BRAIN COMMUNICATIONS

Blood-based protein mediators of senility with replications across biofluids and cohorts

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*The ADNI investigators are listed in the Appendix.

Dementia severity can be quantitatively described by the latent dementia phenotype 'δ' and its various composite 'homologues'. We have explored δ's blood-based protein biomarkers in the Texas Alzheimer's Research and Care Consortium. However, it would be convenient to replicate them in the Alzheimer's Disease Neuroimaging Initiative. To that end, we have engineered a δ homologue from the observed cognitive performance measures common to both projects [i.e. 'd:Texas Alzheimer's Research and Care Consortium to Alzheimer's Disease Neuroimaging Initiative' (dT2A)]. In this analysis, we confirm 13/22 serum proteins as partial mediators of age's effect on dementia severity as measured by dT2A in the Texas Alzheimer's Research and Care Consortium and then replicate 4/13 in the Alzheimer's Disease Neuroimaging Initiative's plasma data. The replicated mediators of age-specific effects on dementia severity are adiponectin, follicle-stimulating hormone, pancreatic polypeptide and resistin. In their aggregate, the 13 confirmed age-specific mediators suggest that 'cognitive frailty' pays a role in dementia severity as measured by δ. We provide both discriminant and concordant support for that hypothesis. Weight, calculated low-density lipoprotein and body mass index are partial mediators of age's effect in the Texas Alzheimer's Research and Care Consortium. Biomarkers related to other disease processes (e.g. cerebrospinal fluid Alzheimer's disease-specific biomarkers in the Alzheimer's Disease Neuroimaging Initiative) are not. It now appears that dementia severity is the sum of multiple independent processes impacting δ. Each may have a unique set of mediating biomarkers. Age's unique effect appears to be at least partially mediated through proteins related to frailty. Age-specific mediation effects can be replicated across cohorts and biofluids. These proteins may offer targets for the re-mediation of age-specific cognitive decline (aka 'senility'), help distinguish it from other determinants of dementia severity and/or provide clues to the biology of Aging Proper.

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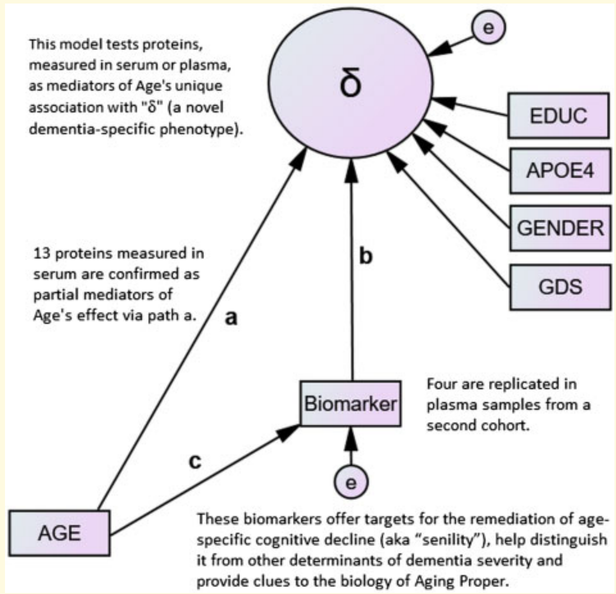
Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; APN = adiponectin; APOE = apolipoprotein E; BMI = body mass index; CSF = cerebrospinal fluid; GDS = geriatric depression scale; IGF-1 = insulin-like growth factor-1; LDD = least detectable dose; MCI = mild cognitive impairment; MIMIC = Multiple Indicators Multiple Causes; MRI = magnetic resonance imaging; NC = normal controls; NHW = non-Hispanic white; PET = positron emission tomography; PPP = pancreatic polypeptide; RBM = Rules-Based Medicine; SAP = serum amyloid protein; TARCC = Texas Alzheimer's Research and Care Consortium

Graphical Abstract



Introduction

Dementia's essential feature is a disruption of the 'cognitive correlates of functional status' (Royall *et al.*, 2007). The assessment of those correlates can be approached by confirmatory factor analysis in a structural equation model framework (Royall *et al.*, 2012). Functional status appears to be linked to cognitive performance through Spearman's general intelligence factor *g* rather than through domain-specific cognitive abilities (Spearman, 1904; Royall and Palmer, 2014). Using bifactor confirmatory factor analysis we can parse *g* into two orthogonal (unrelated) fractions: (i) the psychometric correlates of functional status (i.e. 'δ', for 'dementia') and (ii) g', i.e. residual variance in *g* that is empirically unrelated to instrumental activities of daily living. This approach divorces functionally salient cognitive impairment from cognitive impairment *per se*.

The latent variable δ can be reified as a composite 'd-score' and applied to individuals as an omnibus dementia severity metric, i.e. a dementia-specific phenotype. As *g* is thought to contribute to all cognitive measures, it has proven feasible to construct δ from a wide range of measures/batteries. So many batteries are available that we distinguish each embodiment as a δ 'homologue'. In genetics, a homologue is a gene descended from an ancestral gene in the same species and preserves the original's function.

All validated δ homologues exhibit strong associations with dementia severity (e.g. as measured by the Clinical Dementia Rating Scale 'Sum of Boxes'; Hughes *et al.*, 1982) and achieve high areas under the receiver operating characteristic curve (ROC) for the discrimination of various dementias from normal controls (NC). Moreover, δ appears to be agnostic to dementia's aetiology. Although it has a high area under the receiver operating characteristic curve to discriminate all-cause dementia from NC and cases of mild cognitive impairment (MCI) (Gavett *et al.*, 2015), δ cannot distinguish any two dementing conditions (John *et al.*, 2016).

We have been studying δ homologues and their biomarkers in the Texas Alzheimer's Research and Care Consortium (TARCC). TARCC is a large ($N \cong 3500$), well-characterized, ethnically diverse convenience sample with annual longitudinal follow-up (Waring *et al.*, 2008). Age, APOE ε4 and depressive symptoms are independently associated with δ and may exert their dementing effects through it. Each of their associations is partially mediated by largely nonoverlapping panels of serum protein biomarkers suggesting that they reflect independent dementing processes (Royall *et al.*, 2016, 2017*a*, *b*).

Age's effect was mediated by 22 proteins (Royall *et al.*, 2016). Several were 'somatomedins' including insulin-like growth factor 1 (IGF-1) and IGF-binding protein 2 (IGF-BP2), which had the largest effect. In their aggregate, they implicate 'cognitive frailty' as the cause of age-specific functionally salient cognitive impairment (i.e. 'senility'). Frailty has traditionally been conceived as a strictly physical problem (Fried *et al.*, 2001). Its cognitive aspects are largely unexplored. However, we have been able to show that a frailty index, comprising physical indicators, mediates the majority (51%) of age's effect on δ in a population-based cohort of elderly Mexican-Americans (Palmer and Royall, 2019). Thus, cognitive frailty may be a dementing condition.

Although each risk factor's association with δ is statistically weak to moderate, five proteins rationally selected by our method fully attenuate their 9-fold aggregate 5-year MCI conversion risk (Royall and Palmer, 2019*a*). This suggests first that the protein mediators selected by our methods may offer treatment targets for individual dementia risks (i.e. by a personalized treatment approach) but second that these risk factors are independent dementia-specific processes and do not contribute to a single dementing illness (e.g. Alzheimer's disease) by any final common pathway.

Regardless, our findings await confirmation in other cohorts. To that end, we have developed the ability to replicate TARCC's biomarker findings in the Alzheimer's Disease Neuroimaging Initiative (ADNI). ADNI is a second large, well-characterized convenience sample created to test biomarker findings of relevance to Alzheimer's disease. As it happens, TARCC's methods were largely predicated on ADNI's. Both studies share a common subset of cognitive measures and a panel of ≅100 blood-based biomarkers by a common vendor.

