

Blood-Based Biomarkers

Blood-based protein predictors of dementia severity as measured by δ : Replication across biofluids and cohorts

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Abstract

Introduction: Dementia severity can be empirically described by the latent dementia phenotype “ δ ” and its various composite “homologs”. We have explored δ 's blood-based protein biomarkers in the Texas Alzheimer's Research and Care Consortium (TARCC) study. However, it would be convenient to replicate those associations in the Alzheimer's Disease Neuroimaging Initiative (ADNI). To this end, we recently engineered a δ homolog from observed cognitive performance measures common to both projects (i.e., “dT2A”).

Methods: We used nine rationally chosen peripheral blood-based protein biomarkers as indicators of a latent variable “INFLAMMATION”. We then associated that construct with dT2A in structural equation models adjusted for age, gender, depressive symptoms, and apolipoprotein E (APOE) $\epsilon 4$ allelic burden. Significant factor loadings and INFLAMMATION's association with dT2A were confirmed in random splits of TARCC's relatively large sample, and across biofluids in the ADNI.

Results: Nine proteins measured in serum (TARCC) or plasma (ADNI) explained $\cong 10\%$ of dT2A's variance in both samples, independently of age, APOE, education, and gender. All loaded significantly on INFLAMMATION, and positively or negatively, depending on their known roles are PRO- or ANTI-inflammatory proteins, respectively. The parameters of interest were confirmed across random 50% splits of the TARCC's sample, and replicated across biofluids in the ADNI.

Discussion: These results suggest that SEM can be used to replicate biomarker findings across samples and biofluids, and that a substantial fraction of dementia's variance is attributable to peripheral blood-based protein levels.

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Keywords:

ADNI; Aging; Cognition; Dementia; g; Intelligence; TARCC

¹Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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1. Introduction

While cognitive impairment is widely held to be the hallmark of dementia, three conditions are necessary to that diagnosis [1]:

- (1) There must be acquired cognitive impairment(s).
- (2) There must be functional disability.
- (3) The disability must be related to the cognitive impairment(s) that are observed.

This implies that the essential feature of any dementing process can be resolved to the cognitive correlates of functional status. Explicitly measuring that construct has opened the way to the completely empirical assessment and diagnosis of dementia.

We have used this insight to pioneer the assessment of dementia severity via confirmatory factor analysis in a structural equation model (SEM) framework. By this approach, functional status appears to be linked to cognitive performance largely through Spearman's General Intelligence factor "g" rather than through domain-specific cognitive abilities [2,3]. Our bifactor SEM parses g into two orthogonal (unrelated) fractions: (1) δ that is, "the psychometric correlates of functional status", and (2) g' that is, residual variance in g that is empirically unrelated to Instrumental Activities of Daily Living (IADL). Cognitive variance empirically unrelated to IADL cannot contribute to dementia by our definition. Thus, our method 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, that is, a dementia-specific phenotype. Because g is thought to contribute to all cognitive measures, it has proven feasible to construct δ from a wide range of batteries. This results in multiple d-score composites which comprise a set of δ "homologs". In genetics, a homolog is a gene derived from an ancestral gene and retaining the original's function. 14 homologs have been published to date, and all share similarly strong correlations with dementia severity (e.g., as measured by the Clinical Dementia Rating Scale "Sum of Boxes" [CDR-SB]) [4] and achieve high areas under the receiver operating characteristic curve for the discrimination of various dementias from normal controls (NC).

We have been studying δ homologs and their biomarkers in the Texas Alzheimer's Research and Care Consortium (TARCC) study. The TARCC is a large ($N \cong 3500$), well-characterized, ethnically diverse ($n \cong 1200$ Mexican-American [MA]) convenience sample with annual longitudinal follow-up [5].

We have associated δ with a large number of serum proteins, including pro- and anti-inflammatory cytokines [6–8]. Many of these associations appear to have profound ethnicity effects in the TARCC [9,10]. We have also published the serum protein mediators of δ 's specific associations with several well-recognized dementia risks (including age, depressive symptoms, and the apolipoprotein [APOE] $\epsilon 4$ allele) [11–13]. These associations have been confirmed in random subsets of the TARCC's large sample. Regardless, the TARCC has its limitations. No imaging is available, and its protein biomarkers have been obtained in serum. Biomarker associations may be impacted by the biofluid in which they are measured [14].

The Alzheimer's Disease Neuroimaging Initiative (ADNI) offers an opportunity to replicate our TARCC find-

ings [15]. Its cognitive battery overlaps substantially with the TARCC's, and both have deployed similar blood-based biomarker panels processed by a common vendor (i.e., Rules-Based Medicine [RBM] of Austin Texas). If we can validate δ 's blood-based biomarkers across both studies, we might be able to integrate ADNI's neuroimaging into δ 's growing literature.

To that end, we recently constructed a new δ homolog from a common set of cognitive indicators [16]. In TARCC, "dT2A" targets the IADL from the Older Adults Resources Scale (OARS) [17]. In the ADNI, the Functional Activities Questionnaire (FAQ) [18] was used. The FAQ has been successfully incorporated into some earlier δ homologs [19,20].

dT2A fit the data of both studies well and was strongly correlated with dementia severity, as rated by the CDR-SB (TARCC: $r = 0.99$, $P < .001$; ADNI: $r = 0.96$, $P < .001$). dT2A achieved an area under the receiver operating characteristic curve of 0.981 (0.976–0.985) for the discrimination of Alzheimer's disease (AD) from NC in TARCC, and 0.988 (0.983–0.993) in ADNI [16].

