Barts and The London School of Medicine and Dentistry

Imperial College London

LONDON SCHOOL of HYGIENE & TROPICAL MEDICINE

# A molecular epidemiology approach to cognitive disorders

Valentina Gallo

# Outline

- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

# Outline

- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

# Molecular epidemiology

- In 1982, Perera and Weinstein → "a new paradigm for cancer research that incorporated biomarkers into epidemiological studies to reveal mechanisms and events occurring along the theoretical continuum between exposure and disease" J Chronic Dis 1982;35:581
- In 2007, Vineis and Perera → the new in light of the old

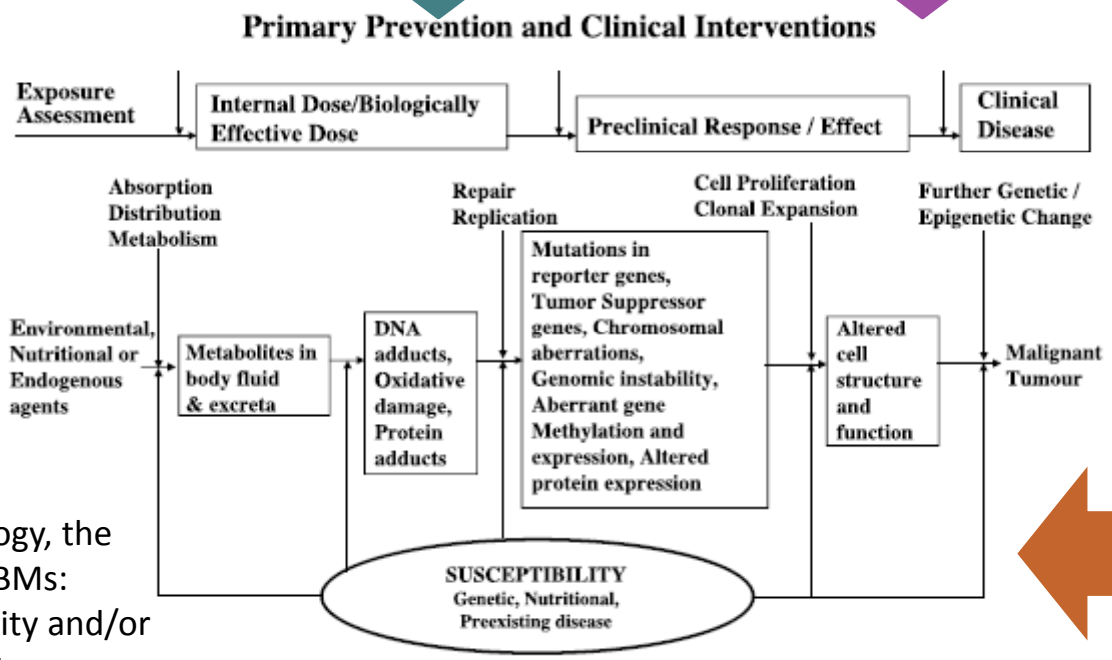
Cancer Epidemiol Biomarker Prev 2007;16:1954

# Meet-in-the-middle

Studies showing overall correlations between DNA or protein adducts level and environmental exposure to carcinogens

Studies confirming the ability of certain carcinogen-DNA adducts and chromosome aberrations to predict cancer

Studies showing a correlation between external measurements of exposure and BMs of biologically effective dose or early biological response/effect




Studies confirming the role of certain genetic variants (SNPs) in modulating the risk of cancer particularly in subjects exposed to carcinogens

In the study of aetiology, the role of intermediate BMs:

1) Increase sensitivity and/or early detectability
2) Show biological plausibility for en exposure proposed to be biologically relevant

Cancer Epidemiol Biomarker Prev 2007;16:1954

# The advent of '–omics' era

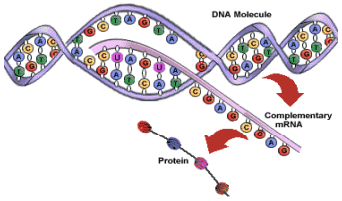
- Global cellular profiling at multiple levels
- Untargeted and simultaneous
- Yields unprecedented views of the cellular inner workings