The 'TARCC to ADNI' δ homologue (dT2A) was engineered from a common set of cognitive performance measures (Royall *et al.*, 2019). In TARCC, dT2A has been reported (i) to have excellent fit, (ii) to exhibit factor equivalence across random subsets of the sample, (iii) to be strongly correlated with dementia severity as measured by the Clinical Dementia Rating Scale and (iv) to exhibit an area under the receiver operating characteristic curve of 0.981 (0.976–0.985) for the discrimination between Alzheimer's disease (AD) and NC. In ADNI, dT2A also had excellent fit, correlated ($r = 0.96$) with Clinical Dementia Rating Scale 'Sum of Boxes' ($P < 0.001$) and achieved an area under the receiver operating characteristic curve of 1.0 (0.995–1.00) for the discrimination of Alzheimer's disease from NC.

Both datasets have limitations that may hinder replications. All δ homologues 'target' a measure of instrumental activities of daily living. TARCC used Lawton and Brody's instrumental activities of daily living index (IADL) (Lawton and Brody, 1969), but ADNI uses the Functional Assessment Questionnaire (FAQ) (Pfeffer *et al.*, 1982). While their biomarker panels were obtained from a common vendor, TARCC measures them in serum, while ADNI measures them in plasma. Some proteins on each study's panel are not available on the other's, and technical problems prevent the analysis of certain proteins in either sample.

On the other hand, each study has unique strengths. ADNI provides access to both structural neuroimaging and functional neuroimaging and so-called Alzheimer's disease-specific cerebrospinal fluid (CSF) biomarkers [e.g. amyloid beta 1–42 ($A\beta_{1-42}$), total tau (t-tau) and phosphorylated tau (p-tau18)]. TARCC is an ethnically diverse cohort. Thirty-six percent of its participants are Mexican-Americans (MA).

We propose to replicate the previously reported mediation effects of 22 age-related serum proteins in ADNI. This will involve confirmations within TARCC across two δ homologues with minimally overlapping cognitive batteries and replications across studies and two biofluids. We will also test several new mediators to provide both discriminant and concordant support for the hypothesis that age's unique effect on dementia severity as measured by δ is mediated via frailty and not by plausible alternative aetiologies (e.g. diabetes mellitus or Alzheimer's disease). We predict that weight and body mass index (BMI) will mediate age's effect, but neither haemoglobin A1c (HgbA1c) nor Alzheimer's disease-specific CSF biomarkers (in ADNI) (etc.). We also predict that treatment with acetylcholinesterase inhibitors will mediate age's effect, as it has been reported to lower serum resistin levels (Satapathy *et al.*, 2011), which have in turn been found to be elevated in Alzheimer's disease patients (Kizilarslanoğlu *et al.*, 2015), confirmed to show a dose-dependent association with clinical diagnoses in TARCC (Royall and Palmer, 2019*b*) and have been identified by us as a mediator of both age's and depression's independent effects on δ (in mutually adjusted models; Royall *et al.*, 2016, 2017*b*).

Materials and methods

Subjects

This is a secondary analysis of data collected by TARCC and ADNI. Informed consent was obtained from all participants (or their legally authorized proxies) before data collection, and both studies were approved by their respective Institutional Review Boards. Because ADNI has few Hispanic subjects, and because ethnicity moderates the association between multiple serum proteins and δ in TARCC (Royall and Palmer, 2015, 2016), we restricted this analysis to non-Hispanic whites (NHW).

Texas Alzheimer's Research and Care Consortium

TARCC is a longitudinally followed convenience sample of elderly volunteers recruited from five Texas medical schools (Waring *et al.*, 2008). Each participant underwent a standardized annual examination that included a medical evaluation, neuropsychological testing and clinical interview. Categorical clinical diagnoses of 'Alzheimer's disease', 'MCI' and 'NC' were established through consensus. The diagnosis of Alzheimer's disease was based on National Institute for Neurological Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (McKhann *et al.*, 1984). Consensus-based clinical diagnoses of 'MCI' were based on all available clinical data. Although TARCC is an ethnically diverse cohort, only NHW participants ($N = 2551$) were included in this analysis.

Alzheimer's Disease Neuroimaging Initiative

ADNI data were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD (Weiner and Veitch, 2015). 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 MCI and early Alzheimer's disease.

The initial 5-year study, ADNI-1, enrolled cognitively normal, MCI and Alzheimer's disease cases. Subsequent studies (ADNI-GO and ADNI-2) added early- and late-MCI cohorts. ADNI has provided a framework for similar initiatives worldwide, including TARCC. Only ADNI's NHW participants were included ($N = 1668$).

Clinical variables

dT2A

dT2A's construction and validation have been recently reported (Royall *et al.*, 2019). Its cognitive indicators were limited to observed measures that are common to both TARCC and ADNI, including the Boston Naming Test (BNT) (Kaplan et al., 1983), Category Fluency (Animals) (Morris *et al.*, 1989), Logical Memory I and II (Wechsler, 1997), the Mini–Mental State Examination (Folstein *et al.*, 1975), and Trail-Making Test Part B (Reitan, 1958).

dT2A's target indicators.ó In TARCC, we used informant-rated instrumental activities of daily living (Lawton and Brody, 1969) as dT2A's target indicator. The Functional Assessment Questionnaire (Pfeffer *et al.*, 1982) was used in ADNI. It is commonly used in dementia evaluations (Juva *et al.*, 1997; Teng *et al.*, 2010). Other investigators have employed Functional Assessment Questionnaire as the target of a δ homologue (Gavett *et al.*, 2015; John *et al.*, 2016).

Observed clinical measures

Observed clinical measures are often used as covariates or to provide external validation. The following measures are available in both TARCC and ADNI.

Self (informant)-reported age and gender are self-explanatory. Education was coded as a continuous measure of subject/informant reported years of formal education.

The Clinical Dementia Rating Scale 'Sum of Boxes' (Hughes *et al.*, 1982): the Clinical Dementia Rating Scale is used to evaluate dementia severity. The rating assesses the patient's cognitive ability to function in six domains—memory, orientation, judgement and problem solving, community affairs, home and hobbies and personal care. Information is collected during an interview with the patient and their caregiver (15 min).

Geriatric depression scale (GDS): depressive symptoms were assessed in both studies by the GDS (Sheikh and Yesavage, 1986; Maixner et al., 1995). GDS scores range from 0 to 30. Higher scores are worse. The GDS is valid in demented persons (Burke *et al.*, 1989).

Apolipoprotein E genotyping

APOEε4 burden was coded zero—two, based on the number of ε4 alleles. TARCC's APOE genotyping was conducted by the Ballantyne Lab at the Baylor College of Medicine in Houston Texas using standard polymerase chain reaction methods (Koch *et al.*, 2002). ADNI's APOE genotyping was performed on DNA extracted from peripheral blood cells and processed by the University of Pennsylvania AD Biofluid Bank Laboratory, as previously described (Saykin *et al.*, 2010).

Mediating variables

Blood-based protein biomarkers from both studies were tested as mediators of age's association with dT2A. Both studies obtained a highly reduplicative panel of blood-based protein biomarkers ($N = 120$) via a multiplexed immunoassay (i.e. the human multi-analyte profile) processed in by a common vendor [i.e. Rules-Based Medicine (RBM) of Austin, TX, USA]. A complete listing of the biomarkers offered by the human multi-analyte profile panel is available at http://www.myriadrbm.com/products-services/humanmap-services/humanmap/ (4 December 2019, date last accessed).

Blood-based biomarker methods

All RBM analyses were run in duplicate, and data were discarded when the duplicate values differed by >5%. All values recorded by RBM as 'LOW' were recorded and analysed. If >50% of the samples for a given analyte were recorded as 'LOW', all readings for that analyte were dropped. If <50% of the analytes were recorded as 'LOW', the LOW values were recorded as the least detectable dose divided by two. Some proteins in the human multi-analyte profile panel are not available to TARCC, ADNI or both.

Raw biomarker data from both studies were inspected to ascertain their normality. Data points beyond 3.0 standard deviations about the mean were labelled as 'outliers' and deleted. Logarithmic transformation was used to normalize highly skewed distributions. The data were then standardized, within each dataset, to a mean of zero and unit variance.