In this study, we associate dT2A with a set of proteins we had previously associated with another δ homolog [9] and attempt to replicate their association in the ADNI. The final obstacle to this replication is that TARCC and ADNI have measured their biomarkers in different biofluids (i.e., serum and plasma, respectively). While biomarker findings have been notoriously difficult to replicate across these biofluids [14], no such study has used our (latent variable) SEM methods. Those can be applied equally to biomarkers as to cognitive batteries.

2. Methods

2.1. 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 are approved by their respective Institutional Review Boards.

2.1.1. Texas Alzheimer's Research and Care Consortium

Subjects included $N = 3502$ TARCC participants. TARCC is a longitudinally followed convenience sample of elderly persons diagnosed with AD ($n = 1275$), "Mild Cognitive Impairment" (MCI) ($n = 723$), or NC ($n = 1445$) (and 58 "others") recruited from five Texas medical schools. Each participant underwent a standardized annual examination that included a medical evaluation, neuropsychological testing, and clinical interview. Categorical clinical diagnoses of "AD", "MCI", and "NC" were established through consensus. The diagnosis of AD was based on National Institute for Neurological Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria [21]. The diagnosis of MCI was based on site-specific

consensus-based clinical diagnoses derived from all available information but without reliance on specific neurocognitive tests and/or cut scores. "All available information" included the results of TARCC's entire neuropsychological battery, clinical evaluations, informant interviews, and any available outside medical records. We could not easily use cut scores because MA norms are not available for many measures.

2.1.2. Alzheimer's Disease Neuroimaging Initiative

The ADNI data used in the preparation of this article 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. The primary goal of ADNI has been to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD.

The ADNI is a well-characterized longitudinal convenience sample developed to validate magnetic resonance, positron emission tomography, cerebrospinal fluid (CSF), and genetic biomarkers for use in AD clinical trials. The initial 5-year study, ADNI-1, enrolled cognitively normal, MCI, and AD subjects, and the subsequent studies (ADNI-GO and ADNI-2) added early- and late-MCI cohorts. The ADNI has provided a framework for similar initiatives worldwide, including the TARCC.

2.2. Clinical variables

2.2.1. *dt2A*, a δ homolog for the ADNI

dt2A's construction has been recently described [16]. Its cognitive indicators were limited to observed measures that are common to both studies, including the Boston Naming Test (BNT) [22], category fluency (animals) [23], Logical Memory I (LMI) and II (LMII) [24], the Mini-Mental State Examination (MMSE) [25], and Trail-Making Part B [26]. All are available in the TARCC in Spanish translation.

BNT [22] is a confrontation naming test that requires the subject to verbally name line drawings of objects of increasingly lower frequency. The TARCC uses 30-item BNT. The ADNI uses 60-item BNT.

Categorical fluency (animals) [23] is a test of verbal fluency that asks subjects to verbally generate as many animal names as they are able in one minute.

LMII [24]: Immediately (LMI), and after a 30-minute delay (LMII), the subject recalls two paragraphs read aloud.

The MMSE [25] is a well-known and widely used test for screening cognitive impairment.

Trail-Making Part B [26] is a timed test of attention, speed, and mental flexibility that requires the subject to alternately connect between numbers and letters. The TARCC reports Trail-Making Part B as scaled scores.

2.2.2. *dt2A*'s target indicators

In the TARCC, we used informant-rated IADL as *dt2A*'s target indicator. Unfortunately, IADL is not available in the ADNI, and so the FAQ [18] was used instead.

IADL is assessed using the OARS [17]. The OARS is a structured clinical interview that provides informant-reported information on 7 IADLs. Each item is scored on a four-point Likert scale with 0 signifying "no impairment".

The FAQ [18] is an informant-rated measure of a participant's ability to perform IADL. The FAQ is commonly used in dementia evaluations because of its reliability, validity, sensitivity, and specificity [27,28].

2.2.3. Observed clinical measures

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

Self (informant)-reported age, education, and gender are self-explanatory. Ethnicity is coded dichotomously according to self-reported Hispanic affiliation. The TARCC has a substantial number of MA participants. MA ethnicity has pronounced effects on serum protein biomarkers in the TARCC [9,10,29]. There are no racial distinctions in the TARCC, and no reported racial effects on plasma protein biomarkers in the ADNI.

The CDR-SB [4] is used to evaluate dementia severity. The rating assesses the patient's cognitive ability to function in six domains—memory, orientation, judgment and problem-solving, community affairs, home and hobbies and personal care. Information is collected during an interview with the patient and their caregiver (15 minutes).

Geriatric Depression Scale (GDS) is used to assess depressive symptoms in both studies [30,31]. GDS scores range from zero to 30. Higher scores are worse. The GDS is valid in demented persons [32].

2.3. Blood-based biomarkers

Blood-based biomarkers were processed in both studies by a common vendor (RBM in Austin, TX). RBM conducted multiplexed immunoassay via their human multianalyte profile (human MAP).

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 analyzed. If more than 50% of the samples for a given analyte were recorded as "LOW", all readings for that analyte were dropped. If less than 50% of the analytes were recorded as "LOW", the LOW values were recorded as the least detectable dose divided by two. As a result, some proteins in the human MAP panel are not available to the TARCC, the 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 labeled as "outliers" and deleted. Logarithmic transformation was used to normalize

highly skewed distributions. The data were then standardized to a mean of zero and unit variance.

