest. Later, another study, published in PLoS ONE, showed that only five proteins (IL-1α, IL-3, EGF, TNF-α and G-CSF) have overall better prediction accuracy. These classifiers are based on the abundance of 120 proteins. Such values were standardised by a Z-score transformation, which means that their values are relative to the average of all others.

Methodology: The original datasets from the Nature Medicine paper are further studied using methods from combinatorial optimisation and Information Theory. We expand the original dataset by also including all pair-wise differences of z-score values of the original dataset ("metafeatures"). Using an exact algorithm to solve the resulting $(\alpha,\beta)-k$ Feature Set problem, used to tackle the feature selection problem, we found signatures that contain either only features, metafeatures or both, and evaluated their predictive performance on the independent test set.

Conclusions: It was possible to show that a specific pattern of cell signalling imbalance in blood plasma has valuable information to distinguish between NDC and AD samples. The obtained signatures were able to predict AD in patients that already had a Mild Cognitive Impairment (MCI) with up to 84% of sensitivity, while maintaining also a strong prediction accuracy of 90% on a independent dataset with Non Demented Controls (NDC) and AD samples. The novel biomarkers uncovered with this method now confirms ANG-2, IL-11, PDGF-BB, CCL15/MIP-1δ; and supports the joint measurement of other signalling proteins not previously discussed: GM-CSF, NT-3, IGFBP-2 and VEGF-B.

Citation: Rocha de Paula M, Gómez Ravetti M, Berretta R, Moscato P (2011) Differences in Abundances of Cell-Signalling Proteins in Blood Reveal Novel Biomarkers for Early Detection Of Clinical Alzheimer's Disease. PLoS ONE 6(3): e17481. doi:10.1371/journal.pone.0017481

2012

PLASMA BIOMARKERS: CONTRASTING RESULTS

Evaluation of a Previously Suggested Plasma Biomarker Panel to Identify Alzheimer's Disease

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1 Brain Disease Biomarker Unit, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University, Lund, Sweden, 2 Computational Biology and Biological Physics, Lund University, Lund, Sweden, 3 Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden, 4 Neuropsychiatric Clinic, Skåne University Hospital, Malmö, Sweden

Abstract

There is an urgent need for biomarkers in plasma to identify Alzheimer's disease (AD). It has previously been shown that a signature of 18 plasma proteins can identify AD during pre-dementia and dementia stages (Ray et al, Nature Medicine, 2007). We quantified the same 18 proteins in plasma from 174 controls, 142 patients with AD, and 88 patients with other dementias. Only three of these proteins (EGF, PDG-BB and MIP-1δ) differed significantly in plasma between controls and AD. The 18 proteins could classify patients with AD from controls with low diagnostic precision (area under the ROC curve was 63%). Moreover, they could not distinguish AD from other dementias. In conclusion, independent validation of results is important in explorative biomarker studies.

PLoS ONE | www.plosone.org January 2012 | Volume 7 | Issue 1 | e29868

PLoS ONE | www.plosone.org 2012 | Volume 7 | Issue 4 | e34341

Multivariate Protein Signatures of Pre-Clinical Alzheimer's Disease in the Alzheimer's Disease Neuroimaging Initiative (ADNI) Plasma Proteome Dataset

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Abstract

Background: Recent Alzheimer's disease (AD) research has focused on finding biomarkers to identify disease at the pre-clinical stage of mild cognitive impairment (MCI), allowing treatment to be initiated before irreversible damage occurs. Many studies have examined brain imaging or cerebrospinal fluid but there is also growing interest in blood biomarkers. The Alzheimer's Disease Neuroimaging Initiative (ADNI) has generated data on 190 plasma analytes in 566 individuals with MCI, AD or normal cognition. We conducted independent analyses of this dataset to identify plasma protein signatures predicting pre-clinical AD.

Methods and Findings: We focused on identifying signatures that discriminate cognitively normal controls ($n = 54$) from individuals with MCI who subsequently progress to AD ($n = 163$). Based on p value, apolipoprotein E (APOE) showed the strongest difference between these groups ($p = 2.3 \times 10^{-13}$). We applied a multivariate approach based on combinatorial optimization (*(α,β)-k Feature Set Selection*), which retains information about individual participants and maintains the context of interrelationships between different analytes, to identify the optimal set of analytes (signature) to discriminate these two groups. We identified 11-analyte signatures achieving values of sensitivity and specificity between 65% and 86% for both MCI and AD groups, depending on whether APOE was included and other factors. Classification accuracy was improved by considering "meta-features," representing the difference in relative abundance of two analytes, with an 8-meta-feature signature consistently achieving sensitivity and specificity both over 85%. Generating signatures based on longitudinal rather than cross-sectional data further improved classification accuracy, returning sensitivities and specificities of approximately 90%.

Conclusions: Applying these novel analysis approaches to the powerful and well-characterized ADNI dataset has identified sets of plasma biomarkers for pre-clinical AD. While studies of independent test sets are required to validate the signatures, these analyses provide a starting point for developing a cost-effective and minimally invasive test capable of diagnosing AD in its pre-clinical stages.

