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RESEARCH ARTICLE

Heritability of Alzheimer's disease plasma biomarkers: A nuclear twin family design

Rebecca Z. Rousset[1] | **Anouk den Braber**[2,3] | **Inge M. W. Verberk**[1] | **Lynn Boonkamp**[1] | **David H. Wilson**[4] | **Lannie Ligthart**[3] | **Charlotte E. Teunissen**[1] | **Eco J. C. de Geus**[3]

[1]Neurochemistry Laboratory, Department of Clinical Chemistry, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Neuroscience, Amsterdam, The Netherlands

[2]Alzheimer Center, Department of Neurology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Neuroscience, Amsterdam, The Netherlands

[3]Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[4]Quanterix Corp, Billerica, Massachusetts, USA

Correspondence
Rebecca Z. Rousset, Neurochemistry Laboratory, Department of Clinical Chemistry, Amsterdam UMC, Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081HV, Amsterdam Neuroscience, Amsterdam, the Netherlands.
Email: r.z.rousset@amsterdamumc.nl

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Abstract

INTRODUCTION: Alzheimer's disease (AD) is a highly heritable disease (60%–80%). Amyloid beta (Aβ) 42/40, neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP) are plasma biomarkers for AD. Clinical biomarker research would be served by an understanding of the sources of variance in these markers.

METHODS: Blood concentrations of Aβ42/40, NfL, and GFAP of twins and their families (monozygotic twins: 1574, dizygotic twins: 1266, other: 3657) were analyzed on the Simoa HD-X. Twin-family models were used to estimate proportional genetic contributions to the variance in biomarker levels.

RESULTS: Heritability estimates were 16% for Aβ42/40, 42% for NfL, and 60% for GFAP. NfL and GFAP were significantly correlated with each other (0.37) but not with Aβ42/40.

DISCUSSION: The heritability of Aβ42/40 (16%) is lower than the heritability of AD, suggesting strong environmental influences on this biomarker. The lack of correlation between NfL/GFAP and Aβ42/40 indicates these markers may be on different biological pathways.

KEYWORDS
Alzheimer's disease, amyloid beta 42/40 ratio, glial fibrillary acidic protein, heritability, neurofilament light chain, twin study

Highlights

- Heritability is found for glial fibrillary acidic protein (60%), neurofilament light chain (42%), and amyloid beta (Aβ) 42/40 (16%) plasma levels.
- Aβ42/40 plasma levels are sensitive to person-specific environmental influences.

1 | BACKGROUND

Alzheimer's disease (AD) is the most common cause of dementia and one of the leading causes of death worldwide.[1,2] The disease is progressive, and biological changes have been reported to start occurring up to 20 years before the appearance of the first cognitive complaints.[3–6] In the early stages of AD, these complaints may be non-specific and difficult to capture on clinical scales, making a timely and accurate diagnosis challenging.[7] Plasma biomarkers have been proposed as a tool to detect AD even in the early symptomatic stages.[3,8,9] Biomarkers of interest include the amyloid beta (Aβ) 42/40 ratio,[8,10] a marker of amyloid burden; neurofilament light chain (NfL), a broad marker of neurodegeneration;[8,10,11] and glial fibrillary acidic protein (GFAP),[8,11–13] a marker which reflects astrocyte activation. These three markers were shown to detect AD pathology with varying accuracy,[4,8,10,11,14,15] although NfL and GFAP levels can also be elevated in other neurological conditions.[16,17] However, biological and lifestyle factors otherwise not related to AD or other neurological conditions have also been suggested to influence these plasma biomarker levels, for example, body mass index and kidney dysfunction,[18–24] and may act as confounders in the interpretation of the relationship between plasma biomarker and AD pathology. Furthermore, it has already been found that AD is a highly heritable disease (60%–80%).[25] If the heritability of the plasma biomarkers is remarkably different from that of AD, this could indicate that the markers are sensitive to environmental influence and susceptible to confounding. Thus, it is important to gain a better understanding of the sources of individual differences in these biomarkers for their use in research and possible clinical implementation.

One approach to reaching a better understanding of individual variation in plasma biomarker levels is to establish the relative contributions of genetic and environmental factors to these levels. These relative contributions to plasma biomarker variation can be assessed using twin-based models.[26,27] The most simple of twin models exploit the different genetic relation between monozygotic (MZ, who share 100% of their genome) and dizygotic (DZ, who share on average 50% of their genome) twins to separate the genetic and environmental contributions to the variance in a phenotypic trait, here the plasma biomarker levels. More complex models than the classical twin design (CTD), such as the extended nuclear twin family design (NTFD), include parents of the twins as well as other family members, such as non-twin siblings.[28]

To our knowledge, only one study has estimated the heritability of AD-related plasma biomarkers using twins,[29] using a CTD with data from older male twins. In this cohort, a significant heritability was reported for NfL, total tau, Aβ42, and Aβ40 plasma levels, but not for the Aβ42/40 ratio. No evidence for shared environmental influences was found for these biomarkers, but the study's power to detect shared environmental contributions was low to modest. If there are shared environmental factors that are not detected, heritability will be overestimated. Non-additive genetic effects were not estimated, as the CTD does not allow for the simultaneous estimation of shared environment and non-additive factors.

Here, we aim to (re-)visit the heritability of NfL, GFAP, and Aβ42/40 ratios, using an extended NTFD in a large cohort of twins, their siblings, and their parents. Compared to the CTD, this NTFD is more strongly powered to estimate the shared environment and can estimate both non-additive genetic and shared environment effects.[30,31] We expand on the previous study[29] by including males and females from young to older adulthood. Based on the high heritability of AD dementia,[25] we expect significant heritability for plasma levels of NfL, GFAP, and Aβ42/40 ratio, and a significant genetic correlation between these markers, reflecting an underlying biological risk for AD.