In the TARCC, these transformations do not affect the performance of the biomarkers. Serum proteins identified to be mediators of certain demographic risk factors and δ in an SEM have been validated by their ability to fully attenuate those same variables as predictors of prospective conversion to clinical "AD" from nondemented states [33].

2.4. Statistical analyses

These analyses were conducted in TARCC's most recent data set ($N = 3502$) and in a combined sample of ADNI-1, ADNI-2, and ADNI-GO data ($N = 1737$).

The analysis was performed using Analysis of Moment Structures software [34]. 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.

A reflective latent variable ("INFLAMMATION") was constructed from nine protein biomarkers available in both the ADNI and TARCC. These were selected from among 12 proteins previously identified as biomarkers of δ (by a different δ homolog) in the TARCC [9]. They, in turn, were selected from 22 serum proteins previously associated with the clinical diagnosis of "AD" by O'Bryant et al. [35], augmented by the TARCC's interleukins, and interferon- γ (IFN- γ). Although identified individually as univariate predictors, they are significantly intercorrelated. In their aggregate, they suggested to us an effect of innate immunity on δ . These findings support our decision to model them as a latent INFLAMMATION construct.

Of the 12 proteins we originally associated with, δ , IFN- γ , and interleukins 10 (IL-10) and 12p40 (IL-12p40) were not available in the ADNI and were dropped from consideration (although they all loaded significantly on INFLAMMATION in the TARCC, data not shown). β 2-Macroglobulin introduced negative variance, and stem cell factor did not load significantly and so they were also dropped.

The 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 all available protein biomarkers. 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.

We had also reported significant ethnicity effects on the association between these proteins and δ . The TARCC has been enriched with MAs, in contrast to the ADNI. Therefore, we also decided to use the BIAS variable to adjust for ethnicity as a systematic influence on within-study biomarker data, particularly in the TARCC.

2.4.1. 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, preserves the overall power of the analysis, and is arguably superior to alternative methods, example, multiple imputation [36,37].

2.4.2. Fit indices

The validity of structural models was assessed using two common test statistics. A nonsignificant chi-square signifies that the data are consistent with the model [38]. However, the ratio of the chi-square to the degrees of freedom in the model is also of interest. A CMIN/DF ratio <5.0 suggests an adequate fit to the data [39]. The comparative fit index (CFI), with values ranging between 0 and 1, compares the specified model with a model of no change [40]. CFI values below 0.95 suggest model misspecification. Values of 0.95 or greater indicate adequate to excellent fit. A root mean square error of approximation (RMSEA) of 0.05 or less indicates a close fit to the data, with models below 0.05 considered "good" fit, and up to 0.08 as "acceptable" [41]. All three fit statistics should be simultaneously considered to assess the adequacy of the models to the data.

2.4.3. Factor equivalence

INFLAMMATION's factor equivalence and the strength of its association with dT2A was tested across two random 50% subsets of the TARCC's participants (i.e., group 1 $N = 1747$; group 2: $N = 1755$). The parameters of interest were constrained to be equal across groups and χ^2 fit was compared in constrained versus unconstrained models.

3. Results

Descriptive statistics are presented in Table 1. Cross-cohort differences exist for almost all the variables, consistent with case-mix and demographic differences between two convenience samples. The ADNI appears to have a relatively high fraction of MCI cases, which were recruited explicitly into ADNI-2 and ADNI-GO. The TARCC has a much higher prevalence of MA participants. Education favors the ADNI, which has a slightly better mean MMSE score. Tables 2 and 3 present the mean concentration for each biomarker in serum (TARCC) and plasma (ADNI), respectively. We lack access to the number of outliers and samples below the limit of quantification. However, the combined effects of both issues cannot have more than 3.5% (i.e., $N = 31/880$ for tumor necrosis factor alpha in the TARCC [Table 2]) as all other biomarkers exhibit less missingness.

The TARCC's model, constrained across random 50% splits, had acceptable fit (chi square = 1264.6 [295], $P < .001$; CFI = 0.95; RMSEA = 0.031) (Fig. 1). All

Table 1
Descriptive statistics by sample

Demographic features	TARCC total N = 3502 mean (SD)	TARCC group 1 N = 1747 mean (SD)	TARCC group 2 N = 1755 mean (SD)	ADNI N = 1738 mean (SD)
AD cases	1275 (37.0%)	613 (35.6)	662 (38.3)	342 (19.7%)
MCI cases	723 (21.0%)	371 (21.6)	352 (20.4)	978 (56.3%)
NC	1445 (41.9%)	734 (42.7)	711 (41.2)	417 (24.8%)
Gender (%♀)	61.6	60.0	63.1	55.1
Ethnicity (%MA)	35.7	36.7	34.7	3.4
Variables	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD/d1)
Age	70.8 (9.6)	70.6 (9.7)	71.0 (9.5)	73.8 (7.2/0.35*)
Education	13.3 (4.3)	13.3 (4.3)	13.3 (4.3)	15.9 (2.9/0.71*)
MMSE	25.6 (4.7)	25.8 (4.6)	25.4 (4.9)	27.2 (2.7/0.42*)
Animals	14.9 (5.5)	15.0 (5.5)	14.9 (5.6)	17.2 (5.9/0.39*)
BNT [†]	7.9 (4.3)	8.0 (4.3)	7.9 (4.2)	26.0 (4.5/ [‡])
CDR-SB	2.4 (3.3)	2.3 (3.2)	2.5 (3.4)	1.6 (1.8/0.28*)
GDS30	5.6 (5.2)	5.6 (5.3)	5.6 (5.1)	1.4 (1.4/1.09*)
LMI	7.9 (4.2)	7.9 (4.2)	7.8 (4.2)	9.3 (4.8/0.30*)
LMII	8.2 (4.6)	8.3 (4.5)	8.2 (4.6)	7.1 (5.3/0.22*)
Trails B (sec)	144.2 (84.1)	8.0 (3.8) [‡]	8.0 (3.9) [‡]	122.2 (75.8/0.27*)

NOTE. d1 = Cohen's d versus TARCC's entire sample.

Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; Animals, Animal Naming; BNT, Boston Naming Test; CDR-SB, Clinical Dementia Rating scale "Sum of Boxes"; GDS, 30-item Geriatric Depression Scale; LMI, Wechsler Logical Memory immediate recall; LMII, Wechsler Logical Memory delayed recall; MA, Mexican-American; MMSE, Mini-mental State Examination; SD, standard deviation; TARCC, Texas Alzheimer's Research and Care Consortium; Trails B, Trail-Making Test Part B.

* $P < .001$.

[†]The TARCC uses 30-item BNT; the ADNI uses 60-item BNT.

[‡]Scaled score.

nine observed serum biomarkers loaded significantly on INFLAMMATION, ranging from von Willebrand factor ($r = 0.27$) to thrombopoietin (THPO) ($r = -0.83$; all $P < .001$). Interestingly, the nine proteins sorted into two sets with inverse loadings on the INFLAMMATION construct, therefore potentially conflicting effects on observed cognitive performance. Alpha 2 macroglobulin, pancreatic polypeptide protein, prolactin, tumor necrosis factor alpha, and von Willebrand factor loaded positively on INFLAMMATION and had adverse associations with cognitive performance (through dT2A), consistent with their roles as proinflammatory cytokines in the literature. The interleukins 3 (IL-3), 13 (IL-3), serum amyloid protein, and THPO loaded inversely on INFLAMMATION and thus had salutary associations with cognitive performance, consistent with their roles as anti-inflammatory cytokines in the literature. INFLAMMATION's factor weights all replicated in a random split of TARCC's sample ($\Delta\text{chi square} = 8.0$ [11], $P > .50$). The INFLAMMATION construct correlated $r = 0.24$ with dT2A ($P < .001$), independently of age, APOE $\epsilon 4$ status, the GDS, and gender. This is consistent with an overall adverse effect on cognitive performance. Their association also replicated across random splits, as evidenced by the insignificant change in chi square in constrained versus unconstrained models.

In the ADNI, the model also fit well (chi square = 363.2 [142], $P < .001$; CFI = 0.94; RMSEA = 0.044) (Fig. 2). The

nine proteins again sorted into two sets with significant inverse loadings on INFLAMMATION. The INFLAMMATION construct correlated $r = 0.31$ with dT2A ($P < .001$), independently of age, APOE $\epsilon 4$ status, the GDS, and gender. Together with covariates, INFLAMMATION explained 38% of dT2A's variance in TARCC and 24% in the ADNI.

4. Discussion

We have used SEM to replicate biomarker findings from the TARCC in the ADNI. The advantages of performing this replication by latent factors in SEM are numerous and likely contributed to this result. First, as latent variables, INFLAMMATION and dT2A are relatively free of nonsystematic measurement error. This might include linguistic, cultural, or educational bias in the cognitive performance measures (or their translations), cross-cohort differences in case-mix and/or diagnostic bias, technical obstacles to the measurement of individual biomarkers and/or biomarker-specific vulnerabilities to unspecified comorbid conditions or medication effects.

Second, we can side-step the remaining systematic bias by specification of the BIAS variable. This construct was introduced to account for biomarker variance related to the different biofluids used in these studies. g' may serve a similar role in relationship to δ . It appears to measure δ 's residual in g , but would also be sensitive to any unspecified

Table 2
Biomarker means in serum (TARCC) (raw unstandardized)

Biomarkers	N	Minimum	Maximum	Mean	SD
Alpha2_Macroglobulin (a2M)*	851	0.55	5.90	1.58	0.72
Interleukin 3 (IL-3) [†]	857	0.001	0.490	0.054	0.09
Interleukin 13 (IL-13) [‡]	865	0.65	160.00	22.02	36.84
Pancreatic_Polypeptide (PPP) [‡]	871	2.30	1210.00	259.66	230.00
Prolactin_(PRL) [†]	875	0.02	23.00	4.76	4.02
Serum_Amyloid_Protein (SAP) [§] _	874	2.90	36.00	17.16	5.75
Thrombopoietin (THPO) [†]	880	0.39	7.60	2.66	1.55
TNF alpha (TNFa) [‡]	849	1.50	58.00	16.98	11.02
von_Willebrand_Factor (vWF) [§] _	877	0.90	139.00	45.86	24.85
Valid N (listwise)	726				

Abbreviations: THPO, thrombopoietin; TNF, tumor necrosis factor; SD, standard deviation.

*mg/ml.

[†]ng/ml.

[‡]pg/ml.

[§]μg/ml.

systematic cognitive bias, example, site-specific investigator or administration bias, etc., acting on all measures collected at an individual site. Additional systematic measurement error can be reintroduced during a factor's reification as a composite index (e.g., when converting δ into a composite d-score homolog). However, we have avoided that too by keeping the latent variables in SEM.