Doecke et al. Arch Neurol. Published online July 16, 2012. doi:10.1001/archneurol.2012.1282

Results A biomarker panel was identified that included markers significantly increased (cortisol, pancreatic polypeptide, insulinlike growth factor binding protein 2, β_2 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide dismutase, and homocysteine) and decreased (apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin) in AD. Cross-validated accuracy measures from the AIBL cohort reached a mean (SD) of 85% (3.0%) for sensitivity and specificity. A second validation using the ADNI cohort attained accuracy measures of 80% (3.0%) for sensitivity and specificity and 85% (3.0).

Plasma multianalyte profiling in mild cognitive impairment and Alzheimer disease

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ABSTRACT

Objectives: While plasma biomarkers have been proposed to aid in the clinical diagnosis of Alzheimer disease (AD), few biomarkers have been validated in independent patient cohorts. Here we aim to determine plasma biomarkers associated with AD in 2 independent cohorts and validate the findings in the multicenter Alzheimer's Disease Neuroimaging Initiative (ADNI).

Methods: Using a targeted proteomic approach, we measured levels of 190 plasma proteins and peptides in 600 participants from 2 independent centers (University of Pennsylvania, Philadelphia; Washington University, St. Louis, MO), and identified 17 analytes associated with the diagnosis of very mild dementia/mild cognitive impairment (MCI) or AD. Four analytes (apoE, B-type natriuretic peptide, C-reactive protein, pancreatic polypeptide) were also found to be altered in clinical MCI/AD in the ADNI cohort (n = 566). Regression analysis showed CSF Aβ42 levels and t-tau/Aβ42 ratios to correlate with the number of *APOE*4 alleles and plasma levels of B-type natriuretic peptide and pancreatic polypeptide.

Conclusion: Four plasma analytes were consistently associated with the diagnosis of very mild dementia/MCI/AD in 3 independent clinical cohorts. These plasma biomarkers may predict underlying AD through their association with CSF AD biomarkers, and the association between plasma and CSF amyloid biomarkers needs to be confirmed in a prospective study. *Neurology*® 2012;79:897–905

Table 4 Linear regression models showing associations between CSF AD biomarker levels (Aβ42 level, ratio of t-tau/Aβ42) and plasma AD biomarkers in all ADNI subjects with CSF and plasma analytes (n = 566)[a]

	Regression coefficients	p
Aβ42		
Male	4.30	0.415
Age	0.68	0.090
No. of *APOE*4 alleles	−45.47	<0.001
BNP	−27.99	<0.001
Pancreatic polypeptide	−18.90	0.007
t-Tau/Aβ42		
Male	0.116	0.04
Age	0.001	0.711
No. of *APOE*4 alleles	0.297	<0.001
Pancreatic polypeptide	0.180	0.015

Abbreviations: Aβ42 = β-amyloid 1–42; AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; BNP = B-type natriuretic peptide; t-tau = total tau.
[a] Similar correlations were observed in ADNI subjects with CSF and plasma analytes not treated with cholinesterase inhibitors (n = 304; see appendix e-1).

These plasma AD biomarkers may thus help predict underlying AD pathology through their relationships to established CSF biomarkers of AD, and could serve as the basis of a plasma-based screening battery for AD.

Neurology 2012;79:897–905

IN SEARCH FOR A RATIONALE OR JUST STATISTIC ASSOCIATION?

Plasma Biomarkers Associated With the Apolipoprotein E Genotype and Alzheimer Disease

Holly D. Soares, PhD; William Z. Potter, MD, PhD; Eve Pickering, PhD; Max Kuhn, PhD; Frederick W. Immermann, MStat; David M. Shera, ScD; Mats Ferm, PhD; Robert A. Dean, MD, PhD; Adam J. Simon, PhD; Frank Swenson, OD, PhD; Judith A. Siuciak, PhD; June Kaplow, PhD; Madhav Thambisetty, MD, PhD; Panayiotis Zagouras, PhD; Walter J. Koroshetz, PhD; Hong I. Wan, PhD; John Q. Trojanowski, MD, PhD; Leslie M. Shaw, PhD; for the Biomarkers Consortium Alzheimer's Disease Plasma Proteomics Project

Background: A blood-based test that could be used as a screen for Alzheimer disease (AD) may enable early intervention and better access to treatment.

Objective: To apply a multiplex immunoassay panel to identify plasma biomarkers of AD using plasma samples from the Alzheimer's Disease Neuroimaging Initiative cohort.

Design: Cohort study.

Setting: The Biomarkers Consortium Alzheimer's Disease Plasma Proteomics Project.

Participants: Plasma samples at baseline and at 1 year were analyzed from 396 (345 at 1 year) patients with mild cognitive impairment, 112 (97 at 1 year) patients with AD, and 58 (54 at 1 year) healthy control subjects.

