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DATA AND MATERIALS AVAILABILITY Data and tissue generated by DIAN are available to qualified investigators according to DIAN's data and tissue sharing policies (http://www.dian-info.org/resourcedb/). The DIAN Genetics Core follows NIH guidelines on the sharing of genetic material and associated data as stated in the Alzheimer's Disease Genetics Sharing Plan (http://www.nia.nih.gov/research/dn/alzheimers-disease-genetics-sharing-plan).

Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer disease

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Abstract

Clinicopathologic evidence suggests the pathology of Alzheimer disease (AD) begins many years prior to cognitive symptoms. Biomarkers are required to identify affected individuals during this asymptomatic ("pre-clinical") stage to permit intervention with potential disease-modifying

therapies designed to preserve normal brain function. Studies of families with autosomal-dominant AD (ADAD) mutations provide a unique and powerful means to investigate AD biomarker changes during the asymptomatic period. In this biomarker study comparing cerebrospinal fluid (CSF), plasma and *in vivo* amyloid imaging, cross-sectional data obtained at baseline in individuals from ADAD families enrolled in the Dominantly Inherited Alzheimer Network (DIAN) demonstrate reduced concentrations of CSF amyloid-β1-42 (Aβ1–42) associated with the presence of β-amyloid plaques, and elevated concentrations of CSF tau, ptau181 and VILIP-1, markers of neurofibrillary tangles and/or neuronal injury/death, in asymptomatic mutation carriers 10-20 years prior to their estimated age at symptom onset (EAO), and prior to detection of cognitive deficits. When compared longitudinally, however, the concentrations of CSF biomarkers of neuronal injury/death within-individuals decrease after their EAO, suggesting a slowing of acute neurodegenerative processes with symptomatic disease progression. These results emphasize the importance of longitudinal, within-person assessment when modeling biomarker trajectories across the course of the disease. If corroborated, this pattern may influence the definition of a positive neurodegenerative biomarker outcome in clinical trials.

INTRODUCTION

Alzheimer disease (AD), the most common cause of dementia in the elderly, is a progressive and fatal neurodegenerative disorder.. AD currently affects ~10.6 million people in the U.S. and Europe, with numbers expected to double every 20 years unless effective disease-modifying treatments are developed (http://www.alz.org/national/documents/Facts_Figures_2011.pdf). Suboptimal clinical diagnostic accuracy (1) and the existence of a long preclinical phase during which the hallmark pathologies (amyloid plaques, neurofibrillary tangles and neuronal injury/death) develop prior to the appearance of clinical symptoms (2-7) has propelled efforts to identify biomarkers to aid disease diagnosis and prognosis, especially during the preclinical and early clinical stages.

Results from scores of biomarker studies have contributed to hypothesized trajectories of fluid and imaging biomarker changes that take place over the natural course of the disease, from the asymptomatic/preclinical stage to the earliest symptomatic stage (variously termed “mild cognitive impairment,” “prodromal AD,” and “very mild AD”) to the end stages characterized by advanced dementia (8, 9). Substantiating the longitudinal change in biomarkers over time will advance our basic understanding of the pathobiology of the disease, and also provide information critical for the design and interpretation of disease-modifying clinical trials that utilize biomarkers for subject enrollment and/or as outcome measures.

In studies of older adults at risk for late-onset AD (LOAD), comparison among independent cohorts with different clinical characteristics has been employed as a proxy for evaluating longitudinal change within individuals over time. For example, CSF concentrations of β-amyloid1-42 (Aβ1-42), the primary component of amyloid plaques, inversely correlate with plaque load (10-16) and are lower at the group level in individuals with clinically expressed AD compared to those with normal cognition. Conversely, CSF levels of the microtubule-associated protein tau and/or hyperphosphorylated forms of tau (p-tau), the primary

constituents of neurofibrillary tangles, positively correlate with brain atrophy measures (17, 18) and tangle load at autopsy (14, 19, 20) , although one study failed to find such associations (21). Mean levels of CSF tau(s) are higher in individuals with clinically expressed AD compared to those with normal cognition, consistent with these analytes being considered markers of neuronal pathologies (tangles, neuronal injury and/or death). However, such a categorical grouping according to cognitive status is only a gross estimate of disease stage during a dynamic neuropathologic process and, furthermore, does not necessarily accurately capture the pathological changes in the earliest preclinical and clinical stages.

Study of autosomal-dominant AD (ADAD) is particularly well-suited to investigations of biomarker trajectories since the 100% penetrance of the mutations and relative consistency of age at symptom onset within families overcomes the limitations of disease uncertainty and unpredictability of symptom onset inherent in studies of LOAD. In our initial cross-sectional study of ADAD individuals enrolled in the Dominantly Inherited Alzheimer Network (DIAN), we analyzed baseline measures of plasma Aβ1-42 and CSF Aβ1-42 and/or tau in 79 mutation carriers (MC) and 34 mutation non-carriers (NC) as a function of their estimated number of years to symptom onset (EYO) (22). Statistical modeling of these cross-sectional measures in the two genetic groups suggested the presence of amyloid and neuronal pathologies at least 10-15 years prior to symptoms, with biomarker abnormalities progressing in severity over time. However, true longitudinal evaluation within individuals with disease progression was not possible in that initial cross-sectional cohort.

To more fully characterize the patterns of fluid marker evidence of amyloid and neuronal pathologies and to test the hypothesis that the degree of biomarker abnormality increases over time with disease progression, the current follow-up study evaluated cross-sectional baseline data for four biomarkers in plasma and five analytes in CSF from a much larger cohort of DIAN participants (146 MCs and 96 NCs) spanning a wide range of EYOs. We also analyzed longitudinal CSF samples collected from a subset of individuals (n=37). In asymptomatic MCs, we observed patterns of longitudinal change in CSF biomarkers within-individuals that were similar to those inferred from cross-sectional analyses based on dementia severity and from modeling cross-sectional baseline measures as a function of EYO (i.e., reductions in CSF Aβ42 and elevations in CSF tau, ptau181 and a novel marker of neuronal injury/degeneration, visinin-like protein-1 (VILIP-1)). However, analysis of longitudinal samples revealed decreases, not increases, in the levels of markers of neuronal injury/death, within individuals over time once they are older than their expected age of symptom onset. These findings have important implications for understanding the dynamics of disease pathobiology and interpreting neuronal injury biomarker levels in response to AD therapies.