2 | METHODS

2.1 | Participants

The participants are part of the Netherlands Twin Register (NTR). Blood was collected from adult twins and their parents, grandparents, non-twin siblings, spouses, and children during a large-scale biobanking effort.[32,33] The first wave of blood collection took place between 2004 and 2008, during which the majority of samples were collected. A second, complementary wave of collection was carried out in 2011 to gather data from additional family members who had not participated during the first collection. Along with blood collection, additional information regarding the participants' health and lifestyle were recorded (current and previous illness, medication use, smoking and alcohol behavior, etc.). Blood was collected for a total of just over 10,000 participants, of which 6497 were included in this study. Twins were included if they were ≥ 30 years of age at time of blood collection, and other family members if they were ≥ 40 years of age. A maximum of two non-twin siblings per family were included, as few families had data available for three siblings or more. Grandparents, spouses, and children of twins were not included. This selection process led to the inclusion of several relatively young parents while their offspring were not old enough to be included, which resulted in a modest mean age difference between the parent generation and offspring generation. The

RESEARCH IN CONTEXT

1. **Systematic review**: Plasma biomarkers are an accessible tool for diagnosing Alzheimer's disease (AD). However, more research is needed to determine the sources of individual differences (i.e., genetic or environmental) in these biomarker levels.
2. **Interpretation**: Heritability of the amyloid beta (Aβ) 42/40 ratio is 16%, heritability of neurofilament light chain (NfL) is 42%, and heritability of glial fibrillary acidic protein (GFAP) is 60%. NfL and GFAP levels being (genetically and environmentally) correlated with each other but not with Aβ42/40 levels indicates that Aβ42/40 reflects a biological process separate from those reflected by NfL and GFAP.
3. **Future directions**: Further research on causes of variation in plasma biomarkers and their genetic and environmental correlations should include other AD biomarkers, such as phosphorylated tau. Genome-wide association studies could help elucidate the shared biological pathway between NfL, GFAP, Aβ42/40, and AD. Attention should also be given to identifying potential environmental factors influencing Aβ42/40, NfL, and GFAP levels.

final sample consisted of 1574 MZ twins of which 638 were complete pairs; 1266 DZ twins of which 432 were complete pairs; 577 non-twin siblings; 1898 mothers and 1182 fathers, for a total of 1047 complete spouse pairs. In families with more than two non-twin siblings available, the two siblings to include were chosen based on how close in age they were to the twins, and if no twins were available (for example, if the twins were < 30 years old at time of collection, if consent was withdrawn, or if blood samples could no longer be analyzed), the two siblings closest in age were chosen. Parents and siblings of twins were included even when the twins' data was not available. Figure S1 in supporting information details the selection process.

2.2 | Biomarker preparation

Blood collection took place between 7 and 10 am in the morning after overnight fasting. Samples were stored at −20°C before use. NfL, GFAP, Aβ42, and Aβ40 were all measured simultaneously using the Neurology 4-Plex E advantage kit (Quanterix) on the Simoa HD-X analyzer, across a total of 33 runs. The inter-assay (NfL: 4.48%, GFAP: 4.63%, Aβ42: 5.19%, and Aβ40: 4.12%) and intra-assay (NfL: 4.46%, GFAP: 4.66%, Aβ42: 5.35%, and Aβ40: 4.13%) coefficient of variability of the quality controls was low for all markers. Samples from members of the same family were randomized across runs to prevent family similarities from being inflated from run-specific influences.

2.3 | Statistical analyses

All statistical analyses were performed in R (version 4.2.1). Outliers, defined as biomarker values ± 3 standard deviations from the mean, were removed. These included 15 NfL measurements, 17 GFAP measurements, 10 Aβ42 measurements, and 16 Aβ40 measurements. NfL and GFAP distributions were right-skewed, which was corrected for by applying a log transformation. The function scale was applied and scaled biomarker levels were corrected for possible age and sex differences using the umx_residualize function from the umx library (version 4.16). After transformation, the data were reorganized in separate data frames based on the zygosity of the twins (MZ male families, MZ female families, DZ male families, DZ female families, and DZ opposite sex families), with each data frame containing all family members related to a twin pair. Plasma Aβ42/40 is the most clinically relevant form of plasma Aβ,[8] and the results of this ratio are reported in the main text. The results of the Aβ42 and Aβ40 isoforms are reported in the Supplements Part I (Figure S2, Table S1–S6) in supporting information. Twin models were fitted to the data using the OpenMx library (version 2.21.8). The first model only used twin data and investigated if there were quantitative or qualitative sex differences in genetic contributions to the biomarker levels between males and females. Quantitative differences present in the form of differences in absolute, but not proportional, contributions, which can occur if there is more variance in a trait in one sex than the other. Qualitative differences are a difference in both absolute and proportional contributions. The second model likewise only used twin data and investigated if there were genetic or environmental influences that were shared between the different markers. The last model used data from all selected family members and assessed the relative genetic and environmental contributions to the biomarker levels, as well as the presence of possible assortative mating. Significance was defined as a p value < 0.05 for all models.

2.3.1 | Decomposition of variance in twin models

The basic twin design aims to estimate the proportional contribution of genetic and environmental factors to the variance in a trait using a path diagram approach, in which path coefficients representing the effect of latent genetic and environmental effects on the observed trait are estimated (Figure 1). Within the CTD, the environmental factors can be further separated into the environment shared by the twins and environmental factors unique to each of them. More complex models integrate additional family members, such as parents and non-twin siblings.[28] This allows the genetic contribution to be further decomposed into additive genetic effects, where the effect of carrying a copy of a specific allele is about half of carrying two copies of that allele, and non-additive effects, where the increase in the effect of carrying two copies of an allele instead of one is not linear (e.g., one allele has very little effect but two have a strong effect). Likewise, the shared environmental contribution can be decomposed into effects of the environment shared by the twins only, the environment shared by twins and their siblings, and the environment shared by parents and their offspring.

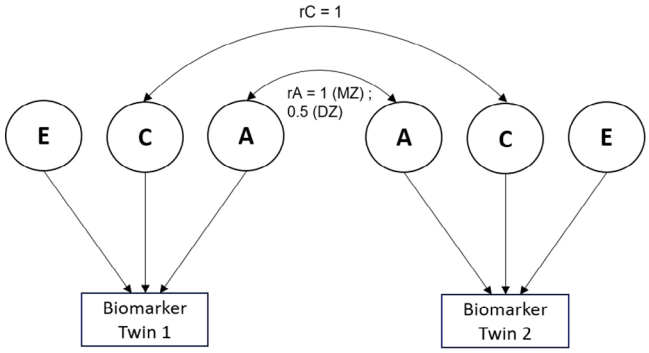

FIGURE 1 Univariate CTD design. Single-headed arrows indicate contributions of a latent factor on a trait. Double-headed arrows indicate a covariance between two latent factors. A, additive genetic effect; C, shared environment effects; CTD, classical twin design; DZ, dizygotic twins; E, unique environment effects; MZ, monozygotic twins; rA = correlation between A factors of Twin 1 and Twin 2; rC = correlation between C factors of Twin 1 and Twin 2.

