Serum transthyretin and risk of cognitive decline and dementia: 22-year longitudinal study

Marzieh Araghi, PhD[a*], Martin J Shipley, MSc[a], Atul Anand, MD[b] Nicholas L Mills, MD[b] Mika Kivimaki, FMedSci [a], Archana Singh-Manoux, PhD[a,c], Adam Tabák, MD[a,d,e], Séverine Sabia, PhD[a,c], and Eric J Brunner, PhD[a]

[a] Institute of Epidemiology and Health Care, University College London, UK

[b] BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

[c] Inserm U1153, Epidemiology of Ageing and Neurodegenerative diseases, Paris, France

[d] Department of Internal Medicine and Oncology, Semmelweis University Faculty of Medicine, Budapest, Hungary

[e] Department of Public Health, Semmelweis University Faculty of Medicine, Budapest, Hungary

***Corresponding author:**

Marzieh Araghi, MPH PhD

Department of Epidemiology and Public Health, University College London, UK

1-19 Torrington Place

London WC1E 7HB

Email address: mary.araghi@hotmail.co.uk

Word count: Abstrct: 163, Main text: 3,231

Abstract

Serum transthyretin (TTR) maybe an early biomarker for Alzheimer's disease and related disorders (ADRD). We investigated associations of TTR measured at baseline with cognitive decline and incident ADRD, and whether TTR trajectories differ between ADRD cases and non-cases, over 22 years before diagnosis. A total of 6,024 adults aged 45-69 in 1997-1999 were followed up until 2019. TTR was assessed three times and 297 cases of dementia were recorded. Higher TTR was associated with higher cognitive function at baseline, however TTR was unrelated to subsequent change in cognitive function. TTR at baseline did not predict ADRD risk (hazard ratio per SD TTR (4.8 mg/dL) = 0.97; 95% confidence interval: 0.94-1.00). Among those later diagnosed with ADRD, there was a marginally steeper downward TTR trajectory than those free of ADRD over follow-up (P=0.050). Our findings suggest TTR is not neuroprotective. The relative decline in TTR level in the preclinical stage of ADRD is likely to be a consequence of disease processes.

Keywords

Dementia; Cognitive decline; Biomarkers; Transthyretin; Prealbumin

Declarations

Funding

The Whitehall II study has been supported by grants K013351, R024227, and S011676 from the UK Medical Research Council (MRC); grants RG/13/2/30098 and RG/16/11/32334 from the British Heart Foundation (BHF); funding from the British Health and Safety Executive; the British Department of Health; grant R01HL036310 from the National Heart, Lung, and Blood Institute; grants R01AG056477 and R01AG034454 from the National Institute on Aging; and grant ES/J023299/1 from the Economic and Social Research Council. MK was supported by NordForsk, MRC, Academy of Finland (311492) and Helsinki Institute of Life Science. AGT was supported by MRC (S011676) and NordForsk (Nordic Research Programme on Health and Welfare, 75021). EJB is supported by BHF and UK Research and Innovation (ES/T014377/1). NLM and AA are supported by the Butler Senior Clinical Research Fellowship (FS/16/14/32023) and a Research Excellence Award (RE/18/5/34216) from the British Heart Foundation.

Conflicts of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Ethics approval

University College London Hospital Committee on the Ethics of Human Research, reference 85/0938.

Consent for publication

The authors give their consent for the publication of identifiable details of this paper.

Availability of data and material

Data cannot be made publicly available because of ethics and institutional review board restrictions. Researchers can apply for data access at ucl.ac.uk/whitehallII/data-sharing.

Introduction

Alzheimer's disease and related disorders (ADRD) are among the great health-care challenges of the 21st century [1]. ADRD is a multifactorial and complex neurodegenerative disorder, progressing gradually but to date no effective pharmacotherapeutic options are available [2]. Thus, early diagnosis and prevention of ADRD is crucial.

Transthyretin (TTR), or prealbumin, is a transport protein for thyroxine and retinol-binding protein in blood and cerebrospinal fluid (CSF) which binds amyloid beta peptide (Aβ) [3]. ADRD is characterized by extracellular deposition of Aβ [4]. TTR is decreased in CSF and plasma of AD patients, strengthening the proposition that TTR may have neuroprotective effects as a result of binding interactions with Aβ and retinoids [5,6]. TTR may be a potentially valuable diagnostic and prognostic tool for dementia and cognitive decline. It has also been used as an indicator of acute malnutrition as low TTR concentrations are associated with inadequate protein calorie consumption and anorexia [7].

The role of TTR in healthy people remains unclear. One hypothesis is that TTR has a neuroprotective effect, such that higher TTR levels during early life and midlife build cognitive reserve which is maintained and delays or prevents the clinical expression of ADRD [8]. However, no study has examined the longitudinal association between TTR levels and cognitive function. Two epidemiological studies have investigated the association between TTR levels and dementia [9,10]. The first followed 135 individuals with mild cognitive impairment (MCI) over 5 years and showed lower plasma TTR levels among MCI patients who converted to dementia compared to those with more stable cognitive function [9]. The second study found that plasma TTR levels were lower in severe MCI cases and ADRD patients with rapid cognitive decline during 6 months follow-up [10].