These methodological advances have allowed us to replicate several findings across samples, and biofluids. As indicators of a latent variable, the nine proteins we have considered share variance which is related to dementia severity, as measured by dT2A. This suggests that they act in concert to achieve their effect on dementia severity. Their raw concentrations differ widely across cohorts (because of the case-mix and demographic differences revealed in Table 1), and across biofluids (Tables 2 and 3). Regardless, the relationships shared by these nine proteins are stable across cohorts and biofluids. This conclusion is supported by their consistent positive and negative loadings on the

INFLAMMATION construct and its significant effect on dT2A across samples.

Regardless, only a fraction of each biomarker's variance is attributable to INFLAMMATION. The rest represents residual variance, orthogonal to INFLAMMATION, and unrelated to its effect on δ . Because only variance shared across all nine proteins can influence INFLAMMATION, any attempt to link dementia risk to the observed concentration of a single protein, or any subset of them, is likely to be unsuccessful. Similarly, an intervention directed against any one of these biomarkers, acting independently of the other eight, is likely to fail to influence INFLAMMATION, just as INFLAMMATION itself resisted differences in case-mix and demographic features across the two cohorts. To effect changes in dementia severity as measured by δ , the interrelationships coded by the INFLAMMATION construct itself must be targeted, and that would require changes to all nine indicator proteins.

Table 3
Biomarker means in plasma (ADNI) (log-transformed and unstandardized)

Biomarkers	N	Minimum	Maximum	Mean	SD
Alpha2_Macroglobulin (a2M)*	1063	-0.25	0.81	0.042	0.10
Interleukin 3 (IL-3) [†]	1063	-2.62	-0.52	-1.62	0.30
Interleukin 13 (IL-13) [‡]	1063	0.20	2.34	1.59	0.18
Pancreatic_Polypeptide (PPP) [‡]	1063	-0.004	3.12	2.13	0.37
Prolactin_(PRL) [†]	1063	-0.66	1.74	0.78	0.21
Serum_Amyloid_Protein (SAP) [§] _	1063	0.71	1.95	1.31	0.12
Thrombopoietin (THPO) [†]	1063	0.30	7.80	2.18	0.99
TNF alpha (TNFa) [‡]	1063	0.11	1.98	0.84	0.23
von_Willebrand_Factor (vWF) [§] _	1063	-0.08	2.44	1.56	0.45
Valid N (listwise)	1063				

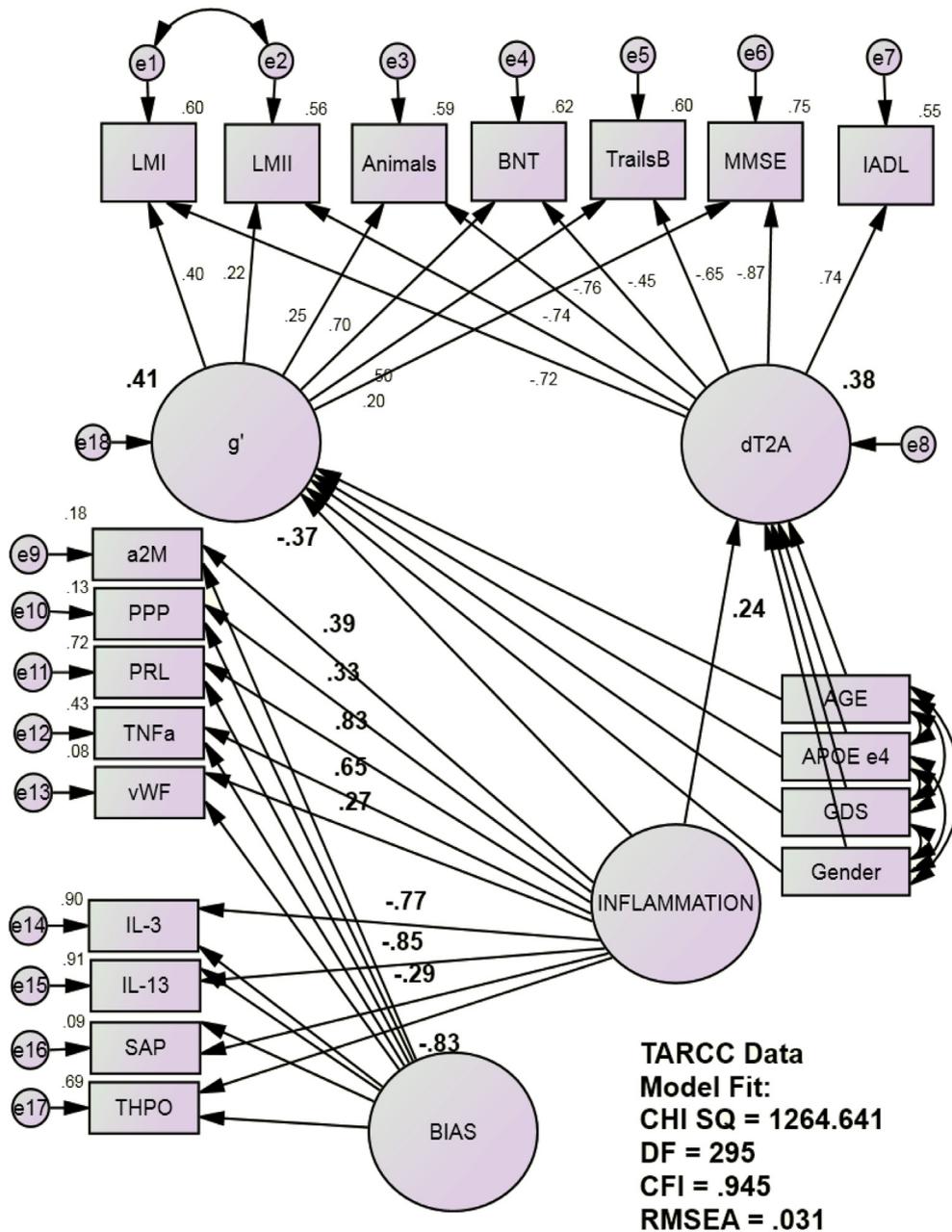
Abbreviations: THPO, thrombopoietin; TNF, tumor necrosis factor; SD, standard deviation.