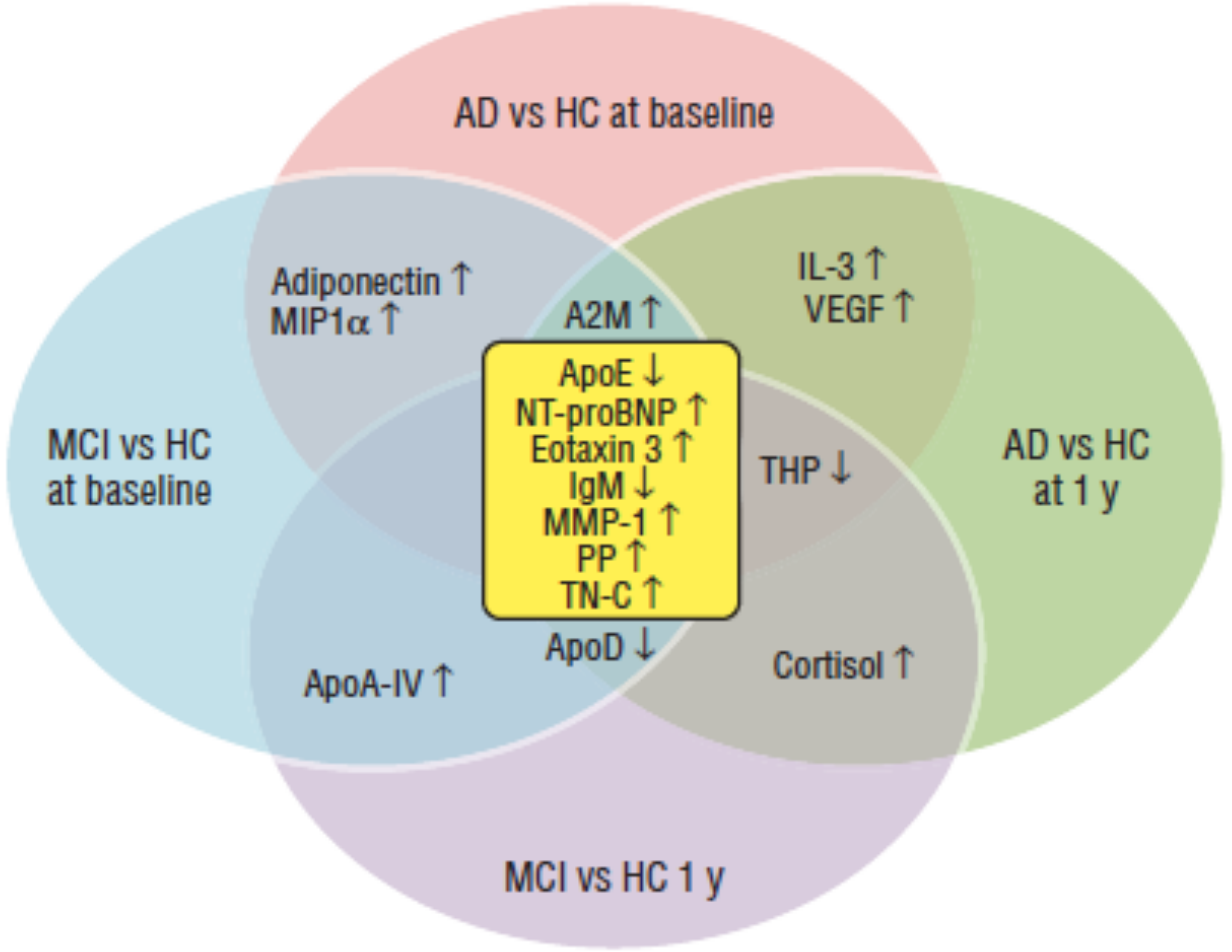
Main Outcome Measures: Multivariate and univariate statistical analyses were used to examine differences across diagnostic groups and relative to the apolipoprotein E (*ApoE*) genotype.

Results: Increased levels of eotaxin 3, pancreatic polypeptide, and N-terminal protein B–type brain natriuretic peptide were observed in patients, confirming similar changes reported in cerebrospinal fluid samples of patients with AD and MCI. Increases in tenascin C levels and decreases in IgM and ApoE levels were also observed. All participants with *Apo* ε3/ε4 or ε4/ε4 alleles showed a distinct biochemical profile characterized by low C-reactive protein and ApoE levels and by high cortisol, interleukin 13, apolipoprotein B, and gamma interferon levels. The use of plasma biomarkers improved specificity in differentiating patients with AD from controls, and ApoE plasma levels were lowest in patients whose mild cognitive impairment had progressed to dementia.

Conclusions: Plasma biomarker results confirm cerebrospinal fluid studies reporting increased levels of pancreatic polypeptide and N-terminal protein B–type brain natriuretic peptide in patients with AD and mild cognitive impairment. Incorporation of plasma biomarkers yielded high sensitivity with improved specificity, supporting their usefulness as a screening tool. The *ApoE* genotype was associated with a unique biochemical profile irrespective of diagnosis, highlighting the importance of genotype on blood protein profiles.

Arch Neurol. Published online July 16, 2012.
doi:10.1001/archneurol.2012.1070

Arch Neurol. Published online July 16, 2012.



Venn diagram of analytes with P .05 based on analyses of covariance across groups. Diagnostic categorization was based on diagnosis at the time of plasma sample collection. Analytes highlighted in center (yellow) were common across group comparisons. AD indicates Alzheimer disease; A2M, alpha-2-macroglobulin; ApoA-IV, apolipoprotein A-IV; ApoD, apolipoprotein D; ApoE, apolipoprotein E; HC, healthy control subject; IL-3, interleukin 3; MCI, mild cognitive impairment; MIP1, macrophage inflammatory protein 1; MMP-1, matrix metalloproteinase 1; NT-proBNP, N-terminal protein B–type brain natriuretic peptide; PP, pancreatic polypeptide; TN-C, tenascin C; THP, Tamm-Horsfall protein; VEGF, vascular endothelial growth factor.