In this long-term longitudinal study, we use complementary analytic approaches with three objectives. First, to estimate the association of TTR in midlife with trajectories of cognitive function. Second, to estimate the effect of TTR level at baseline on risk of ADRD over the follow-up period. Third, to

model trajectories of TTR for more than two decades, using repeat data and a backward timescale anchored to the year of ADRD diagnosis. This method allows differences in TTR trajectories to be estimated between those who receive a diagnosis and those who remain free of disease.

Methods

Study design and participants

The Whitehall II study is a closed cohort of British civil servants (6,895 men and 3,413 women, aged 35–55) recruited in London between 1985 and 1988, with a response rate of 73% [11]. Follow-up clinical examinations have taken place approximately every 5 years since recruitment. TTR level was first measured at the 1997-1999 examination (Clinic 3), and this is the baseline of the present study. Informed consent and research ethics are renewed at each contact; the most recent approval was from the University College London Hospital Committee on the Ethics of Human Research, reference 85/0938.

Measurement of TTR

Serum TTR level was determined at Clinic 3 (1997-1999), Clinic 5 (2007-2009) and Clinic 6 (2012-2013), see flow chart in **Supplementary Fig 1**. Blood was collected by venepuncture and centrifuged at 2500g for 15 min at 10 °C. Serum samples were stored at −80°C until assay [12]. TTR concentrations were determined using the Siemens Atellica® CH analyser (Siemens Healthineers, Erlangen, Germany). This assay has a limit of detection of 1.8 mg/dL and an inter-assay coefficient of variation of 2.6% at a mean concentration of 20.8 mg/dL.

Cognitive function

The cognitive test battery was administered at five clinical examinations: Clinic 3 (1997-1999), Clinic 4 (2002-2004), Clinic 5 (2007-2009), Clinic 6 (2012-2013), and Clinic 7 (2015-2016). Test–retest reliability was assessed in 556 participants within 3 months and showed moderate to high reliability (range 0.6–0.9). The cognitive tests covered the following domains: Memory (participants were presented with a 20-word list of one or two syllable words at two second intervals, with 2 minutes to

write down as many words as they can recall, regardless of word order); reasoning (participants had 10 min to complete the Alice Heim 4-I (AH4-I) test including a series of 65 verbal and mathematical reasoning items of increasing difficulty); and verbal fluency (phonemic fluency was assessed via the "S" words, and semantic fluency via "animal" words tests; one minute was allowed for each test). To ease comparison between tests with different score ranges, we standardized all raw test scores for these four measures to z-scores (mean=0, standard deviation [SD]=1) using the means and SDs of the Clinic 3 cognitive tests. To reduce measurement error, these z-scores were summed and re-standardized to yield a global cognitive score, as in previous studies [13,14].

Dementia

We used the national hospital episode statistics (HES) database and death certificates for dementia ascertainment. Diagnostic data are routinely extracted from hospital medical records and coded by trained National Health Service (NHS) clinical coders using the 10th Revision of the International Classification of Diseases (ICD-10). For this study, dementia in HES records and in death certificates was defined as any of the following ICD-10 codes: F00, F01, F02, F03, G30 and G31.

Covariates

We included covariates measured at Clinic 3 in the analysis (ethnicity, education, employment grade, smoking, alcohol consumption, moderate or vigorous physical activity and body mass index (BMI)).

Ethnicity was reported by the participant and regrouped into two levels: White, and non-white.

Education was reported by the participant as the highest level of education achieved and regrouped into three standard hierarchic levels: no formal education/lower secondary education, higher secondary education, university degree/higher university degree.

Employment grade was reported by the participant and regrouped into three standard hierarchic levels: high, intermediate, and low representing income and status at work.

Smoking was reported by the participant and regrouped into three levels: never smokers, ex-smokers and current smokers.

Alcohol consumption was reported by the participant based on the number of alcoholic drinks consumed in the previous days, converted to units of alcohol consumed in a week and regrouped into three levels: no alcohol consumption, moderate alcohol consumption (1–13 units/week), and heavy alcohol consumption (≥14 units/week).

Moderate or vigorous physical activity was reported by the participant, based on duration of physical activities over the week regrouped into three levels: <1 hour per week, 1-6.9 hours per week and ≥ 7 hours per week.

BMI was calculated using weight and height: (weight in kilograms/height in metres squared). Weight was measured in underwear to the nearest 0.1 kg on Soehnle electronic scales with digital readout (Leifheit AS, Nassau, Germany). Height was measured in bare feet to the nearest 1 mm using a stadiometer with the participant standing erect with head in the Frankfurt plane.

Statistical analysis

We undertook three sets of analyses; first, linear mixed models to estimate both the cross-sectional and the longitudinal association of TTR with the global cognitive score. Second, Cox proportional hazards regression to examine associations of TTR with subsequent dementia. Third, changes in TTR (trajectories) in those individuals who were later diagnosed with dementia were compared with the trajectories in other participants using mixed models with a backward timescale.