*mg/ml.

[†]ng/ml.

[‡]pg/ml.

[§]μg/ml.



*Model parameters constrained across random 50% splits of TARCC's sample.

Fig. 1. Inflammation's association with δ in the TARCC*. *Model parameters constrained across random 50% splits of the TARCC's sample. Abbreviations: A2M, alpha 2 macroglobulin; Animals, Animal Naming; BNT, Boston Naming Test; CHI QS, chi square; CFI, comparative fit index; EDUC, education (years); GDS, 30-item Geriatric Depression Scale; IADL, Instrumental Activities of Daily Living; IL-3, interleukin 3; IL-13, interleukin 13; LMI, Wechsler Logical Memory immediate recall; LMII, Wechsler Logical Memory delayed recall; MMSE, Mini-Mental State Examination; PPP, pancreatic polypeptide; PRL, prolactin; RMSEA, Root Mean Square Evaluative Assessment; SAP, serum amyloid protein; TARCC, Texas Alzheimer's Research and Care Consortium; THPO, thrombopoietin; TNFa, tumor necrosis factor alpha; Trails B, Trail-Making Test part B; vWF, von Willebrand factor.

We interpret the INFLAMMATION construct as an aspect of inflammation, most likely innate immunity, although that has not been directly confirmed here. Not only do the proteins sort themselves out across INFLAMMATION's loadings consistently with their recognized PRO- and ANTI-inflammatory effects, but most can also

be shown in the literature to be related to IL-10, which loads significantly on INFLAMMATION in the TARCC (but cannot be modeled in the ADNI).

IL-10 is a key ANTI-inflammatory cytokine, associated with multiple other pro- and anti-inflammatory proteins in a complex network [42]. IL-12 is a similarly important

