


RESEARCH ARTICLE

Abnormal cerebrospinal fluid levels of amyloid and tau are associated with cognitive decline over time in cognitively normal older adults: A monozygotic twin study

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Abstract

Introduction: The contribution of genetic and environmental factors to the relation between cerebrospinal fluid (CSF) biomarkers and cognitive decline in preclinical Alzheimer's disease remains unclear. We studied this in initially cognitively normal monozygotic twins.

Methods: We included 122 cognitively normal monozygotic twins (51 pairs) with a follow-up of 4.3 ± 0.4 years. We first tested associations of baseline CSF A β 1-42/1-40 ratio, total tau (t-tau), and 181-phosphorylated-tau (p-tau) status with subsequent cognitive decline using linear mixed models, and then performed twin specific analyses.

Results: Baseline abnormal amyloid- β and tau CSF markers predicted steeper decline on memory ($p \leq .003$) and language ($p \leq 0.04$). Amyloid- β and p-tau markers in one twin predicted decline in memory in the co-twin and tau markers in one twin predicted decline in language in the co-twin (r range $-0.26, 0.39$; p 's $\leq .02$).

Discussion: These results suggest that memory and language decline are early features of AD that are in part determined by the same genetic factors that influence amyloid- β and tau regulation.

KEYWORDS

amyloid-beta, biomarkers, cerebrospinal fluid, cognition, cognitive decline, longitudinal design, monozygotic twins, neuropsychology, preclinical Alzheimer's disease, tau

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by amyloid- β and tau accumulation in the brain.¹ AD has a long preclinical stage of around 10 years in which amyloid- β is abnormal while cognition is unimpaired.²⁻⁶ Recent studies indicated

that cerebrospinal fluid (CSF) tau levels may already be abnormally increased in preclinical AD.^{7,8} It is a major question which of these pathologies drive cognitive decline and which cognitive domain is most sensitive for decline in this early stage. Such information is necessary for the design of secondary prevention trials, that aim to prevent onset of cognitive impairments in individuals with preclinical AD.

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Several studies have examined the association of single^{9–15} or combined^{16–18} CSF amyloid- β and tau markers with subsequent cognitive decline among cognitively normal individuals, but findings have been inconsistent. These discrepancies may reflect the variability of cognitive tasks or composite measures used, size and composition of participant samples, and variability in relative proportion of abnormal AD biomarkers present in groups. In addition, it is unclear how genetic or environmental factors influence the relation between amyloid- β and tau and cognitive decline. Twin studies allow determining the contribution of genetic and environmental influences on diseases such as AD and, therefore, can be an important scientific tool contributing to improved understanding of how AD develops. We previously showed in monozygotic twins that the cross-sectional relation between amyloid- β pathology and cognition was explained by shared genetic and environmental factors.¹⁹ How genetic and environmental factors further influence the association between CSF amyloid- β , t-tau, and p-tau and cognitive decline over time remains unknown.

Our aim was to investigate the relationship of AD CSF markers for amyloid- β and tau pathology and subsequent cognitive decline in a wide range of cognitive domains in cognitively normal older monozygotic twins. We further studied the contribution of genetic and environmental influences on those relations with twin specific statistical analyses. First, within twin pair correlations, to test genetic and environmental contributions to cognitive decline, where similarities in monozygotic twins reflect genetic contribution and differences can be attributed to unique environmental factors. Second, cross-twin cross-trait (CTCT) analyses, which provide the opportunity to investigate whether CSF amyloid- β and tau levels of one twin can predict cognitive decline in their co-twin. Because monozygotic twin pairs share 100% of their genetic material and are raised in a partly shared environment, significant CTCT correlations point toward genetic or shared environmental factors as the cause of the relation between CSF markers of amyloid- β and tau pathology and subsequent cognitive decline. Because evidence for shared family environment on either neurodegenerative disorders or cognition in the elderly is absent,^{20–23} a shared genetic source for the relation between CSF biomarkers and cognitive decline would be more likely. Third, we tested whether within twin pair differences in CSF biomarkers were related to within twin pair differences in cognitive decline over time. Because differences within monozygotic twin pairs can only be explained by subject specific exposure to unique environmental factors, a significant association of such twin difference analyses indicates that the relation between CSF biomarkers and cognitive performance over time is partly driven by unique environmental factors that influence both amyloid- β and tau regulation and cognitive decline.

2 | METHODS

2.1 | Participants

For the present study, we included cognitively normal monozygotic twins from the longitudinal Amsterdam sub-study of the EMIF-AD Pre-

RESEARCH IN CONTEXT

- 1. Systematic Review:** We reviewed the literature using traditional sources (e.g., PubMed, Google Scholar). Literature provided inconsistent results regarding the relationship between cerebrospinal fluid (CSF) amyloid- β and tau markers and subsequent cognitive decline in preclinical Alzheimer's disease (AD). No studies were found that investigated the effect of genetic or environmental factors on the association between amyloid- β and tau and cognitive change over time in older monozygotic twins.
- 2. Interpretation:** Our findings suggest that memory and language decline are early features of AD, abnormal levels of both amyloid- β and tau effects the rate of cognitive decline, and the relation between CSF AD biomarkers and memory decline is in part driven by shared genetic factors.
- 3. Future Directions:** Our results should be replicated in larger twin datasets, and, in particular, in longitudinal settings.

clinAD cohort.²⁴ Currently, twins have been followed for an average of 4.3 years with two follow-up visits (FU1, FU2, after 2.3 and 4.3 years, respectively). For this study, we included twins who had CSF assessment available at their first (i.e., baseline) visit and at least one cognitive follow-up ($n = 122$). All participants underwent an extensive baseline assessment including neuropsychological assessment, years of education, buccal cell collection, lumbar puncture, and blood sampling. At baseline neuropsychological testing was performed at home, and at both follow-up visits testing was performed at either the VU medical center or at home when participants were unable to come to the hospital (FU1 $n = 3$, FU2 $n = 7$). DNA analysis confirmed twin zygosity. The research was performed according to the principles of the Declaration of Helsinki and was approved by the Medical Ethics Review Committee of the VU University Medical Center, and all participants gave written informed consent.

