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# Plasma proteins predict conversion to dementia from prodromal disease

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**Abstract**

**Background:** The study aimed to validate previously discovered plasma biomarkers associated with AD, using a design based on imaging measures as surrogate for disease severity and assess their prognostic value in predicting conversion to dementia.

**Methods:** Three multicenter cohorts of cognitively healthy elderly, mild cognitive impairment (MCI), and AD participants with standardized clinical assessments and structural neuroimaging measures were used. Twenty-six candidate proteins were quantified in 1148 subjects using multiplex (xMAP) assays.

**Results:** Sixteen proteins correlated with disease severity and cognitive decline. Strongest associations were in the MCI group with a panel of 10 proteins predicting progression to AD (accuracy 87%, sensitivity 85%, and specificity 88%).

**Conclusions:** We have identified 10 plasma proteins strongly associated with disease severity and disease progression. Such markers may be useful for patient selection for clinical trials and assessment of patients with predisease subjective memory complaints.



*Keywords:* Plasma; Mild cognitive impairment; Pathology; Alzheimer's disease; Biomarker; Prediction and magnetic resonance imaging

Conflicts of interest: SL has patents filed jointly with Proteome Sciences plc related to these findings. IP, HDZ, and MW are employees of Proteome Sciences plc. JR is a full-time employee of Glaxo Smith-Kline. AH, JRC, AB, NA, CB, RL, EW, AS, RD, MS, ML, KL, AK, DP, WZ, AT, SG, HS, IK, PM, MT, and BV have no conflicts of interest.

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## 1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the aging population; usually affecting people over the age of 65 years and resulting in progressive cognitive and functional decline. Detecting AD at the earliest possible stage is vital to enable trials of disease modification agents and considerable efforts are being invested in the identification and replication of biomarkers for this purpose.

Such biomarkers currently include measures of tau and amyloid beta (Aβ) in cerebrospinal fluid (CSF), brain atrophy using magnetic resonance imaging (MRI), and measures of Aβ pathological load using positron emission tomography (PET). All these approaches are promising, although molecular imaging is currently a costly procedure available in relatively few centers and lumbar puncture is moderately invasive. Furthermore, repeated measures are problematical in both cases.

Blood (plasma) on the other hand is a more accessible biofluid suitable for repeated sampling. This led many groups including ours to investigate the potential of a diagnostic signal in blood. Using a case-control study design with a gel-based approach (two-dimensional gel electrophoresis and liquid chromatography tandem mass spectrometry) two proteins (complement factor H [CFH] and alpha-2-macroglobulin [A2M]) were observed as potential markers of AD [1], both of which were subsequently replicated by independent groups [2,3]. In the same study we observed changes in serum amyloid P (SAP), complement C4 (CC4), and ceruloplasmin, all of which have been implicated in AD pathogenesis [4–6]. However, case-control studies are problematical when there is a long prodromal disease phase. In such instances a large proportion of apparently normal controls already harbors the disease processes and hence may already have a peripheral biomarker disease signature. To overcome the limitations of case-control design, we searched for proteins associated with surrogates of disease severity (hippocampal atrophy and clinical progression), and identified Clusterin as a marker associated with both these surrogate measures [7]. Building on this "endophenotype" discovery approach we subsequently found transthyretin (TTR) and apolipoprotein A1 (ApoA1) to be associated with faster declining AD subjects and increased plasma apolipoprotein E (ApoE) levels related to increased Aβ burden in the brain [8,9].

These studies, and those from other groups, have identified a set of proteins that might act as biomarkers relevant to AD. However such findings require replication, in large studies, ideally using samples drawn from more than one cohort source and using a platform that enables multiplexing. We therefore developed multiplex panels using our discovery proteins together with additional putative candidate biomarkers that have been implicated in AD and neurodegeneration (Supplementary Table S1).

The aims of the current study were (1) to validate a set of blood-based biomarkers in a large multicenter cohort with specified *a priori* outcome variables of the disease endophenotype measure of atrophy on MRI and of clinical severity and (2) to determine the accuracy of a multiplexed panel of disease relevant biomarkers in predicting conversion of mild cognitive impairment (MCI) to dementia in a defined time period.

## 2. Methods

### 2.1. Subjects and clinical classification

Plasma samples from AD, MCI subjects and elderly nondemented controls were selected from three independent studies. AddNeuroMed (ANM) a multicenter European study [10], Kings Health Partners-Dementia Case Register (KHP-DCR) a UK clinic and population based study and Genetics AD Association (GenADA) a multisite case-control longitudinal study based in Canada [11]. The diagnosis of probable AD was made according to *Diagnostic and Statistical Manual for Mental Diagnosis*, fourth edition and National Institute of Neurological, Communicative Disorders and Stroke–Alzheimer's disease and Related Disorders Association criteria. MCI was defined according to Petersen criteria [12]. Standardized clinical assessment included the Mini-Mental State Examination (MMSE) for cognition and for global levels of severity the Clinical Dementia Rating (ANM and KHP-DCR only). The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

In total we examined plasma samples from 1148 subjects: 476 with AD, 220 with MCI, and 452 elderly controls with no dementia (Table 1). The APOE single nucleotide polymorphisms (SNPs) rs429358 and rs7412 were genotyped using Taqman SNP genotyping assays (determined by allelic discrimination assays based on fluorogenic 5′ nuclease activity) and the allele inferred.

### 2.2. Cognitive decline

Cognitive decline, as determined by the slope of change in cognition, was calculated for a subset of AD subjects (n = 342) who had a minimum of three separate MMSE assessments. The rate of cognitive decline was calculated separately for ANM because it had a different following up interval (every 3 months for 1 year) in comparison to DCR and GenADA, which were followed up yearly for a period of at least 3 years. Linear mixed effect models were generated using the package "nlme" in R. We estimated the rate of change using a multilevel linear model with random intercepts and random slopes adjusted for subject and center level clustering. Covariates including age at baseline, gender, APOE ε4 allele presence, and years of education were investigated for their effect on the rate of decline. Age at baseline and years of education had a significant effect on the rate ($P$ value $< .05$) and thus were included as fixed effects in the final model. The slope coefficient obtained from the final model for each sample was then used as a rate of cognitive change, defined as the change in MMSE score per year.