RESULTS

Baseline demographics and cross-sectional biomarker data are presented in Table 1. Two-hundred and forty-two participants had at least one plasma or CSF sample evaluated (n=237 with plasma, n=206 with CSF). For analysis purposes, participants were grouped by genetic (MC, mutation carrier; NC, mutation non-carrier) and cognitive (asymptomatic,

symptomatic) status. The cognitively normal (defined by a Clinical Dementia Rating score, CDR, of 0) MC group is termed MC-AS (asymptomatic), and MCs with CDR >0 are termed MC-S (symptomatic). CDR scores in the MC-S group ranged from 0.5 (very mild) to 3 (severe), although the majority (65%) was CDR 0.5. The CDR- Sum of Boxes (CDR-SB) in the symptomatic group ranged from 0.5 to 17 (with 18 indicative of worst performance), and the Mini-Mental State Exam (MMSE) (23) scores ranged from 3 to 30 (with 30 indicative of perfect performance). As a group, MC-ASs were younger than NCs ($p<0.05$), who were in turn younger than MC-Ss ($p<0.05$), although the mean parental age at symptom onset (AAO) was not different among the groups (45-48 years) ($p>0.05$). The estimated years to symptom onset (EYO) for the entire cohort ranged from approximately −30 (30 years before the parental AAO) to +30 (30 years after the parental AAO). Nearly 60% of the participants were female, and ~25% of individuals carried at least one *APOE* ε4 allele. Five participants were ε4 homozygotes. The majority of participants (70%) were from families with mutations in *PSEN1*.

Trajectories of plasma analytes estimated from baseline cross-sectional data

In contrast to LOAD in which disease certainty is unknown and age of symptom onset is unpredictable, asymptomatic individuals with deterministic mutations will develop dementia and at an age that is relatively predictable within a given family. To take advantage of this characteristic of ADAD, a metric defined as the estimated years to symptom onset (EYO) for each DIAN participant was calculated as the participant's age at clinical evaluation subtracted from the reported parental AAO, thus providing an estimate of where along the disease trajectory a specific individual falls at a given point in time, regardless of their chronological age. This allows for comparison among individuals from different families with different parental AAOs. General linear mixed models, accounting for within-family dependencies, were used to estimate the trajectory of biomarker concentrations in the two genetic groups in order to derive predicted mean biomarker concentrations at defined EYOs (−25 to +10). Estimated mean concentrations of plasma Aβ1-40 in MCs did not differ significantly from those of NCs at any EYO point ($p=0.8720$) (Figure 1A, see Table S1 for EYO-specific statistical differences). In contrast, while plasma Aβ1-42 concentrations in the two groups were stable across EYO points (trajectories not different from 0, NC, $p=0.2903$; MC, $p=0.7710$), overall concentrations in MCs were significantly higher at all but the earliest (EYO −25) EYO point evaluated (all $p<0.0343$) (Figure 1B, Table S1). However, small participant numbers at this early EYO warrant statistical caution. Similar results were obtained for the ratio of plasma Aβ1-42/1-40 (Figure 1C, Table S1). The estimated trajectories and the group differences for plasma Aβx-40, Aβx-42 and the Aβx-42/x-40 ratio were virtually identical to those of the full-length species (Aβ1-40 and Aβ1-42) (Table S1). Plasma concentrations of VILIP-1, a novel marker of neuronal injury/death, did not differ significantly between the two groups ($p=0.0881$), nor consistently as a function of EYO (Figure 1D, Table S1). However, at every time point examined, mean concentrations of plasma VILIP-1 were higher in MCs compared to NCs (Table S1). For all analytes, statistical comparisons could not be made at the far extremes of the EYO distribution (earlier than EYO −25 and later than EYO +10) due to the small number of individuals at these points.

Trajectories of CSF amyloid-related analytes estimated from baseline cross-sectional data

The changes in the various CSF biomarkers estimated from cross-sectional baseline measures were more complex than those for plasma (Figure 2). CSF Aβ1-40 concentrations did not consistently differ as a function of EYO between the NC and MC groups. While the overall approximate F-test (p-value=0.0021) suggested that concentrations were not equal between the groups over the EYO range, the approximate t-tests at 5 year intervals did not reveal persistent differences across the entire EYO range (Figure 2A, Table S2). In contrast, levels of CSF Aβ1-42 in MCs were significantly lower than those in the NC group at least 10 years prior to their parental AAO (EYO −10), whereas levels were stable over the range of EYOs in NCs (linear trajectory=-0.1534, p=0.9088) (Figure 2B, Table S2). In agreement with results from our initial report evaluating a smaller subset of this DIAN cohort (22), the Aβ1-42 concentrations appeared to diverge from one another even earlier (earlier than EYO −10), with concentrations in the MC group higher than those in NCs at very early EYO points. However, there were too few individuals at these early EYO points to make any statistical conclusions. Similar to what was observed for CSF Aβ1-42, the ratios of CSF Aβ1-42/1-40 in MCs were lower than those of NCs in individuals who were approaching or surpassing their EAO (EYO 0) (Table S2). Consistent with previous studies (24-26), absolute concentrations of CSF Aβ1-42 obtained by the two assay platforms (INNOTEST vs AlzBio3) were different but highly correlated (Pearson r=0.8559, p<0.0001).

Relationship between fluid Aβ analytes and *in vivo* amyloid imaging

In order to better understand the potential etiology of the alterations in fluid Aβ measures in MCs, concentrations of CSF and plasma Aβ1-40 and Aβ1-42 were compared to cortical retention of the β-amyloid-binding agent, Pittsburgh Compound B (PIB), a marker of fibrillar Aβ deposition, as assessed by positron emission tomography (PET). This cohort consisted of DIAN participants who had undergone both baseline fluid collection and PET PIB procedures (82 NC, 61 MC-AS and 35 MC-S with CSF and PIB; 94 NC, 68 MC-AS and 43 MC-S with plasma and PIB) within a short time interval (mean [SD] = 22.7 [36.9] days). A preliminary analysis estimated PIB positivity in this ADAD cohort to be a standardized uptake value ratio (SUVR) ≥0.85 when normalized to brainstem (mean cortical SUVR) using a *k*-means clustering algorithm (see Materials and Methods). As shown in Figure 3 (**left panels A, C, E, G**), all NCs were PIB-negative as expected given their young age (mean [SD] = 39.6 [9.8] years). Concentrations of plasma and CSF Aβ species differed up to ~6-fold among these normal individuals. In contrast, 58% of MCs were considered to be PIB+ (53/93), including 40% (23/59) of those who were asymptomatic and 88% (30/34) of those who were symptomatic. PIB+ individuals generally had low concentrations of CSF Aβ1-42 (Figure 3D), whereas concentrations of CSF Aβ1-40 (Figure 3B) and plasma Aβ species (Figure 3F, H) were not associated with cortical PIB retention. Similar to what is observed in LOAD, the distribution of biomarker values in the MC group included both normal (high CSF Aβ1-42, low PIB) and AD-like (low CSF Aβ1-42, high PIB) patterns. The normal pattern was observed more often in individuals who were farthest from their EAO (i.e., earlier in the disease course, smaller symbols), whereas the AD pattern was more common in individuals closer to their parental AAO (i.e., later in the course of the disease, larger symbols). However, in individuals who were considered PIB+, concentrations of CSF

Aβ1-42 were only weakly negatively correlated with amyloid load (Pearson r=-0.3008, p=0.0120; r=-0.2569, p=0.0470 when the high outlier is omitted).