Other mediators

Other variables of interest were tested as potential mediators of age's association with dT2A. Some (e.g. BMI) were chosen to provide construct validity for the hypothesis that age's effect on dementia severity is mediated through cognitive 'frailty'. Others (e.g. HgbA1c) were chosen to provide discriminant validity. Some variables were selected from TARCC while others were available only in ADNI.

Texas Alzheimer's Research and Care Consortium mediators

Serum cholesterol, C-peptide, homocysteine, HgbA1c, high-density lipoprotein, low-density lipoprotein (calculated), lipoprotein-associated phospholipase A2 and triglycerides were obtained from the Ballantyne Lab. HgbA1c was measured in whole blood by the turbidimetric inhibition immunoassay. Homocysteine was measured in serum using the recombinant enzymatic cycling assay (i.e. Roche Hitachi 911).

Height and weight were obtained at all TARCC sites, and BMI was calculated from those variables. The presence of diabetes mellitus, hyperlipidaemia and/or hypertension were obtained from the subject or their informant and coded dichotomously. The duration of smoking exposure was obtained from the subject or their informant and coded continuously.

Alzheimer's Disease Neuroimaging Initiative mediators

CNS structural and functional neuroimaging biomarkers and certain 'Alzheimer's disease-specific' biomarkers measured in CSF were tested as potential mediators of age's association with dT2A in ADNI.

Imaging biomarkers

Magnetic resonance imaging data

The participants underwent a standardized 1.5-T MRI protocol (www.loni.ucla.edu/ADNI/Research/Cores/index.shtml (4 December 2019, date last accessed)), which included 2 T1-weighted MRI scans using a sagittal volumetric magnetization prepared rapid gradient echo sequence with the following acquisition parameters: echo time 4 ms, repetition time 9 ms, flip angle 8° and acquisition matrix size $256 \times 256 \times 166$ in the *x*-, *y*- and *z*-dimensions with a nominal voxel size of $0.94 \times 0.94 \times 1.2$ mm^3. Only one of the magnetization prepared rapid gradient echo sets was used for the analysis. The ADNI MRI quality control centre at the Mayo Clinic selected the magnetization prepared rapid gradient echo image with higher quality and corrected for system-specific image artefacts, as described in Jack *et al.* (2008). Further details are described in Wyman *et al.* (2013).

18F-Fluorodeoxyglucose positron emission tomography imaging data

All fluorodeoxyglucose PET scans were acquired using ADNI's standardized fluorodeoxyglucose PET acquisition protocols. Raw PET data were uploaded to the University of Michigan for preprocessing to correct for differences in the PET scanners used across ADNI sites. During preprocessing, each of the 5-min emission frames acquired in every scan were co-registered and then averaged to the first frame. The image was then reoriented such that the anterior–posterior axis of the subject ran parallel to the anterior commissure–posterior commissure line and interpolated onto a uniform $60 \times 160 \times 96$ voxel image grid, with 1.5-mm cubic voxels (http://adni.loni.usc.edu/methods/pet-analysis/pre-processing (4 December 2019, date last accessed)). Finally, a subject-specific mask was applied for intensity normalization (where average in the mask was one). Further details are described in Jagust *et al.* (2010).

Cerebrospinal fluid biomarkers

CSF was collected from the lower spine by lumbar puncture after an overnight fast as described in the ADNI procedures manual (www.adni-info.org). In brief, CSF was transferred in polypropylene tubes on dry ice within 1 h after collection and shipped overnight to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center. $A\beta_{1-42}$, t-tau and p-tau18 were measured in each aliquot using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium) immunoassay kit-based reagents. Full details of ADNI baseline CSF biomarker measurements are provided by Shaw *et al.* (2009).

Statistical analyses

These analyses were conducted in the NHW subset of TARCC's most recent dataset ($N = 2551$) and in a combined sample of ADNI-1, ADNI-2 and ADNI-GO data ($N = 1668$).

The analysis was performed using Analysis of Moment Structures software (Arbuckle, 2006). The maximum likelihood estimator was chosen for these models. Covariances between the residuals were allowed to be estimated if they were significant and improved model fit.

Analysis sequence

We used the 'TARCC to ADNI' δ homologue (dT2A) as previously described (Royall *et al.*, 2019). dT2A was constructed from baseline data using unadjusted indicators.

First, we constructed Multiple Indicators Multiple Causes (MIMIC) models (Muthén, 1979) of age's effect on cognitive performance. By that approach, the latent dT2A construct acted as a mediator of age's direct effects on dT2A's observed indicators. The MIMIC model thereby distinguishes age's direct effects on individual cognitive measures from its δ-specific indirect effects on all of them. Only age's δ-related effects are likely to be disabling and therefore potentially 'dementing'.

Next, we converted age's association with dT2A in the MIMIC model into the 'direct' effect (i.e. 'path a') of a mediation model by introducing an observed biomarker (Fig. 1). Path 'a' represents age's direct association with dT2A. Path 'b' represents the proposed mediator's independent effect on dT2A. Because these models were hypothesis driven, no Bonferroni correction was applied. Path 'c' represents age's effect on the proposed mediator. When both paths b and c are significant, the mediator's effect on age's direct association can be calculated by MacKinnon's method (MacKinnon, 1994).

Age's effect by path a and indirect effect via paths c–b were adjusted for APOE ε4 burden, education, gender and depressive symptoms (i.e. variables previously shown to exert age-independent effects on δ). We restricted this analysis to NHW only. Gender had no significant effect on δ in TARCC's NHW, and so it was omitted from TARCC's model (to improve fit).

TARCC's RBM biomarkers are known to exhibit significant batch effects. In the past, we have adjusted each TARCC biomarker with dichotomous dummy variables coding batch. However, in this analysis, batch effects were assumed to be a source of 'systematic' error and were adjusted by the introduction of a latent 'BIAS' variable, indicated by the proposed mediator and multiple other proteins (i.e. cluster of differentiation 40, fibrinogen, ferritin, human C–C motif chemokine-4, immunoglobulin E, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β and prostatic acid phosphatase). The latter biomarkers were chosen for their lack of associations with either age or δ in TARCC (when measured in serum; Royall *et al.*, 2016). The same proteins, measured in plasma, were used as indicators of a BIAS construct in ADNI. The BIAS variable should also account for biofluid-specific measurement bias across studies, as that too is a systematic source of variance across all protein biomarkers. The BIAS construct and its

