lar dementia compared to those with AD [17], indicating that changes in plasma AP may indeed occur as a result of neurodegenerative disease; however, this study [17] did not compare plasma AP activity in AD subjects with that of age-matched controls. Therefore, to determine whether plasma AP activity is altered in AD we designed the present study which involved the analysis of clinical data from the Oxford Project to Investigate Memory and Aging (OPTIMA). Our first aim was to establish whether AD patients have altered plasma AP activity and our second aim was to examine the relationship between plasma AP activity and cognitive function. We established that plasma AP activity is increased in AD patients and is inversely correlated with cognitive function.

Materials and methods

Subject characteristics

Samples obtained from subjects recruited for OPTIMA were used. All procedures received prior approval from the Central Oxford Research Ethics Committee and all participants and their carers gave prior informed consent. The present study included 121 National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disor- Association work group (NINCDS-ADRDA) 'Probable' or pathologically confirmed AD patients, 89 MCI patients and 180 cognitively-screened non-demented controls assessed using the Cambridge Examination for Mental Disorders (CAMCOG) [18]. The study excluded patients with NINCDS-ADRDA diagnoses of 'Possible' AD. Analysis was restricted to participants who were over 60 at their first assessment, with at least 2 assessments with qualifying CAMCOG scores and plasma AP measurements. The number of follow-up assessments ranged from 2 to 12 (median 3) and the total duration of follow-up in each group was as follows (median (lower quartile, upper quartile): control subjects 2.05 (2.00, 3.00) years; MCI patients 2.03 (1.94, 2.16) years; AD subjects 2.01 (1.05, 3.06) years.

Biochemical analyses

Blood samples were taken between 9 am and 1 pm and sent to the local clinical biochemistry laboratory to be assayed for AP. AP activity was measured with *p*-nitrophenyl phosphate as substrate [19] and activity is shown as International Units (IU; the amount of AP that catalyses the transformation of 1 µmol *p*-nitrophenyl phosphate/min). As increases in plasma AP activity can occur in other diseases, most notably liver and bone disease, as well as in acute inflammation. We also assessed the levels of a number of other clinically used markers to assess any changes observed in plasma AP activity as a result of such conditions. The markers assessed were γ-glutamyl transferase (γ-GT), calcium, inorganic phosphate, albumin and erythrocyte sedimentation rate (ESR). γ-GT was assayed with L-γ-glutamyl-3-carboxy-4-nitroanilide as substrate with glycylglycine as the acceptor for the γ-glutamyl residue [19]. The liberated 5-amino-2-nitro-benzoate was measured at 410 nm. Calcium ions were measured by their formation of a complex with Arsenazo III at pH 5.9 whose absorbance was monitored at 658 nm [20]. Inorganic phosphate was measured through its reaction with ammonium molybdate in the presence of sulphuric acid and monitored at 340 nm [20]. Albumin was measured by its quantitative binding to bromocresol green to form a complex that absorbs at 596 nm [21]. In addition, due to changes in AP activity that can result from acute inflammation, we also assessed the use of non-steroidal anti-inflammatory drugs (NSAIDs) in the subject groups. As apolipoprotein E (*APOE ε4*) is a known genetic risk factor for AD [22] we also assessed the percentage of *APOE ε4* carriers in the subject groups and finally, within the AD and MCI groups, we also determined the number of participants taking cholinesterase inhibitors and memantine, both used in the treatment of AD. Demographic and clinical profiles of the subjects who participated in this study are summarised in **Table 1**.

Statistical analysis

All Analyses used the open-source statistical programming language 'R' (www.R-project.org.) (http://CRAN.R-project.org/package=lme4). All demographic data to compare control, MCI and AD subjects was analysed using the Kruskal-Wallis rank sum test, except for gender, *APOE ε4* status and NSAID use which were analysed by Fisher's exact test for count data. All data are presented as dian (lower quartile, upper quartile) (**Table1**). To analyse the relationships between plasma AP activity, diagnosis and cognition, mixed effects models were constructed; details of these are given with the relevant re-