**DNA**

**Epigenetics**

**Illumina Infinium Human Methylation 450 BeadChip**

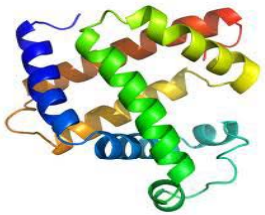
**RNA**




**Transcriptomics**

**Agilent 44K DNA mircroarray**

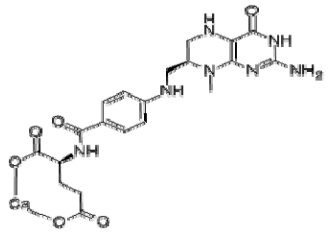
**PROTEINS**



**Proteomics**

**Luminex Multianalyte Profiling system**

**METABOLITES**

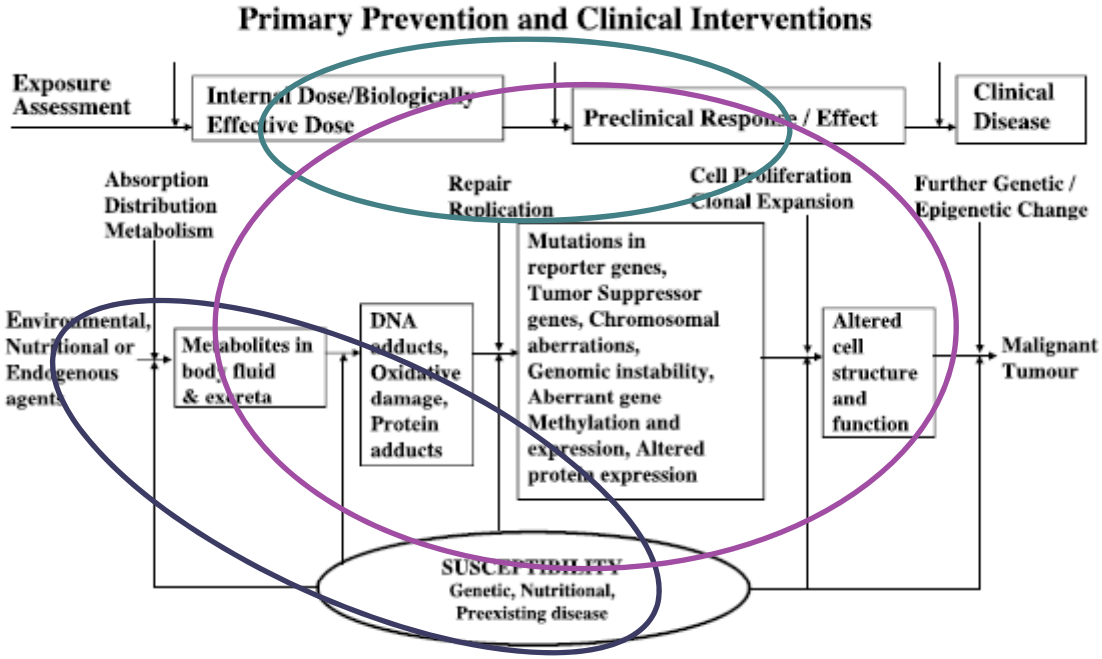


**Metabolomics**

**Ultra-High Performance Liquid chromatography mass-spectrometry**

# The New in light of the Old

**METABONOMICS**
The study of the complete set of low-molecular weight metabolites

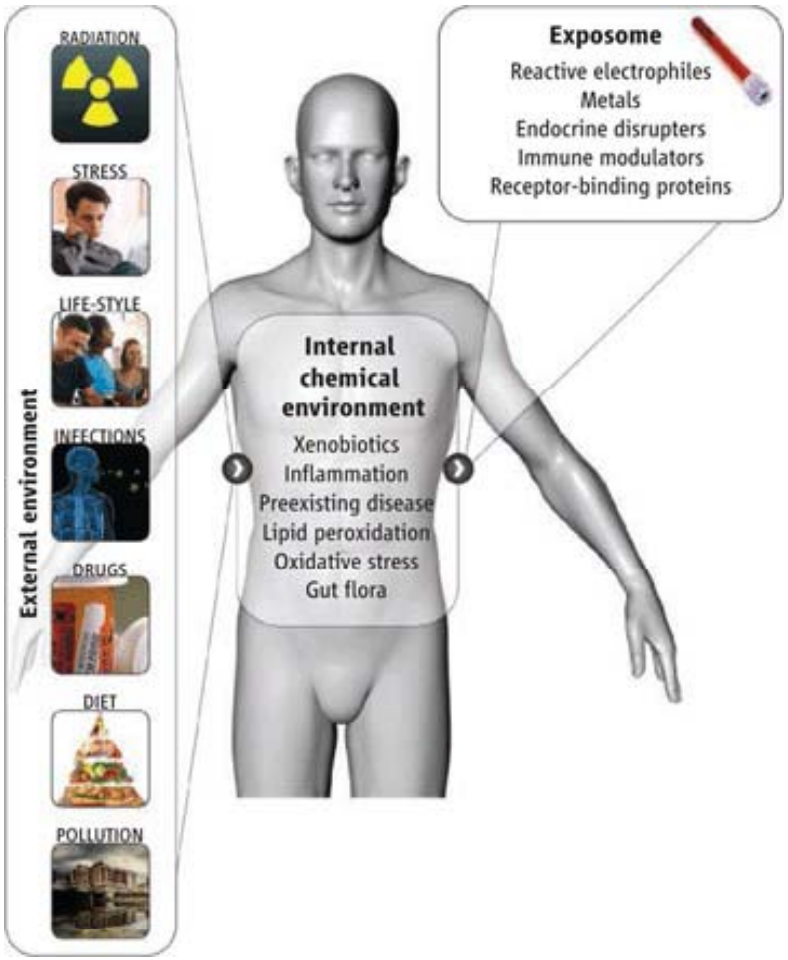



**PROTEOMICS**
The analysis of the total protein output encoded by the genome

**EPIGENETICS**
Mechanisms which do not depend on structural changes in DNA but on functional regulation such as DNA methylation

Cancer Epidemiol Biomarker Prev 2007;16:1954

# Exposomics

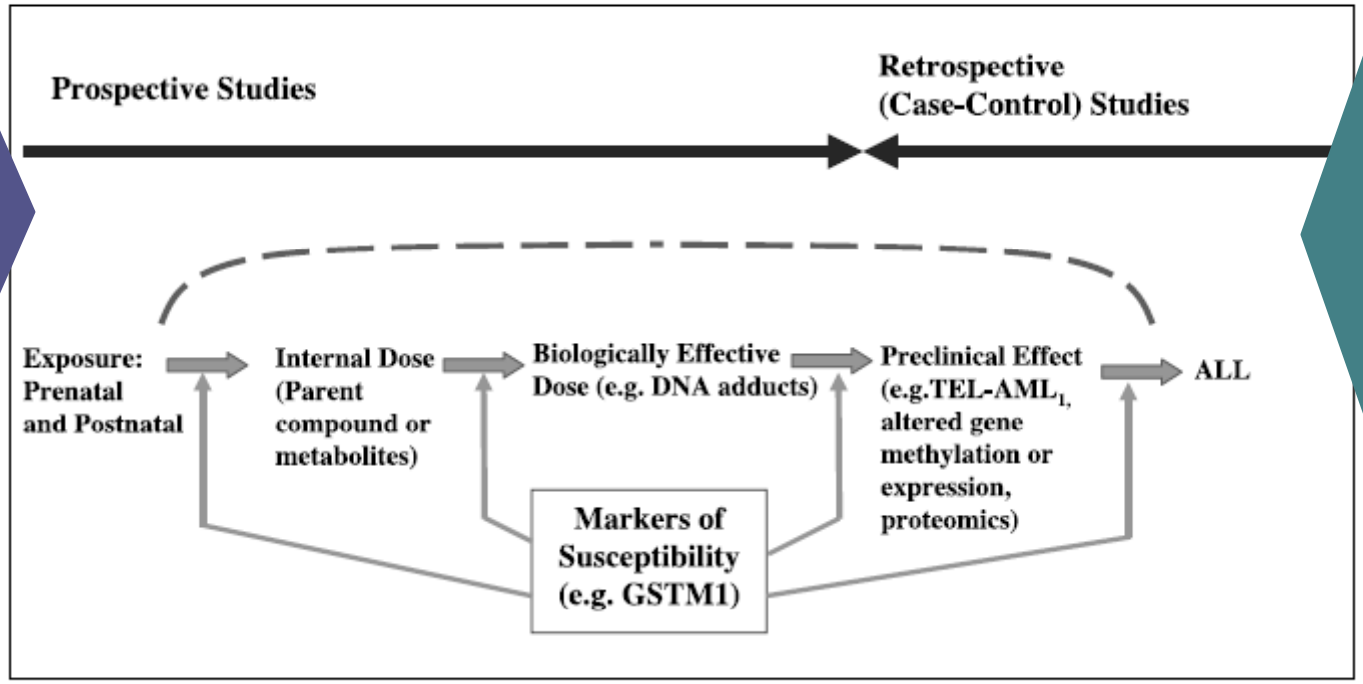



Science 2010;330:460-461

# Meet-in-the-middle

**Is there an aetiological role for *in utero* and childhood exposure to environmental pollutants and childhood leukemia?**

Pre-diagnostic biomarkers (internal dose of toxicants, DNA adducts, gene mutations, chromosomal aberrations, gene methylation) but reduced sample of outcomes




BMs in cases and controls coupled with environmental exposure information from questionnaire