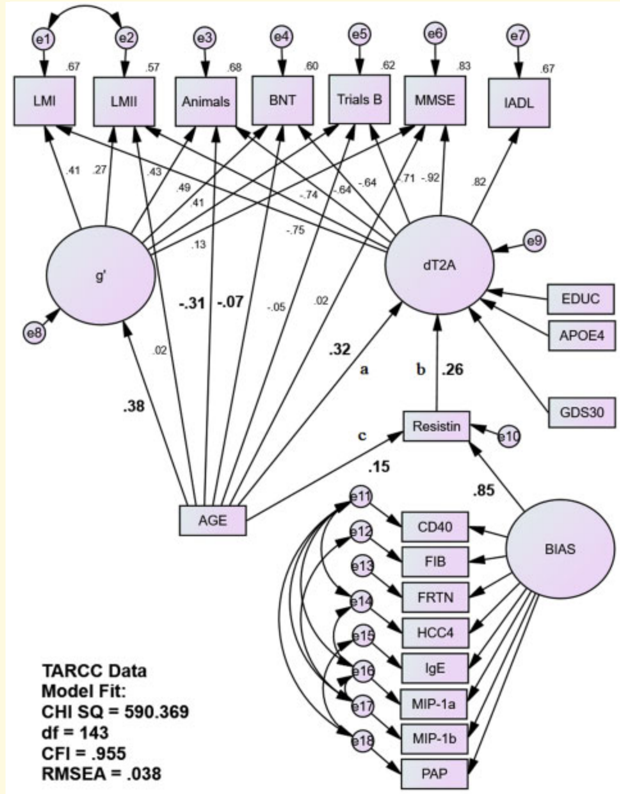


Figure 1 Fully adjusted MIMIC mediation model of serum resistin as a mediator of age's unique association with the latent dT2A homologue in TARCC. Age's association with the bifactor dT2A δ homologue is being partially mediated by serum resistin in TARCC's NHW sample (*N* = 2251) independently of age's direct effects on individual cognitive performance measures. Gender had no significant independent association with dT2A and was omitted from the model. AGE is correlated to EDUC, APOE4, GENDER and GDS (paths not shown). EDUC is also correlated with e8. Statistically significant structural paths of interest are in bold. Animals = animal naming; BNT = Boston Naming Test; CHI SQ = chi square; CD40 = cluster of differentiation 40; CFI = comparative fit index; EDUC = education (years); FIB = fibrinogen; FRTN = ferritin; HCC4 = human C–C motif chemokine-4; IADL = instrumental activities of daily living; IgE = immunoglobulin E; LMI = Wechsler Logical Memory immediate recall; LMII = Wechsler Logical Memory delayed recall; MIP-1a = macrophage inflammatory protein 1 alpha; MIP-1b = macrophage inflammatory protein 1 beta; MMSE = Mini–Mental State Examination; PAP = prostatic acid phosphatase; RMSEA = root-mean-square evaluative assessment; Trails B = Trail-Making Test Part B.

indicators were dropped from models that tested non-protein mediators.

Missing data

We used full information maximum likelihood methods to address missing data. Full information maximum likelihood uses the entire observed data matrix to estimate parameters with missing data. In contrast to listwise or pairwise deletion, full information maximum likelihood yields unbiased parameter estimates and preserves the overall power of the analysis (Schafer and Graham, 2002; Graham, 2009).

Fit indices

The validity of structural models was assessed using two common test statistics. A non-significant chi square signifies that the data are consistent with the model (Bollen and Long, 1993). The ratio of the chi square to the degrees of freedom in the model is also of interest. A chi square/degrees of freedom ratio of <5.0 suggests an adequate fit to the data (Wheaton *et al.*, 1977). The comparative fit index, with values ranging from 0 to 1, compares the specified model with a model of no change (Bentler, 1990). Comparative fit index values <0.95 suggest model misspecification. Values ≥ 0.95 indicate adequate to excellent fit. A root-mean-square error of approximation of ≤ 0.05 indicates a close fit to the data, with models <0.05 considering as 'good' fit and models up to 0.08 considering as 'acceptable' (Browne and Cudeck, 1993). All three fit statistics should be simultaneously considered to assess the adequacy of the models to the data.

Data availability

The data underlying the results presented in the study are available from TARCC and ADNI. Requests for TARCC data should be made to http://www.txalzresearch.org/research/ (4 December 2019, date last accessed). Requests for ADNI data should be made to adni.loni.usc.edu.

Results

Descriptive statistics is presented for two cohorts in Table 1 and by diagnoses in Table 2 (TARCC) and Table 3 (ADNI). The cohorts differed significantly on all measures. ADNI has a relatively high fraction of MCI cases, which were recruited explicitly into ADNI-2 and ADNI-GO. TARCC has a much higher prevalence of Mexican-American participants.

Technical issues precluded the testing of 6/22 proteins in ADNI. We can assume that >50% of those analytes' samples were reported to be 'LOW' by RBM. We had no access to the unprocessed biomarker data and could not determine how many samples were removed from each cohort because their duplicate values differed by >5%, or because they were assessed as 'outliers' prior to analysis. However, an upper bound for these issues can be estimated by the number of cases with missing data when biomarker data were available and cannot have been more than $123/886 = 13.9\%$ (i.e. for the unconfirmed biomarker glutathione *S*-transferase in TARCC). No data were missing in ADNI's ($N = 566$) samples. The eight discordant confirmed biomarkers, i.e. angiopoetin-2N,

Table 1 Descriptive statistics by sample (raw scores except where indicated)

	TARCC NHW ($N = 2251$)	ADNI NHW ($N = 1668$)
Alzheimer's disease cases, *n* (%)	1100 (49.7)	342 (19.7)
MCI cases, *n* (%)	395 (17.8)	978 (56.3)
NC, *n* (%)	718 (32.4)	417 (24.8)
Gender, female, *n* (%)	1288 (57.2)	919 (55.1)
	Mean (SD)	Mean (SD/*d*1)
Age	73.1 (8.96)	73.8 (7.2/0.09**)
Education	15.1(2.78)	15.9 (2.9/0.28**)
MMSE	25.2 (5.04)	27.2 (2.7/0.49**)
Animals	14.4 (6.04)	17.2 (5.9/0.47**)
BNTa	9.2 (4.19)	26.1 (4.51/b)
CDR-SB	3.1 (3.59)	1.6 (1.8/0.53**)
GDS	4.9 (4.10)	1.4 (1.4/1.14**)
LMI	7.7 (4.43)	9.3 (4.8/0.35**)
LMII	7.5 (4.75)	7.1 (5.3/0.08**)
Trails B	8.0 (4.08)	122.2 (75.8/c)

*d*1 = Cohen's *d* versus TARCC. Animals = animal naming; BNT = Boston Naming Test; CDR-SB = Clinical Dementia Rating scale 'Sum of Boxes'; LMI = Wechsler Logical Memory immediate recall; LMII = Wechsler Logical Memory delayed recall; MA = Mexican-American; MMSE = Mini–Mental State Examination; SD = standard deviation; Trails B = Trail-Making Test Part B.
[a]Scaled scores.
[b]TARCC uses 30-item BNT, and ADNI uses 60-item BNT.
[c]TARCC uses scale scores, and ADNI uses in seconds.
**$P < 0.001$.

creatinine kinase-MB, epidermal growth factor receptor 1, FAS, plasminogen activator inhibitor type 1, serum amyloid protein (SAP), thyroxine-binding globulin, and von Willebrand factor had no more than $15/886 = 1.7\%$ missing values (i.e. for creatinine kinase-MB) in TARCC.

Age was significantly associated with dT2A in both cohorts (TARCC: $r = 0.36$, $P < 0.001$); ADNI: $r = 0.16$, $P < 0.001$) (by unadjusted path a). All mediation models had acceptable fit (e.g. resistin; Figs 1 and 2). Significant mediation effects were found for 13/22 of TARCC's serum proteins (Table 4). Of the 16 potential mediators available in ADNI, significant mediation effects were found for four including adiponectin (APN; $Z = 2.32$, $P = 0.02$; 12.0%), FSH (FHS; $Z = 2.21$, $P = 0.03$; 8.2%), pancreatic polypeptide (PPP; $Z = 2.00$, $P = 0.05$; 14.2%) and resistin ($Z = 2.01$, $P = 0.04$; 8.3%). All mediation effects in both cohorts were partial, ranging from 3.3% (creatinine kinase-MB in TARCC) to 32.8% (IGF-binding protein 2 in TARCC). Near significant mediation effects were observed for plasma complement 3 ($Z = -1.82$, $P = 0.07$) and thyroxine-binding globulin ($Z = -1.83$, $P = 0.07$) in ADNI.

