Supplementary Online Content

Lautner R, Palmqvist S, Mattsson N, et al. Apolipoprotein E genotype and the diagnostic accuracy of cerebrospinal fluid biomarkers for Alzheimer disease. *JAMA Psychiatry*. Published online August 27, 2014. doi:10.1001/jamapsychiatry.2014.1060.

eMethods 1. Detailed description of the cohorts

eMethods 2. [^{18}F]Flutemetamol postitron emission tomography (PET) acquisition and analysis

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Detailed description of the cohorts

Cohort A:

Four memory clinics in Sweden, Finland and Germany took part in the study. The total cohort comprised 251 controls, 399 patients with stable mild cognitive impairment (sMCI), 287 patients with prodromal AD (MCI-AD), 309 demented patients with AD, and 99 patients with other dementias than AD. Patients with MCI were followed for at least 2 years or until they progressed to AD dementia or developed other dementias. MCI was diagnosed according to the Petersen criteria including objective decline in memory or in other cognitive domains but with only limited impairment of activities of daily living and without fulfilling criteria for full-blown dementia.[1-3] AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Relates Disorders Association (NINCDS-ADRDA) criteria.[4] Participants diagnosed with other known causes of cognitive impairment, such as cerebral tumors, subdural hemorrhage or alcohol abuse, were excluded from the study. Participants in the control group did not show any signs of objective cognitive symptoms and they were not diagnosed with any active neurologic disorder. Parts of cohort A, including 186 patients from the ongoing prospective clinical longitudinal Gothenburg MCI study[5], have been included in earlier publications from our groups.[6-9]

Cohort B:

The study also included a separate cohort comprising 105 individuals younger than 35 years (mean age 27.7 ± 3.8 years) without neurodegenerative conditions (67 patients with bipolar disorder and 38 healthy controls). This cohort was only used to assess the association of *APOE* ε4 with CSF biomarker levels but was not included in the studies of the diagnostic accuracy of the biomarkers due to their low age.

Cohort C:

These subjects were included from the larger BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably), which enrolls patients from three memory clinics in Sweden (www.biofinder.se). The cohort consisted of patients who were referred to these memory clinics due to cognitive complaints (subjective or objective) and were included between 2010 and 2013. The inclusion criteria were: 1) referred due to cognitive complaints experienced by the patient and/or informant; 2) not fulfilling dementia criteria; 3) a Mini-Mental State Examination (MMSE) score[10] between 24 and 30 points; 4) aged 60 to 80 years and; 5) adequate knowledge of the study language (Swedish). The exclusion criteria were: 1) the cognitive impairment could be explained by another condition or disease (other than prodromal dementia); 2) severe somatic disease and; 3) refusing lumbar puncture. A physician with special interest in cognitive disorders assessed the patients at the clinical baseline visit. Cognitive tests, analysis of *APOE* genotype, lumbar puncture and brain imaging were performed. From the BioFINDER study, we selected 118 patients who had undergone both [^{18}F]flutemetamol PET imaging and CSF taps (age 71.1 (mean) ± 5.4 (SD); MMSE: 27.8 (mean) ± 1.6 (SD); male/female: n=68/50; *APOE* ε4 negative/positive: n=66/52).

ADNI cohort:

53 subjects (9 with AD, 33 with MCI and 11 healthy controls) with data on both CSF analysis and ^{11}C-PiB scans were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects originally

recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see www.adni-info.org.

eMethods 2

[^{18}F]Flutemetamol postitron emission tomography (PET) acquisition and analysis

[^{18}F]Flutemetamol injection was manufactured at the radiopharmaceutical production site in Risø, Denmark, using a FASTlab synthesizer module (GE Healthcare).[11] PET/CT scanning of the whole brain was conducted at two sites (Malmö and Lund in Sweden) using the same type of scanner, a Philips Gemini TF 16. The dynamic PET acquisition (6 frames, 5 min/frame) in 3D mode was started 90 min post-injection. The generation of PET sum images and further image processing have been described previously.[12] A composite cortical volume of interest (VOI)[12] was created from the prefrontal, parietal, anterior cingulate, precuneus/posterior cingulate and lateral temporal cortex. The standardized uptake value ratio (SUVR) was defined as the regional tracer uptake in this composite VOI normalized for the mean uptake in a reference region, which in this case was the cerebellar cortex. To obtain unbiased cut offs for [^{18}F]flutemetamol cortical uptake a mixture modeling analysis was performed using the normalmixEM function implemented in the mixtools package[13] installed in the statistical freeware R (version 3.0.2). Briefly, mixture modeling is a two-step iterative procedure based on an expectation maximization algorithm and, in the current setting, it is assumed that the PET data are sampled from a population that has a mix of two different normal distributions. The use of mixture modeling in a similar context has previously been described in detail.[14]

References

1. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* Sep 2004;256(3):183-194.
2. Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med.* Sep 2004;256(3):240-246.
3. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.* Mar 1999;56(3):303-308.
4. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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.* Jul 1984;34(7):939-944.
5. Nordlund A, Rolstad S, Hellstrom P, Sjogren M, Hansen S, Wallin A. The Goteborg MCI study: mild cognitive impairment is a heterogeneous condition. *J Neurol Neurosurg Psychiatry.* Nov 2005;76(11):1485-1490.
6. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *Jama.* Jul 22 2009;302(4):385-393.
7. Hertze J, Minthon L, Zetterberg H, Vanmechelen E, Blennow K, Hansson O. Evaluation of CSF biomarkers as predictors of Alzheimer's disease: a clinical follow-up study of 4.7 years. *J Alzheimers Dis.* 2010;21(4):1119-1128.
8. Andreasson U, Lautner R, Schott JM, et al. CSF biomarkers for Alzheimer's pathology and the effect size of APOE ε4. *Mol Psychiatry.* Feb 19 2013.
9. Mattsson N, Rosen E, Hansson O, et al. Age and diagnostic performance of Alzheimer disease CSF biomarkers. *Neurology.* Feb 14 2012;78(7):468-476.
10. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research.* Nov 1975;12(3):189-198.
11. Nelissen N, Van Laere K, Thurfjell L, et al. Phase 1 study of the Pittsburgh compound B derivative 18F-flutemetamol in healthy volunteers and patients with probable Alzheimer disease. *J Nucl Med.* Aug 2009;50(8):1251-1259.
12. Lundqvist R, Lilja J, Thomas BA, et al. Implementation and validation of an adaptive template registration method for 18F-flutemetamol imaging data. *J Nucl Med.* Aug 2013;54(8):1472-1478.
13. Benaglia T, Chauveau D, Hunter DR, Young DS. mixtools: An R Package for Analyzing Mixture Models. *Journal of Statistical Software.* 2009;32(6).
14. De Meyer G, Shapiro F, Vanderstichele H, et al. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. *Arch Neurol.* 2010;67(8):949-956.