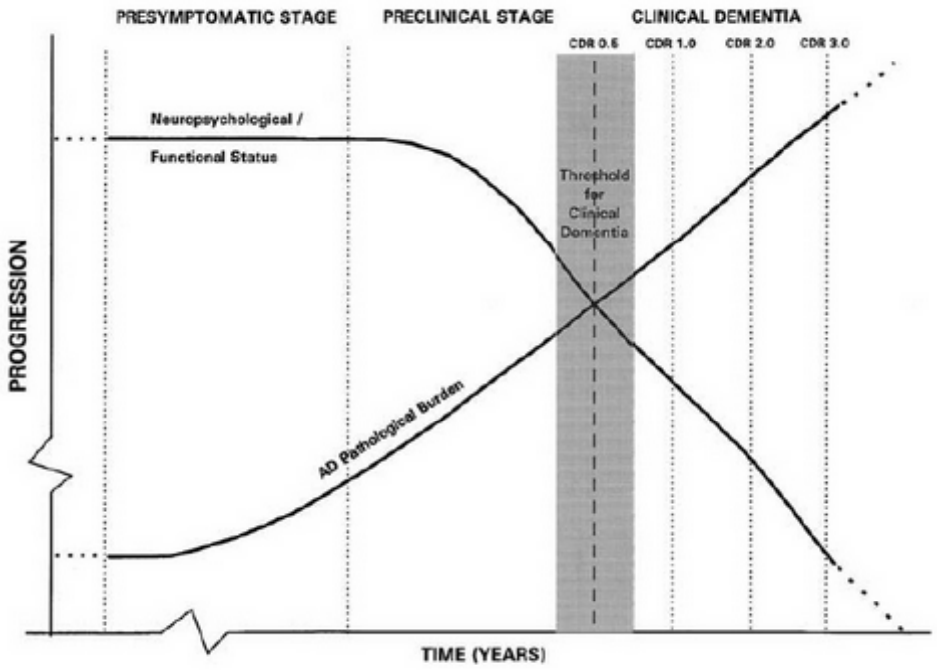
**TEL-AML1 was found in 25% of cases in retrospective studies and in neonatal bloodspots of health babies who would have developed leukemia: "first hit". Different methylation patterns of TEL-AML1 were observed between different cytogenetic groups. Links with environmental pollutants remain to be established.**

Cancer Epidemiol Biomarker Prev 2007;16:1954

# Outline

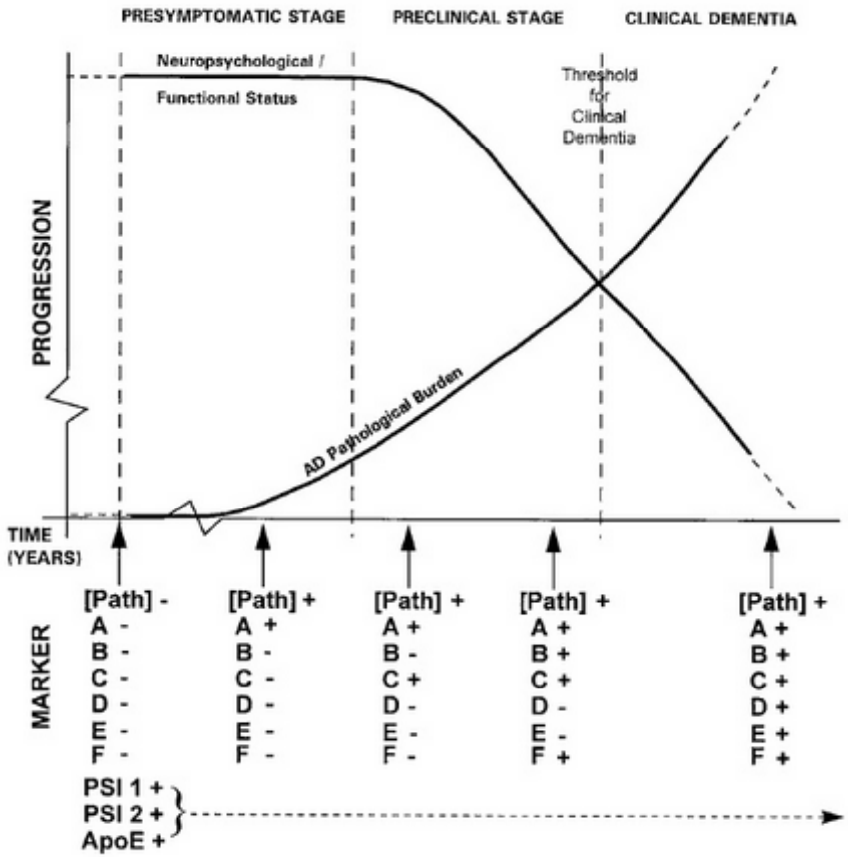
- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

# Theoretical timeline



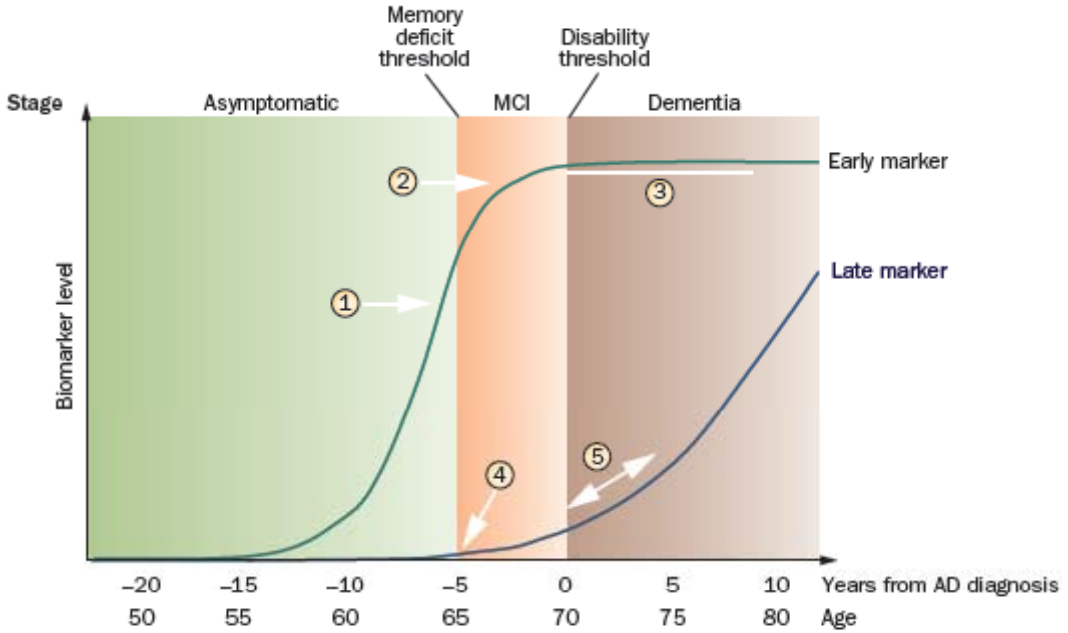

Percy ME et al in Scinto LFM and Daffner KR: Early Diagnosis of AD. Human press: 2000