2.3.2 | Correlations between family members

Phenotypic correlations were computed for each of the possible family member pairings (MZ twins, DZ twins, Twin–Sibling, Sibling–Sibling, Father–Offspring, Mother–Offspring, Father–Mother). This pattern of correlations gives a first indication of the contribution of additive and non-additive genetic influence, sibling-shared environmental influence, and parent–offspring shared environmental contribution to the variance in biomarker levels. If the influences on a marker are mostly from additive genetic contributions, it is expected that the MZ twin correlations will be highest (as MZ share 100% of their genes), and that the DZ, Twin–Sibling, Sibling–Sibling, Father–Offspring, and Mother–Offspring are roughly half of the MZ and similar to each other (as all these pairs share ≈ 50% of their genes). If the MZ correlations are more than twice that of the DZ twins, this is suggestive of non-additive genetic contributions to the marker. If the DZ, Twin–Sibling, and Sibling–Sibling correlations are higher than the Father–Offspring and Mother–Offspring correlations, this is suggestive of contributions from an environment that is shared by all siblings but not by the parents. If the correlations between DZ are higher than the Twin–Sibling and Sibling–Sibling correlations, this is suggestive of contributions from an environment that is shared only by the twins. If the Father–Offspring and Mother–Offspring correlations are more than half that of the MZ twins, this is suggestive of the influence of an environment that is passed from parents to their offspring.

2.3.3 | Univariate CTD

We first tested several assumptions made in twin models about equal variances and means in first and second-born twins, MZ and DZ twins, and males and females. The majority of these assumptions held for each biomarker (see Tables S7–S9 in supporting information). A univariate CTD, only including the twins, with five zygosity groups (MZ males, MZ females, DZ males, DZ females, DZ opposite sex) was fit to decompose the variance in plasma biomarker levels into additive genetic effects (A), effects of the environment shared by twins (C), and unshared environmental effects (E; Figure 1). Differences in pre-analytical handling and measurement errors are also captured by the E component. Definitions of the different parameters of the CTD are explained in Box 1. The additive genetic effects A represent the heritability, and the shared environment C specifically reflects environmental factors that make members of a family resemble each other more than they resemble members of other families. We choose to model an ACE model, rather than a model with Dominance (ADE) based on the pattern of twin correlations. Quantitative sex differences in these parameters were tested by comparing models that equated these parameters in males and females to models that estimated sex-specific parameters. The best-fitting model was chosen based on model simplicity (number of parameters). Qualitative sex differences in additive genetic factors were tested by computing the genetic correlation between male and female twins in opposite-sex pairs, which should not differ from 0.5 when sex does not have an effect. If the fit of the simpler model without sex-specific parameters was not worse (as defined by a p value > 0.05) than that of the model with sex-specific parameters, the model without sex-specific parameters was chosen. The A and C components were then dropped one at a time from the model, and the simpler submodels (AE, CE, and E) were compared to the complete ACE model to identify the parsimonious model.

AD is a disease of old age, which suggests the heritability of plasma biomarkers NfL, GFAP, and Aβ42/40 ratio might be subject to change as one gets older. To test this, we performed a CTD with an age moderation parameter. To ensure we had enough power to detect possible age moderation effects, we added two siblings per family to our model, for a maximum of four members per family (two twins and two non-twin siblings). In this model, we fit the A, C, and E parameters that were suggested to be significant by the CTD, as well as age moderation on each of these parameters (Figure S3 in supporting information). We then tested if age moderation of the A, C, or E paths could be removed one at a time without the fit of the model significantly worsening.

2.3.4 | Multivariate CTD

The univariate CTD model can be extended to a multivariate design, which estimates the same parameters as the univariate model (A, C, and E) but also tests the degree to which the same A, C, and E factors influence all biomarkers. This is illustrated for a bivariate design on GFAP and NfL in Figure 2. When the model fit is worsened by dropping the cross-path between the latent A1 factor influencing NfL to GFAP, this means that some of the genetic factors are shared between the two traits. In a similar vein, the cross-paths between the shared environmental factors and unique environmental factors between the biomarkers can be computed. The so-called Cholesky design in Figure 2 can be used to estimate the genetic and environmental correlation between NfL and GFAP, as well as the percentage of the phenotypic correlation (the overall correlation, as opposed to the correlation

Box 1: Twin model parameters and their interpretation

Parameter	*Model*	*Interpretation*
Additive genetic influence (A)	Classical twin design (CTD); nuclear twin family design (NTFD)	The genetic influence that is additive, in which the effect conferred by homozygous carriage of an allele is twice that of the effect conferred by heterozygous carriage of that allele.
Dominance genetic influence (D)	CTD; NTFD	Genetic influence that is non-additive, in which the effect conferred by homozygous carriage of an allele is more (or less) than twice that of heterozygous carriage of that allele.
Shared environment influence (C)	CTD	Environmental influences that are shared by two twins, and that make them resemble each other more than they resemble twins from other families. Within the NTFD model, this factor is split into S, T, and F.
Sibling-shared environment influence (S)	NTFD	Environmental influences that are shared by all siblings (twins and non-twins) of a family, making them resemble each other more than they resemble siblings of other families. This is measured as part of C within the CTD.
Twin-shared environment influence (T)	NTFD	Environmental influences are shared only by the twins within a family and not shared by their siblings. This is measured as part of C within the CTD.
Family-shared environment influence (F)	NTFD	Environmental influences that are passed from parents to offspring. This is measured as part of C within the CTD.
Unique environment influence (E)	CTD; NTFD	Environmental influences that are unique to an individual and not shared by any other family member.
Assortative mating (μ)	NTFD	Behavior through which an individual consciously or unconsciously chooses their mates based on their similarities or dissimilarities.

between specific sources of variance) between the two traits that is accounted for by the sharing of genetic (A), shared (C), and unique (E) environmental factors (see de Vries et al. for details).[34]