Perhaps the most notable finding from the ADNI cohort was the identification of a protein profile associated with ApoE allelic status, a known risk factor for AD. For example, ApoE protein, C-reactive protein, and gamma interferon plasma protein levels were lowest in Apo 4 carriers, while IL-13 levels were elevated.

The present data support a phenotypic plasma signature associated with the ApoE genotype and could provide some explanation for the biological variability of blood-based biomarkers of AD described in the literature.

THREE COMMON BIOLOGICAL THEMES SEEMED TO BE ASSOCIATED WITH THE TOP PLASMA BIOMARKER CHANGES IN THE ADNI COHORT.

The first biological theme appeared to be associated with metabolic markers that might be altered by cholinergic tone but most likely were driven by concomitant medication use (eg, PP)

The second biological theme appeared to be linked to vascular pathologic conditions (eg, TN-C, MMP-1, and NTproBNP), including endothelial remodeling

The third biological theme seemed to revolve around the phenotypic signature of an ApoE genotype, perhaps best characterized by ApoE protein levels, the acute-phase C-reactive protein, and a subset of interleukins, The ApoE genotype also seems to be loosely associated with increases in several inflammatory cytokines.

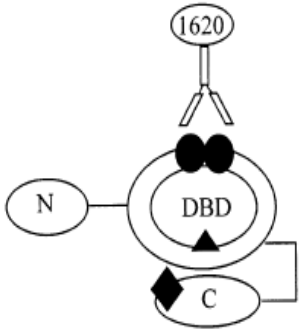
PROVIDED THIS CONTEXT…

our experience with P53

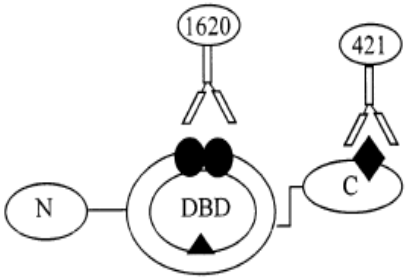
The fibroblast model for Alzheimer's disease

- Fibroblasts are a peripheral cellular model that shows some of the biochemical abnormalities detected in the brain of AD patients
- These cells can be replicated *in vitro* a sufficient number of times to allow collaborative studies
- Their features are mostly independent from the "environment" of the patient
- Their initial use was aimed specifically at the characterization of potentially useful diagnostic markers

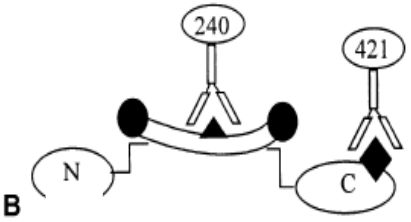
p53 conformational state in fibroblasts from AD and non-AD patients