# Theoretical sequence of BMs




Percy ME et al in Scinto LFM and Daffner KR: Early Diagnosis of AD. Human press: 2000

# … 12 years later



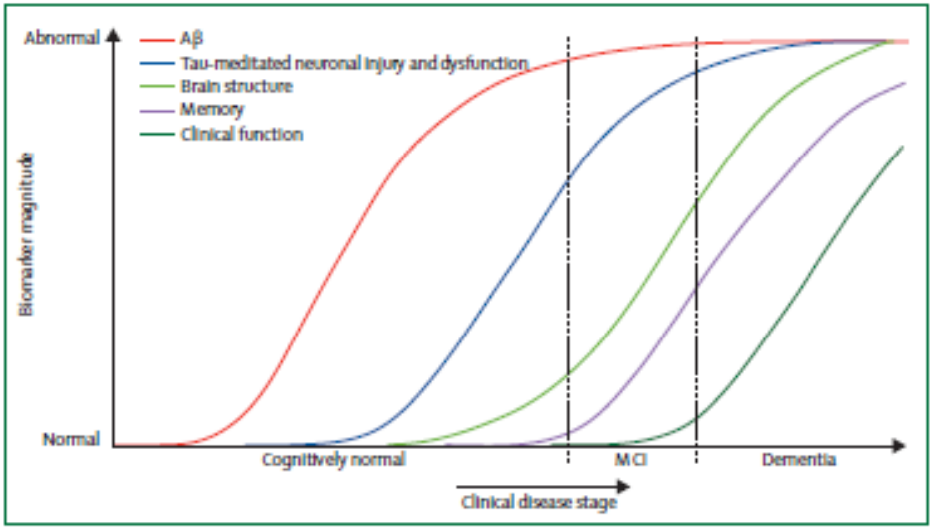

Nat Rev Neurol 2012;8:299

# Biomarkers of dementia

- Biomarkers of exposure
  - Guide the understanding of environmental exposure risk in aetiology
- Biomarkers of early biological effect
  - Longitudinal studies of dementia biomarkers take many years to show the full pathological cascade of events that lead to dementia
  - Trial of disease-modifying agents require large numbers of patients over extended periods owing to the slow progression of the cognitive symptoms

# Dynamic BMs: hypothetical model

- Biomarkers of Aβ plaque deposition
  - Decreased CSF-$A\beta_{42}$
  - PET amyloid imaging (PiB-PET)
- Biomarkers of neurodegeneration
  - Increased CSF-tau
  - Decreased fluordeoxyglucose uptake on PE (FDG-PET)
- Structural MRI measure of cerebral atrophy



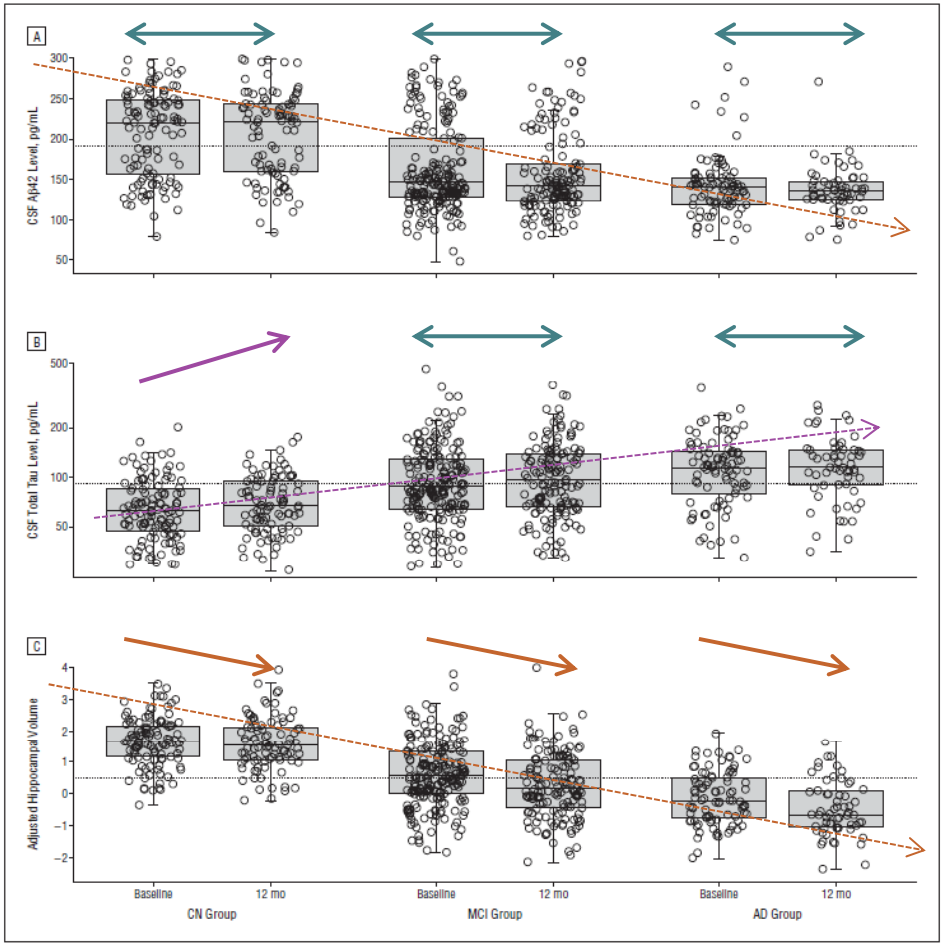

Lancet Neurol 2010;9:119

# Seven Principles

1. The BMs become abnormal in a temporarily ordered manner as disease progresses
2. Aβ plaques BMs are dynamic early in the disease before the appearance of clinical symptoms and have largely reached a plateau by the time clinical symptoms appear
3. BMs of neuronal injury, dysfunction, and degeneration are dynamic later in the disease and correlate with clinical disease severity
4. MRI is the last BM to become abnormal, however it retains the closer relationship with cognitive performance later into disease
5. None of the BMs is static, rates of changes change over time, and follow a sigmoid shaped course
6. Anatomical information from imaging BM provide crucial disease-staging information
7. Lag phase between Aβ-plaque formation and the neurodegenerative cascade of unknown duration (interacting variables?)