Trajectories of CSF neuronal injury-related analytes estimated from baseline cross-sectional data

The observed changes in CSF tau and ptau181 estimated from cross-sectional baseline measures were similar to each other. Consistent with our earlier report in a much smaller cohort (22), concentrations of tau in MCs were significantly higher than those of NCs as early as 15 years prior to their EAO (EYO −15), with levels even higher in individuals who were approaching or who had surpassed their EAO (linear trajectory=3.7394, p<0.0001) (Figure 2C, Table S2). Similar patterns were observed for ptau181 (Figure 2D), although concentrations in MCs were higher than NCs even earlier (EYO −20) (Table S2). The changes in the ratios of tau/Aβ1-42 (Figure 2E) and ptau181/Aβ1-42 (Figure 2F, Table S2) were similar to those for tau and ptau181. In contrast to the pattern of VILIP-1 in plasma, but similar to those of CSF tau and ptau181, concentrations of CSF VILIP-1 (Figure 2G) in MCs were significantly higher than those of NCs as early as EYO −15 (Table S2), with concentrations even higher in individuals who were approaching or had surpassed their EAO (EYO 0) (linear trajectory=2.3050, p=0.0004). In addition, the estimated trajectory of the ratio of VILIP-1/Aβ1-42 (Figure 2H) and group differences as a function of EYO were similar to those observed for the tau(s)/Aβ1-42 ratios (Table S2). Concentrations of CSF VILIP-1 were strongly correlated with both tau (Spearman correlation = 0.7610, p<0.0001) and ptau181 (Pearson correlation = 0.7320, p<0.0001), and less so with Aβ40 (Pearson correlation = 0.2429, p=0.0001) and Aβ42 (Pearson correlation = −0.1694, p<0.0001). Trajectories of cognitive measures estimated from cross-sectional data revealed differences between mutation groups later than differences in biomarker measures (CDR-SB and MMSE at EYO −5, compared to CSF biomarkers at EYOs −10 and earlier) (Table S2).

Longitudinal change in CSF biomarkers within individuals over time

The biomarker trajectories estimated from the cross-sectional baseline data from MCs (Figure 2) over a range of EYOs support a dynamic model of AD neuropathologic changes characterized by early reductions in CSF Aβ1-42 (marker of amyloid) and continued elevations in tau, ptau181 and VILIP-1 (markers of neurofibrillary tangles and/or neuronal injury/death), a pattern that suggests an increase in biomarker abnormality with disease progression. This pattern is consistent with what has been proposed from cross-sectional analyses in LOAD in which mean biomarker concentrations are compared as a function of clinical diagnosis and/or staging (e.g., AD vs controls, CDR 0 vs CDR>0, decreasing MMSE, etc.) (27, 28). To investigate this pattern in an ADAD cohort, we analyzed the baseline cross-sectional DIAN data as a function of CDR as opposed to EYO. Similar to what has been reported in LOAD, mean concentrations of CSF Aβ1-42 in MCs appear to decrease with advancing dementia severity (especially at early stages) (Figure 4A), whereas concentrations of the neurodegenerative markers tau, ptau181 and VILIP-1 appear to increase as dementia progresses (Figure 4B-D). However, given the fairly wide distribution and overlap between individual CSF analyte values among the groups, we sought to better understand the nature of true longitudinal change within individuals by analyzing 2-3

samples obtained from the same person over time (LP interval ranged from 10-38 months, mean [SD] = 16.7 [8.9]).

The longitudinal cohort (n=37) was comprised of 11 NCs and 26 MCs (9 MS-AS and 17 MC-S at baseline), with demographics similar to the larger cross-sectional cohort (with the exception of a higher than expected percentage of APOE4+ individuals in the small MC-AS group [7/9, 78%]) (Table 2). Due to current limitations regarding biomarker assay precision and reproducibility (29-31), longitudinal samples from a given individual were analyzed together on the same assay plate, and all plates were from a single lot number so to eliminate between-plate and -batch variability as potential confounders. As expected, the mean baseline concentration of CSF Aβ1-42 in MCs in this longitudinal cohort was lower than that observed in NCs ($p<0.0001$), whereas baseline concentrations of tau, ptau181 and were higher ($p<0.0001$). Levels of VILIP-1 in MC-Ss were significantly higher than those in NCs ($p<0.0050$). When comparing within-person biomarker concentrations, these relatively young NCs (mean age [SD] = 40.0 y [8.3 y]) exhibited little change in their high levels of CSF Aβ1-42 and low levels of tau, ptau181 and VILIP-1 over the 1-3 year time period, although changes in Aβ1-42 concentrations were more variable (Figure 5A). In general, concentrations of Aβ1-42 in MCs appeared to decrease, albeit to differing degrees, across the range of baseline EYOs (Figure 5A, **top**), consistent with what we observed in cross-sectional analyses (Figure 2B). However, we observed an interesting finding with respect to longitudinal changes in markers of neuronal pathology and injury in MCs. In contrast to a pattern of steady increase in tau, ptau181 and VILIP-1 with disease progression that was inferred from cross-sectional analyses (Figure 2C, D, G) or CDR (Figure 4B-D), the within-person longitudinal changes of these markers differed in direction depending on where a person fell with respect to their baseline EYO. Specifically, concentrations of tau, ptau181 and VILIP-1 *increased* longitudinally in those at early stages of the disease (EYO≤0) but *decreased* in those at later stages (EYO>0) (Figure 5 B-D, **tops**).