2.3.5 | Univariate NTFD

The final model fit was a univariate NTFD,[30] which includes twins, parents of twins, and siblings of twins. The parameters estimated in this NTFD were the additive genetic effects (A), the non-additive genetic effects (D), the environment shared by twins and siblings (S), the environment shared only by twins (T), the unique environmental effects (E), and assortative mating (μ; Figure 3). A description of the parameters of the NTFD model is in Box 1. The A and E parameters are the same as in the CTD, while the S and T parameters are what constitute the C component of the CTD. This model includes two types of genetic contribution (A and D), the combination of which makes up the heritability estimate. Assortative mating is the concept that people do not choose their mates at random, but rather based on traits that they do (not) share. We choose to model an ADSTE model rather than alternative models based on the observed pattern of correlations between various types of family members. Submodels, in which combinations of A, D, S, and T were dropped, were then compared to the full model in which all parameters were freely estimated. If dropping one (or multiple) parameters did not significantly worsen the model fit, that model was considered acceptable. The parsimonious model among all acceptable models was chosen as the best model. Once this model was identified, it was further tested whether μ could be dropped from the model without worsening the fit.

3 | RESULTS

Family demographic information and biomarker distributions can be found in Table 1.

3.1 | Phenotypic correlations between family members

Family correlations for NfL, GFAP, and the Aβ42/40 ratio are shown in Figure 4. Few complete Sibling–Sibling pairs were available, leading to consistently non-significant correlations and very large confidence intervals. For NfL and GFAP, the strongest correlation was observed in MZ twins (NfL: 0.46 and GFAP: 0.58) and was roughly twice that of DZ twins (NfL: 0.24 and GFAP: 0.30). For these two markers, the

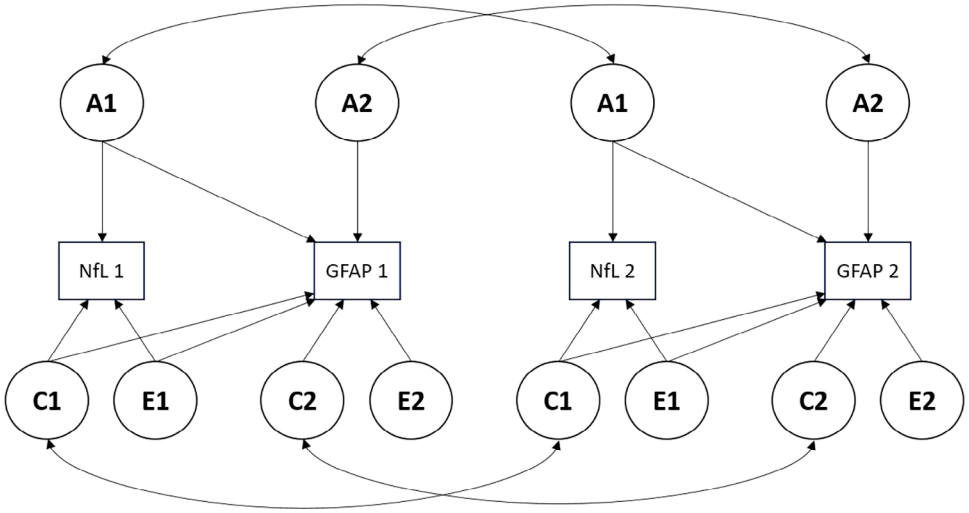

FIGURE 2 Bivariate model between plasma NfL and plasma GFAP. Twin 1 and Twin 2 are denoted as "1" and "." Single-headed arrows indicate contributions of a latent factor on a trait. Double-headed arrows indicate a covariance between two latent factors. A, additive genetic effect; C, shared environment effects; E, unique environment effects; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain.