2.2 | Neuropsychological assessment

We assessed cognitive functioning with a standardized neuropsychological test battery covering four domains (memory, attention, executive function, and language). For memory, we used the total immediate recall and delayed recall of the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT),^{25,26} the 3 and 20 minute recall of the Rey Complex Figure Test (RCFT)^{27,28} and the total score of the FNAME-names and -occupation delayed recall.^{29,30} For attention, we used the Trail Making Test (TMT) part A³¹, the Digit Symbol Substitution Test³² and the forward condition of the Digit Span.³² For executive function, we used TMT part B³¹ (corrected for TMT part A), the

backward condition of the Digit Span,³² the Dutch version of the Controlled Oral Word Association Test (letter fluency), with letters D A T.³³ For language, we used the category fluency (animal fluency) 1 minute and the graded naming test (GNT).^{34–37} FNAME data were missing in nine participants (7%) at baseline, in nine participants (7%) at FU1 and four participants (4%, of $n = 102$) at FU2 due to lack of time or fatigue effects, seeing this was the last test in the test battery. On the other tests, 0% to 4% of the test scores were missing.

2.3 | Cerebrospinal fluid analysis

At baseline CSF was obtained through lumbar puncture.²⁴ Levels of A β 1-40, 1-42, total-tau (t-tau), and 181-phosphorylated-tau (p-tau) were measured with kits from the same batch according to manufacturer instructions (ADx Neurosciences/Euroimmun).³⁸ Intra-assay coefficients of variation (CVs) were less than 3% (A β 1-40 2.2%; A β 1-42 2.5%; t-tau 2.4%; p-tau 2.9%) (average %CV of clinical samples analyzed over 4 runs in total). Inter-assay CVs were below 14% (A β 1-40 10.3%; A β 1-42 7.5%; t-tau 13.2%; p-tau 10.8%) (average %CV of low and high quality control samples analyzed over 4 runs in total). We used the CSF A β 1-42/1-40 ratio as markers for amyloid- β aggregation, with lower values indicating abnormality.³⁹ CSF t-tau and p-tau levels were used as biomarkers for tau pathology, with higher levels of t-tau being indicative for more tau-based neurodegeneration, and higher levels of p-tau levels being indicative for more AD related tau aggregation.^{40–42} Additionally, we used the CSF p-tau/A β 1-42 ratio, with higher values indicating abnormality. We used Gaussian mixture modeling to determine cutoffs for CSF A β 1-42/1-40 ratio, t-tau, p-tau, and p-tau/A β 1-42 ratio. For every CSF marker two distributions showed the best fit, and we used the point of intersection between these distributions as a cutoff to indicate abnormality (CSF A β 1-42/1-40 ratio <0.066; CSF t-tau >593 pg/ml; CSF p-tau >109 pg/ml; CSF p-tau/A β 1-42 ratio >0.104; Figure S1). Additionally, A/T (A-T-, A+T-, A+T+) and A/P (A-P-, A+P-, A+P+) subgroups were determined by using CSF A β 1-42/1-40 ratio cutoff (A), CSF t-tau cutoff (T), and CSF p-tau cutoff (P).

2.4 | APOE genotyping

To assess APOE ϵ 4 allele carriership, all participants were genotyped using Illumina Global Screening array (GSA) with shared custom content (Illumina, Inc) and established quality control measures were applied.⁴³ Genotype imputation was previously described.⁴⁴ In short, we used high-quality genotyping in all individuals (individual call rate > 98%, variant call rate > 95%) and Hardy-Weinberg equilibrium-departure was considered significant at $p < 1 \times 10^{-6}$. Genotypes were prepared for imputation using bcftools (v1.9).⁴⁵ This was followed by haplotype phasing using SHAPEIT⁴⁶ and imputation of unobserved genotypes using Minimac3 and a precompiled Haplotype Reference Consortium (HRC) reference panel.⁴⁷

2.5 | Statistical analysis

Baseline (first visit), year 2 follow-up (FU1), and year 4 follow-up (FU2) individual neuropsychological tests were standardized against the baseline mean and standard deviation of each test from the entire Amsterdam EMIF-AD PreclinAD cohort ($n = 204$),²⁴ and domain specific tests were subsequently averaged into four cognitive composite scores (i.e., memory, attention, executive function, and language). For each participant, results of at least two-thirds of the cognitive tasks used for a specific domain had to be available to construct a cognitive composite score for this participant, otherwise data were missing. TMT part A and B scores were inverted by multiplying the z-score with -1, so that, for all domains, lower scores reflect worse performance. At baseline and FU1, cognitive data were available for 122 participants (51 complete twin pairs) and at FU2 for 102 participants (41 complete twin pairs). CSF tau data were log-transformed to improve normal distribution of the data.

We used generalized estimating equations (GEE) models including random effect for family, thereby correcting for clustering in the data, to compare demographic characteristics between amyloid- β abnormal and amyloid- β normal participants. We applied linear mixed models (LMMs) to estimate the effects of dichotomous baseline CSF markers (separate models for each marker), on baseline cognitive performance (separate models per cognitive composite score) and change in cognition over time by including an interaction term CSF marker \times time. Models included subject-specific random intercepts and slopes, a random effect for family, thereby correcting for clustering in the data, and were adjusted for age, sex, and years of education. Next, for significant overall group relations, we assessed combined effects of CSF markers on cognition, by repeating analyses using A/T and A/P groups as predictors (separate model per group and cognitive composite score). Estimated marginal means and contrasts were used to determine group differences at baseline and over time. We corrected for multiple testing with the false discovery rate (FDR) procedure using a q-value of 0.05.⁴⁸ Post-hoc tests were done for associations between dichotomous CSF markers and individual neuropsychological test scores (separate models per CSF marker and neuropsychological test scores).