In order to quantify and statistically evaluate these observations, we fitted linear random coefficients models (32) that permitted different longitudinal rates of change (i.e., slopes) as a function of whether participants' EYO was ≤ 0 or >0 at baseline, thereby facilitating the comparisons of the longitudinal rate of change in biomarker concentrations across the groups. Additional models including *APOE* genotype (E4+ vs E4-) and gender were also implemented to examine the effects of these covariates. Statistically, NCs exhibited no significant longitudinal change in CSF Aβ1-42 concentrations prior to their EAO (EYO≤0) (−3.12 [9.33] pg/mL/yr, $p=0.7406$) but a small (4.8%), albeit significant, decrease after their EAO (EYO>0) (−24.96 [11.89], $p=0.0432$) (Figure 5A, **bottom;** Table S3A). Mutation carriers exhibited a trend towards decreases in CSF Aβ1-42, both prior to (−16.14 [10.04]) and after (−17.86 [12.45]) EYO 0, although these trajectories did not reach statistical significance ($p=0.1170$ and $p=0.1607$, respectively) (Figure 5A, **bottom;** Table S3A).

As expected, concentrations of tau in NCs did not change significantly before (−3.00 [3.73] pg/mL/yr, $p=0.4251$) or after (−0.61 [4.70], $p=0.8970$) their EAO (Figure 5B, **bottom;** Table S3A). In contrast, MCs displayed a trend towards *increasing* tau concentrations (+6.91 [3.72], $p=0.0699$) prior to their EAO, but a significant *decrease* in tau concentrations later in the disease course (EYO>0) (−10.78 [4.70], $p=0.0270$) (Figure 5B, **bottom;** Table

S3A). Similar patterns of longitudinal decreases at later stages were observed for the other markers of neuronal pathologies (ptau181 and VILIP-1) in MCs but not NCs. Concentrations of ptau181 decreased significantly by an estimated −9.52 [3.37] pg/mL/yr (p=0.0088), and VILIP-1 by an estimated −14.61 [3.83] pg/mL/yr (p<0.0005), in MCs with baseline EYO>0 (Figure 5B-D, **bottoms;** Table S3A). However, the changes prior to their EAO were not statistically significant (ptau181, +0.588 [2.81] p=0.8356; VILIP-1, −0.885 [3.09] p=0.7766) (Table S3A). These markers did not change longitudinally in NCs at either time (EYO≤0 or EYO>0; all p>0.4231) (Table S3A). Virtually identical results were obtained when models were adjusted for gender and *APOE* genotype (Table S3B). Even though the magnitude of change in neuronal injury markers between longitudinal samples was relatively small (ranges [pg/mL]; tau (−52 to +49), ptau181 (−41 to +21), VILIP-1 (−54 to +21)), the co-efficients of variation (% CV) between longitudinal samples from a given individual were significantly greater than those within each sample analyzed in duplicate (tau, p=0.0004; ptau181, p<0.0001; VILIP-1, p=0.0069; Figure S1). In contrast, there was no significant difference between the within-sample and between-sample % CVs for Aβ1-42 (p=0.3241) (Figure S1). These data provide support for the idea that the longitudinal changes we observe in tau, ptau181 and VILIP-1 are not due to assay imprecision.

In order to put these longitudinal biomarker changes into the context of cognitive performance in this sub-cohort, we evaluated a composite of three psychometric test measures that were recently reported to be sensitive to cognitive decline in asymptomatic MCs (CDR 0) in DIAN (33). These tests include Logical Memory IA-Immediate Recall and Logical Memory IIA-Delayed Recall, both tests of episodic memory (34) and Digit Symbol, a measure of speeded visual spatial processing (35). Cognitive performance in NCs across the range of sampled EYOs did not change significantly before (z=0.0259 [0.0608], p=0.6721) or after (z=0.0636 [0.0778], p=0.4179) their EAO (Figure S2). In contrast, MCs who were after their EAO (EYO>0) exhibited lower mean baseline scores (worse performance) than those who were prior to their EAO (EYO≤0) (EYO>0 z=-0.9305 [0.2247]; EYO≤0 z=0.2018 [0.1902], p=0.0004). However, performance was quite variable in MCs who were close to their EAO (EYO ~−5 to 0), with some performing within the range of NCs and others already impaired to the level of those past their EAO (Figure 2S). Furthermore, while the mean estimated change in this composite was not significantly different from zero in MCs who were prior to their EAO (EYO≤0) (z=0.0144 [0.0601], p=0.8119), performance decreased in MCs who were after their EAO (EYO>0) (z=−0.2815 [0.0647], p<0.0001). Virtually identical results were obtained when analyses were adjusted for gender and APOE4 status (data not shown).

DISCUSSION

Our understanding of AD has evolved greatly over the past two decades with the advent of fluid and imaging biomarkers that permit the *in vivo* detection of underlying disease pathologies. Data from clinicopathologic and biomarker studies have converged to support the existence of a long preclinical stage during which AD pathologies develop prior to the appearance of cognitive symptoms. As a consequence, individuals in this preclinical stage are receiving intense focus as a targeted population for AD secondary prevention trials. In such trials, biomarkers are being used for subject enrollment, proof of therapeutic target

engagement and as surrogates for assessing downstream effects on disease pathology. Thus, it is critical to elucidate the trajectories of biomarker changes during the natural course of the disease, especially during this dynamic preclinical phase.

Studies of families harboring known ADAD mutations provide a unique and powerful means by which to investigate AD biomarker changes that take place as the disease progresses from its initial asymptomatic/preclinical phase through its symptomatic phase characterized by cognitive decline and eventual dementia. These individuals have long been excluded from observational studies and clinical trials due to the genetic mechanism of their disease. However, despite potential differences in the mechanisms by which Aβ accumulates to form amyloid in the brain (hypothesized to be due to an increase in Aβ1-42 production in ADAD compared to more complex and still poorly understood mechanism(s) in LOAD) (36), the hallmark pathology of amyloid plaques and neurofibrillary tangles and associated neuronal loss and brain atrophy is similar between the two groups. Yet individuals with ADAD often exhibit pathologies not frequently observed in LOAD including “cotton wool” plaques, severe cerebral amyloid angiopathy and Lewy bodies (37) and these differences may impact biomarker profiles. In general, ADAD pathologies develop much earlier in life and to a greater extent than in LOAD, clinically manifesting as faster cognitive decline, additional non-memory and non-cognitive neurological symptoms (e.g., apraxia, aphasia, myoclonus and spastic paraparesis), and higher mortality (38-43). However, despite these differences, investigation of the asymptomatic period in ADAD overcomes the three biggest challenges facing similar studies in LOAD: 1) knowing whether future dementia will develop (a virtual certainty in ADAD mutation carriers); 2) the unknown age at symptom onset (relatively predictable estimates within ADAD families); and 3) possible confounds due to age-related co-morbidities (relative lack of other diseases in the younger ADAD individuals). Comparing MCs with NCs (as controls) of similar ages within the same families also minimizes possible genetic influences beyond the disease-causing mutations themselves.