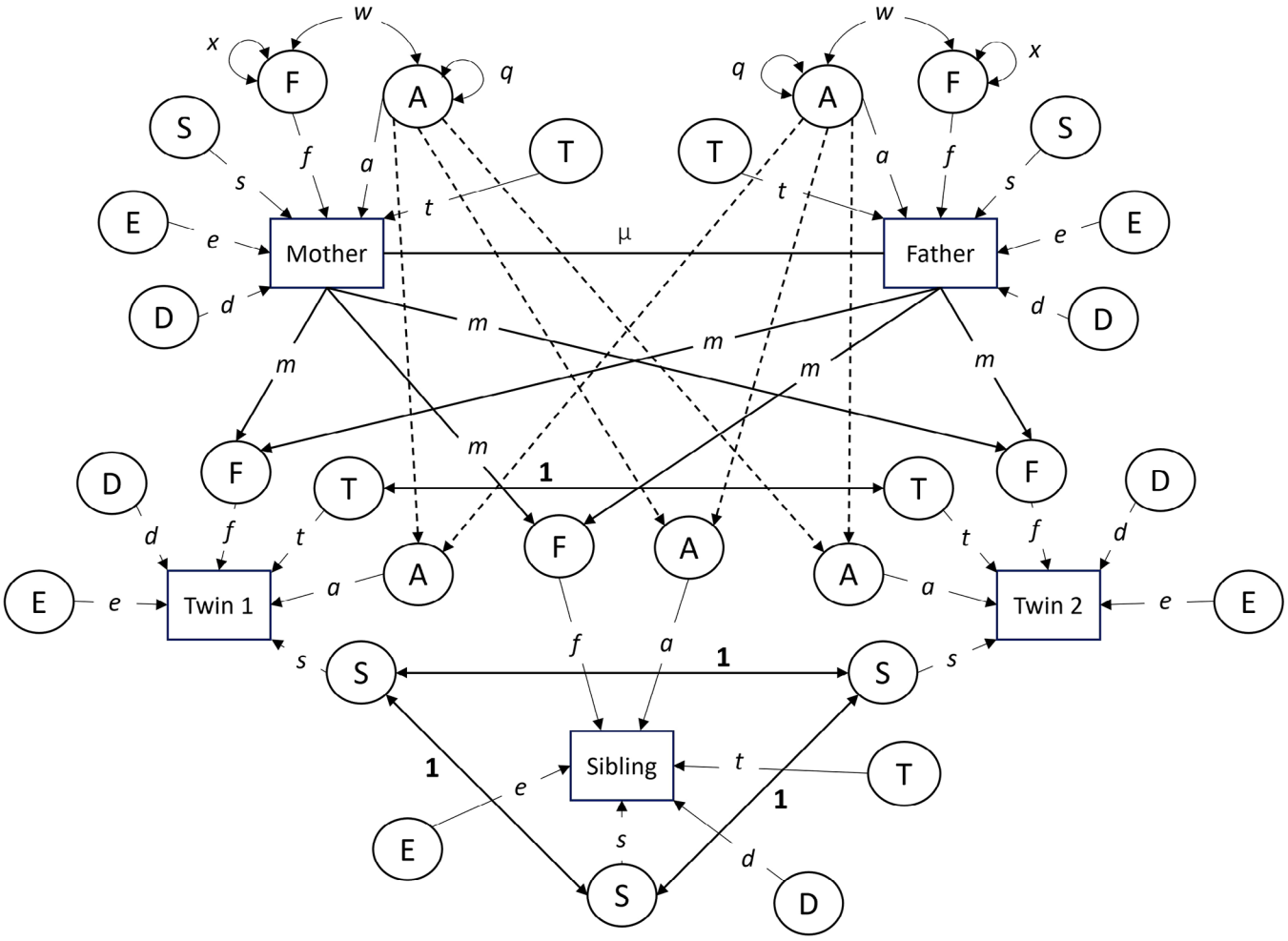

FIGURE 3 Simplified NTFD with siblings. To reduce complexity, the second sibling was not depicted. A, additive genetic effects; E, unique environment; F, family environment; m, familial transmission; NTFD, nuclear twin family design; S, sibling environment; T, twin-specific environment; w, A–F covariance; μ, assortative mating.

TABLE 1 Family demographics.

	Age Mean (SD)	N (Complete pairs)	NfL Mean (SD)	GFAP Mean (SD)	Aβ42/40 Mean (SD)
MZ twins	42 (11)	1574 (638)	9.5 (5.8)	71.8 (35.5)	0.08 (0.02)
Males	41 (12)	469	9.7 (6.1)	66.5 (34.1)	0.08 (0.02)
Females	42 (11)	1105	9.4 (5.7)	74 (35.9)	0.07 (0.02)
DZ twins	40 (10)	1266 (432)	8.4 (5.1)	64.7 (32.8)	0.07 (0.01)
Males	38 (10)	478	8 (5)	59.3 (28.9)	0.08 (0.02)
Females	40 (10)	788	8.6 (5.1)	68.1 (34.5)	0.07 (0.01)
Siblings	52 (10)	577 (-)	11.9 (6.5)	80.8 (37.6)	0.07 (0.02)
Males	54 (10)	197	12.9 (6.6)	79.1 (38.9)	0.07 (0.01)
Females	51 (10)	380	11.4 (6.4)	81.7 (36.9)	0.07 (0.02)
Mothers	57 (9)	1898 (1047)	13.6 (7)	96.6 (45.6)	0.07 (0.02)
Fathers	61 (7)	1182 (1047)	16.1 (8.1)	96.1 (46)	0.07 (0.02)
Total	50 (13)	6497	11.9 (7.1)	82.8 (42.5)	0.07 (0.02)

Abbreviations: Aβ, amyloid beta; DZ, dizygotic; GFAP, glial fibrillary acidic protein; MZ, monozygotic; NfL, neurofilament light chain; SD, standard deviation.

DZ twin correlation was similar to the Twin–Sibling correlation (NfL: 0.17 and GFAP: 0.27). The DZ twin and Twin–Sibling correlations were not much higher than the Parent–Offspring correlations (NfL: 0.12–0.19 and GFAP: 0.22–0.28). Because MZ twins share 100% of their genes and other relationship pairs (aside from Father–Mother) share ≈ 50%, observing MZ correlations that are about twice those of DZ twins, Twin–Sibling, and Parent–Offspring is indicative of mainly additive genetic influences on these biomarkers. Both markers had low Father–Mother correlations (NfL: 0 and GFAP: 0.06), which are indicative of the absence of assortative mating. The family correlations for the Aβ42/40 ratio were overall low, with the highest correlations being observed for MZ twins (0.23) and Father–Mother (0.21). The correlations for DZ twins, Twin–Sibling, and Parent–Offspring are close to 0 (0.03–0.08), indicating very low overall genetic and shared-environment contributions to the Aβ42/40 ratio. The relatively high Father–Mother correlation could indicate some type of assortative mating is taking place.

3.2 | Testing for qualitative and quantitative sex differences—univariate CTD

3.2.1 | NfL

Constraining the additive genetic (A), shared environment (C), and unique environment (E) estimates to be the same across sexes did not significantly worsen the fit, indicating that there were no significant quantitative sex differences for this biomarker. The genetic correlation between twins of opposite sex pairs was not found to be significantly different from 0.5, suggesting no qualitatively different genetic factors are at play in males or females. Dropping A or both A and C from the model significantly worsened the fit; however, only dropping C did not worsen the fit. The AE model without sex differences was thus retained as the best-fitting model, with 47% of the variance being explained by A (additive genetic effects) and the remaining variance explained by E (unique environmental effects). We then a fit an age moderation model with non-twin siblings to estimate if these A and E influences were stable over age. We found evidence of age moderation effects on E, but not A, specifically leading E to be less influent as one gets older. In return, this led to A being more influent with age, with a relative increase in heritability of ≈ 2% per 5 years (Figure S4 in supporting information).

3.2.2 | GFAP

Similar to NfL, no evidence was found for quantitative or qualitative sex differences. When testing whether the A or C parameters could be dropped from the model, it was found that dropping A worsened the model but dropping C did not, and the AE model also had the overall lowest Akaike information criterion (AIC). The AE model without sex differences was then concluded to be the best-fitting model, attributing 60% of the variance in GFAP levels to A (additive genetic effects). After fitting the age moderation model with siblings, we found evidence of age moderation effect on both A and E, specifically increasing the influence of A and decreasing the influence of E with age. This led to a relative increase in heritability of ≈ 1.5% per 5 years (Figure S5 in supporting information).

3.2.3 | Aβ42/40

No evidence was found for quantitative or qualitative sex differences in A, C, or E effects on Aβ42/40. As for NfL and GFAP, the parsimonious model was the AE model without sex differences, with 21% of the variance being attributed to additive genetic effects. The age moderation model with siblings showed evidence of an age moderation effect on E, but not A, increasing the influence of E as age progressed, and thus decreasing the heritability. This decrease was very small, with a relative decrease of ≈ 0.6% per 5 years (Figure S6 in supporting information).