Analysis 1: Associations of TTR at baseline (1997-1999) with cognitive trajectories between 1997-1999 and 2015-2016

The mixed model takes into account the fact that repeated measures on the same individual are correlated with each other. The intercept and the slope were fitted as random effects, allowing individuals to have different cognitive scores at baseline and different rates of cognitive decline over the follow-up. The TTR levels were categorized into three groups based on tertiles and the mixed models were fitted using these categories of TTR with global cognitive score, and also for each cognitive domain, as the dependent variable. The non-significant TTR × time × sex interaction terms suggested no sex differences in the TTR cross-sectional associations with cognitive function, or with

cognitive decline and, therefore, all analyses were conducted using men and women combined. Analyses were adjusted for sex, age, ethnicity by fitting these terms and their interactions with follow-up time.

Analysis 2: Associations of TTR at baseline (1997-1999) with incident dementia

The age range of participants at Clinic 3 was 45 to 69 years. We used TTR levels measured at Clinic 3 as the exposure measure. With dementia as outcome, we used Cox regression with date of entry being the date of clinical assessment at which TTR was measured. Those who died free of dementia were censored at date of death, so that participants were followed until date of dementia diagnosis, death, or March 31, 2019, whichever came first. Analyses were adjusted for age, sex, and ethnicity. In Supplementary Tables, we show analyses with further adjustment. We used cubic-spline regression to assess the shape of the association using *xblc* command in STATA.

Analysis 3: Trajectories of TTR before dementia

Trajectories of TTR were modelled for 22 years using a backward timescale such that year 0 was year of dementia diagnosis for dementia cases, year of death for those who died during the follow-up, and March 31, 2019 (end of follow-up) for all others. TTR in each of the preceding 22 years (years 0 to -22) was estimated from mixed effects models with the intercept and slope as random effects and a backward timescale using TTR measurements made at three occasions (Clinics 3, 5 and 6) [15]. Dementia (coded as 1 for cases and 0 for others) and its interaction with time and time squared (to allow for nonlinear changes) were added to the model to test for differences in TTR trajectories between dementia cases and others. This modelling strategy implies that year 0 (the index date; the year of dementia diagnosis for cases) was the intercept in the analysis and the coefficient associated with the dementia term estimates the difference in TTR between cases and others at dementia diagnosis. The slope terms (time and time squared) allow assessment of changes in TTR over the period of observation. To test the difference in cases and others, we examined whether the terms dementia × time and dementia × time squared improved the fit of the model using likelihood ratio tests. Analyses were adjusted for age, sex, ethnicity, their interactions with time and time squared.

The software SAS 9.4 (SAS Institute, NC, USA) was used for data management and STATA 14 (StataCorp LP, College Station, TX, USA) for analysis. A two-sided P value <.05 was considered statistically significant.

Results

The study baseline was 1997 to 1999 (Clinic 3) and the age range of participants was 45 to 69 years. In total, 6,024 individuals had TTR measured at baseline. Follow up continued for almost 22 years. Over follow-up 297 individuals (277 of whom had TTR measured at baseline) developed dementia (**Supplementary Fig 1). Table 1** presents the characteristics of the participants in the study sample according to the thirds of TTR at baseline. The levels of TTR were significantly lower in women compared to men (**Supplementary Fig 2**). The age of participants in the lowest third of TTR was higher, compared to the middle and highest thirds of TTR. The highest third of TTR had lower percentages of participants with low employment grade and those who developed dementia. There were associations between smoking, alcohol consumption, physical activity and BMI with TTR (**Table 1**).

Associations of TTR with cognitive function

Cross-sectional and longitudinal associations between TTR and global cognitive score and individual cognitive domains are shown in **Table 2**. In the cross-sectional analysis, global cognitive score was significantly higher in the highest third of TTR compared to the lowest third. There was no association between TTR and change in cognitive function, adjusting for age, sex, and ethnicity. Similar effects were seen for all the separate cognitive domains: scores were higher in the highest third of TTR at baseline but subsequent cognitive declines in each domain were not associated with baseline TTR. In **Supplementary Table 1**, analyses with further adjustment for socioeconomic and health behaviour factors diminished the association between TTR and cognitive score at baseline and the absence of longitudinal association between TTR and change in cognitive function. Declines in global cognitive score over time, adjusted for sex, age and ethnicity are similar for the three TTR categories (**Fig 1)**.

Association of TTR with dementia

The 6,024 participants were followed up from the baseline to 31st March 2019 and 277 individuals developed dementia over 22 years. Compared to the lowest third of TTR, individuals in the middle and highest thirds of TTR had similar risks of dementia (**Table 3)**. As continuous measure, TTR was also not associated with incident dementia across the distribution (HR per standard deviation increase in TTR = 0.97; 95% CI: 0.94 to 1.00)). The finding was unchanged by further adjustment for socioeconomic and health behaviour factors (**Supplementary Table 2**). We confirmed the absence of association between TTR and incident dementia using cubic spline analysis (**Supplementary Fig 3)**.