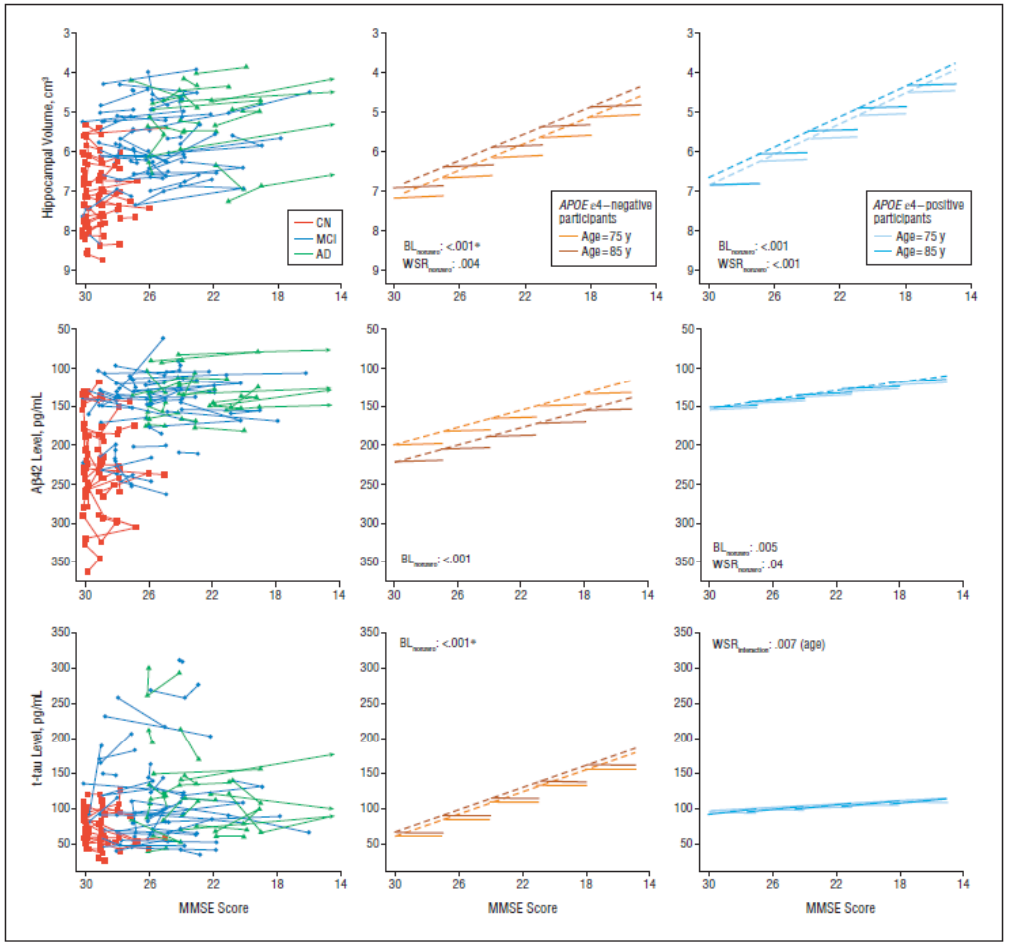
Lancet Neurol 2010;9:119

# Evidence for ordering AD BMs



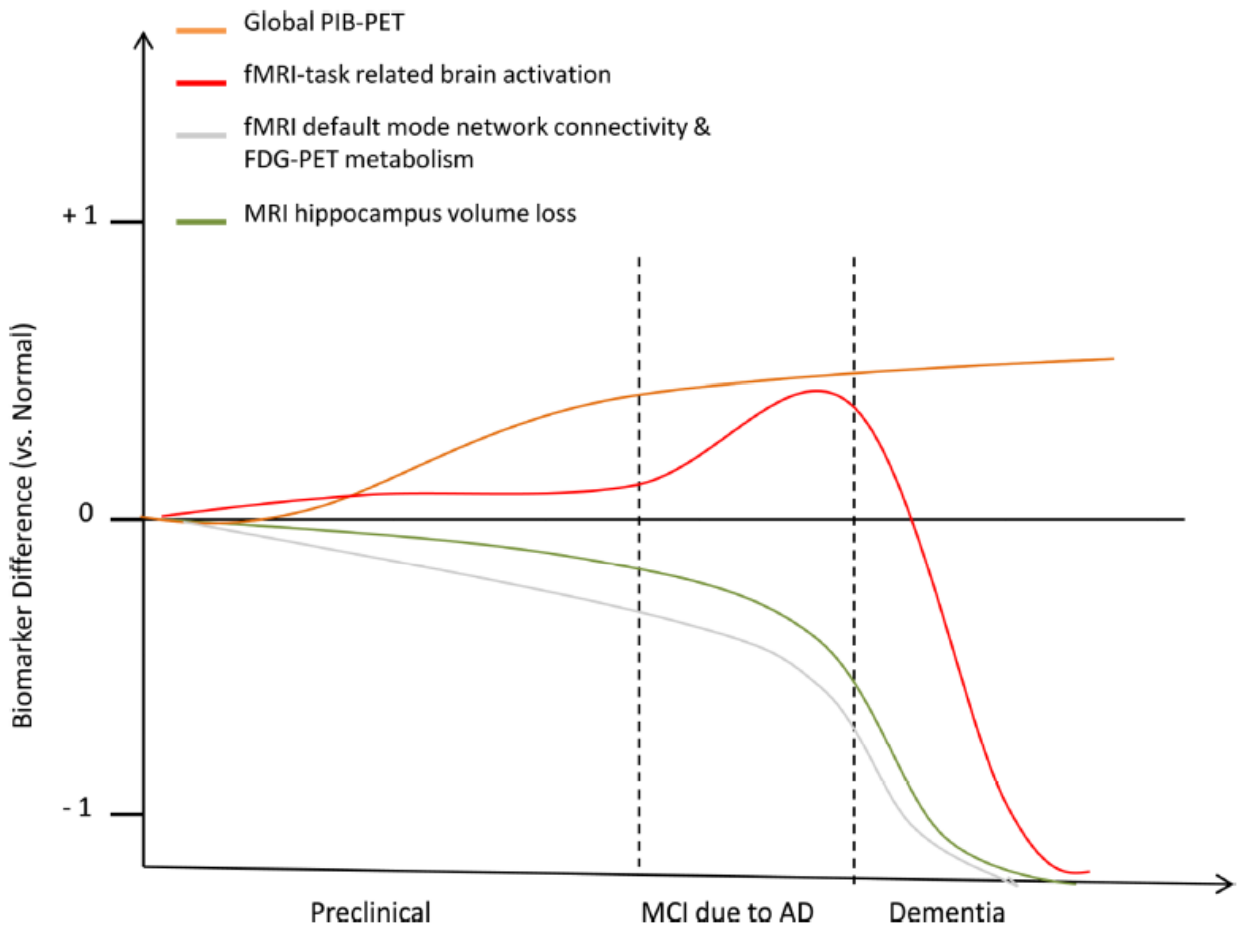

Arch Neurol 2011;68:1526

# Shaping the trajectories of AD BMs



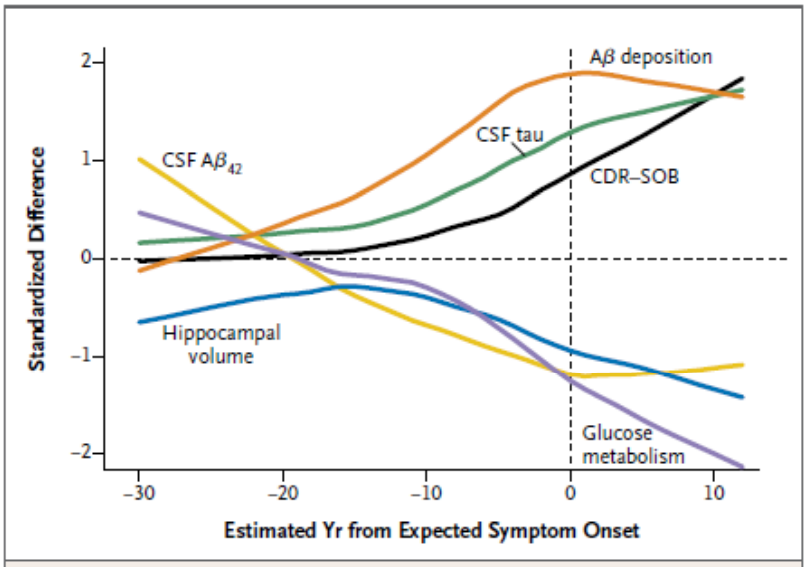

Arch Neurol 2012;69:856

# Dynamic neuroimaging model




Trends Neurosci 2011;34:430

# BMs in dominant Alzheimer disease




N Engl J Med 2012;367:795

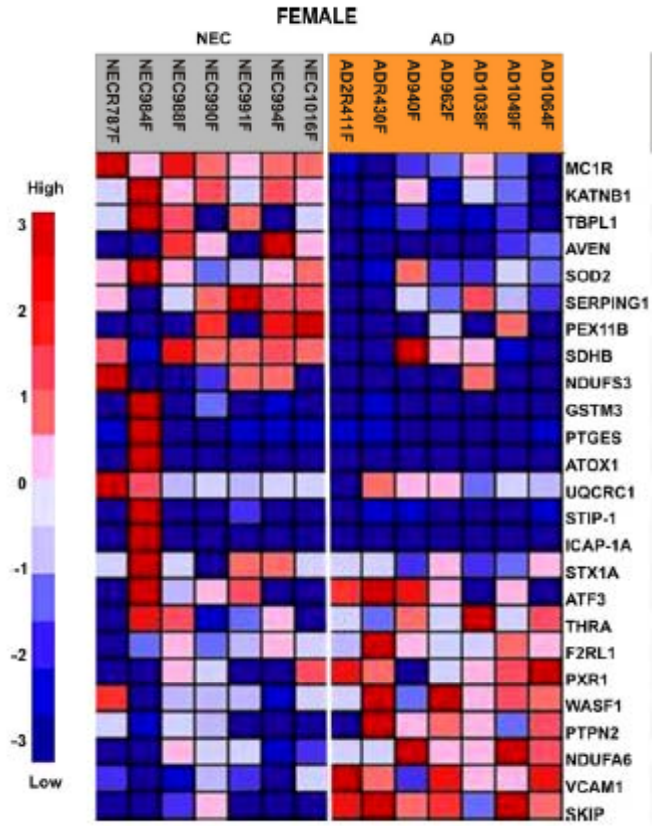
- $A\beta_{42}$ in CFS decline in carriers when compared to non carriers
- Aβ as measured by PIB-PET was detected at least 15 yrs before expected symptoms onset (ESO)
- Increase levels of tau in CFS and brain atrophy were detected approximately 15 yrs before ESO