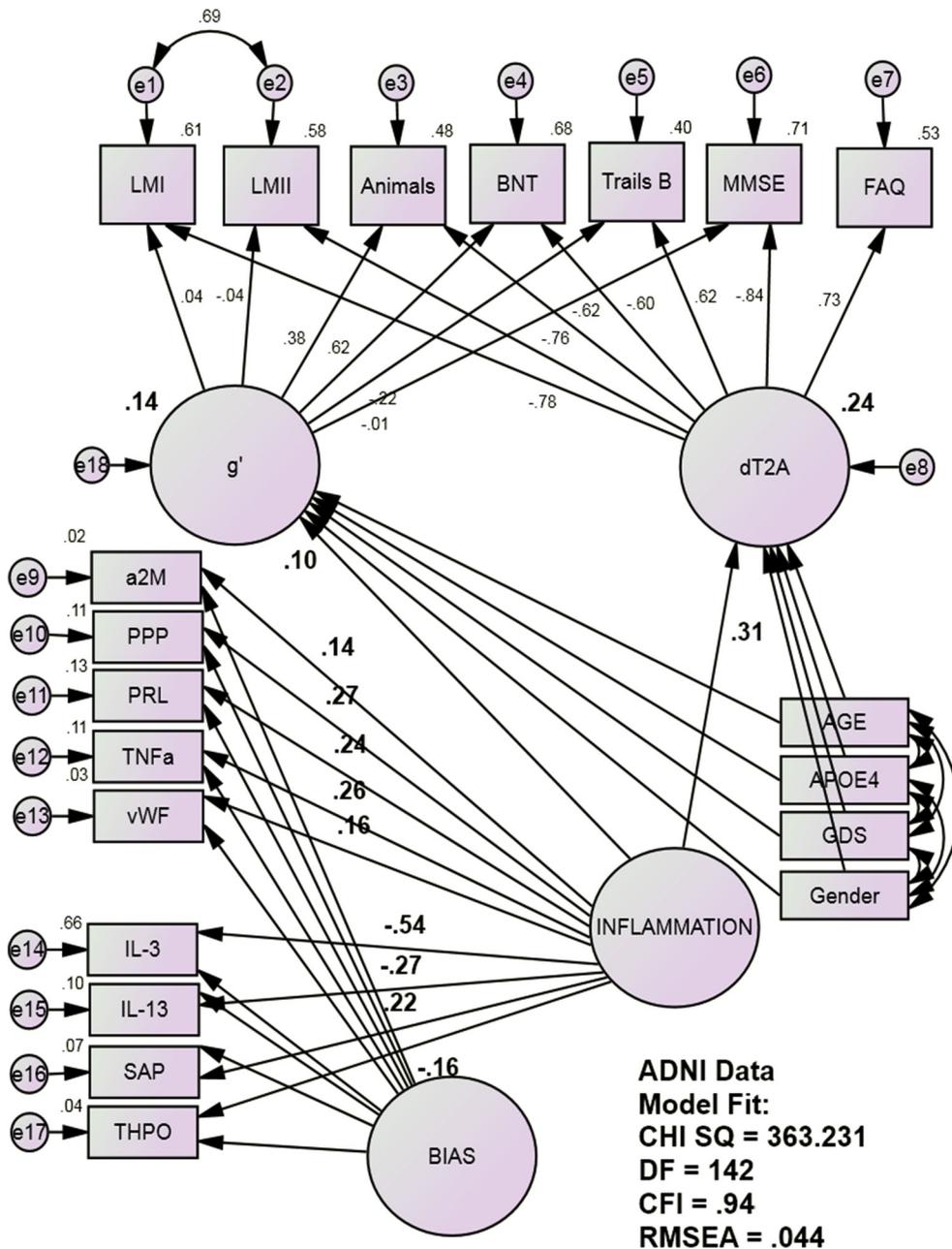


Fig. 2. Inflammation's association with δ in the ADNI. Abbreviations: A2M, alpha 2 macroglobulin; Animals, Animal Naming; BNT, Boston Naming Test; CHI QS, chi square; CFI, comparative fit index; EDUC, education (years); GDS, 30-item Geriatric Depression Scale; IADL, Instrumental Activities of Daily Living; IL-3, interleukin 3; IL-13, interleukin 13; LMI, Wechsler Logical Memory immediate recall; LMII, Wechsler Logical Memory delayed recall; MMSE, Mini-Mental State Examination; PPP, pancreatic polypeptide; PRL, prolactin; RMSEA, Root Mean Square Evaluative Assessment; SAP, serum amyloid protein; TARCC, Texas Alzheimer's Research and Care Consortium; THPO, thrombopoietin; TNFa, tumor necrosis factor alpha; Trails B = Trail-Making Test part B; vWF = von Willebrand factor.

PRO-inflammatory protein, and antagonizes many of IL-10's effects. Both are associated with amyloid deposition in the Australian Imaging, Biomarker & Lifestyle Flagship Study on Ageing (ABIL) [43]. IL-12 is also unavailable in the ADNI, but loads significantly on INFLAMMATION in the TARCC, and inversely to IL-10 (data not shown). Other proteins in IL-10's network also load on INFLAMMATION in the TARCC (e.g., interleukin 1 receptor agonist

[IL-1ra] and IFN- γ), but were not used here because they are unavailable in the ADNI. Regardless, their significant loadings on INFLAMMATION in TARCC provides hypothesis-driven validation of our latent INFLAMMATION construct as an IL-10-related network of blood-based proteins.

INFLAMMATION accounts for 5%–10% of dT2A's variance, which we have previously shown to be strongly

associated with CDR-SB and the diagnosis of clinical "AD" [16]. Its effect on δ is independent of other recognized dementia risks (e.g., age, APOE, and depression) which are mediated by yet other serum proteins [11–13]. In their aggregate, INFLAMMATION and those dementia risks account for between 24% (ADNI) to 38% (TARCC) of dT2A's variance. These are clinically salient fractions. Each quintile change in the d-scores of nondemented persons triples MCI conversion risk, while crossing a d-score threshold for dementia conversion increases the risk of "AD"'s diagnosis by 73-fold [44,45].

Other blood-based protein biomarkers, perhaps acting through independent and as yet unspecified latent processes, might further increase the explained variance in dementia severity. For example, O'Bryant et al., [46] from the beginning in the TARCC's data, have proposed a blood-based biomarker panel for AD-specific diagnosis composed of eight proteins. Interestingly, their set also replicates in the ADNI [35]. Because O'Bryant's set is largely nonoverlapping with INFLAMMATION's indicators, it seems likely that an even greater fraction of dementia's severity will eventually be accounted for on the basis of easily measured blood-based protein biomarkers. However, that also suggests that neither INFLAMMATION nor O'Bryant's panel is likely to offer a comprehensive explanation for δ 's variance, or even an AD-specific one.

How blood-based protein biomarkers effect changes in cognitive performance cannot be addressed in these data, and would be speculative at this point. However, future analyses of the ADNI's data can address relationships between these protein-mediated dementia risks (including also age, APOE, and depression, etc.) and AD's neuroimaging and cerebrospinal biomarkers. As both the ADNI and TARCC are longitudinal studies, we can also test the association of blood-based protein biomarkers to δ 's temporal evolution and the role of AD's neuroimaging and cerebrospinal biomarkers as potential mediators of their relationship. The ABIL study also collects plasma protein biomarkers by RBM and neuroimaging data and may be able to replicate INFLAMMATION's impact on dT2A and their associations with neuroimaging data in the ADNI.

Hippocampal atrophy and AD-specific CSF biomarkers have already been related to δ [47]. Regardless, there are reasons to suspect that the effects of many blood-based protein biomarkers on δ are not mediated through AD-specific pathology(ies). First, δ itself is agnostic to dementia's etiology [19]. A biomarker's association with δ , even in clinically diagnosed "AD" cases, does not necessarily implicate AD, as "AD" is often diagnosed in the absence of AD-specific pathology(ies) [48,49]. Other processes must determine the d-score in such cases.

Etiology-specific cognitive variance is residual (i.e., orthogonal) to δ [20]. This suggests that attempts to define disease-specific biomarker panels relating to etiology-specific variance in cognitive performance will not be asso-

ciated with dementia severity. By contrast, δ 's biomarkers, very likely to explain dementia severity and conversion risks, are unlikely to be disease-specific.