Trajectories of TTR before dementia

The trajectory of TTR, modelled backwards in time from dementia diagnosis was marginally steeper downwards in those diagnosed with dementia compared with dementia-free participants (**Fig 2**). The mean difference in TTR between the two groups according to number of years before dementia diagnosis was significant (P<0.05) at -5 to 0 years (**Supplementary Table 3)**. These results, using a backward timescale, showed that compared to the dementia-free group, those who develop dementia had similar TTR in midlife, tending to accelerate decline in the decade before dementia diagnosis.

Discussion

Prior studies of individuals with mild cognitive impairment or ADRD indicated that a low TTR level in CSF and blood was a predictor of disease severity [9,10]. We replicate this finding in a population-scale sample, showing that serum TTR is lower in those who receive a dementia diagnosis within five years compared to those remaining dementia-free. Our long-term longitudinal study, with measurements of TTR on three occasions and cognitive function on five occasions, extends observation back to age 45. This study design enabled trajectories of serum TTR and cognition to be visualised between mid-life and old age, hypothesising that low TTR predicts accelerated cognitive decline and ADRD in those without mild cognitive impairment.

We show first that serum TTR in mid-life is not a predictor of the rate of decline of cognitive function. Second, survival analysis finds that TTR level in mid-life does not predict incident dementia. Third, divergence of the trajectories of TTR over the decades prior to dementia diagnosis, compared to participants who remain dementia-free, provides clear evidence that the relative decline in TTR in dementia cases is a prodromal sign of disease development. Taken together, the study findings suggest TTR is a biomarker of disease severity, but they do not support the hypothesis that TTR is neuroprotective.

In parallel with increasing size of the ageing population, it is not surprising that the number of people with dementia is increasing [16]. Up to now, there has been no effective therapy or diagnostic marker for dementia [2,17] Finding one or more blood-based diagnostic markers would be valuable for early detection, and TTR may serve as one marker in this respect. Our evidence suggests, however, that circulating TTR may not represent an effective target for preventive drug treatment. Together with the important roles as a carrier for thyroxine and retinol, recent studies [9,10] have linked TTR to ADRD through its binding affinity for β-amyloid (Aβ), thought to be a causal factor in ADRD [3]. *In vitro* studies [18-20], suggest that TTR has a neuroprotective role against Aβ toxicity by binding to Aβ and inhibiting its aggregation [21]. Our study finds, contrary to the neuroprotective hypothesis, that circulating TTR levels are uncorrelated with cognitive decline between mid-life and old age. It may be implied that TTR levels are dependant from the Aβ pathological activity and maybe associated with Aβ dysmetabolism [22]. This consideration is important because it might boost the use of pharmaceutical agents that in the early phases of the disease, could potentially revert the Aβ dysmetabolism [23].

Clinical studies have shown decreased TTR levels in the CSF of ADRD patients [24-27]. CSF and blood plasma are both in close contact with the central nervous system and are the most promising sources of biomarkers in ADRD, compared to brain tissues. However, CSF sampling is invasive and our large-scale study is limited to serum measurements of TTR. It may be that mid-life TTR levels in CSF would be related to future cognitive decline and incident ADRD, unlike the serum levels we have studied.

At study baseline, when participants were at mean age 55, higher serum TTR was linked with higher global and domain-specific cognitive scores. This finding is subsidiary to our hypothesis that TTR level in midlife is associated with subsequent trajectories of cognitive function. The explanation of the cross-sectional association may be behavioural. In our study, TTR level at baseline was associated with physical activity, smoking, alcohol consumption and adiposity. Further, TTR levels were linked to education level and socioeconomic position. All of these factors influence cognitive function in mid-life, however it is beyond the scope of the present study to analyse the confounding structure in the data. The central finding in respect of the TTR-cognitive function hypothesis is that there was no association between baseline TTR and change in any of the four cognitive measures analysed in our powerful longitudinal study.

Circulating TTR concentrations differ by ethnic group. A study by Tien and colleagues [9] based on 184 Taiwanese individuals showed mild cognitive impairment was associated, paradoxically, with higher plasma TTR levels compared with cognitively healthy controls, although dementia was linked with low TTR levels. Compared to African-Americans, Caucasians have higher serum TTR levels [28], while Japanese have yet higher TTR levels [29]. Whitehall II study participants are predominantly Caucasian; we adjusted for ethnicity in our analyses. Further, serum TTR levels were lower in women than in men, consistent with other studies [28]. Our models were adjusted for sex, having confirmed that the effects of interest were similar in men and women.