We performed the following three twin specific analyses (1) Monozygotic twin pair intraclass correlations of cognitive slopes, extracted from longitudinal models, to investigate the relative contribution of genetic and environmental influences on cognitive decline. Correlations were obtained both with and without adjusting for age, sex, and years of education. (2) CTCT analysis, testing whether CSF markers in one twin can predict cognitive decline in the co-twin, and vice versa. Significance suggests genetic factors underlie this association. (3) Within twin pair difference analyses, testing the association between within twin pair difference in CSF markers and within twin pair difference in cognitive decline. Significance suggests that the unique environmental factors influencing CSF marker and cognitive decline are correlated. For these analyses continuous CSF markers were used. The data were reordered, with participants classified as Twin 1 (lowest CSF A β 1-42/1-40 ratio levels) or Twin 2 (highest CSF

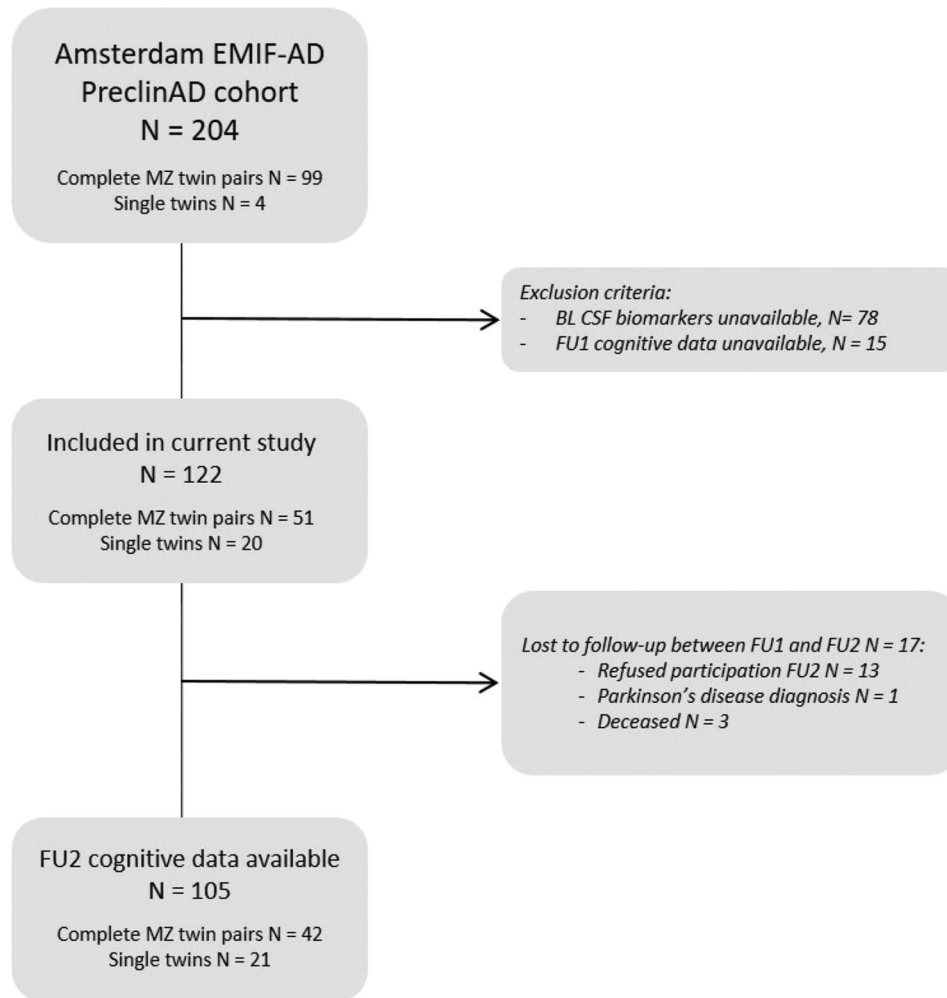


FIGURE 1 Flowchart of participant selection. None of the participants developed AD dementia or MCI between BL and FU2. EMIF-AD, Innovative Medicine Initiative European Medical Information Framework for Alzheimer's Disease; PreclinAD, preclinical Alzheimer's disease; BL, Baseline; FU, follow-up; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MZ, monozygotic

$A\beta_{1-42}/1-40$ ratio levels) within twin pairs. For twin specific analyses, we extracted subject slopes for cognitive performance over time from LMMs including subject-specific random intercepts and slopes, adjusted for age, sex, and years of education, to be analyzed as dependent variables. Statistical analyses were performed in RStudio (version 3.6.1, 'Action of the Toes', <http://www.r-project.org/>), using mixtools, lme4, lmerTest, emmeans, ggplot2, and ICC packages.

3 | RESULTS

3.1 | Sample description

We included 122 cognitively normal older participants, comprising of 51 monozygotic twin pairs and 20 individuals from pairs in which CSF was only measured in one twin (mean age 68.8 ± 6.74 , 53% female, 11.4 ± 2.7 years of education, mean follow-up 4.3 ± 0.4 years) (Table 1, Figure 1). Twenty participants (16%, 5 complete pairs) were classified

as amyloid- β abnormal based on CSF $A\beta_{1-42}/1-40$ ratio cutoff, and 47 participants (39%, 20 complete pairs) carried at least one *APOE* $\epsilon 4$ allele. Amyloid- β abnormal participants were older, more often *APOE* $\epsilon 4$ carriers, had lower baseline memory composite scores, and higher t-tau, p-tau, and p-tau/ $A\beta_{1-42}$ ratio levels compared to individuals classified as amyloid- β normal (Table 1). Twelve participants (10%, 2 complete twin pairs) were classified as t-tau abnormal, 15 (12%, 1 complete twin pair) as p-tau abnormal and 27 (22%, 7 complete twin pairs) as p-tau/ $A\beta_{1-42}$ ratio abnormal.