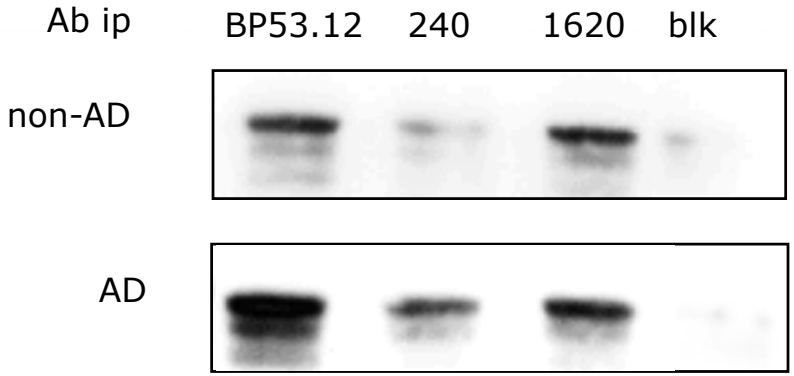
Wild-type, latent

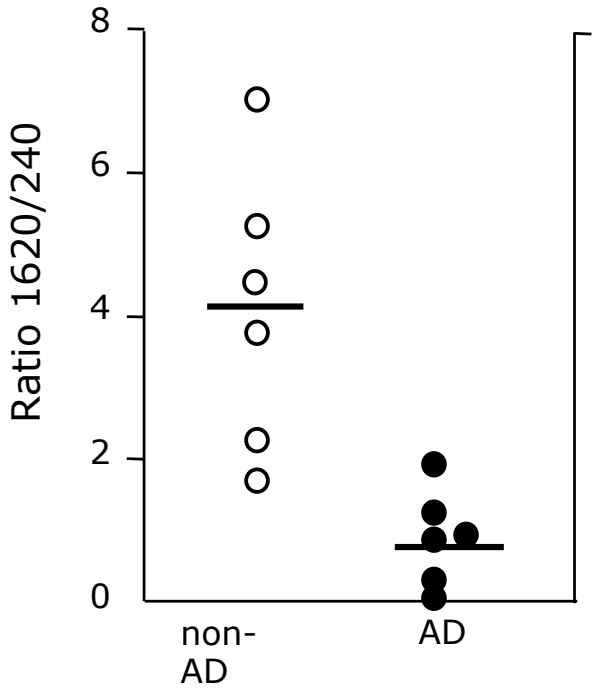


Wild-type, active



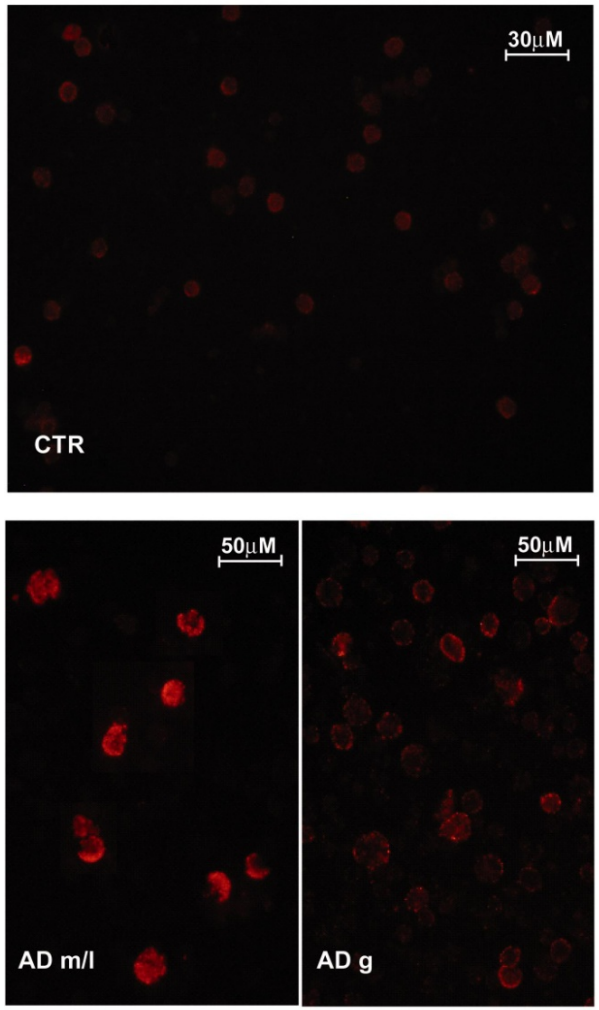
Mutant





Uberti et al. Neurobiol Aging, 2006

a



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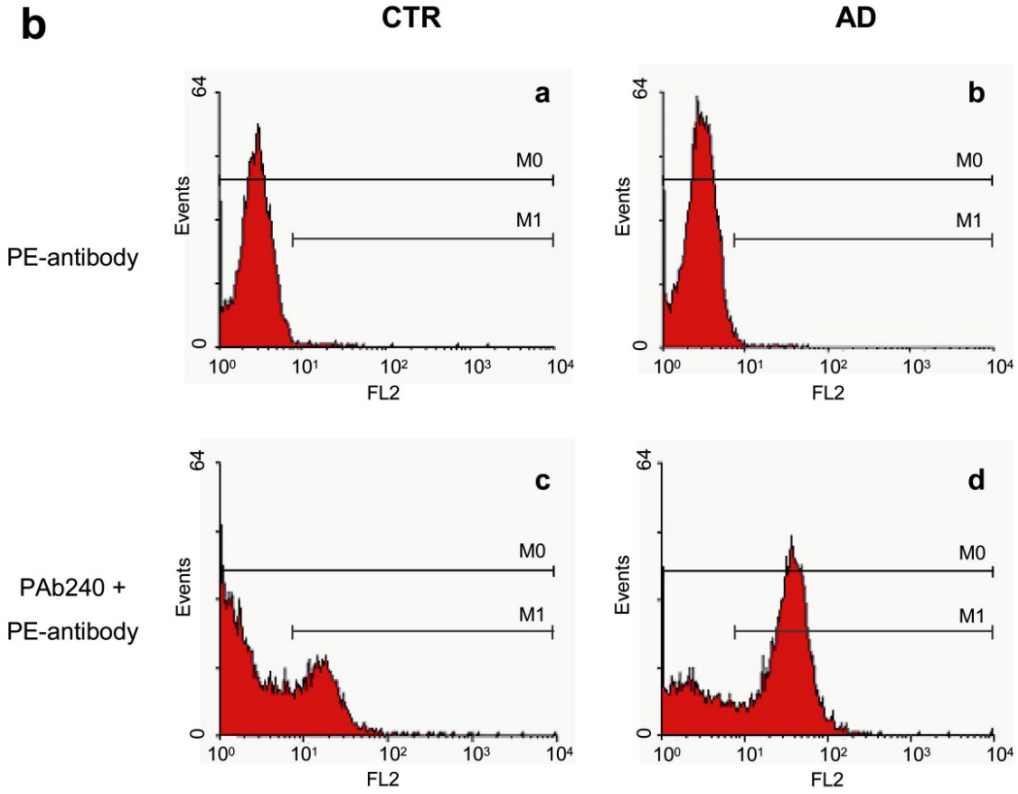


CTR (PE-antibody)

Region	Events	%Total
M0	18903	100.00
M1	38	0.21

AD (PE-antibody)

Region	Events	%Total
M0	20445	100.00
M1	35	0.17

CTR (PAb240+PE-antibody)