Our study benefitted from a large sample size, multiple repeat measures of cognitive function and three longitudinal measures of TTR over a period of 22 years. This design allowed us to assess three critical dimensions related to dementia in association with TTR, as we were able to analyse TTR as a risk factor for dementia, to compare the trajectories of TTR in those who later received a diagnosis of dementia with those free of dementia for more than 22 years, and to examine trajectories of cognitive function over 22 years according to the baseline level of TTR. Our Clinic 3 serum samples had been stored at -80^{o}C for 20 years. Although no formal stability studies have been conducted, absolute levels of TTR, and their relation with multiple covariates suggest the protein was stable under the storage conditions we employed. Using death certificate for dementia case ascertainment have the potential

advantage of providing complete follow-up, but with limitations. The information on clinical subtype is not available in death certificates, therefore our study sample may include non-related Alzheimer's disease dementias such as dementia with Lewy bodies and Parkinson's dementia.

In conclusion, our longitudinal study based or serum TTR levels among more than 6000 men and women increases understanding of the role of TTR in ADRD. Our findings do not support the TTR neuroprotective hypothesis. The biomarker role of TTR was confirmed, as levels were lower among individuals given a dementia diagnosis in the subsequent five years. The relative decline in TTR level in the preclinical stage of ADRD is likely to be a consequence of disease processes.

References

1. Collaborators GBDD (2019) Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18 (1):88-106. doi:10.1016/S1474-4422(18)30403-4

2. Kumar A, Singh A, Ekavali (2015) A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacol Rep 67 (2):195-203. doi:10.1016/j.pharep.2014.09.004

3. Sousa JC, Cardoso I, Marques F, Saraiva MJ, Palha JA (2007) Transthyretin and Alzheimer's disease: where in the brain? Neurobiol Aging 28 (5):713-718. doi:10.1016/j.neurobiolaging.2006.03.015

4. Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R, Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 14 (4):535-562. doi:10.1016/j.jalz.2018.02.018

5. Alemi M, Gaiteiro C, Ribeiro CA, Santos LM, Gomes JR, Oliveira SM, Couraud PO, Weksler B, Romero I, Saraiva MJ, Cardoso I (2016) Transthyretin participates in beta-amyloid transport from the brain to the liver--involvement of the low-density lipoprotein receptor-related protein 1? Sci Rep 6:20164. doi:10.1038/srep20164

6. Corcoran JP, So PL, Maden M (2004) Disruption of the retinoid signalling pathway causes a deposition of amyloid beta in the adult rat brain. Eur J Neurosci 20 (4):896-902. doi:10.1111/j.1460-9568.2004.03563.x

7. Delliere S, Cynober L (2017) Is transthyretin a good marker of nutritional status? Clin Nutr 36 (2):364-370. doi:10.1016/j.clnu.2016.06.004

8. Giao T, Saavedra J, Cotrina E, Quintana J, Llop J, Arsequell G, Cardoso I (2020) Undiscovered Roles for Transthyretin: From a Transporter Protein to a New Therapeutic Target for Alzheimer's Disease. Int J Mol Sci 21 (6). doi:10.3390/ijms21062075

9. Tien YT, Lee WJ, Liao YC, Wang WF, Jhang KM, Wang SJ, Fuh JL (2019) Plasma Transthyretin as a Predictor of Amnestic Mild Cognitive Impairment Conversion to Dementia. Sci Rep 9 (1):18691. doi:10.1038/s41598-019-55318-0

10. Velayudhan L, Killick R, Hye A, Kinsey A, Guntert A, Lynham S, Ward M, Leung R, Lourdusamy A, To AW, Powell J, Lovestone S (2012) Plasma transthyretin as a candidate marker for Alzheimer's disease. J Alzheimers Dis 28 (2):369-375. doi:10.3233/JAD-2011-110611

11. Marmot M, Brunner E (2005) Cohort Profile: the Whitehall II study. Int J Epidemiol 34 (2):251-256. doi:10.1093/ije/dyh372

12. Pasella S, Baralla A, Canu E, Pinna S, Vaupel J, Deiana M, Franceschi C, Baggio G, Zinellu A, Sotgia S, Castaldo G, Carru C, Deiana L (2013) Pre-analytical stability of the plasma proteomes based on the storage temperature. Proteome Sci 11 (1):10. doi:10.1186/1477-5956-11-10

13. Sommerlad A, Sabia S, Singh-Manoux A, Lewis G, Livingston G (2019) Association of social contact with dementia and cognition: 28-year follow-up of the Whitehall II cohort study. PLoS Med 16 (8):e1002862. doi:10.1371/journal.pmed.1002862

14. Sabia S, Dugravot A, Dartigues JF, Abell J, Elbaz A, Kivimaki M, Singh-Manoux A (2017) Physical activity, cognitive decline, and risk of dementia: 28 year follow-up of Whitehall II cohort study. BMJ 357:j2709. doi:10.1136/bmj.j2709

15. Amieva H, Le Goff M, Millet X, Orgogozo JM, Peres K, Barberger-Gateau P, Jacqmin-Gadda H, Dartigues JF (2008) Prodromal Alzheimer's disease: successive emergence of the clinical symptoms. Ann Neurol 64 (5):492-498. doi:10.1002/ana.21509