3.2 | Association between CSF markers and cognitive performance

At baseline, abnormal CSF markers were not associated with baseline composite cognitive scores. For single tests, we observed that an abnormal CSF $A\beta_{1-42}/1-40$ ratio was related to lower baseline RCFT 3 and 20 min recall scores, an abnormal CSF p-tau/ $A\beta_{1-42}$ ratio was

TABLE 1 Baseline and follow-up characteristics

Baseline characteristics	Overall	Amyloid- β normal	Amyloid- β abnormal
N	122	102	20
Complete MZ pairs	51	39	5
Age, mean (SD)	68.8 (6.74)	67.8 (6.29)	74.0 (6.69)**
Female, n (%)	65 (53)	51 (50)	14 (70)
APOE ϵ 4 carrier, n (%)	47 (38.8)	34 (33.7)	13 (65.0)*
Years of education, mean (SD)	11.43 (2.68)	11.55 (2.54)	10.85 (3.36)
MMSE, mean (SD)	28.93 (1.22)	29.03 (1.11)	28.45 (1.61)
Memory (z score composite), mean (SD)	0.06 (0.72)	0.17 (0.70)	-0.49 (0.57)**
Attention (z score composite), mean (SD)	0.12 (0.67)	0.15 (0.67)	-0.08 (0.68)
Executive Function (z score composite), mean (SD)	0.03 (0.68)	0.04 (0.69)	-0.01 (0.64)
Language (z score composite), mean (SD)	0.03 (0.85)	0.07 (0.84)	-0.14 (0.88)
CSF A β 1-42/1-40 ratio, mean (SD)	0.10 (0.03)	0.11 (0.02)	0.05 (0.01)**
CSF t-tau, mean (SD), pg/ml	416.17 (143.59)	372.40 (94.38)	639.40 (145.69)**
CSF t-tau cutoff, abnormal, n (%)	12 (10)	0 (0)	12 (60)
CSF p-tau, mean (SD), pg/ml	76.98 (44.39)	62.52 (18.35)	150.70 (62.55)**
CSF p-tau cutoff, abnormal, n (%)	15 (12)	2 (2)	13 (65)
CSF p-tau/A β 1-42 ratio, mean (SD)	0.11 (0.10)	0.07 (0.02)	0.30 (0.13)**
CSF p-tau/A β 1-42 ratio cutoff, abnormal, n (%)	27 (22)	7 (7)	20 (100)
A/T groups			
A-T-, n (%)	102 (84)	-	-
A+T-, n (%)	8 (6)	-	-
A+T+, n (%)	12 (10)	-	-
A/P groups			
A-P-, n (%)	100 (82)	-	-
A+P-, n (%)	7 (6)	-	-
A+P+, n (%)	13 (10)	-	-
Follow-up characteristics			
Follow-up, mean (SD), y	4.29 (0.42)	4.27 (0.41)	4.43 (0.47)
Last known status, n (%)			
Active	105 (86)	90 (88)	15 (75)
Lost to follow-up	14 (11)	10 (10)	4 (20)
Death	3 (2)	2 (2)	1 (5)

Amyloid- β groups were based CSF A β 1-42/1-40 ratio using cutoff <0.066 . Differences between baseline amyloid- β normal and amyloid- β abnormal participants were assessed using generalized estimating equation models corrected for family relatedness.

Abbreviations: APOE, apolipoprotein E; A, amyloid- β groups normal/abnormal based on CSF A β 1-42/1-40 ratio cutoff <0.066 ; T, t-tau groups normal/abnormal based on CSF t-tau cutoff >593 pg/ml; P, p-tau groups normal/abnormal based on p-tau cutoff >109 pg/ml; MZ, monozygotic; MMSE, mini mental state examination; CSF, cerebrospinal fluid; A β , amyloid beta; t-tau, total-tau; p-tau, 181-phosphorylated-tau; SD, standard deviation.

* $p < .05$

** $p < .01$ different from normal amyloid- β group.

related to lower baseline RCFT 20 min recall scores, and abnormal CSF p-tau was related to lower baseline FNAME occupation recall scores (Table S1). Longitudinally, abnormal CSF A β 1-42/1-40 ratio, t-tau, p-tau, and p-tau/A β 1-42 ratio at baseline were associated with steeper decline in memory (all $p < .001$) and language (all $p < .01$) composite scores (Table S1, Figure 2). In addition, abnormal CSF p-tau/A β 1-42

ratio was associated with steeper decline in executive function composite scores ($p = .03$). No significant associations were found for CSF biomarker abnormality with composite attention. When combining amyloid- β and tau status, we observed that over time A-T- subjects improved in memory and language, A+T- subjects remained stable, and A+T+ subjects declined. The A+T+ group also showed a steeper