ADNI also confirmed that 6/22 proteins were not likely to be mediators of senile cognitive decline in TARCC, at least in NHW. In every case, both models agreed which path (b or c) remained significant and which did not. In half of the cases (3/6), the proposed mediator was related to age but not to δ (in NHW) (i.e. complement 3, glutathione *S*-transferase and thyroxine-binding globulin). IGF-1, myoglobin and von Willebrand factor were related to

Table 2 Descriptive statistics by diagnosis (TARCC)

	TARCC total NHW sample (*N* = 2251), mean (SD)	TARCC NHW Alzheimer's disease (*N* = 1100), mean (SD)	TARCC NHW MCI (*N* = 395), mean (SD)	TARCC NHW controls (*N* = 718), mean (SD)
Gender, female (%)	57.2	54.8	51.9	63.9
Age	73.1 (8.97)	75.46 (8.51)	72.4 (8.54)	69.8 (8.81)
Education	15.1 (2.79)	14.75 (2.95)	14.9 (2.59)	15.6 (2.54)
MMSE	25.2 (5.04)	21.68 (4.87)	27.5 (2.15)	29.3 (0.97)
Animals	14.3 (6.05)	10.40 (4.28)	15.8 (5.52)	19.6 (3.90)
BNT[a]	9.2 (4.19)	6.88 (3.55)	10.0 (3.43)	12.4 (3.11)
CDR-SB	3.1 (3.59)	5.82 (3.29)	1.3 (0.98)	0.0 (0.08)
GDS30	4.9 (4.08)	5.59 (4.20)	5.6 (4.43)	3.3 (3.21)
LMI	7.7 (4.43)	4.42 (2.65)	8.3 (3.01)	12.3 (2.68)
LMII	7.5 (4.75)	3.88 (2.53)	8.2 (3.21)	12.6 (2.71)
Trails B (s)	8.0 (4.08)	5.34 (3.37)	9.1 (3.03)	11.3 (2.53)

Animals = animal naming; BNT = Boston Naming Test; CDR-SB = Clinical Dementia Rating scale 'Sum of Boxes'; LMI = Wechsler Logical Memory immediate recall; LMII = Wechsler Logical Memory delayed recall; MA = Mexican-American; MMSE = Mini–Mental State Examination; SD = standard deviation; Trails B = Trail-Making Test Part B.
[a]TARCC uses 30-item BNT.

Table 3 Descriptive statistics by diagnosis (ADNI)

	ADNI total NHW sample (*N* = 1668), mean (SD)	ADNI NHW Alzheimer's disease (*N* = 342), mean (SD)	ADNI NHW MCI[a] (*N* = 978), mean (SD)	ADNI NHW controls (N = 417), mean (SD)
Gender, female (%)	55.1	44.7	42.8	49.9
Age	73.8 (7.19)	75.03 (7.79)	72.91 (7.42)	74.76 (5.73)
Education	15.91 (2.86)	15.18 (2.99)	16.00 (2.82)	16.28 (2.73)
MMSE	27.17(2.67)	23.22 (2.07)	27.75 (1.81)	29.07 (1.12)
Animals	17.15 (5.93)	12.25 (4.98)	17.39 (5.22)	20.60 (5.50)
BNT[b]	25.97 (4.51)	22.24 (6.05)	26.43 (3.68)	27.94 (2.66)
CDR-SB	1.64 (1.79)	4.39 (1.67)	1.36 (0.95	0.03 (0.13)
GDS30	1.42 (1.40)	1.65 (1.44)	1.63 (1.41)	0.75 (1.12)
LMI	9.28 (4.83)	4.08 (2.80)	9.10 (3.91)	13.98 (3.25)
LMII	7.07 (5.33)	1.37 (1.89)	6.46 (4.10)	13.18 (3.33)
Trails B (s)	122.23 (75.78)	191.46 (89.69)	113.61 (65.42)	85.68 (43.18)

Animals = animal naming; BNT = Boston Naming Test; CDR-SB = Clinical Dementia Rating scale 'Sum of Boxes'; LMI = Wechsler Logical Memory immediate recall; LMII = Wechsler Logical Memory delayed recall; MA = Mexican-American; MMSE = Mini–Mental State Examination; SCI = subjective cognitive impairment; SD = standard deviation; Trails B = Trail-Making Test Part B.
[a]All subtypes and SCI.
[b]ADNI uses 60-item BNT.

δ but not age (in NHW). Thus, the TARCC and ADNI models agreed with regard to 8/16 testable proteins.

Table 5 presents additional mediation effects. In TARCC, age's association with dT2A was not mediated by serum cholesterol, C-peptide, diabetes mellitus, homocysteine, HgbA1c, hyperlipidaemia, hypertension, lipoprotein-associated phospholipase A2, serum triglycerides or years of smoking history. It was partially mediated by high-density lipoprotein C ($Z = 2.38$, $P = 0.02$; 3.5%), low-density lipoprotein (calculated) ($Z = -2.24$, $P = 0.03$; 3.4%), weight ($Z = 3.62$, $P < 0.001$; 5.1%) and BMI ($Z = 4.28$, $P < 0.001$; 5.4%). Age's effect was also partially mediated by both treatment by galantamine ($Z = 2.51$, $P = 0.01$; 1.0%), which has been associated with change in resistin levels (Satapathy *et al.*, 2011), and treatment by any acetylcholinesterase inhibitors ($Z = 7.76$, $P < 0.001$; 8.5%).

Age's association with dT2A in ADNI was not mediated by CSF t-tau, p-tau18, $A\beta_{1-42}$ or the t-tau/$A\beta_{1-42}$ ratio but was severely attenuated by whole brain atrophy ($Z = 10.39$, $P < 0.001$; 66.0%), hippocampal atrophy, ventricular size ($Z = 8.88$, $P < 0.001$; 70.5%), fluorodeoxyglucose ($Z = 4.83$, $P < 0.001$; 47.4%) and AV45 PET ($Z = 7.12$, $P < 0.001$; 55.8%). SAP mediated age's effect in serum (TARCC; $Z = 3.35$, $P < 0.001$; 12.3%) but not in plasma (ADNI; $Z = -0.86$, $P = 0.39$).

Discussion

We have replicated age-specific mediation effects (or the lack thereof) for eight blood-based biomarkers across two independent cohorts representing convenience samples with differing case mixes. Our analysis also produced confirmations of 13/22 mediation effects in TARCC across two δ homologues with few common indicators. Finally, the present analysis replicates the mediation effects of four protein biomarkers across biofluids.

In TARCC, we confirm 13 of the 22 previously reported mediation effects. Our original reports were

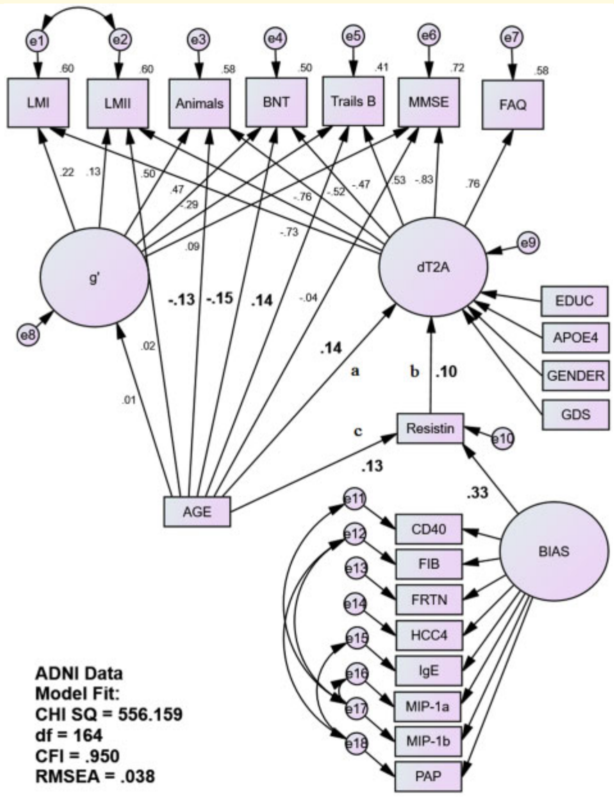


Figure 2 Fully adjusted MIMIC mediation model of plasma resistin as a mediator of age's unique association with the latent dT2A homologue in in ADNI. Age's association with the bifactor dT2A δ homologue is being partially mediated by plasma resistin in ADNI's NHW sample ($N = 1668$) independently of age's direct effects on individual cognitive performance measures. AGE is correlated to EDUC, APOE4, GENDER and GDS (paths not shown). EDUC is also correlated with e8. Statistically significant structural paths of interest are in bold. Animals = animal naming; BNT = Boston Naming Test; CHI SQ = chi square; CD40 = cluster of differentiation 40; CFI = comparative fit index; EDUC = education (years); FAQ = Functional Abilities Questionnaire; FIB = fibrinogen; FRTN = ferritin; HCC4 = human C–C motif chemokine-4; IgE = immunoglobulin E; LMI = Wechsler Logical Memory immediate recall; LMII = Wechsler Logical Memory delayed recall; MIP-1a = macrophage inflammatory protein 1 alpha; MIP-1b = macrophage inflammatory protein 1 beta; MMSE = Mini–Mental State Examination; PAP = prostatic acid phosphatase; RMSEA = root-mean-square evaluative assessment; Trails B = Trail-Making Test Part B.

exploratory analyses of an *ad hoc* panel in Bonferroni corrected models. The current replications are now hypothesis driven and relate to a second δ homologue. Moreover, the MIMIC model better distinguishes age's δ-specific effect on cognitive performance from its other effects on individual measures. Age's δ-independent effects on animal naming, Boston Naming Test and Trail-Making Test Part B were also confirmed. However, only age's δ-specific effect is likely to be functionally salient and it is precisely that effect, which is being mediated by the biomarkers.