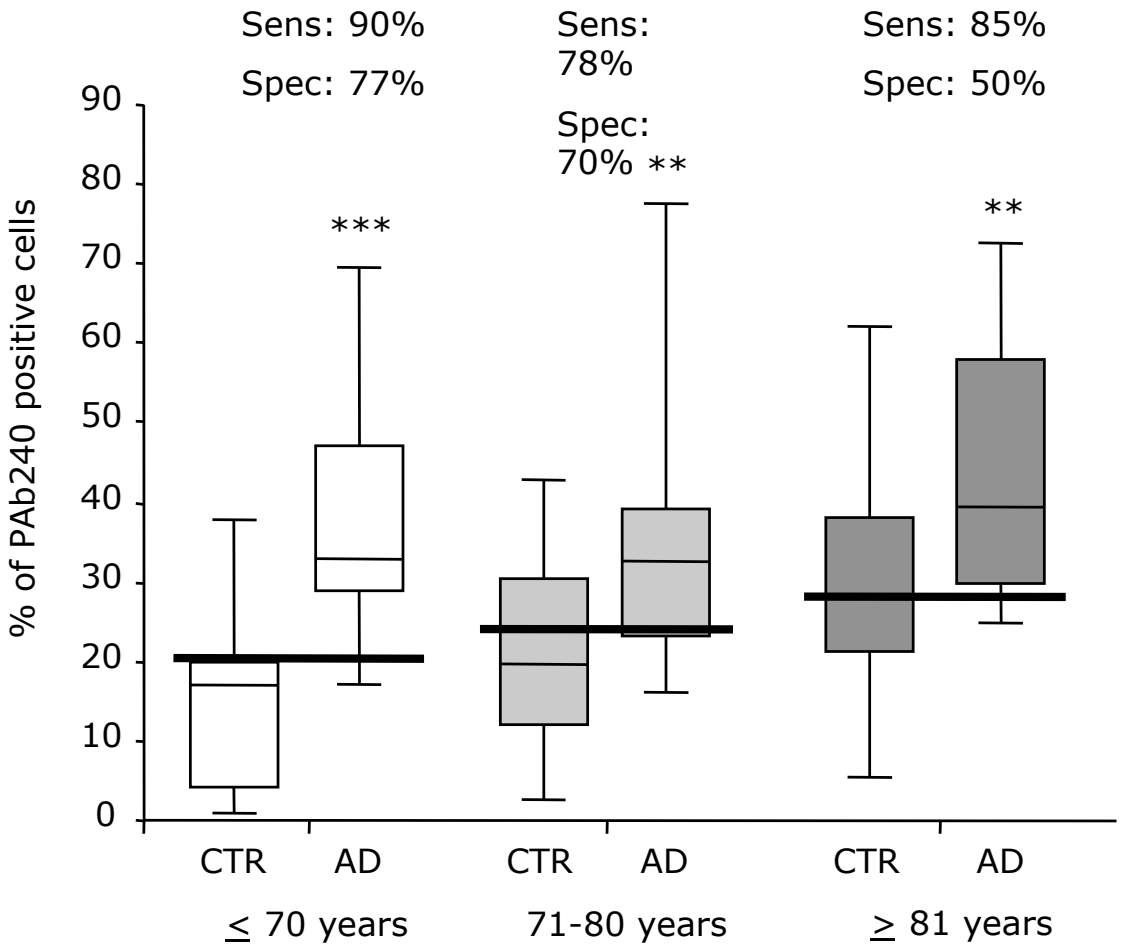
Region	Events	%Total
M0	22273	100.00
M1	4949	22.22

AD (PAb240+PE-antibody)

Region	Events	%Total
M0	22801	100.00
M1	14775	64.80

Lanni et al. Molec Psych, 2008

p53 (PAb 240) distribution in mononuclear blood cells from AD and non-AD patients



subjects up to 70 years of age

subjects between 71-80 years

subjects over 80 years

Lanni et al. Molec Psych, 2008

Conformationally Altered p53 compared with CSF Protein Biomarkers for Alzheimer's Disease