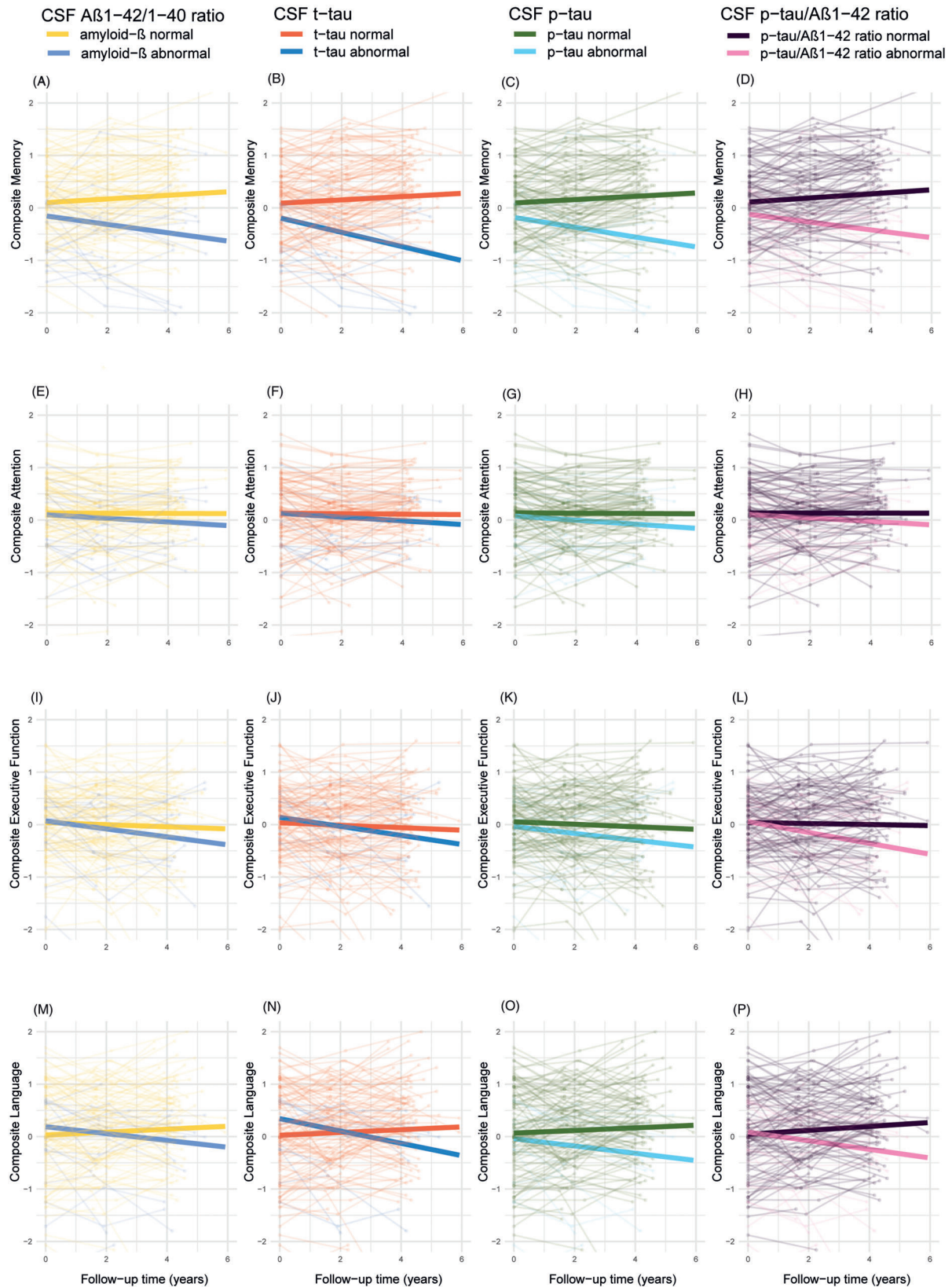


FIGURE 2 Effects of CSF biomarkers on changes in cognitive composite scores. Neuropsychological tests were z-transformed and averaged for each cognitive domain. Linear mixed models were adjusted for age, sex, and years of education. After correcting for multiple testing (FDR) results remained the same. Cutoffs based on Gaussian Mixture Modelling (CSF $A\beta_{1-42/1-40}$ ratio abnormal < 0.066 ; CSF t-tau abnormal > 593 pg/ml; CSF p-tau abnormal > 109 pg/ml; CSF p-tau/ $A\beta_{1-42}$ ratio abnormal > 0.104). Number of participants per group: CSF $A\beta_{1-42/1-40}$ ratio normal $n = 102$, abnormal $n = 20$; CSF t-tau normal $n = 110$, abnormal $n = 12$; CSF p-tau normal $n = 107$, abnormal $n = 15$; CSF p-tau/ $A\beta_{1-42}$ ratio normal $n = 95$, abnormal $n = 27$. $A\beta$, Amyloid-beta; CSF, cerebrospinal fluid; t-tau, total-tau; p-tau, 181-phosphorylated-tau.

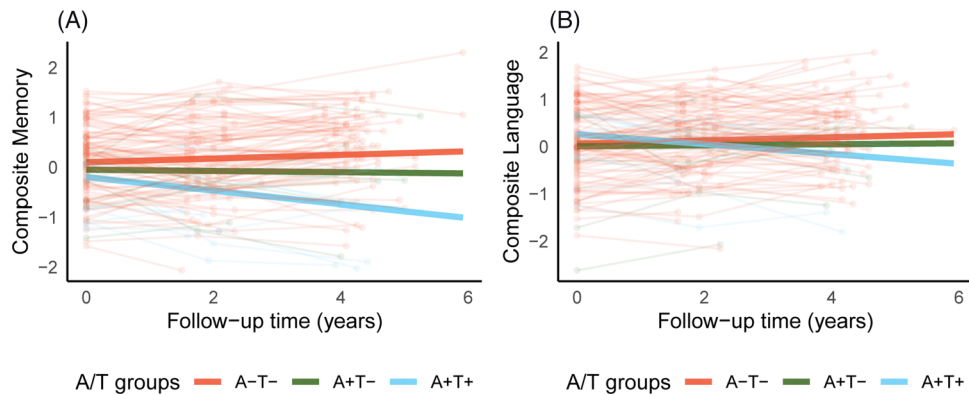


FIGURE 3 Effects of A/T groups on changes in memory and language composite scores. Spaghetti plots show individual trajectories on two domains: (A) Composite memory; (B) Composite Language. Separate lines represent the adjusted mean trajectory of A/T groups. CSF cutoffs based on Gaussian Mixture Modelling (CSF A β 1-42/1-40 ratio abnormal < 0.066; CSF t-tau abnormal > 593 pg/ml). Number of participants per group: A-T- $n = 102$, A+T- $n = 8$, A+T+ $n = 12$. A/T, Amyloid- β and t-tau groups; CSF, cerebrospinal fluid; t-tau, total-tau.

decline in memory and language over time compared to A-T- group (Table S2, Figure 3). Results were similar when combining amyloid- β and p-tau groups (Table S2, Figure S2).