TABLE 2 Parsimonious univariate CTD model for each plasma biomarker and corresponding standardized estimate with confidence intervals.

	A (95% CI)	C (95% CI)	E (95% CI)
NfL	0.47 (0.4–0.52)	—	0.53 (0.48–0.52)
GFAP	0.60 (0.55–0.64)	—	0.40 (0.36–0.45)
Aβ42/40	0.21 (0.14–0.28)	—	0.79 (0.72–0.86)

Abbreviations: A, additive genetic effect; Aβ, amyloid beta; C, shared environment effects; CI, confidence interval; CTD, classical twin design; E, unique environment effects; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain.

Estimates for A, C, and E for each biomarker from the best-fitting univariate CTD model without sex differences are summarized in Table 2. There was an age moderation effect on E for all markers and on A for GFAP only, leading to a small increase in relative heritability with age for NfL and GFAP and a small decrease for the Aβ42/40 ratio.

3.3 | Testing for shared genetic and environmental contributions between the biomarkers—multivariate CTD

The phenotypic correlation between NfL and GFAP was 0.37 (95% confidence interval [CI]: 0.35–0.39), while the phenotypic correlation of NfL and GFAP with the Aβ42/40 ratio was 0.03 (95% CI: 0–0.05) for both. We initially intended to run a trivariate model with NfL, GFAP, and the Aβ42/40 ratio, but the absence of any phenotypic correlation with the Aβ42/40 ratio rendered this uninformative. Instead, a bivariate model was fit with NfL and GFAP only. Because no sex differences were found for any of the three biomarkers in the CTD, we narrowed the five zygosity groups down to two zygosity groups: MZ twins (MZ males and MZ females) and DZ twins (DZ males, DZ females, and opposite-sex twins). We fit a bivariate Cholesky ACE model, and compared it to models in which the C component was dropped for both NfL and GFAP and their cross-path. This parsimonious model did not lead to a significant loss of fit to the data, suggesting that variances in NfL and GFAP as well as their covariance was explained by additive genetic and unique environmental factors. The A and E estimates to each biomarker were identical to those produced from the univariate CTD model, with a genetic contribution of 47% to NfL and 60% to GFAP. Fixing the genetic cross-path between NfL and GFAP to 0 strongly worsened the model fit, and the same was found when fixing the unique environment cross-path between NfL and GFAP. As a result, the AE model with shared A and E contributions on NfL and GFAP was chosen as the best fit. The shared genetic influence contributed 48% of the phenotypic correlation between NfL and GFAP, and the remaining 52% was explained by environmental contributions shared by both traits. The genetic correlation between NfL and GFAP was 0.32 (95% CI: 0.24–0.40), and the unique environmental correlation was 0.40 (95% CI: 0.33–0.46). These correlations point to a shared biological pathway.

3.4 | Assessing the proportional genetic and environmental contributions to the variance in biomarker levels—univariate NTFD

Because no sex differences were found for any of the three traits, the extended NTFD model was restricted to two zygosity groups, which strongly decreases the complexity of the model. Because the pattern of correlations, as presented in Figure 4, showed no evidence of parent–offspring environment influences, we fit a base model that allowed the family resemblance to be determined by additive genetic (A), non-additive genetic (D), sibling-shared environmental (S), twin-shared environmental (T), and unique environmental (E) effects. Although no evidence of shared environment (C) was detected using the CTD, the inclusion of more participants and new types of family correlations in the NTFD model makes it better suited to detect small sources of influence that may have gone undetected in the CTD. We then fit reduced models, for example, ADSE, ADE, and AE models.

3.4.1 | NfL

The ADSE, ADTE, ASTE, ADE, ASE, ATE, and AE models all provided acceptable fits compared to the full ADSTE model (Table S10 in supporting information). The AE model was the parsimonious model as it included the fewest number of parameters of all acceptable models. Compared to the estimates from the univariate CTD model, the estimates of A and E were relatively similar, with 42% of the proportion of variance being attributed to A and the remaining 58% being attributed to E. No significant assortative mating was found for NfL.

3.4.2 | GFAP

The acceptable models for GFAP were ADSE, ADTE, ASTE, ADE, and ATE (Table S11 in supporting information). The ADE model was chosen as the best-fitting model based on the number of parameters and having a lower AIC than the ATE model. The E estimate from the NTFD model was the same as the one from the CTD model (40%), while the 60% estimate for the A parameter of the CTD model was split into 43% for the A parameter and 17% for the D parameter in the NTFD model. The broad sense heritability was thus the same as that of the CTD (60%), but the NTFD was more precise in identifying the course of that heritability. No significant assortative mating was found for this biomarker.

3.4.3 | Aβ42/40

The acceptable models for the Aβ42/40 ratio were ADSE, ADTE, ASTE, ADE, ASE, ATE, and AE (Table S12 in supporting information). The AE model had the fewest number of parameters of all acceptable models. This AE model attributed 16% of the contribution to the Aβ42/40 ratio variance to genetic effects, which is comparable to the 21% estimate