As in our earlier report, all 13 confirmed proteins were partial mediators of age's effect on δ. Once again, IGF-binding protein 2 emerged with the strongest effect. Unfortunately, technical issues prevented a test of that biomarker in ADNI. However, the effect of IGF-1 could be neither confirmed in TARCC nor replicated in ADNI. IGF-1 was associated with age by path c in both cohorts but with δ in neither. This does not preclude age-specific effects on other organs or on less functionally salient cognitive domains and/or measures.

There was considerable concordance across the cohorts. We replicated 8/16 testable effects (or the lack thereof). In 8/8 discordant cases, we replicated at least one of the intervening paths (e.g. paths b or c). All the discordant dyads included a confirmed mediation effect in TARCC. No protein measured in plasma was found to be a mediator in ADNI but not in TARCC. In every case where mediation was not confirmed in TARCC, the ADNI model was concordant. Moreover, both models agreed on which paths were significant and which were not. The 2/8 discordant dyads involved near significant plasma effects in ADNI. These findings lend credibility to TARCC's serum findings and suggest that the measurement of these proteins in serum may be more clinically meaningful and generalizable than their measurement in plasma.

Several issues may have contributed to our failure to achieve confirmations in some of the previously reported effects. The current analysis involves a new δ homologue with a minimally overlapping set of indicators. We also limited these analyses to NHW. This reduced our sample size and may have undermined some replications. Our use of the MIMIC model has narrowed the focus of this analysis to age's unique effect on δ. Our use of the BIAS variable or our selection of its indicators may have inadequately accounted for batch effects. Finally, our earlier models targeted a *d*-composite. That may have introduced a reification bias relative to the latent constructs being targeted in the present work.

Regardless, we have replicated four of TARCC's 13 confirmed mediation effects across cohorts and biofluids. Such replications are difficult to achieve in the opinion of many experts (Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials *et al.*, 2012; O'Bryant et al., 2015). However, we may have been advantaged by our use of a latent variable approach, which has not been previously applied to blood-based protein biomarkers.

The replicated proteins were APN, follicle-stimulating hormone (FSH), PPP and resistin. APN and resistin are so-called 'apidokines' associated with adipocytes and their related secretome (Arai *et al.*, 2019). We recently demonstrated that APN's effect on cognitive performance to be fully mediated via δ (in TARCC; Benavente *et al.*, 2019). In addition, APN, resistin and PPP have each been previously associated with 'Alzheimer's disease' (Kiliaan *et al.*, 2014). However, 20% of so-called 'Alzheimer's disease'

Table 4 ADNI replication of age's mediators on dT2A in TARCC

Biomarker	TARCC (serum)	ADNI (plasma)
APN	$r = 0.33$ ($P < 0.001$) $Z = 2.50$ ($P = 0.01$) 8.8%	$r = 0.14$ ($P < 0.001$) $Z = 2.32$ ($P = 0.02$) 12.0%
ANG-2	$r = 0.33$ ($P < 0.001$) $Z = 3.52$ ($P < 0.001$) 9.6%	$r = 0.16$ ($P < 0.001$) $Z = -1.01$ ($P = 0.31$) c
C3	$r = 0.36$ ($P < 0.001$) $Z = -0.17$ ($P = 0.87$) b	$r = 0.17$ ($P < 0.001$) $Z = -1.82$ ($P = 0.07$) b, c
CK-MB	$r = 0.35$ ($P < 0.001$) $Z = 1.93$ ($P = 0.05$) 3.3%	$r = 0.15$ ($P < 0.001$) $Z = 0.42$ ($P = 0.68$) c
EGFR	$r = 0.26$ ($P < 0.001$) $Z = 5.20$ ($P < 0.001$) 26.9%	$r = 0.17$ ($P < 0.001$) $Z = -1.09$ ($P = 0.28$) C
FAS	$r = 0.35$ ($P < 0.001$) $Z = 2.37$ ($P = 0.02$) 4.3%	$r = 0.15$ ($P < 0.001$) $Z = 0.80$ ($P = 0.42$) c
FSH	$r = 0.34$ ($P < 0.001$) $Z = 2.77$ ($P = 0.006$) 7.1%	$r = 0.14$ ($P < 0.001$) $Z = 2.21$ ($P = 0.03$) 8.2%
GST	$r = 0.37$ ($P < 0.001$) $Z = -0.38$ ($P = 0.70$) b	$r = 0.15$ ($P < 0.001$) $Z = 1.40$ ($P = 0.16$) b
G-CSF	–	–
IGF-1	$r = 0.40$ ($P < 0.001$) $Z = -1.35$ ($P = 0.18$) c	$r = 0.15$ ($P < 0.001$) $Z = 0.20$ ($P = 0.84$) c
IGF-BP2	$r = 0.23$ ($P < 0.001$) $Z = 5.80$ ($P < 0.001$) 32.8%	–
IL-5	$r = 0.34$ ($P < 0.001$) $Z = 1.59$ ($P = 0.11$) b	–
MyG	$r = 0.38$ ($P < 0.001$) $Z = -1.53$ ($P = 0.13$) c	$r = 0.16$ ($P < 0.001$) $Z = -0.04$ ($P = 0.99$) c
PP	$r = 0.32$ ($P < 0.001$) $Z = 2.96$ ($P = 0.003$) 10.2%	$r = 0.13$ ($P < 0.001$) $Z = 2.00$ ($P = 0.05$) 14.2%
PAI-1	$r = 0.32$ ($P < 0.001$) $Z = 3.64$ ($P < 0.001$) 12.5%	$r = 0.16$ ($P < 0.001$) $Z = -1.57$ ($P = 0.12$) b
PDGF	$r = 0.36$ ($P < 0.001$) $Z = P\ 0.48$ ($P = 0.63$) c	–
Progesterone	$r = 0.36$ ($P < 0.001$) $Z = 0.51$ ($P = 0.61$)	–
Resistin	$r = 0.32$ ($P < 0.001$) $Z = 4.02$ ($P < 0.001$) 10.7%	$r = 0.14$ ($P = 0.001$) $Z = 2.01$ ($P = 0.04$) 8.3%
S100b	$r = 0.37$ ($P < 0.001$) $Z = -1.25$ ($P = 0.21$) b	–
SAP	$r = 0.32$ ($P < 0.001$) $Z = 3.35$ ($P < 0.001$) 12.3%	$r = 0.16$ ($P < 0.001$) $Z = -0.86$ ($P = 0.39$) b
TBG	$r = 0.34$ ($P < 0.001$) $Z = 2.22$ ($P = 0.03$) 5.4%	$r = 0.17$ ($P < 0.001$) $Z = -1.83$ ($P = 0.07$) b
vWF	$r = 0.34$ ($P < 0.001$) $Z = 1.82$ ($P = 0.07$) 6.3%	$r = 0.16$ ($P < 0.001$) $Z = 0.10$ ($P = 0.92$) c