16. Ahmadi-Abhari S, Guzman-Castillo M, Bandosz P, Shipley MJ, Muniz-Terrera G, Singh-Manoux A, Kivimaki M, Steptoe A, Capewell S, O'Flaherty M, Brunner EJ (2017) Temporal trend in dementia incidence since 2002 and projections for prevalence in England and Wales to 2040: modelling study. BMJ 358:j2856. doi:10.1136/bmj.j2856

17. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, Chai X, Proctor NK, Eichenlaub U, Zetterberg H, Blennow K, Reiman EM, Stomrud E, Dage JL, Hansson O (2020) Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis,

neuropathology and longitudinal progression to Alzheimer's dementia. Nat Med 26 (3):379-386. doi:10.1038/s41591-020-0755-1

18. Cascella R, Conti S, Mannini B, Li X, Buxbaum JN, Tiribilli B, Chiti F, Cecchi C (2013) Transthyretin suppresses the toxicity of oligomers formed by misfolded proteins in vitro. Biochim Biophys Acta 1832 (12):2302-2314. doi:10.1016/j.bbadis.2013.09.011

19. Giunta S, Valli MB, Galeazzi R, Fattoretti P, Corder EH, Galeazzi L (2005) Transthyretin inhibition of amyloid beta aggregation and toxicity. Clin Biochem 38 (12):1112-1119. doi:10.1016/j.clinbiochem.2005.08.007

20. Li X, Zhang X, Ladiwala AR, Du D, Yadav JK, Tessier PM, Wright PE, Kelly JW, Buxbaum JN (2013) Mechanisms of transthyretin inhibition of beta-amyloid aggregation in vitro. J Neurosci 33 (50):19423-19433. doi:10.1523/JNEUROSCI.2561-13.2013

21. Mangrolia P, Murphy RM (2018) Retinol-Binding Protein Interferes with Transthyretin-Mediated beta-Amyloid Aggregation Inhibition. Biochemistry 57 (33):5029-5040. doi:10.1021/acs.biochem.8b00517

22. Martorana A, Di Lorenzo F, Belli L, Sancesario G, Toniolo S, Sallustio F, Sancesario GM, Koch G (2015) Cerebrospinal Fluid Abeta42 Levels: When Physiological Become Pathological State. CNS Neurosci Ther 21 (12):921-925. doi:10.1111/cns.12476

23. Martorana A, Di Lorenzo F, Manenti G, Semprini R, Koch G (2014) Homotaurine induces measurable changes of short latency afferent inhibition in a group of mild cognitive impairment individuals. Front Aging Neurosci 6:254. doi:10.3389/fnagi.2014.00254

24. Hansson SF, Andreasson U, Wall M, Skoog I, Andreasen N, Wallin A, Zetterberg H, Blennow K (2009) Reduced levels of amyloid-beta-binding proteins in cerebrospinal fluid from Alzheimer's disease patients. J Alzheimers Dis 16 (2):389-397. doi:10.3233/JAD-2009-0966

25. Ribeiro CA, Santana I, Oliveira C, Baldeiras I, Moreira J, Saraiva MJ, Cardoso I (2012) Transthyretin decrease in plasma of MCI and AD patients: investigation of mechanisms for disease modulation. Curr Alzheimer Res 9 (8):881-889. doi:10.2174/156720512803251057

26. Han SH, Jung ES, Sohn JH, Hong HJ, Hong HS, Kim JW, Na DL, Kim M, Kim H, Ha HJ, Kim YH, Huh N, Jung MW, Mook-Jung I (2011) Human serum transthyretin levels correlate inversely with Alzheimer's disease. J Alzheimers Dis 25 (1):77-84. doi:10.3233/JAD-2011-102145

27. Castano EM, Roher AE, Esh CL, Kokjohn TA, Beach T (2006) Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented elderly subjects. Neurol Res 28 (2):155-163. doi:10.1179/016164106X98035

28. Buxbaum J, Koziol J, Connors LH (2008) Serum transthyretin levels in senile systemic amyloidosis: effects of age, gender and ethnicity. Amyloid 15 (4):255-261. doi:10.1080/13506120802525285

29. Buxbaum J, Anan I, Suhr O (2010) Serum transthyretin levels in Swedish TTR V30M carriers. Amyloid 17 (2):83-85. doi:10.3109/13506129.2010.483118

Table 1. Baseline characteristics of 6,024 participants and dementia proportion at end of follow-up in the study sample according to the thirds of transthyretin

	[Mean (SD) or %]			
	Thirds of baseline transthyretin (mg/dL)			P value for heterogeneity
	Lowest third (<26.4)	Middle third (26.4-<29.6)	Highest third (≥29.6)	
Number	2,049	2,000	1,975	
Age (y)	56.3 (6.1)	55.8 (6.1)	54.9 (5.6)	<0.001
Female (%)	49.5	25.7	11.5	<0.001
Non-White (%)	13.3	7.3	4.4	<0.001
Education (%)				<0.001
≤ Lower secondary	22.6	20.6	15.3	
Higher secondary	20.1	18.0	19.0	
≥ Degree	57.2	61.2	65.6	
Employment grade (%)				<0.001
High	32.8	44.0	53.8	
Intermediate	45.7	44.1	39.7	
Low	21.4	11.8	6.4	
Smoking (%)				