The EYO construct in DIAN permits evaluation of biomarker concentrations as a function of where along the disease trajectory an individual falls, independent of the actual age of dementia onset of their parent. With this strength in mind, the present study demonstrates fluid biomarker profiles consistent with the presence of AD pathologic abnormalities 10 to 20 years prior to the estimated age at symptom onset in MCs and prior to significant impairments in cognitive performance as measured by CDR-SB, MMSE and a psychometric composite recently reported to be a sensitive measure of cognition in MCs prior to the onset of dementia (33), in agreement with our initial report from an earlier, smaller group of DIAN participants (22) and a young cohort of individuals from families with the *PSEN1 E280A* mutation (the Colombian kindred) (44). Although we cannot formally rule out possible aging-related reductions in CSF production and/or turnover (45) as a contributor to the alterations in CSF analytes in MCs, the young age of the DIAN cohort and the lack of change in NCs makes this an unlikely possibility. Cross-sectional analyses suggest that concentrations of CSF Aβ1-42 in asymptomatic MCs drop as individuals approach their parental AAO, becoming significantly lower than NCs by ~10 years prior to their estimated age of dementia onset. Consistent with what has been reported in LOAD (10-14, 24, 46, 47)

and in cognitively normal individuals at risk for LOAD by virtue of their advanced age (10, 24, 47), low concentrations of CSF Aβ1-42 were associated with PIB-positivity, despite the overproduction of Aβ42 in these mutation carriers (10-14, 24, 46, 47). However, also similar to what is observed in LOAD, the relationship is not perfect; there are several members of the DIAN cohort, including NCs, who exhibit relative low concentrations of CSF Aβ1-42 in the absence of detectable PIB retention in the selected cortical regions. Whether this apparent discrepancy reflects a true pathobiological process (e.g., concentrations of CSF Aβ1-42 dropping prior to β-amyloid becoming detectable by PIB), simple biological variability across individuals, or a more technical limitation of the fluid assays and/or imaging protocols is the subject of ongoing investigation. In general, the “normal” pattern (high CSF Aβ42, PIB-negativity) was observed more often those who were farthest from their EAO (i.e., earlier in the disease course), whereas the AD pattern (low CSF Aβ42, PIB-positivity) was more often observed in those closer to their EAO (i.e., later in the course of the disease). Evaluation of the relationship between longitudinal changes in CSF Aβ42 and longitudinal PIB measures within a given individual will lead to a better understanding of the actual timing of these biomarker changes early in the disease process and whether such trajectories differ between individuals with ADAD compared to LOAD. Although the trajectories estimated by cross-sectional baseline data suggested *higher* concentrations of CSF Aβ1-42 in MCs compared to NCs even earlier in the disease process (~10-25 years prior to their EAO), the statistics did not bear this out, likely due to the small number of participants at these extreme age points. Such CSF changes appear to parallel those observed in Down Syndrome in which the eventual development of AD occurs due to the presence of three copies of the *APP* gene on chromosome 21. Elevated concentrations of CSF Aβ1-42 are observed very early in life in DS (48), while concentrations are reduced at later ages (49). Higher concentrations of CSF Aβ1-42 have also been reported in early adulthood (age 18-26 years) in the Colombian ADAD kindred (44).

Although MCs exhibited elevated concentrations of plasma Aβ1-42 throughout the course of the disease (consistent with the known stimulatory effect of these mutations on Aβ1-42 production) (50), in contrast to CSF Aβ1-42, concentrations in plasma did not appear to change as a function of EYO. In addition, plasma Aβ1-42 (and Aβ1-40) concentrations were not related to cortical PIB retention, suggesting elevated plasma Aβ1-42 may be a systemic effect of ADAD mutations and does not reflect underlying cortical β-amyloid pathology in the brain.

Consistent with what we observed in the initial DIAN cohort (22), cross-sectional analyses suggest CSF tau and ptau181 (and their respective ratios with Aβ1-42) begin to increase in MCs in the pre-symptomatic period (~15 years prior to their EAO), and continue to increase further with disease progression as has been reported in cross-sectional studies of LOAD (8, 51, 52) and ADAD (53). Mutation carriers also displayed elevations in CSF concentrations of the novel marker VILIP-1, similar to what has been observed in LOAD (54, 55). VILIP-1 is a neuron-specific intracellular calcium sensor protein that is not a component of neurofibrillary tangles and, thus, when measured in fluids, is considered to be a marker of neuronal cell injury and/or death. Concentrations of CSF VILIP-1 are positively correlated with tau (55), and both increase in response to stroke and acute traumatic brain injury (56).

When combined with Aβ1-42, VILIP-1 is a strong predictor of cognitive decline in cognitively normal elders (55) and in persons with very mild symptomatic AD (57), performing as well as or slightly better than the tau/Aβ1-42 ratio. The observation of elevations in CSF VILIP-1 in MCs at least 15 years prior to their EAO, with concentrations even higher in individuals who are closer to their EAO, suggests a robust phase of neuronal injury and/or death that begins prior to the onset of cognitive symptoms. Concomitant statistical changes in VILIP-1 in plasma in MCs vs NCs were not observed, though concentrations in MCs appeared to be higher than in NCs, thus warranting further investigation. Although CSF VILIP-1 is not specific for AD, it could be used as a neurodegeneration biomarker outcome in AD clinical trials, especially those targeting tau processing or tangle formation that require assessment of tau-independent markers of neuronal cell injury or death.

In general, biomarker trajectories are typically based on the premise that cross-sectional age-related and/or clinical stage-related changes represent changes that would occur over time with disease progression in single individuals. Indeed, a wealth of cross-sectional studies in LOAD has provided the basis for the proposed patterns of reductions in Aβ42 and elevations in tau and ptau that are currently accepted in the field (8, 9). The modeling of cross-sectional baseline data in the current DIAN cohort supports this idea. However, the premise that once tau or other neurodegeneration markers are elevated, they continue to increase or stay the same as degeneration progresses, is not supported by our longitudinal data. Analyses of within-person change in LOAD have demonstrated little or no change in biomarker levels over relatively short time periods (6 months to 2 years) (58-62). Whether such findings reflect true biology, the short interval assessed or methodologic shortcomings in fluid collection procedures and/or analysis of longitudinal samples remains to be determined. A closer review of the data, however, reveals between-subject variability in the patterns of tau changes that may be biologically relevant (63-66). For example, longitudinal increases in tau have been observed in persons with LOAD who had low tau at baseline (presumably early in the course of the disease), but no difference or even decreases in tau in those with high baseline levels (presumed to be later in the disease process) (65, 67). Consistent with this finding, statistical modeling in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort revealed similar reductions in tau in individuals with AD dementia, but not in those exhibiting mild cognitive impairment (68). Reductions in CSF ptau181 have also been reported in demented persons who were followed for ~3 years (69).