- cerebral hypometabolism ~ 10 yrs before ESO
- Global cognitive impairment started 5 yrs before ESO

# Outline

- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

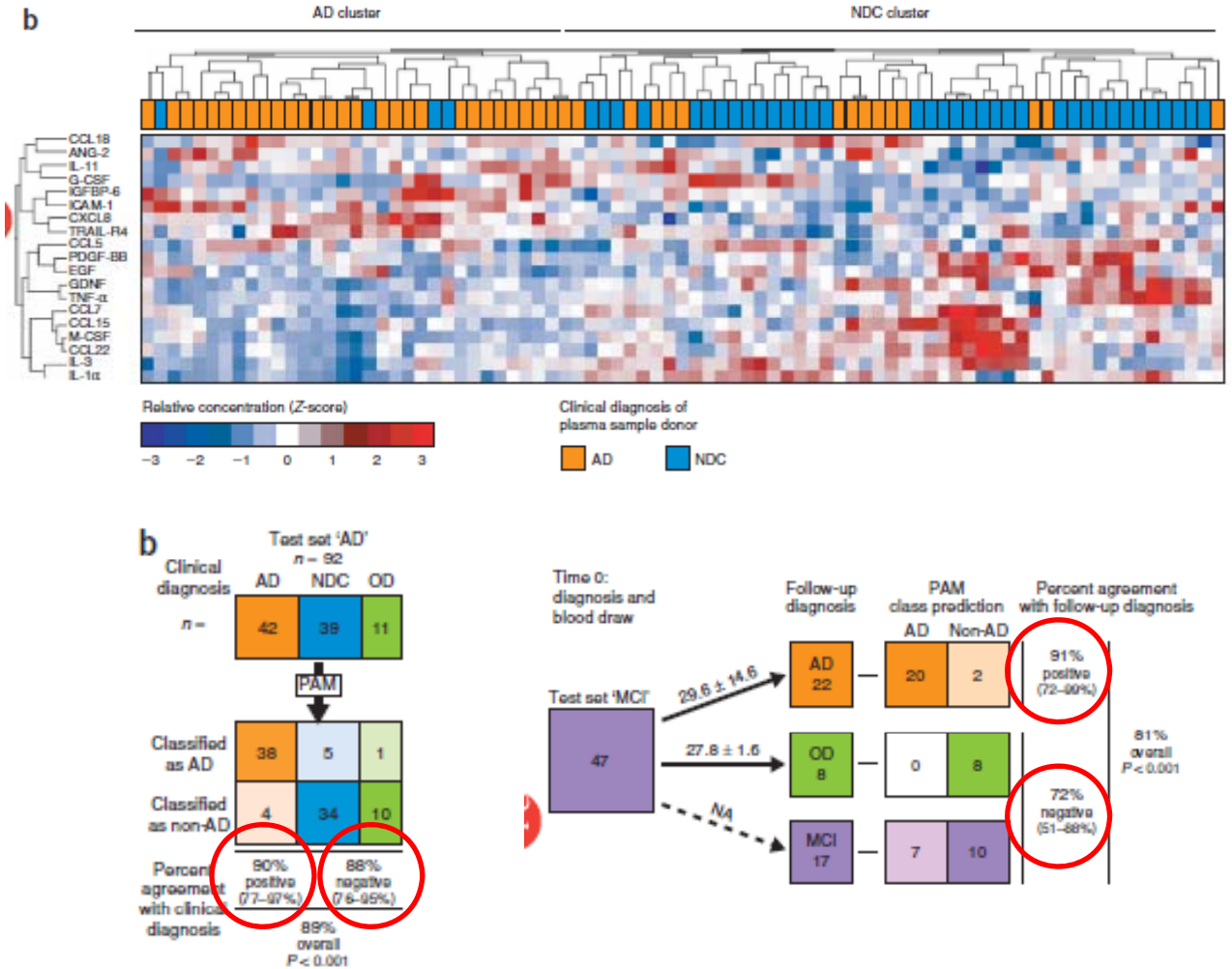
# Gene expression of MNC

- Use of MNC: a window into the CNS
  - Easy to access
  - Communication CNS-immune system
  - Abnormal APP expression, altered level of antioxidant, and increased rates of apoptosis share by AD brain and lymphocytes
- 28% of the up-regulated genes and 16% of the down-regulated in MNC had been reported to exhibit similar expression patterns in AD brains
- In 4% of affected genes there was a divergent regulation between MNC and brain