Never	51.3	50.6	48.1	<0.001
Ex-smoker	35.7	41.0	44.1	
Current	12.8	8.2	7.6	
Alcohol consumption (%)				
No alcohol	22.5	13.7	8.0	<0.001
Moderate alcohol	50.0	46.5	38.7	
Heavy alcohol	27.4	39.8	53.2	
Moderate or vigorous physical activity				
<1 h/wk	22.0	19.8	17.9	0.060
1-6.9 h/wk	65.0	66.6	68.5	
≥ 7 h/wk	12.9	13.4	13.4	
BMI (kg/m^2)	29.9 (4.9)	26.5 (4.0)	26.2 (3.4)	<0.001
Dementia (%)	6.0	3.9	3.7	<0.001

Table 2. Cross-sectional and longitudinal association between the thirds of transthyretin (mg/dL) and standardized scores of global cognitive score and individual cognitive domains (N=6,024)*

Cognitive domain outcome	Transthyretin (mg/dL)	Standardized cognitive score at baseline		Change in standardised cognitive score	
		Difference (95% CI)[a]	*P* value	Difference (95% CI)[a]	*P* value
Global cognitive score					
	Lowest third	Ref	/	Ref	/
	Middle third	0.06 (0.01 to 0.12)	0.020	-0.01 (-0.07 to 0.04)	0.698
	Highest third	0.13 (0.07 to 0.19)	<0.001	-0.04 (-0.10 to 0.02)	0.214
Memory					
	Lowest third	Ref	/	Ref	/
	Middle third	0.07 (0.01 to 0.13)	0.009	-0.01 (-0.09 to 0.07)	0.797
	Highest third	0.09 (0.03 to 0.16)	0.002	-0.05 (-0.14 to 0.03)	0.261
AH4-I					
	Lowest third	Ref	/	Ref	/
	Middle third	0.06 (0.01 to 0.12)	0.015	-0.00 (-0.05 to 0.04)	0.804
	Highest third	0.11 (0.05 to 0.17)	<0.001	0.00 (-0.05 to 0.05)	0.958
Phonemic fluency					

	Lowest third	Ref	/	Ref	/
	Middle third	0.04 (-0.01 to 0.10)	0.177	0.00 (-0.07 to 0.07)	0.960
	Highest third	0.09 (0.03 to 0.15)	0.003	-0.04 (-0.12 to 0.03)	0.307
Semantic fluency					
	Lowest third	Ref	/	Ref	/
	Middle third	0.03 (-0.02 to 0.09)	0.274	-0.00 (-0.07 to 0.06)	0.840
	Highest third	0.10 (0.04 to 0.16)	0.001	-0.01 (-0.09 to 0.05)	0.598

[a]Adjusted for age, sex and ethnicity

* 6,024 participants had a TTR measure at baseline (Clinic 3), and of these 5,418 had ≥1 measure of global cognitive score over the follow-up period

Table 3. Association of transthyretin and dementia over 22 years of follow-up (N=6,024)

	N	Risk of dementia	
		Hazard ratio[a] (95% CI)	***P* value**
TTR as a categorical variable (in tertiles)			
Lowest third	2,049	Ref	
Middle third	2,000	0.94 (0.71 to 1.25)	0.702
Highest third	1,975	0.79 (0.57 to 1.11)	0.188
TTR as a continuous variable	6,024	0.97 (0.94 to 1.00)	0.114

[a]Adjusted for sex, age and ethnicity, hazard ratio per 1 unit standard deviation increase in TTR (4.8 mg/dL) for continuous TTR