3.3 | Twin specific analyses

We next performed twin specific analyses to estimate contributions of genetic or environmental factors to cognitive decline in early AD stages and to its association with CSF biomarkers. We first studied monozygotic twin pair correlations and found that all baseline composite scores correlated across twin pairs (r ranging between 0.41 and 0.75, Figure 4, Table S3). Changes in memory, attention, and language performance over time were also correlated across twin pairs (r ranging between 0.23 and 0.76, Figure 4, Table S3), while change in executive function was not ($r = 0.08$, $p = .28$). Correlation coefficients remained similar when correcting for age, sex, and years of education (r range 0.31-0.59), except for language performance over time that became somewhat stronger after correction for covariates (Table S3). We then performed CTCT analyses, and observed that lower, more abnormal CSF A β 1-42/1-40 ratios and higher, more abnormal t-tau, p-tau, and p-tau/A β 1-42 ratio levels in one twin predicted worse memory scores in their co-twin at baseline (CSF A β 1-42/1-40 ratio: $r = 0.30$, $p = .005$; t-tau: $r = -0.39$, $p < .001$; p-tau: $r = -0.33$, $p = .002$; p-tau/A β 1-42 ratio: $r = -0.21$, $p = .04$). In addition, lower CSF A β 1-42/1-40 ratios, higher p-tau levels and higher p-tau/A β 1-42 ratio levels in one twin predicted steeper decline in memory over time in their co-twin (CSF A β 1-42/1-40 ratio: $r = 0.39$, $p < .001$; p-tau: $r = -0.23$, $p = .02$; p-tau/A β 1-42 ratio: $r = -0.29$, $p = .003$) (Figure 5A, Table S4A). Also, higher levels of CSF t-tau and p-tau and higher CSF p-tau/A β 1-42 ratios in one twin could predict steeper decline in language over time in the co-twin (t-tau: $r = -0.24$, $p = .02$; p-tau: $r = -0.26$, $p = .008$; p-tau/A β 1-42 ratio: $r = -0.21$, $p = .04$), but not CSF A β 1-42/1-40 ratio ($r = 0.18$, $p = .08$) (Figure 5A, Table S4A). CSF markers in one twin did not predict lower scores or decline in composites attention and executive function in their co-twin. Finally, we performed within twin pair differ-

ence analyses, restricted to complete twin pairs (for all markers $n = 51$ twin pairs), which did not show any significant associations between CSF markers and cognitive composite scores (Figure 5B, Table S4B).

4 | DISCUSSION

We found that older individuals with normal cognition who have abnormal CSF biomarkers for AD pathology showed steeper decline in memory and language functioning over time, and that these associations are in part determined by the same genetic factors.

We observed that abnormal CSF amyloid- β was associated with steeper decline in memory performance over time, which is in line with previous studies.^{9-13,15} We also observed that abnormal levels of CSF t-tau and p-tau were related to steeper decline in memory functioning over time compared to normal levels of CSF t-tau and p-tau, which also replicates previous findings in cognitively normal individuals.¹⁶ All CSF markers were also associated with steeper decline in language functioning over time. Repeating analyses using the CSF p-tau/A β 1-42 ratio generated similar results. Individuals who had both abnormal CSF amyloid- β and tau levels showed the steepest decline in memory and language functioning, compared to individuals with both markers being normal, who showed improvement in cognitive functioning reflecting learning effects.⁴⁹ This learning effect was absent in individuals who had abnormal CSF amyloid- β levels only, although this slope did not significantly differ from individuals with normal CSF amyloid- β and tau. This suggests that changes in learning may be a very early effect of amyloid- β aggregation, and future research should include tests that target learning effects to further investigate very early amyloid- β effects on cognition. Together, our findings align with previous studies showing a combination of both markers being abnormal to be associated with steeper decline in cognitive composite,¹⁷ visuospatial episodic memory,¹⁶ and higher risk to decline on global Clinical Dementia Rating-scale.¹⁸ We found no relation between CSF AD markers and decline on attention. Abnormal CSF p-tau/A β 1-42 ratios were related to a steeper decline in executive function over time. Possibly, with a