The current observations of increases in tau in MCs early in their disease course (EYO≤0) but decreases in those at later (typically symptomatic) stages (EYO>0) lend strong support to the general concept that biomarker trajectories may differ as a function of where an individual falls in the neuropathological cascade, and specifically, that markers of the acute phase of neuronal injury increase in early, preclinical stages of AD but then later fall from their peak levels as cellular neurodegeneration slows. Consistent with this pattern, a previous report of a single asymptomatic ADAD (*APP* V717I) MC showed substantial increases in tau and ptau181 over a 4.5 year period very early in the disease process (between 19 and 14 years prior to their EAO) (70), whereas a longitudinal decrease (or a

lack of increase) over 5 years in levels of CSF ptau181 have been observed in a small Japanese cohort (n=4) of symptomatic *PSEN1* MCs (71).

The present study is not without weaknesses. Since ADAD and LOAD are similar but not identical in their underlying pathologies, symptoms and clinical course, the generalizability of the present findings to the more common “sporadic” form remains to be determined. Also, the number of participants in the longitudinal cohort is small, as is the number of repeat CSF samples. Future analyses in the growing DIAN cohort, with greater numbers of longitudinal samples collected per person, will allow us to evaluate the validity and robustness of these biomarker changes. Although the consistency in the patterns of the three different neurodegenerative markers (tau, ptau181, and VILIP-1) lends strong support to the validity of the trajectories, collection of additional samples will permit more elaborate modeling, such as one that does not assume linear changes over time. The small number of participants in the longitudinal cohort is especially problematic for assessing cognitive change over time since evaluation of only two time points does not permit an accurate characterization of the rate of cognitive decline (72). Analyses over longer follow-up times and that account for different levels of cognitive impairment among individuals will permit appropriate modeling of potential nonlinear patterns of cognitive decline. Such data will also allow conclusions to be drawn regarding the temporal relationship between cognitive change and fluid biomarker trajectories over the course of the disease, an evaluation that is not possible in the present small cohort. Another limitation is that we did not examine mutation-specific effects. Evaluation of a larger cohort will permit assessment of mutation-specific effects, as well as possible influences of *APOE* genotype on biomarker patterns.

In conclusion, the current data suggest a model of disease pathogenesis in the asymptomatic period of ADAD in which MCs display elevated concentrations of Aβ1-42 in the plasma and perhaps in CSF very early in the pre-symptomatic phase. Subsequently, Aβ1-42 begins to aggregate and accumulate in the brain until a critical threshold is reached, at which time concentrations in the CSF decrease as Aβ1-42 is sequestered in β-amyloid plaques in brain parenchyma, as has been reported in transgenic mouse models of ADAD (73, 74). Concentrations of Aβ1-42 in the plasma remain high in MCs over the course of the disease, likely due to the sustained contribution of peripheral over-expression of Aβ1-42 in these individuals (75). As plaques continue to develop during the long asymptomatic phase, tangles form, and neurons begin to undergo a robust phase of injury and cell death, as evidenced by increases in the CSF concentrations of tau, ptau181 and VILIP-1. Neurodegeneration continues as the disease progresses through its symptomatic phase, but its rate may be slower relative to earlier stages. This may result in a decrease in absolute concentrations of tau, ptau181, and VILIP-1 in CSF over time in symptomatic individuals.

Although this general model is consistent with data obtained from cross-sectional studies in LOAD (8, 9, 76, 77) and suggests a common pathophysiology for AD due to mutations and the much more common “sporadic” form, the current longitudinal data suggest a potential modification to the proposed model to incorporate an eventual slowing down of the rate of neuronal injury and death such that the release of these proteins from cells slows relative to its peak release, resulting in lower absolute concentrations in the CSF over time. However, it is also possible that early increases in these markers followed by later decreases may reflect

differences in the size of neuronal populations undergoing acute injury/death during the different disease stages (e.g., larger populations early followed by smaller populations later). Early elevations may also be due to acute neuronal cell death, while apparent later reductions reflect the death of a smaller number of neurons that remain. Finally, it is also possible that the early elevations in CSF tau and VILIP-1 are due to cellular stress that leads to an increase in the normal release of these proteins, and that this stress decreases later in the disease. None of these scenarios necessarily contradicts the findings of accelerations of rates of brain atrophy with disease progression in mild cognitive impairment and LOAD (78, 79) and in ADAD (80, 81), but instead speaks to the possible relationship between the appearance of fluid indicators of cell injury and death and the subsequent structural sequelae of such processes, namely tissue shrinkage. If corroborated in additional cohorts, this pattern of marker change will likely have an impact on the definition of a positive neurodegenerative biomarker outcome in clinical trials, especially during the symptomatic phase. For example, depending on where a person falls along the pathologic cascade, a slowing of the course of neuronal injury/death may be indicated by a slowing of the rate of increase in neurodegenerative markers in individuals who are early in the disease process, but perhaps a stabilization or even a slowing or reversal of the downward trajectory later in the disease, potentially reflected as an increase in these markers. This is an important issue to consider as trials move forward.

MATERIALS AND METHODS

Study design

The objectives of this study were to characterize the patterns of fluid biomarker evidence of amyloid and neuronal pathologies in a large research cohort of individuals from families known to carry autosomal-dominant AD mutations and to test the hypothesis that the degree of biomarker abnormality increases over time with disease progression. Cross-sectional baseline data for five analytes in plasma and five analytes in CSF were obtained by immunoassay and compared in mutation carriers (n=146) and non-carriers (n=96) as a function of the participants' expected age of symptom onset. Longitudinal data from a subset of individuals (n=37) was obtained for 4 analytes in CSF to evaluate within-person change in biomarker concentrations over time.

Cohort

Participants at 50% risk of carrying an autosomal-dominant AD mutation in one of three genes (*APP*, *PSEN1*, *PSEN2*) were enrolled in the Dominantly Inherited Alzheimer Network (DIAN, NIA U19 AG032438, JC Morris, PI) at one of 11 performance sites (www.dian-info.org, clinicaltrials.gov number NCT00869817) (22). All study procedures were approved by the Washington University Human Research Protection Office and the local institutional review boards of each participating site. Written informed consent (or assent with proxy consent if capacity to consent was impaired) was obtained from all participants prior to their participation. Data in the present follow-up study are from participants with clinical, genetic, amyloid imaging (PIB PET), and fluid (CSF and plasma) measures that were collected and passed quality control (QC) standards as of Data Freeze 4 (DF4) (June 30, 2012). This cohort of 242 participants includes 110 individuals described in our initial cross-sectional

study (82) (n=29 NCs and n=62 MCs with CSF, and n=34 NCs and n=76 MCs with plasma). No longitudinal data were presented in our earlier study.