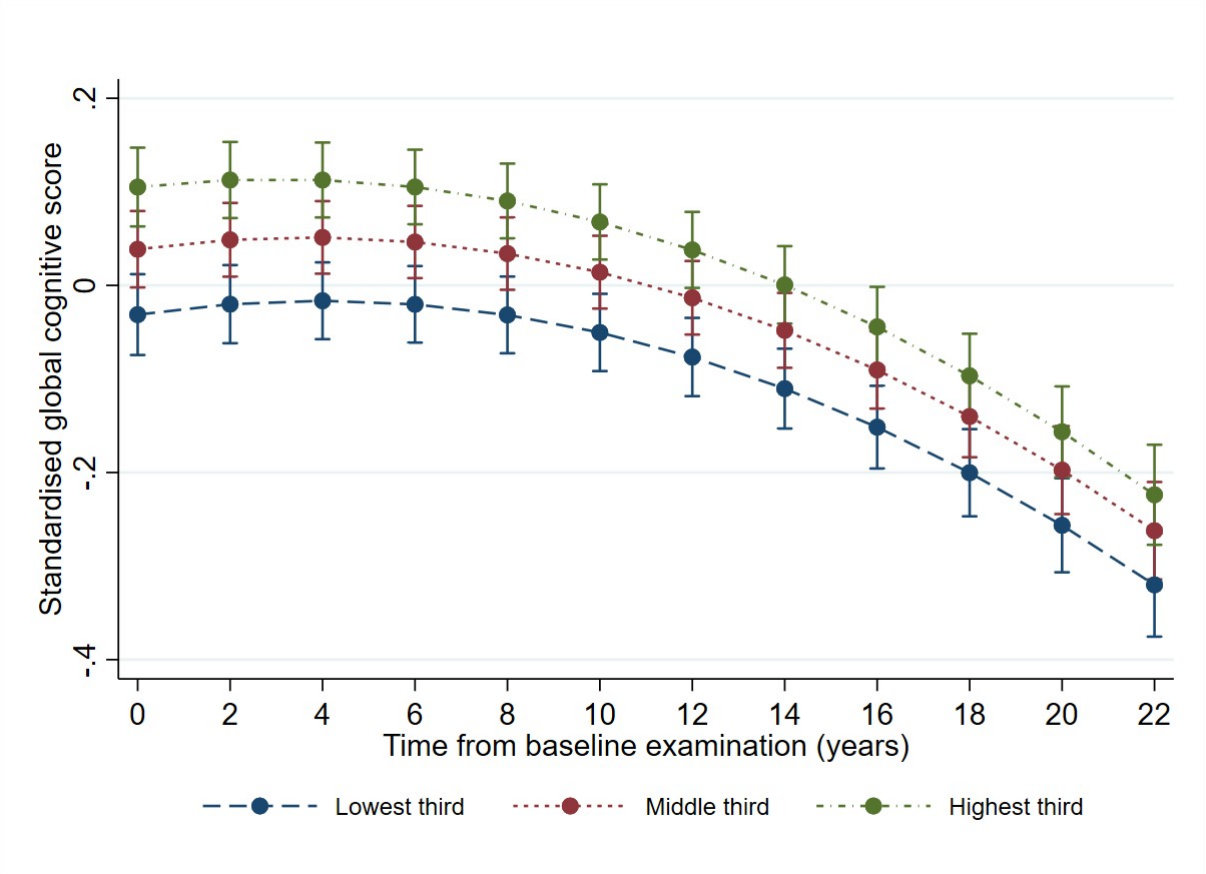

Fig 1. Trajectories (predicted values from mixed model of global cognitive score over follow-up time in prospective analysis from baseline examination according to thirds of serum transthyretin

	Number of observations in the analysis										
Years	-20 to -22	-18 to -20	-16 to -18	-14 to -16	-12 to -14	-10 to -12	-8 to -10	-6 to -8	-4 to -6	-2 to -4	0 to -2
Dementia cases	4,889	128	112	87	690	4,311	238	5,015	287	79	62
Dementia free	44	67	54	33	25	47	93	76	117	263	238

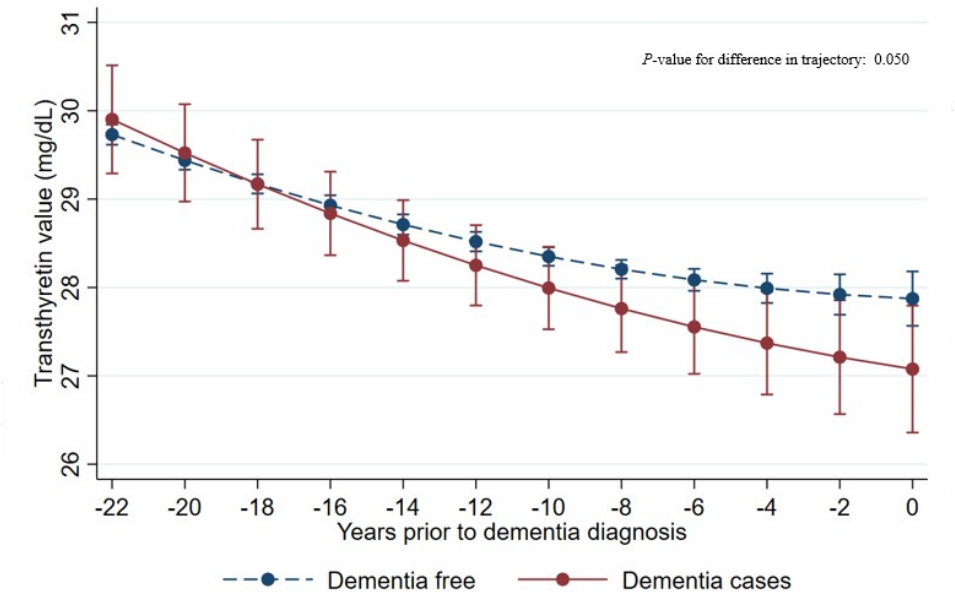

Fig 2. Trajectories of TTR with 95% confidence interval in retrospective analyses over the 22 years before dementia, adjusted for sex, age, and ethnicity (difference in trajectory (time × caseness interaction) P=0.050)