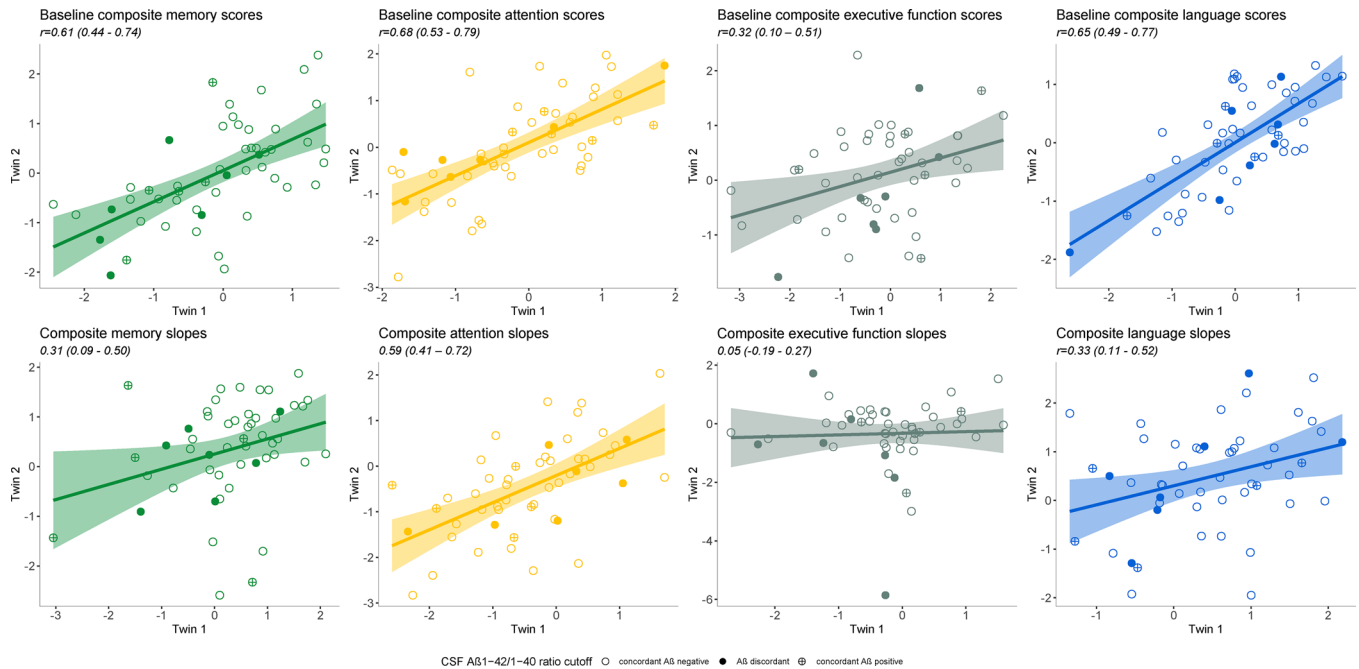


FIGURE 4 Monozygotic twin pair correlations. Intra class correlation values for association of memory and language composite scores between one twin and their co-twin (Model 2); all $p < .0001$. Each dot represents one twin pair, open circles indicate twin pairs who are concordant normal based on CSF $A\beta_{1-42}/1-40$ ratio cutoff ($n = 39$ twin pairs), circles with a cross inside indicate twin pairs who are concordant abnormal based on CSF $A\beta_{1-42}/1-40$ ratio cutoff ($n = 5$ twin pairs), and solid filled circles indicate discordant twin pairs based on CSF $A\beta_{1-42}/1-40$ ratio cutoff ($n = 7$ twin pairs).

longer follow-up period, these individuals may start to show decline in these other domains as well. Furthermore, our post-hoc analyses suggest that in individuals with normal cognition, composite measures may not be sensitive enough to pick up cognitive alterations associated with CSF AD markers. While amyloid- β did not correlate with baseline composite scores for memory, we did find associations with the RAVLT immediate and delayed recall (episodic memory) and FNAME-names (associative memory) scores, and in language by the object naming task (GNT), as we previously reported in this cohort.¹⁹ Deficits in object naming are related to episodic memory⁵⁰ and have previously been associated with preclinical AD.^{12,51} Together, these findings suggest that single tests may be more sensitive to pick up decline in very early AD than a composite in an initially normal population.

Due to our unique monozygotic twin design, we were further able to test the contribution of genetic and environmental factors on the relationship between CSF AD markers and cognitive decline. We found moderate monozygotic twin pair correlations for baseline memory and language composite scores and rates of decline in memory and language (r ranging between 0.31 and 0.68), suggesting that, in addition to genetic factors, unique environmental factors substantially affect baseline performance and decline in memory and language over time. This is in line with previous studies that found that unique environmental factors influence late-life cognitive performance and decline.^{22,23,52} A meta-analysis further showed a decrease in heritability for verbal ability after age 60.⁵³ Heritability estimates for episodic memory also showed a slight decline after 60 years, but this was not significant. Our results suggest that the presence of abnormal amyloid- β may

explain at least part of the decrease in heritability with age, bigger twin studies including AD biomarkers are necessary to further study this. In addition, we previously showed both genetic and environmental factors to influence CSF biomarker levels (monozygotic twin pair correlation $A\beta_{1-42}/1-40$ ratio: 0.50; t -tau: 0.73; p -tau: 0.64).⁸ For preventive purposes, future research should focus on identifying these possible modifiable risk factors. For this, longitudinal studies in large genetically informative preclinical AD cohorts are needed to further investigate causal effects of modifiable environmental risk factors on AD biomarkers (e.g., Mendelian randomization).

We further studied whether rates of cognitive decline are determined by the same genetic and environmental factors that influence amyloid- β and tau regulation. CTCT analyses showed that CSF markers in a twin could predict cognitive decline in the co-twin and vice versa, suggesting the relationship between CSF markers and cognitive decline to be largely driven by shared genetic pathways. The within twin pair difference analyses were, however, not significant, suggesting that unique environmental factors that explain differences in CSF marker levels do not resemble those explaining differences in cognitive decline. This may reflect that the effect of amyloid- β and tau pathology on cognitive decline in this very early stage is still subtle, and that larger samples would be required to detect significant within twin pair difference associations. It must be noted that the sample size is halved for this twin specific analyses, which may have decreased statistical power. Also, possibly, within twin pair differences may become more pronounced at longer follow-up, especially when investigating preclinical AD.⁵⁴