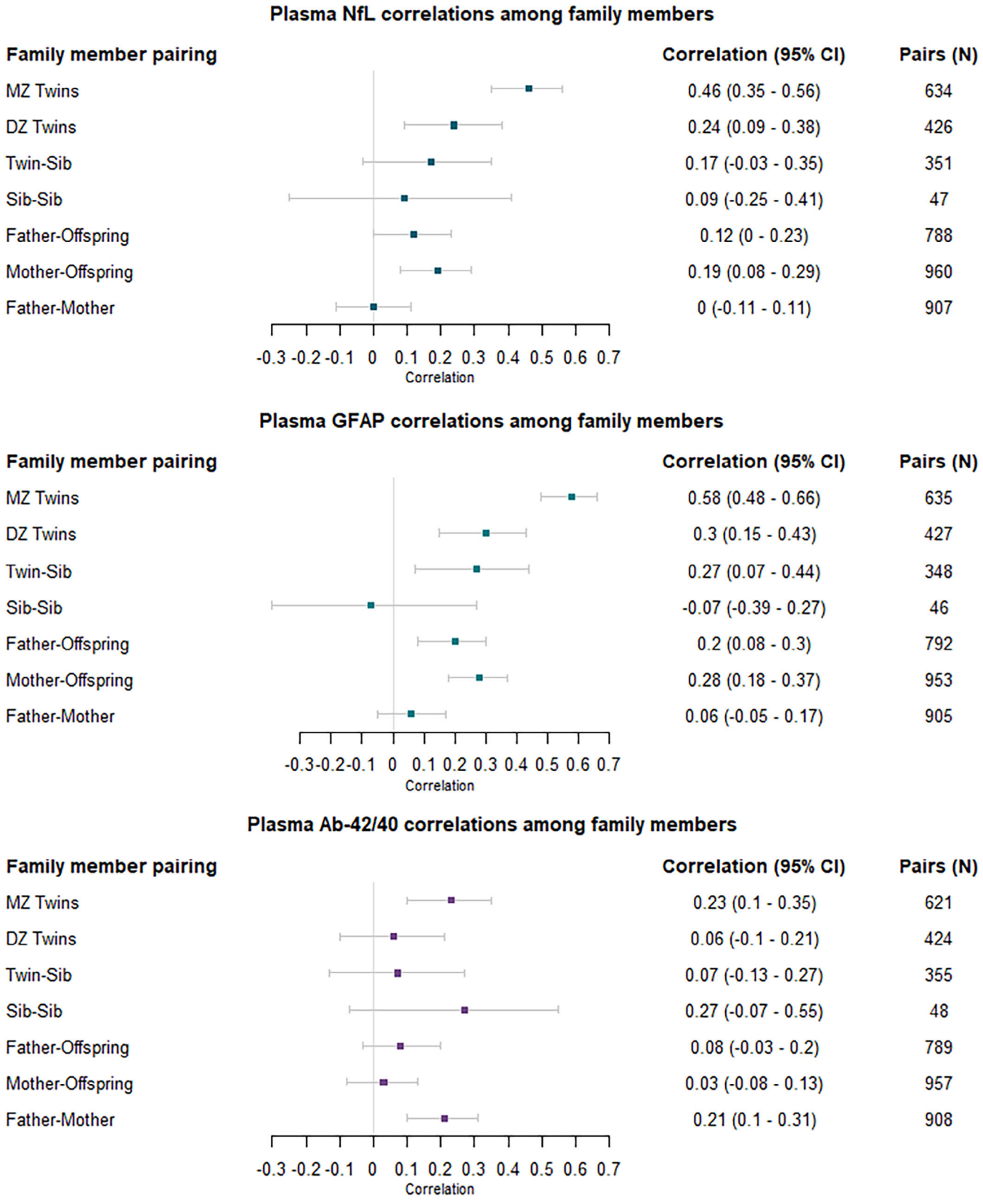

FIGURE 4 Correlations of plasma biomarkers between different family members. Ab, amyloid beta; CI, confidence interval; DZ, dizygotic twins; GFAP, glial fibrillary acidic protein; MZ, monozygotic twins; NfL, neurofilament light chain.

TABLE 3 Parsimonious NTFD model for each plasma biomarker and corresponding standardized estimate with confidence intervals.

	A (95% CI)	D (95% CI)	E (95% CI)
NfL	0.42 (0.37–0.47)	—	0.58 (0.53–0.63)
GFAP	0.43 (0.34–0.51)	0.17 (0.08–0.26)	0.40 (0.36–0.45)
Aβ42/40	0.16 (0.11–0.21)	—	0.84 (0.79–0.89)

Abbreviations: A, additive genetic effect; Aβ, amyloid beta; CI, confidence interval; D, the non-additive genetic effects; E, unique environment effects; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; NTFD, nuclear twin family design.

of the CTD. As anticipated from the spouse correlation, significant assortative mating was found for this marker.

The best-fitting NTFD models for all biomarkers are reported in Table 3.

4 | DISCUSSION

Using twins, siblings, and parents from a large number of twin families, we tested the contribution of genetic and environmental effects on NfL, GFAP, and Aβ42/40 ratio plasma levels. We separated genetic influences into additive and non-additive effects and the shared environmental effects into sibling-shared and twin-specific environmental effects. We found consistent evidence for heritable variation in these AD-related plasma biomarkers. The strongest heritability was found for GFAP (60%; 43% additive and 17% non-additive), followed by NfL (42%, all additive), and the Aβ42/40 ratio (16%, all additive). We did not find any sex differences in the genetic contributions to the plasma biomarker levels. Sibling-shared and twin-specific environmental influences were not observed.

Previous studies using twins only already reported genetic influences on the plasma biomarkers GFAP, NfL, and Aβ42/40 ratio,[9,29,35] and remaining causes of individual differences were attributed to unique environmental factors. Results from our CTD mostly converge with these previous findings. Heritability estimates for NfL were highly similar to those observed before.[29,35] Heritability of GFAP had not been investigated yet, but our MZ twin correlations closely match those of a previous study.[9] In contrast to the results in a previous CTD in a smaller cohort of older male twins that reported no heritable variance in the Aβ42/40 ratio, we were able to detect a low but significant heritability for this marker.[29] Performing a CTD with siblings and age moderation parameters, we were able to detect small age moderation effect on E for all markers, as well as on A for GFAP. These influences led to small increases in relative heritability over age for NfL and GFAP. From age 40 to 70, the heritability of NfL increased from 44% to 56%, while the heritability of GFAP increased from 60% to 69%. The heritability of Aβ42/40 ratio remained stable at ≈ 16% across this age span.

Adding many more family members in the NTFD compared to the CTD also enabled us to detect and account for other sources of variance in biomarker levels. First, this extended model enabled the detection of non-additive genetic effects for GFAP. Of note is that these non-additive effects are modeled as dominance effects in the NTFD.[30] If there is non-additivity through gene–gene interaction (epistasis) then dominance may be overestimated (and the sibling-shared environmental effects underestimated). This is not possible to test for in the NTFD. Second, we found non-negligible spousal correlations for the Aβ42/40 ratio. When we modeled this as a primary phenotypic assortment, a small but significant effect of assortative mating was found. Assortative mating is the behavior by which people choose their mates based on their (dis)similarities. Assortative mating can occur on traits that cannot be consciously perceived, such as the immune system,[36] but this is quite rare and so it is unlikely that there is assortative mating on Aβ42/40 directly. Instead, it is more likely that this assortment is indirect through correlated traits. For example, there is a known assortment for smoking, alcohol consumption, and physical activity,[37,38] and if these traits influence Aβ42/40 levels, they could induce a spousal correlation. This would further support the possibility of the Aβ42/40 ratio being sensitive to environmental effects. An additional advantage of the NTFD over the CTD is that we could take such an assortment into account. Investigating the nature of the observed spousal correlation could be a possible next step.