Instead, the largely nonoverlapping biomarkers we have associated with independent δ -related risk factors may be mediating unique, competitive, and independent dementia-related processes which should interact summatively to determine dementia severity, as measured by δ . Dementia severity is therefore likely to be "overdetermined" by multiple competing and independent δ -related processes of which age, APOE, depression, and INFLAMMATION are a subset [50]. It remains to be seen (i.e., in the ADNI) whether AD-specific neuroimaging and/or CSF biomarkers mediate those effects on δ , or whether they contribute independently to δ 's variance. Age and depression (at least) do not appear to be mediated through AD-specific neurodegeneration [51,52]. δ 's vulnerability to multiple independent processes provides a rationale for cognitive "reserve" and intelligence's relevance to that concept.

Moreover, INFLAMMATION's indicators might also be contributing to and/or influenced by other processes, unmodeled by this analysis. Those effects would appear in the indicators' residuals. Unmodeled influences on the observed protein concentrations could explain nondementing effects of the same biomarker in other organs or even in brain, given that not all cognitive domains are functionally salient [3,45]. Several δ -related serum protein biomarkers mediate both age and depression's independent and mutually adjusted effects on δ (apparently as contributors to independent competing processes) [11,13].

SEM can also address the temporal associations among δ -related biomarkers. For example, we have shown that THPO, which partially mediates gender's association with δ , is associated with δ 's intercept (i.e., in a latent growth curve model) but not its slope [10]. Thus, that serum protein may initiate prospective changes in dementia severity as measured by δ without prosecuting them. It becomes an empirical question whether AD-related neuroimaging and/or CSF biomarkers in the ADNI act independently of INFLAMMATION, mediate its prospective association with δ , or engender prospective changes in INFLAMMATION downstream of their central nervous system effects.

Finally, our model says nothing regarding the possible contributions of other, unmodeled, INFLAMMATION-independent processes impacting δ . If such processes can be defined, they should increase the explained variance in δ while improving model fit, or at least without sacrificing fit. O'Bryant's work with a minimally overlapping set of blood-based proteins suggests that peripheral blood-based protein biomarkers may eventually account for a sizable fraction of δ 's variance.

Moreover, because δ is almost uniquely responsible for dementia severity, any δ -related biomarker is potentially clinically salient. Serum resistin, which has been shown to be elevated in AD [53,54], fully attenuates the GDS' 2.3-

fold 5 yr prospective MCI conversion risk in the TARCC [33], even though it correlates only $r = -0.33$ with δ and mediates but 33% of GDS' effect on δ [13]. Ironically perhaps, serum resistin levels are lowered by galantamine [55]. Thus, the impact of acetylcholinesterase inhibitors on dementia severity might be mediated by effects on blood-based proteins and limited to the cognitive effects of depressive symptoms (or age [11]) and not AD-specific neurodegeneration.

In summary, by SEM, we can replicate the latent interactions of multiple blood-based protein biomarkers across cohorts and biofluids. The association of these biomarkers with dementia severity is an empirical fact, now replicated across cohorts and biofluids. The mechanisms by which these proteins exert these effects are still unclear. As they are acting via a latent construct, their interrelationships may be more important to dementia severity than their observed concentrations. Attempts to link observed concentrations with clinical outcomes are likely to be undermined by measurement bias, cohort differences, and biomarker contributions to other unmodeled outcomes. Few of INFLAMMATION's indicator proteins would make attractive individual targets for antidementia interventions. It may be more effective to modulate the entire latent INFLAMMATION process via intervention on key upstream proteins (e.g., IL-10 or IL-12) and we should be prepared for the unintended consequences of those interventions in other organ systems (witness AD's "inverse" association with cancer risk). Conversely, because δ is agnostic to dementia's etiology, successful interventions on functionally salient cognitive impairment in other (i.e., non-AD) conditions may generalize to δ in AD (e.g., the tumor necrosis factor alpha antagonist etanercept's purported salutary effects on dementia due to multiple causes) [56].

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(www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. The ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Dr. Royall has disclosed his co-invention of δ , its homologs and orthologs 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 δ 's construction and biomarkers.

Dr. Palmer has disclosed his co-invention of δ , its homologs and orthologs to the UTHSCSA.

These data were previously presented at the 2018 International Congress on Alzheimer's Disease (ICAD). Chicago, IL, 2018.

Royall DR, Bishnoi R, Palmer RF. [abstract] Blood-based protein predictors of dementia severity as measured by δ : Replication across biofluids and cohorts. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*. 2018;14:P649-650.

RESEARCH IN CONTEXT

1. Systematic review: Blood-based biomarkers have been associated with the omnibus dementia severity metric, δ . All relevant citations have been appropriately cited.
2. Interpretation: Nine proteins measured in serum (Texas Alzheimer's Research and Care Consortium) or plasma (Alzheimer's Disease Neuroimaging Initiative) explained $\cong 10\%$ of δ 's variance in both samples, independently of age, APOE, education, and gender. All loaded significantly on a latent INFLAMMATION construct, and positively or negatively, depending on their known roles are PRO- or ANTI-inflammatory proteins, respectively. The parameters of interest replicated across random 50% splits of the Texas Alzheimer's Research and Care Consortium's sample, and across biofluids in the Alzheimer's Disease Neuroimaging Initiative.
3. Future directions: This analysis strengthens the case for using latent variables in a structural equation model framework to understand biomarker associations with dementia severity.

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