Neurobiol Aging 2007;28:1795

# Signalling proteins: “cellular communicome”




- 120 known signalling proteins
- 19 proteins identified via a clustering algorithm
- 18 AD predictors identified via predictive analysis of microarrays (PAM)
- PAM used for classifying subjects with MCI who had different clinical diagnoses 2-7 yrs later
- Networks of TNF-α + M-CSF and EGF → role of immune response, hematopoiesis and apoptosis

Nat Med 2007;13:1359

# Outline

- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

# STROBE-ME

OPEN ACCESS Freely available online

PLoS MEDICINE

Guidelines and Guidance

## STrengthening the Reporting of OBservational studies in Epidemiology – Molecular Epidemiology (STROBE-ME): An Extension of the STROBE Statement

**Valentina Gallo[1,2*], Matthias Egger[3], Valerie McCormack[4], Peter B. Farmer[5], John P. A. Ioannidis[6,7], Micheline Kirsch-Volders[8], Giuseppe Matullo[9,10], David H. Phillips[11], Bernadette Schoket[12], Ulf Stromberg[13], Roel Vermeulen[14], Christopher Wild[4], Miquel Porta[15], Paolo Vineis[9,16]**

**1** Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom, **2** Social and Environmental Health Research, London School of Hygiene and Tropical Medicine, London, United Kingdom, **3** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland, **4** International Agency for Research on Cancer (IARC) Lyon, France, **5** Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom, **6** Stanford Prevention Research Centre, Stanford University School of Medicine, Stanford, California, United States of America, **7** Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece, **8** Laboratory for Cell Genetics, Vrije Universiteit Brussel, Brussels, Belgium, **9** HuGeF Human Genetics Foundation, Turin, Italy, **10** Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy, **11** Institute of Cancer Research, Sutton, United Kingdom, **12** National Institute of Environmental Health, Budapest, Hungary, **13** Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden, **14** Institute for Risk Assessment Sciences (IRAS), Division Environmental Epidemiology, Utrecht University, Utrecht, the Netherlands, **15** Institut Municipal d'Investigacio Medica (IMIM), Universitat Autonoma de Barcelona, Barcelona, Spain, **16** MRC-HPA Centre for Environment and Health, School of Public Health, Imperial College London, London, United Kingdom

# Collection, handling and storage of biological samples

## Source of variability

- Type of sample
  - Blood stored as whole or separated
- Timing of collection (e.g. hormones, vitamin D)
- Narrow needles causes haemolysis
- Blood additives (heparin, EDTA, etc.)

## Proper sample storage

- Standardised precedures in all phases
- Minimal loss or degrading of material
- Otpimal preservation of material
- Blinding
- Easy access to material
- Easy matching of biological material with subjects
- Respect of confidentiality
- Anticipation of emergencies

# Biomarker validity and reliability

## Validity and reliability

- Validity: lack of systematic measurement error when comparing to a standard (i.e., the “truth”)
- Validity: the extent to which any measuring procedure yields to the same results in repeated experiments

## Sources of variation

- Intra-subject variability (i.e. diurnal, monthly, seasonal variability)
- Biological sampling variation (i.e. colonic mucosa sampling)
- Laboratory variations within and between batches

# Special sources of selection bias

- Poor cognitive performance (low MMSE and high ADAS-Cog) was predictive of missing data even for the NC group
- Depression was associated with drop outs MCI
- Family history of AD and higher CDR scores characterised AD pts who stayed in the study

Table 4 Univariate association with missing MRI during follow-up[a]