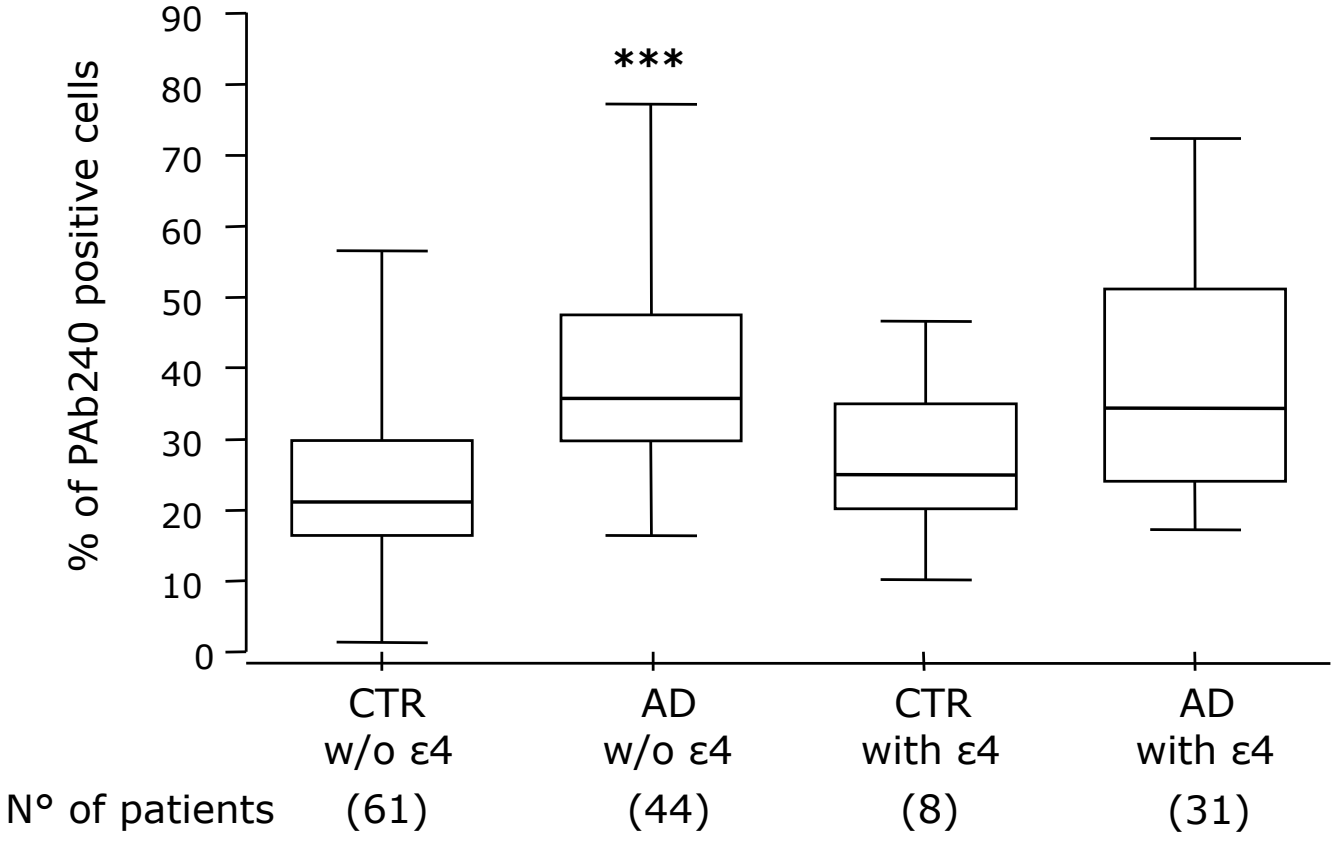
	Study number	Number of AD cases	AD Sensitivity	Number of Controls	Controls Specificity
Total tau	20	2022	81,4	1005	91,5
Phospho tau	16	1084	81,3	504	91,2
Aβ 1-42	16	660	85,9	541	88,5
CA p53 in subjects ≤70 years			**90,0**		**77,0**

Modified from Blennow K., NeuroRx, 2004

Current Pharmaceutical Design, 2008, *14*, 000-000

Pharmacogenetics and Pharmagenomics, Trends in Normal and Pathologic al Aging Studies: Focus on p53

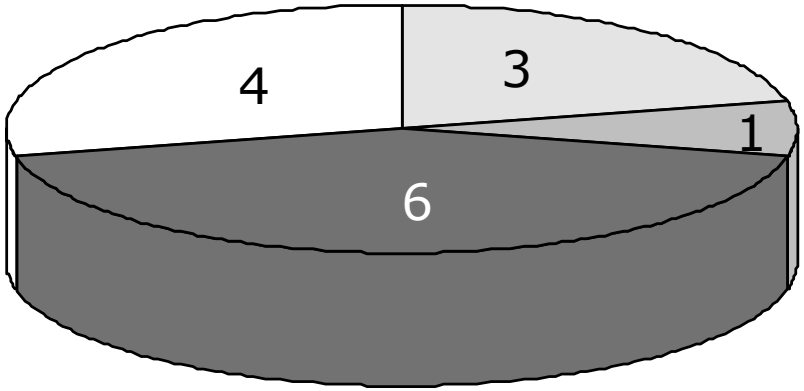
C. Lanni[1], M. Racchi[1], D. Uberti[2], G. Mazzini[3], S. Stanga[1], E. Sinforiani[4], M. Memo[2] and S. Govoni[1,*]



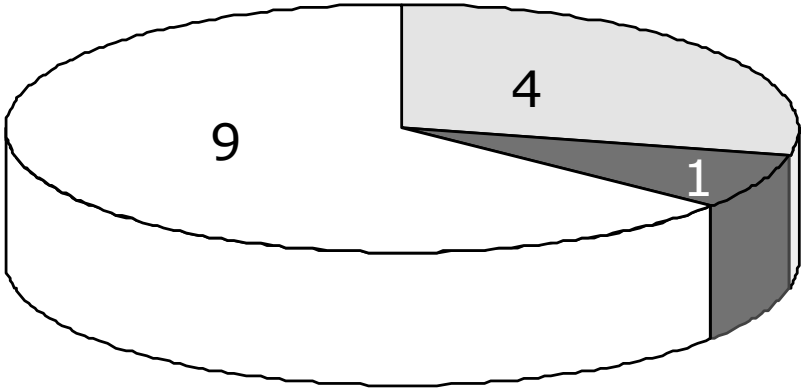
Lanni et al. Curr Pharm Des, 2008

Scheme of converted and not-converted MCI patients based on unfolded p53 analysis and APOEε4 genotype

A) MCI with progression to Alzheimer's disease



B) MCI without progression to Alzheimer's disease



- APOEε4 positive
- high unfolded p53
- both APOEε4 positive and high unfolded p53
- none of these 2 parameters

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PLoS one

Homeodomain Interacting Protein Kinase 2: A Target for Alzheimer's Beta Amyloid Leading to Misfolded p53 and Inappropriate Cell Survival

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Abstract

Background: Homeodomain interacting protein kinase 2 (HIPK2) is an evolutionary conserved serine/threonine kinase whose activity is fundamental in maintaining wild-type p53 function, thereby controlling the destiny of cells when exposed to DNA damaging agents. We recently reported an altered conformational state of p53 in tissues from patients with Alzheimer's Disease (AD) that led to an impaired and dysfunctional response to stressors.