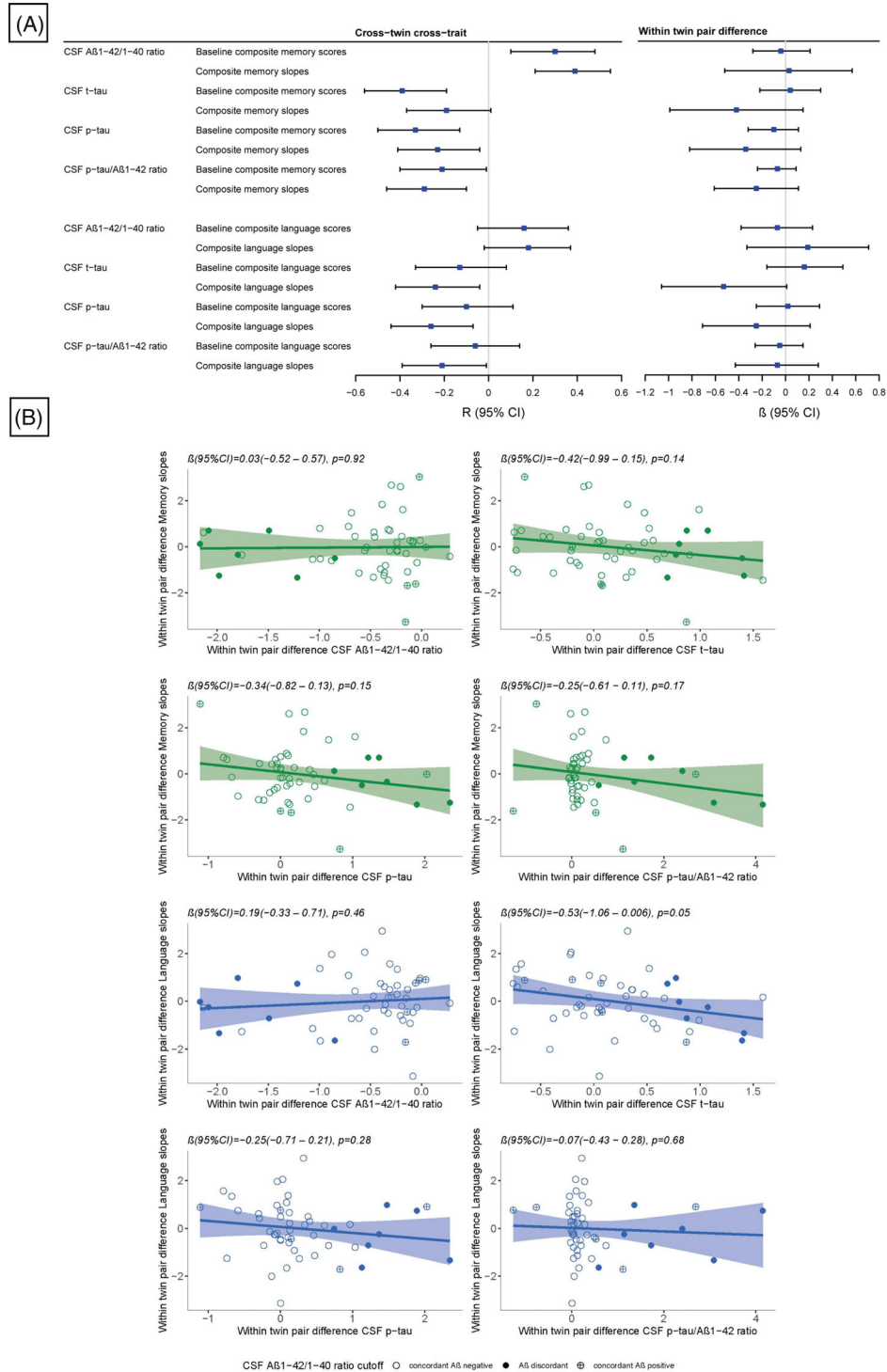


FIGURE 5 Cross-twin cross-trait and within twin pair difference analyses. (A) Left: Cross-twin cross-trait; Data are displayed as correlation coefficient (SE). Correlation coefficient indicates the correlation of the CSF marker in one twin with the cognitive composite score in its co-twin. Cross-twin cross-trait analyses are shown for variables that had a statistically significant association in the whole cohort (Table S1). For exact numbers see Table S4A. Right: Within twin pair difference; Linear regression results are shown for the relation between the standardized difference scores (z-scores) within a twin pair per CSF marker. Data are displayed as beta (95% CI). Beta indicates the association between the within twin pair difference in the CSF marker and the within twin pair difference in the cognitive composite score. Within twin pair difference analyses are shown for variables that had a statistically significant association in the whole cohort (Table S1). For exact numbers see Table S4B. (B) Within twin pair differences in CSF Aβ1-42/1-40 ratio, CSF t-tau, CSF p-tau, and CSF p-tau/Aβ1-42 with within twin pair differences in composite memory and language slopes. Each dot represents one twin pair. Open circles indicate twin pairs who are concordant normal based on CSF Aβ1-42/1-40 ratio cutoff ($n = 39$ twin pairs), circles with a cross inside indicate twin pairs who are concordant abnormal based on CSF Aβ1-42/1-40 ratio cutoff ($n = 5$ twin pairs), and solid filled circles indicate discordant twin pairs based on CSF Aβ1-42/1-40 ratio cutoff ($n = 7$ twin pairs). CSF, cerebrospinal fluid; Aβ, amyloid beta; t-tau, total-tau; p-tau, 181-phosphorylated-tau

A strength of this study is the large sample of cognitively normal older monozygotic twins with CSF AD markers and extensive repeated neuropsychological data over a time period of four years. As we did not include dizygotic twins in our study, we could not exclude that the significant CTCT effects resulted from shared environmental factors between twins. However, shared environmental factors have been shown to have a limited effect on neurological traits,^{20,21} amyloid- β pathology⁵⁵ and cognitive decline.^{22,23} Other possible limitations are that the cutoffs used for CSF markers need validation, because these are only based on the current cognitively normal sample. Future research should aim to further validate cutoffs using either amyloid- β PET or pathology data. Furthermore, the relatively small number of individuals with abnormal amyloid- β may have made it difficult to detect changes in other domains. None of our participants have progressed to mild cognitive impairment or dementia yet. One explanation might be attrition bias, in which the best performing individuals remain in the study. In our study, 17 participants did not complete all follow-up assessments and their current cognitive status is unknown. Comparing their baseline characteristics to remaining individuals showed that they were older, had fewer years of education, and had lower baseline memory, attention, executive function and language composite scores compared to the 105 individuals who were assessed at FU2 (see Table S5). Since these are risk factors for decline, the possibility of attrition bias in our study cannot be excluded. Another explanation for the lack of clinical progression in our cohort is that the follow-up period of 4 years might be too short to capture clinical progression. Indeed, previous studies suggest that it may take an average of 6 years to show clinical progression from preclinical to prodromal AD or dementia.⁵⁴ We are currently collecting 6-year follow-up measures to further investigate cognitive decline and biomarker changes in this cohort. Still, the notion that memory and language showed such strong associations with abnormal CSF biomarker levels in cognitively unimpaired individuals, and these results are in line with those of previous studies, supports the robustness of results and thus the idea that these are the very first cognitive changes in the early stages of the disease.

5 | CONCLUSION

Our findings provide further support that the earliest cognitive changes in AD are found in the memory and language domains. This cognitive decline is foreshadowed by abnormal amyloid- β and tau levels in CSF. Furthermore, the association between CSF markers and cognitive decline is in part determined by shared genetic factors, which warrants further research into specific genes that contribute to both AD pathology and subsequent cognitive decline.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

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