There was no phenotypic association between either NfL or GFAP with the Aβ42/40 ratio. This indicates that changes in these markers' levels are most likely part of different biological pathways. Indeed, elevated levels of NfL and GFAP have been observed in multiple other neurodegenerative and neurological conditions,[39–43] which indicates that this biomarker change may not be specific to AD. In contrast, we did observe a significant phenotypic correlation between NfL and GFAP with a moderate effect size ($r = 0.37$). This replicates previous correlations of NfL and GFAP in both cerebrospinal fluid (CSF) and serum reported in non-AD cohorts.[44–47] We now show that 48% of this correlation can be explained by genetic factors shared by NfL and GFAP, and 52% of this correlation by environmental factors influencing both of these biomarkers. In particular, the shared genetic factors indicate that NfL and GFAP could be part of a common biological pathway. We also found a small shared genetic influence on NfL and the Aβ42 and Aβ40 isoforms, although none were found between the two isoforms and GFAP. This could indicate that there is a biological process implicating NfL/Aβ42/Aβ40, and another implicating only NfL/GFAP (Supplement Part I).

AD is a highly heritable disease, with heritability estimates ranging from 60% to 80%.[25] In comparison, our heritability estimates for the plasma Aβ42/40 ratios are only 16%. For reference, the estimated heritability of amyloid burden as measured by amyloid positron emission tomography is ≈ 52%.[48] This suggests that the plasma Aβ42/40 ratios are influenced by environmental factors, which is in line with the varying sensitivity of this marker for the detection of AD pathology in biomarker studies.[8,15,49,50] The role of measurement errors may be due to the relatively modest level of natural variation in the plasma Aβ42/40 ratio.[51] For this reason, it is possible that the genetic effects on the Aβ42/40 ratio were underestimated. Of note, compared to the Aβ42/40 ratio in CSF, the plasma Aβ42/40 ratio is a relatively weak predictor of amyloidosis.[52–54] As the E component also captures measurement error, there could be artificial inflation of E if the assay is not

specific enough, in which case A will be proportionally smaller. Repeating this analysis using a different Aβ assay might lead to different results.[55]

Regarding the comparatively higher heritability of NfL (42%) and GFAP (60%), it is worth mentioning that these two markers are not as specific to AD, in particular NfL, which is quite a broad marker of neurodegeneration.[16,17] Therefore, the high heritability observed here might not entirely be a reflection of the heritability of AD (60%–80%).[25] It does however indicate that levels of NfL and GFAP are less likely to be influenced by environmental factors, particularly at older ages.

A logical next step is to identify the genetic variants for these biomarkers to shed light on the biological processes underlying the heritability of these markers. Combining this with the known genetic variants influencing AD[56–60] could further clarify the extent of overlap in the biological pathways of these biomarkers and AD pathology. A significant genetic correlation of a plasma biomarker level to AD would confirm the validity of this biomarker to signal AD risk at an early moment. Mendelian randomization[61] could furthermore be performed to test for a direct causal effect of NfL, GFAP, or Aβ42/40 on AD outcomes.

4.1 | Strengths and limitations

The main strength of our study is the large sample size of our community-dwelling cohort, consisting of just under 6500 individuals. Additionally, the inclusion of parents and siblings of twins enabled us to expand the CTD to an NTFD, which allowed for a better characterization of non-additive genetic effects, shared environmental effects, and less biased estimation of the heritability than in the CTD. Even so, there are various limitations inherent to the use of the NTFD model, including a number of assumptions that cannot be tested. First, this model operates under the assumption that the correlation observed between spouses is due to primary phenotypic assortment, and cannot detect other types of assortative mating. Violation of that assumption can lead to both over- and underestimation of any of the estimated parameters. Second, it is assumed that there are no genetic–environment interactions and if this assumption is violated, either the additive genetic or environmental contribution can be inflated. Third, it is assumed that there are no gene-by-age interactions, meaning that the genes carrying variants relevant to these biomarkers are not differentially expressed in the parent and offspring generations. If that assumption is violated, the sibling-shared and non-additive effects will be overestimated and the additive genetic effects underestimated. Finally, we did not model sex differences in the NTFD model because we had no power to estimate the > 100 parameters of such a model. If the correlations between females (e.g., female siblings, mother–daughter) and males (e.g., male siblings, father–son) differ from the inter-sex correlations (e.g., opposite sex siblings, mother–son, father–daughter) our estimates would not have reflected this sex difference.

5 | CONCLUSION

We find that genetic factors contribute to about half of the variance in levels of plasma NfL and GFAP. There is also a correlation between both genetic and environmental contributions to these two markers, indicating they may be part of a common biological pathway. In contrast, unique environmental factors that are not correlated across family members primarily account for the variance in the Aβ42/40 ratio, although heritable factors still exert a small, but significant, effect. NfL/GFAP and Aβ42/40 levels appear to reflect two different biological processes, as suggested by the lack of correlation between NfL/GFAP and Aβ42/40.

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CONFLICT OF INTEREST STATEMENT

CET has research contracts with Acumen, ADx Neurosciences, AC-Immune, Alamar, Aribio, Axon Neurosciences, Beckman-Coulter, BioConnect, Bioorchestra, Brainstorm Therapeutics, Celgene, Cognition Therapeutics, EIP Pharma, Eisai, Eli Lilly, Fujirebio, Instant Nano Biosensors, Novo Nordisk, Olink, PeopleBio, Quanterix, Roche,

Toyama, Vivoryon. She is editor in chief of *Alzheimer Research and Therapy* and serves on editorial boards of Medidact Neurologie/Springer, and *Neurology: Neuroimmunology & Neuroinflammation*. She had consultancy/speaker contracts for Eli Lilly, Merck, Novo Nordisk, Olink, and Roche. D.W. is an employee of Quanterix. R.R., A.dB., I.V., L.B., L.L., and E.dG. have nothing to disclose. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All participants gave consent during the biobank collection to have their samples and information used in epidemiological research.

ORCID

Rebecca Z. Rousset https://orcid.org/0000-0001-9355-7286

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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