Clinical Evaluation

Participants underwent an extensive clinical evaluation which included family history of AD, personal medical history, and physical and neurologic examination. Cognitive status was determined with the Clinical Dementia Rating (CDR) in accordance with standard protocols and criteria (83-85). CDR 0 indicates cognitive normality (referenced as asymptomatic, AS), whereas CDRs of 0.5, 1, 2 or 3 correspond to cognitive impairments that are considered to be very mild, mild, moderate or severe, respectively (referenced as symptomatic, S). A clinical diagnosis of AD in individuals who are CDR 0.5 or greater was based on NINCDS-ADRDA criteria (86). The CDR Sum of Boxes (CDR-SB) and scores on the Mini-Mental State Exam (MMSE) (23) were also evaluated with standardized protocols. DIAN investigators involved in all clinical, cognitive, and other assessments were without knowledge of the mutation status of the participant. No research data, including genetic status, were provided to participants as part of the DIAN study, and special care was taken to prevent unblinding of participants to their mutation status (actual or perceived) in the presentation of all data (as is required by DIAN). Participants wishing to know their status are offered private genetic counseling and testing at no cost and no disclosure to any other entity. The parental age at symptom onset (AAO) was determined by a semi-structured interview with family members to estimate the age of the first progressive cognitive decline. The age at clinical onset of ADAD is similar between generations (87) and is affected mostly by mutation type and background family genetics (88).

Genetic Analysis

DNA sequencing for familial AD mutations and *APOE* genotyping was performed by the DIAN Genetics Core at Washington University. Ambient blood samples were shipped from the DIAN performance sites to both the National Cell Repository for Alzheimer's Disease (NCRAD) and the DIAN Genetics Core, and DNA was extracted at both sites using standard procedures. DNA sequencing of *APP*, *PSEN1* and *PSEN2* was performed by DIAN Genetics Core personnel, using Sanger sequencing methods on an ABI 3130xl, to determine the presence/absence of a disease-causing mutation (89). *APOE* genotyping was performed using an ABI predesigned real time TaqMan assay "rs7412 & rs429358" according to the manufacturer's protocol (ABI, Foster City, CA). DNA fingerprinting was performed with the Cell ID kit, using short tandem repeat (STR) analysis of 10 specific loci in the human genome, nine STR loci and Amelogenin for gender identification (Promega #G9500, Madison, WI) in order to confirm that DNA samples obtained by NCRAD and the DIAN Genetics Core were from the same individual. DNA sequencing, fingerprinting and genotyping was performed on DNA from NCRAD and the DIAN Genetics Core in parallel for each individual, and the data were compared for QC purposes. All individuals included in this analysis have 100% concordant data for each DNA sample and are defined as mutation non-carriers (NC) or mutation carriers (MC).

Fluid Collection

Protocols for the collection of blood (for plasma) and cerebrospinal fluid (CSF) are consistent with the biofluid protocol of the Alzheimer's Disease Neuroimaging Initiative (ADNI) (http://www.adni-info.org/) in order to facilitate comparisons between biomarker measures in LOAD and ADAD. Briefly, fluids were collected at 8:00 AM after overnight fasting. Blood was obtained by venipuncture into polypropylene tubes. Plasma (in EDTA) was prepared by standard methods, transferred to polypropylene transfer tubes and immediately frozen on dry ice. Following blood draw, CSF (≥15mL) was collected by standard lumbar puncture (LP) (typically L4/L5) using sterile technique. CSF was collected into polypropylene tubes and immediately frozen on dry ice. Frozen samples were shipped on dry ice the same day (or batch shipped from non-US sites after storing at −80°C) to the DIAN Biomarker Core at Washington University. Frozen samples were subsequently thawed, aliquoted (300-500uL) into polypropylene tubes, and stored at −84°C until analyzed.

Fluid Analysis

All fluid samples were analyzed by the DIAN Biomarker Core at Washington University. CSF concentrations of Aβ1-42, tau and ptau181 were measured by immunoassay using Luminex bead-based multiplexed xMAP technology (INNO-BIA AlzBio3™, Innogenetics, Ghent, Belgium). The assay has intra-assay precisions of 2.5-6% for Aβ42, 2.2-6.3% for tau and 2-11% for ptau181) (68, 90). Concentrations of CSF Aβ1-40 were measured by plate-based enzyme-link immunosorbant assay (ELISA) (research prototype INNOTEST™ Aβ1-40, Innogenetics, Ghent, Belgium), as were concentrations of Aβ1-42 (INNOTEST™ Aβ1-42, Innogenetics, Ghent, Belgium) in order to permit comparison of Aβ1-42 concentrations in the DIAN cohort to those obtained in the many published biomarker studies in LOAD which use the INNOTEST kit (14, 91-97). Concentrations of plasma Aβ species (Aβ1-40, Aβ1-42, Aβx-40, and Aβx-42) were measured by xMAP (INNO-BIA Plasma Aβ Forms Multiplex Assay™, Innogenetics, Ghent, Belgium). Fluid samples were also analyzed for visinin-like protein 1 (VILIP-1) using a two-site immunoassay implemented via a microparticle-based immunoassay system (Erenna, Singulex, Alameda, CA). This assay utilizes a monoclonal antibody coated on magnetic beads for the "capture" step and affinity purified sheep antibody labeled with AlexaFluor-647 for the detection step. The assay has an intra-assay precision of 4.4%, an inter-assay precision of 6.2%, and a lower limit of quantitation of 2.1 pg/mL in CSF and 3.9 pg/mL in plasma. For all assays, values had to meet QC standards, including co-efficients of variation (CV) ≤ 25%, kit "controls" within the expected range as defined by the manufacturer, and measurement consistency of two common CSF samples that were included on each plate. Two-hundred and thirty-seven plasma samples and 206 CSF samples passed analytical QC and, therefore, contributed to the current dataset.

Longitudinal CSF samples collected over 2-3 years after baseline were available from a subset of participants (n=37) at the time of DF4. For longitudinal analyses, samples from the same individuals were reanalyzed for Aβ1-42 (by AlzBio3 only), tau, ptau181 and VILIP-1 after being loaded onto the same assay plate in order to compare within-person change in analyte concentrations over time.