Methodology/Principal Findings: Here we examined the molecular mechanisms underlying the impairment of p53 activity in two cellular models, HEK-293 cells overexpressing the amyloid precursor protein and fibroblasts from AD patients, starting from recent findings showing that p53 conformation may be regulated by HIPK2. We demonstrated that beta-amyloid 1–40 induces HIPK2 degradation and alters HIPK2 binding activity to DNA, in turn regulating the p53 conformational state and vulnerability to a noxious stimulus. Expression of HIPK2 was analysed by western blot experiments, whereas HIPK2 DNA binding was examined by chromatin immunoprecipitation analysis. In particular, we evaluated the recruitment of HIPK2 onto some target promoters, including hypoxia inducible factor-1α and metallothionein 2A.

Conclusions/Significance: These results support the existence of a novel amyloid-based pathogenetic mechanism in AD potentially leading to the survival of injured dysfunctional cells.

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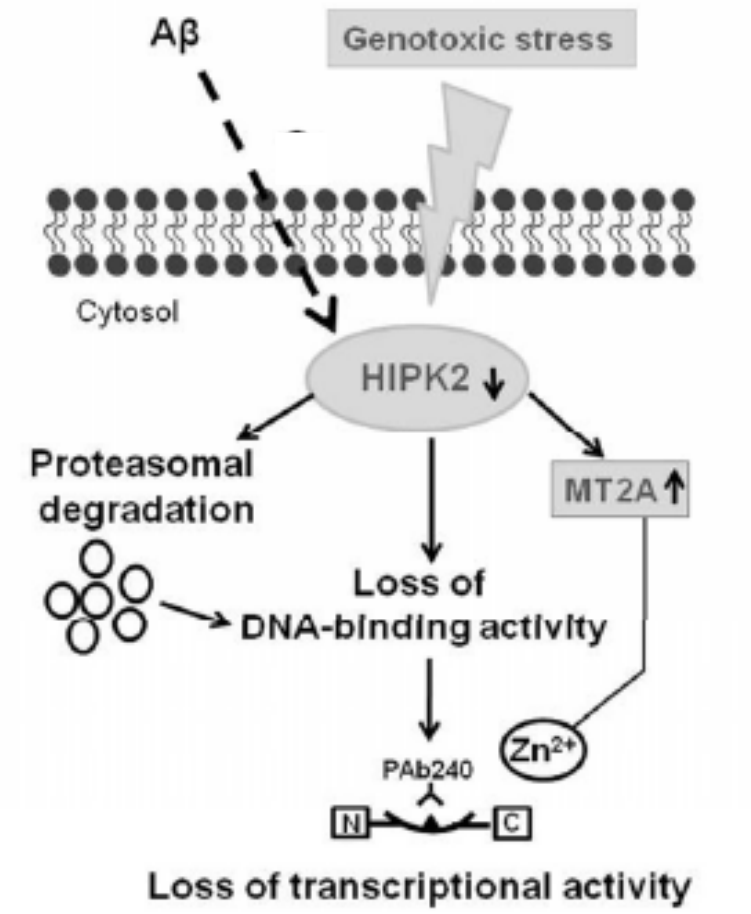
Aβ
Genotoxic stress
Cytosol
HIPK2 ↓
Proteasomal degradation
MT2A ↑
Loss of DNA-binding activity
PAb240
Zn2+
N
C
Loss of transcriptional activity

Zyxin Is a Critical Regulator of the Apoptotic HIPK2-p53 Signaling Axis

Johanna Crone, Carolina Glas, Kathrin Schultheiss, Jutta Moehlenbrink, Eva Krieghoff-Henning, and Thomas G. Hofmann

Abstract

HIPK2 activates the apoptotic arm of the DNA damage response by phosphorylating tumor suppressor p53 at serine 46. Unstressed cells keep HIPK2 levels low through targeted polyubiquitination and subsequent proteasomal degradation. Here we identify the LIM domain protein Zyxin as a novel regulator of the HIPK2-p53 signaling axis in response to DNA damage. Remarkably, depletion of endogenous Zyxin, which colocalizes with HIPK2 at the cytoskeleton and in the cell nucleus, stimulates proteasome-dependent HIPK2 degradation. In contrast, ectopic expression of Zyxin stabilizes HIPK2, even upon enforced expression of its ubiquitin ligase Siah-1. Consistently, Zyxin physically interacts with Siah-1, and knock-down of Siah-1 rescues HIPK2 expression in Zyxin-depleted cancer cells. Mechanistically, our data suggest that Zyxin regulates Siah-1 activity through interference with Siah-1 dimerization. Furthermore, we show that endogenous Zyxin coaccumulates with HIPK2 in response to DNA damage in cancer cells, and that depletion of endogenous Zyxin results in reduced HIPK2 protein levels and compromises DNA damage-induced p53 Ser46 phosphorylation and caspase activation. These findings suggest an unforeseen role for Zyxin in DNA damage-induced cell fate control through modulating the HIPK2-p53 signaling axis. *Cancer Res; 71(6); 2350–9. ©2011 AACR.*

WHICH PATHOGENESIS?

and consequently

WHICH MARKERS?