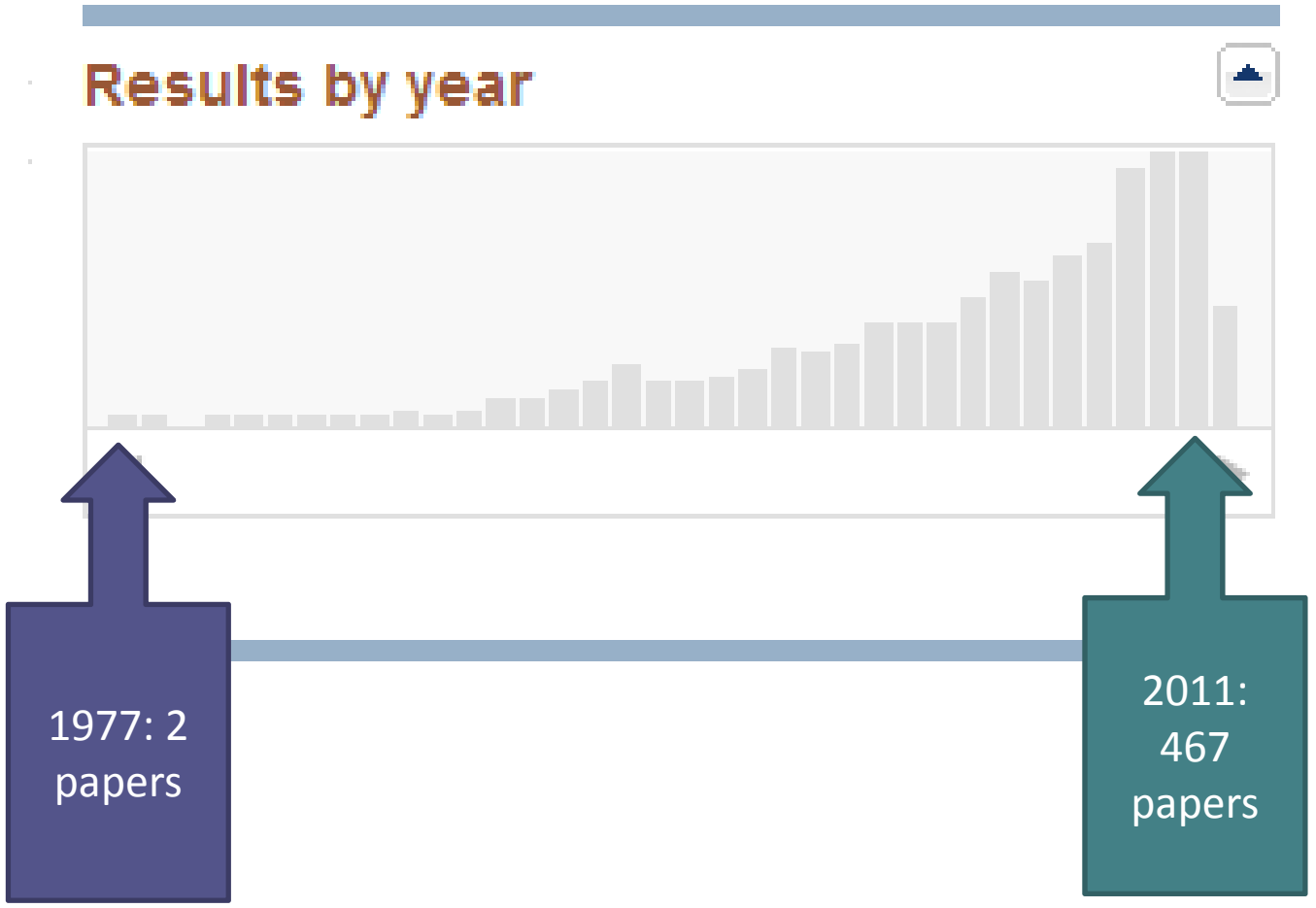
| | Odds ratios (95% CI) | | |
|---|---|---|---|
| | NC | MCI | AD |
| Missing n/total | 47/228 | 85/393 | 86/193 |
| Demographic features | | | |
| Age, y | 1.03 (0.97–1.10) | 1.01 (0.98–1.05) | 0.99 (0.96–1.03) |
| Female | 1.15 (0.61–2.20) | 0.87 (0.52–1.44) | 1.04 (0.59–1.84) |
| Education, y | 0.93 (0.83–1.04) | 0.93 (0.86–0.99)[b] | 0.93 (0.85–1.02) |
| Occupation type | 1.29 (0.85–1.93) | 1.12 (0.83–1.50) | 1.24 (0.88–1.76) |
| Smoking | 0.84 (0.42–1.62) | 1.28 (0.78–2.07) | 1.15 (0.64–2.06) |
| Family history of AD | 0.36 (0.13–0.85)[b,c] | 0.67 (0.36–1.18) | 0.36 (0.17–0.74)[b,c] |
| APOE4 carrier | 0.59 (0.25–1.25) | 1.15 (0.71–1.88) | 0.59 (0.32–1.08) |
| ANART error, n | 1.01 (0.98–1.05) | 1.01 (0.98–1.03) | 0.99 (0.97–1.02) |
| General clinical features | | | |
| Body mass index | 0.93 (0.86–1.01) | 1.02 (0.96–1.08) | 0.96 (0.89–1.04) |
| Comorbidity, n | 0.99 (0.88–1.10) | 0.97 (0.89–1.05) | 0.96 (0.88–1.05) |
| CVD risk score | 0.97 (0.89–1.06) | 1.02 (0.96–1.09) | 1.04 (0.97–1.12) |
| FAQ score | 0.67 (0.20–1.29) | 1.01 (0.96–1.06) | 0.99 (0.95–1.03) |
| GDS score | 1.03 (0.77–1.35) | 1.23 (1.03–1.45)[b,c] | 0.97 (0.79–1.19) |
| NPI-Q score | 0.96 (0.63–1.34) | 1.09 (1.01–1.19)[b] | 1.02 (0.94–1.11) |
| Abnormal gait | 0.76 (0.11–3.01) | 1.04 (0.43–2.27) | 0.51 (0.23–1.08) |
| Cognitive performance | | | |
| CDR scale | NA | NA | 0.28 (0.09–0.89)[b,c] |
| MMSE score | 0.69 (0.51–0.93)[b,c] | 0.93 (0.81–1.06) | 1.01 (0.88–1.16) |
| ADAS-Cog | 1.21 (1.08–1.36)[b,c] | 1.06 (1.01–1.12)[b] | 0.99 (0.95–1.04) |
| Baseline MRI hippocampal volume, $mm^3$ | 1.00 (0.99–1.00) | 1.00 (0.99–1.00) | 0.99 (0.99–1.00) |

Neurology 2012;78:1376

# Outline

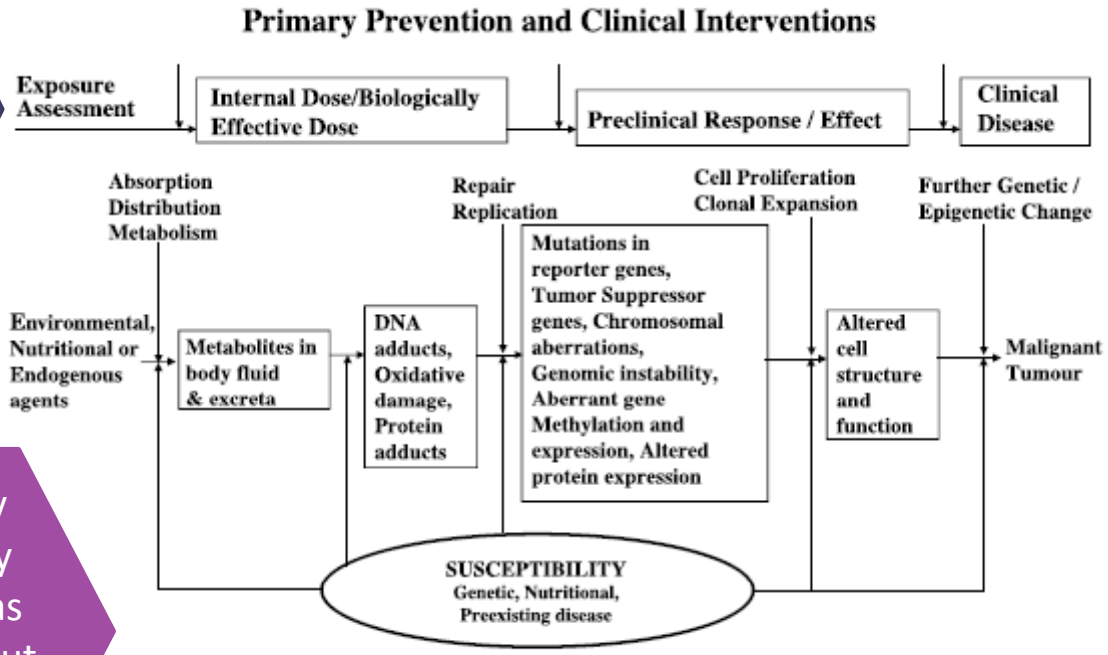
- The concept of Meet-in-the-middle
- Dynamic models in Alzheimer disease dementia
- New approaches: the -omics
- STROBE-ME recommendations
- Has epidemiology complied with its role in understanding dementia?

# Dementia & biomarkers [MeSH]



# Has epidemiology complied with its role in understanding dementia?

The majority of environmental factors still remain elusive




Prognostic models more than aetiopathological models have been studied

Susceptibility modulated by APOE SNPs has been studies but not completely elucidated

# Thank you for you attention!

v.gallo@qmul.ac.uk