(continued)

Table 4 Continued

b: path b (mediator to dT2A) is significant at $P < 0.05$; c: path c (age to mediator) is significant at $P < 0.05$. ANG-2 = angiopoetin-2N; C3 = complement 3; CK-MB = creatinine kinase-MB; EGFR = epidermal growth factor receptor 1; FSH = follicle-stimulating hormone; G-CSF = granulocyte colony-stimulating factor; GST = glutathione *S*-transferase; IGF-BP2 = IGF-binding protein 2; IL-5 = interleukin 5; MyG = myoglobin; PAI-1 = plasminogen activator inhibitor type 1; PDGF = platelet-derived growth factor; PP = pancreatic polypeptide; S100b = S100 calcium-binding protein B; TBG = thyroxine-binding globulin; vWF = von Willebrand factor.

cases do not exhibit amyloidosis by PET (Witte *et al.*, 2014; Degenhardt *et al.*, 2016; Landau *et al.*, 2016). It cannot be assumed that APN's, resistin's and PPP's associations with δ in the current analysis are mediated by Alzheimer's disease pathology. δ is 'agnostic' to dementia's aetiology (Gavett *et al.*, 2015; John *et al.*, 2016).

Instead, APN resistin and PPP have been shown to be mediators of age's unique effect on dementia severity. Alzheimer's disease-specific CSF biomarkers were not. The latter biomarkers impacted δ independently of age's effect. This suggests that the replicated biomarkers' associations with clinical 'Alzheimer's disease' in other studies could have been attributable to age's effect (i.e. senility) rather than Alzheimer's disease's.

Resistin is associated with resistance to oral hypoglycemics in diabetes mellitus (Acquarone *et al.*, 2019). However, we did not confirm either serum insulin (Royall *et al.*, 2016) or HgbA1c (in the present analysis) as mediators of age's effect. Resistin must be acting here through other mechanisms.

Resistin levels have been shown to be elevated in Alzheimer's disease (Kizilarslanoğlu *et al.*, 2015) and to rise progressively in MCI and Alzheimer's disease relative to NC (in TARCC; Royall and Palmer, 2019*b*). However, resistin has been shown here to be a mediator of age's effect. Its association with clinical dementia in other studies should not be attributed to Alzheimer's disease in the absence of a formal test of mediation via an Alzheimer's disease-specific biomarker.

Ironically perhaps, serum resistin levels can be shown to be modulated by treatment with acetylcholinesterase inhibitors (Satapathy *et al.*, 2011). This opens the door to possibly modulation of δ by acetylcholinesterase inhibitors in demented persons with elevated resistin levels. Our results suggest that these can be found among the fraction of demented persons with advanced age and/or depressive symptoms (Royall *et al.*, 2017*b*). Serum resistin levels fully attenuate the GDS' nearly 3-fold prospective 5-year MCI conversion is risk in an age-adjusted TARCC model (Royall and Palmer, 2019*a*). We have recently proposed a method to select individuals who might revert to non-demented states after the correction of any

Table 5 **Other mediators of age's effect in TARCC and ADNI**

Mediator	TARCC	ADNI
Hyperlipidaemia	$r = 0.36$ ($P < 0.001$) $Z = -0.97$ ($P = 0.33$)	
Hypertension	$r = 0.37$ ($P < 0.001$) $Z = -1.29$ ($P = 0.20$)c	
Diabetes mellitus	$r = 0.36$ ($P < 0.001$) $Z = 0.87$ ($P = 0.38$)	
Years smoked	$r = 0.36$ ($P < 0.001$) $Z = 0.15$ ($P = 0.88$)	
Cholesterol	$r = 0.37$ ($P < 0.001$) $Z = -0.97$ ($P = 0.33$)c	
Triglycerides	$r = 0.36$ ($P < 0.001$) $Z = 0.17$ ($P = 0.87$)	
HDL-c	$r = 0.35$ ($P < 0.001$) $Z = 2.38$ ($P = 0.02$) 3.5%	
LDL-c	$r = 0.37$ ($P < 0.001$) $Z = -2.24$ ($P = 0.03$) 3.4%	
LpPLA2	$r = 0.35$ ($P < 0.001$) $Z = 1.66$ ($P = 0.10$) b, c	
HCY	$r = 0.35$ ($P < 0.001$) $Z = 1.58$ ($P = 0.11$) c	
C-peptide	$r = 0.37$ ($P < 0.001$) $Z = -0.78$ ($P = 0.44$) b	
HgbA1c	$r = 0.36$ ($P < 0.001$) $Z = -0.49$ ($P = 0.62$)	
Weight	$r = 0.34$ ($P < 0.001$) $Z = 3.62$ ($P < 0.001$) 5.1%	
BMI	$r = 0.34$ ($P < 0.001$) $Z = 4.28$ ($P < 0.001$) 5.4%	
On galantamine	$r = 0.36$ ($P < 0.001$) $Z = 2.51$ ($P = 0.01$) 1.0%	
On any AChEI	$r = 0.33$ ($P < 0.001$) $Z = 7.76$ ($P < 0.001$) 8.5%	
CSF $A\beta_{1-42}$		$r = 0.16$ ($P < 0.001$) $Z = -0.33$ ($P = 0.74$) b
CSF t-tau		$r = 0.14$ ($P < 0.001$) $Z = 0.92$ ($P = 0.36$) b
CSF p-tau18		$r = 0.15$ ($P < 0.001$) $Z = 0.16$ ($P = 0.88$) b
CSF t-tau/$A\beta_{1-42}$		$r = 0.17$ ($P < 0.001$) $Z = -1.00$ ($P = 0.87$) b
AV45 PET		$r = 0.06$ ($P = 0.052$) $Z = 7.12$ ($P < 0.001$) 55.8%
FDG PET		$r = 0.08$ ($P = 0.009$) $Z = 4.83$ ($P < 0.001$) 47.4%
Whole brain		$r = 0.05$ ($P = 0.14$) $Z = 10.39$ ($P < 0.001$) 66.0%
Ventricular size		$r = 0.05$ ($P = 0.13$) $Z = 8.88$ ($P < 0.001$) 70.5%
Hippocampal volume		$r = -0.10$ ($P < 0.001$) $Z = 14.47$ ($P < 0.001$) 100%

b: path b (mediator to dT2A) is significant at $P < 0.05$; c: path c (age to mediator) is significant at $P < 0.05$. AChEI = acetylcholine esterase inhibitors; FDG = fluorodeoxyglucose; LDL-c = low-density lioprotein (calculated); LpPLA2 = lipoprotein-associated phospholipase A2; HCY = homocysteine; HDL-c, high-density lipoprotein C.

prespecified δ-related condition or risk factor (Royall and Palmer, 2019*b*). Demented cases selected by that approach on the basis of their depressive symptoms have demonstrably higher serum resistin levels, as we predict similarly selected age-specific dementias would be.

Resistin, APN and PPP have been proposed as biomarkers of weight loss and frailty in older persons (Cardoso *et al.*, 2018). We have confirmed weight and BMI as mediators of age's effect on δ (in TARCC). In contrast, alternative disease-specific conditions and biomarkers did not mediate that effect, notably HgbA1c, homocysteine and lipoprotein-associated phospholipase A2 (an ischaemic vasculopathy-associated risk factor).

Alzheimer's disease-specific CSF biomarkers were also not found to be mediators of age's effect. Given that CSF concentrations of $A\beta_{1-42}$, t-tau and p-tau18 have been shown to provide high sensitivity and specificity for diagnosing Alzheimer's disease, predict conversion from MCI to a diagnosis of probable Alzheimer's disease and identify non-demented elderly persons likely to progress (Blennow and Zetterberg, 2009), the failure of these analytes to exhibit mediation effects helps distinguish Alzheimer's disease from the dementing effects of Aging Proper (i.e. senility) under consideration here. This is consistent with the relative paucity of Alzheimer's disease neuropathology among centenarians and the oldest old (von Guten *et al.*, 2010).

Regardless, age's association with δ has been shown to be fully attenuated by a latent variable indicated by Alzheimer's disease pathology in autopsy-proven Alzheimer's disease cases of the National Alzheimer's Coordinating Center's Unified Dataset (at a mean age of 81.6 ± 10.6 years; Gavett *et al.*, 2016). However, Alzheimer's disease neuropathology was 'inversely related to age' in that sample ($r = -0.49$, $P < 0.001$), as was NIA-Reagan AD staging (National Institute on Aging, 1997). Thus, it is more properly stated that the 'lack of AD pathology' mediates age's association with δ in the National Alzheimer's Coordinating Center.

Although CSF $A\beta_{1-42}$, t-tau, p-tau18 and the t-tau:$A\beta_{1-42}$ ratio were related to δ by path b, none were related to age by path c, and so they cannot mediate age's effect on dementia severity. These biomarkers may be sensitive to clinical and pre-clinical Alzheimer's disease and contribute to its dementing effects, but Alzheimer's disease may not be entirely responsible for an individual's *d*-score and, therefore, for conversion risk (Ritchie *et al.*, 2017). Age's independent effect on δ might instead manifest as an erosion of the 'cognitive reserve' that has been proposed to explain the empirically weak relationships between many Alzheimer's disease-specific biomarkers (including autopsy findings) and measures of dementia severity (Stern, 2012). Our structural equation model approach has the potential to distinguish each compartment of δ's variance and to identify its unique biomarker profile.

In contrast to the CSF biomarkers, CNS amyloidosis by AV45 florbetapir PET was a strong mediator of age's association with δ. PiB-PET was available in only a small minority of ADNI participants and so could not be tested. There are subtle differences in the distributions of these radioligands. Florbetapir is more strongly associated with $A\beta_{1-40}$ than with $A\beta_{1-42}$ (Beach *et al.*, 2018). $A\beta_{1-40}$ is associated with diffuse plaque while the latter is more closely associated with NP. AV45's mediation effect in the absence of effects via CSF Alzheimer's disease-specific biomarkers might be explained by an $A\beta_{1-40}$ amyloidosis rather than $A\beta_{1-42}$ and NP formation.

In fact, extreme old age is associated with both a diminished NP burden and a reduced distribution of neurofibrillary tangles, limited largely to the mesiotemporal region, i.e. 'primary age-related tauopathy' (Crary *et al.*, 2014). As a result, most primary age-related tauopathy cases present at lower Braak stages (Besser *et al.*, 2019). Such a restricted distribution is unlikely to impact general cognition and hence *g* and δ, suggesting that the reserve of demented primary age-related tauopathy cases may be eroded by some process other than primary age-related tauopathy-related neurofibrillary tangle formation.

The age-specific AV45 signal might also be influenced by CNS amylin deposition (Jackson *et al.*, 2013). Amylin (i.e. insular amyloid polypeptide) is secreted by the pancreas and contributes to the pancreatic amyloidosis of type 2 diabetes. AV45 labels pancreatic amylin deposits (Templin *et al.*, 2018). A genome-wide association study (GWAS) using AV45 as an endophenotype (presumably of 'Alzheimer's disease') found single-nucleotide polymorphisms in the insular amyloid polypeptide gene to be predictive 'of CNS amyloisdosis' (Roostaei *et al.*, 2017). Amylin deposits can be demonstrated in the brains of demented persons with AODM, and even in the absence of that condition (Fawver *et al.*, 2014).

Plasma SAP levels are associated with Alzheimer's disease in Han Chinese (Cheng *et al.*, 2018). However, that finding was age-adjusted and may not be relevant to Aging Proper. However, elevated plasma SAP concentrations are also associated with cognitive impairment in centenarians (Nybo *et al.*, 1998). SAP was associated with δ in both TARCC and ADNI but was found to be a mediator of age's effect in TARCC's serum data only.

SAP is co-localized with a variety of amyloids, including both $A\beta_{1-42}$ and amylin. It co-localizes with florbetapir both in and outside the CNS (Wagner *et al.*, 2018). SAP is thought to accelerate $A\beta_{1-42}$ deposition in NP (Hamazaki, 1995; Tennent *et al.*, 1995). However, it 'interferes' with insular amyloid polypeptide deposition in amylin deposits (Gao *et al.*, 2015). In TARCC's data, SAP is associated with lower *d*-scores (a salutary effect). Age's adverse effect is mediated by 'lower' serum SAP levels in advancing age but not higher levels.

Plasma PPP levels are also predictive of CNS amyloidosis, both by PiB-PET (Kiddle *et al.*, 2012) and by AV45 (Voyle *et al.*, 2015). PPP levels rise exponentially with age (Brimnes et al., 1997) and are associated with anorexia and weight loss in older persons (Moss *et al.*, 2012). High plasma PPP levels are associated with both weight loss and MCI and moderate the association of diabetes with that diagnosis (Roberts *et al.*, 2015).

In summary, we cannot be certain that the CNS AV45 PET signal is specific to $A\beta_{1-42}$ and therefore that AV45's mediation effect involves that aggregate. Age's effect on δ could be mediated instead by $A\beta_{1-40}$ in the absence of NP formation or by CNS amylin deposition, accelerated by falling SAP levels, in parallel with rising PPP and in the presence of PPP-induced anorexia and weight loss. Since we are addressing age-specific contribution to dementia severity, neither scenario would conflict with AV45's co-localization at autopsy with NP 'in age-adjusted' models (Clark *et al.*, 2012).

We also found important mediation effects by other less Alzheimer's disease-specific structural and functional imaging modalities (e.g. fluorodeoxyglucose PET and whole brain atrophy by voxel-based morphometry). These were consistently stronger mediators than the blood-based protein biomarkers. An earlier ADNI analysis has associated CSF $A\beta_{1-42}$ and t-tau concentrations with the same structural features (Tosun *et al.*, 2010). However, as that analysis was age-adjusted, it cannot speak to the effects of Aging Proper. Brain atrophy's mediation of age's unique effect on δ is likely to be independent of CSF $A\beta_{1-42}$ and t-tau concentrations, as they were not themselves mediators.

Low high-density lipoprotein C and high low-density lipoprotein (calculated) were found to be partial mediators of age's effect on δ. They might offer potentially modifiable risk factors for age-specific cognitive decline and frailty (Ramsay *et al.*, 2015). Both were found to be associated with clinical 'Alzheimer's disease' in an early TARCC report (Warren *et al.*, 2012). However, those effects were age-adjusted once again. The present results suggest that high-density lipoprotein C and low-density lipoprotein (calculated) may additionally affect δ by an 'age-specific' mechanism. C-peptide, diabetes mellitus and LpLA2 were not mediators, but they were associated with dementia severity by path b, suggesting that they may be involved in 'age-independent' dementing processes.

Finally, FSH is associated with frailty in older men participating in the European Male Aging Study (Tajar *et al.*, 2011) and the Concord Health and Ageing in Men Project (Travison *et al.*, 2011). FSH's unadjusted effect on frailty in the Concord Health and Ageing in Men Project is fully attenuated by adjustment for age.

In summary, several blood-based protein biomarkers related to frailty have been shown to mediate age-specific differences in dementia severity as measured by δ. The 13 proteins have been confirmed by a second δ homologue in TARCC (serum). Four have been replicated across cohorts and biofluids. These may offer targets for the remediation of age-specific cognitive decline (aka 'senility'), help distinguish it from other determinants of dementia severity and/or provide clues to the biology of Aging Proper.

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Competing interests

Dr. D.R.R. has disclosed his co-invention of δ, its homologues and orthologues to the University of Texas Health Science Center at San Antonio (UTHSCSA), which has filed patent application 2012.039.US1.HSCS and provisional patents 61/603,226 and 61/671,858 relating to the latent variable *d*'s construction and biomarkers. Dr. R.F.P. has disclosed his co-invention of δ, its homologues and orthologues to UTHSCSA.

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