PET PIB Image Analysis

Participants were evaluated for fibrillar brain β-amyloid using positron emission tomography (PET) with the amyloid imaging agent Pittsburgh Compound B (PIB) (98) within 3 months of clinical evaluation and fluid collection. All images underwent QC inspection and pre-processing at a DIAN centralized site. Magnetic resonance imaging (MRI) was performed on qualified 3T scanners at each site with initial and ongoing QC and matching between site scanners performed according to the ADNI protocol and QC (see http://www.adniinfo.org). The volumetric scan consisted of an 8-10 minute 3D T1-weighted image (i.e., MP-RAGE) with 1.0 × 1.0 × 1.2 mm resolution. β-amyloid imaging was performed with [^{11}C] PIBPET scans, acquired with a 30 minute dynamic (4 × 5 minute frames), 3D acquisition, beginning 40 minutes after a bolus injection of 15 mCi of PIB. The T1-weighted MRI scans were processed through the FreeSurfer image analysis suite Version 5.1 using Dell PowerEdge 1950 servers with Intel Xeon processors running CentOS 5.5 Linux. FreeSurfer involves cortical reconstruction and volumetric segmentation (http://surfer.nmr.mgh.harvard.edu/). The processing routine includes segmentation of the subcortical white matter and deep gray matter volumetric structures, extraction of the cortical surfaces, and parcellation of cortical regions. PET frame-to-frame motion correction and PET-MRI alignment was accomplished through standard registration techniques written with in-house software (99). PET images were then transformed into each individual participant's MRI space. For each FreeSurfer region-of-interest, standardized uptake value ratios (SUVR) were calculated using a brainstem (pons) grey matter reference. To minimize the impact of partial volume effects on the PET signal, a regional spread function-based approach for partial volume correction was used for all regional measurements (100). The mean cortical SUVR was calculated as the summary of regions within the prefrontal cortex, gyrus rectus, lateral temporal and precuneus regions. As a preliminary analysis in this ADAD cohort, a *k*-means clustering algorithm (101) implemented in R (http://www.R-project.org/) was used to estimate a cut-off point for PIB positivity. Three clusters were defined, and the threshold between the first and second cluster, 0.85, was considered the cut-off for the current study.

Longitudinal Cognitive Performance Measures

Within-individual performance over time on a composite of three psychometric measures shown to be sensitive to cognitive decline in asymptomatic MCs (CDR 0) in DIAN (33) was evaluated in the longitudinal cohort (n=36). These standardized tests included measures of episodic memory (Logical Memory IA-Immediate Recall and Logical Memory IIA-Delayed Recall from the Wechsler Memory Scale-Revised) (34) and a measure of speeded visual spatial processing (Digit Symbol Coding from the Wechsler Adult Intelligence Scale-Revised) (35). Z scores for each individual measure were derived from the longitudinal sample and averaged to form a composite. Higher z scores are indicative of better performance.

Statistical Analyses—The estimated years to symptom onset (EYO) for each DIAN participant was calculated as the participant's age at clinical evaluation minus the reported parental age at symptom onset (AAO). For example, if a participant's age was 35 and the reported parental AAO was 45, then the EYO for this participant would be −10. Participant characteristics of the cohort at the data freeze conducted on June 30, 2012, as well as at

baseline for the subset of participants with longitudinal data, were summarized as mean ± standard deviation for continuous characteristics and as N (column percent) for categorical characteristics. Continuous participant characteristics were compared between groups with general linear mixed models, dichotomous characteristics were compared with mixed-effects logistic regression models (102), and family mutations were compared with a mixed-effects multinomial logistic regression model (103). All of the above models incorporated a random intercept at the family level in order to account for within-family dependencies. Pairwise testing was conducted only after an omnibus test suggested significant ($p<0.05$) differences between groups.

In general, a biomarker "trajectory" is defined as the pattern of biomarker change over time. In DIAN cross-sectional analyses, participants' EYO serves as a proxy for time. In order to explore the trajectories of the biomarkers over the range of EYOs, non-parametric curves were fit to the cross-sectional (baseline) data using a locally weighted regression method (LOESS) (104). General linear mixed models, with linear, quadratic or cubic trends where appropriate, were then utilized to estimate the trajectories of the biomarkers (and CDR-SB and MMSE) over EYO and subsequently test for differences in mean values between the participant groups (NC, MC) at 5 year intervals along the EYO distribution, using approximate t-tests. Comparisons between the participant group biomarker concentrations and trajectories were also made through approximate t- or F-tests on appropriate interactive effects in these models. Additionally, the trajectories in both participant groups were tested relative to zero with approximate t-tests for linear trajectories, or with approximate F-tests for non-linear trajectories. In order to account for the within-family dependencies of the biomarkers, these models incorporated random intercepts at the family level which also allowed differential variances across the participant groups (NC, MC). Given that the current study aimed to generate scientific hypotheses that will be critically tested in the future, values were not adjusted for multiple comparisons.

Longitudinal analyses employed general linear mixed models with random intercepts/slopes (32) at the subject level and random intercepts at the family level in order to quantify and statistically evaluate the within-person rate of change in biomarker concentrations and psychometric test performance. The fixed effects of these models included the participant groups (NC, MC) and where participants fell with respect to their estimated years to symptom onset (EYO≤0 vs EYO>0) at baseline, as well as their interactions with time. Consequently, different slopes across participant groups prior to and after baseline EYO 0 were estimated, thus facilitating the comparisons of the longitudinal annual rate of change across the two groups. All general linear mixed models in the longitudinal analyses were estimated using restricted maximum likelihood estimation, with the approximate F-test denominator degrees-of-freedom based on the method of Kenward and Roger (105). To protect participant confidentiality regarding mutation status and investigator blinding (as required by the DIAN protocol), longitudinal individual data points are plotted with reference to relative EYO (EYO≤0 vs EYO>0), not specific EYO. P-values reported for the Pearson or Spearman correlation coefficients were computed based on 2000 bootstrap replications using the percentile-t method (105). All statistical analyses were performed

using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA), and statistical significance was defined as $p < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AAO	age at symptom onset
AD	Alzheimer disease
ADAD	autosomal-dominant Alzheimer disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
APOE	apolipoprotein E
APP	amyloid precursor protein
CDR	Clinical Dementia Rating
CDR-SB	CDR Sum of Boxes
CSF	cerebrospinal fluid
CV	co-efficient of variation
DIAN	Dominantly Inherited Alzheimer Network
EAO	estimated age at symptom onset
EDTA	ethylenediaminetetraacetic acid
EYO	estimated years to symptom onset
ELISA	enzyme-linked immunosorbant assay
LOAD	late-onset Alzheimer disease
LP	lumbar puncture
MC	mutation carrier

MC-AS	asymptomatic mutation carrier
MC-S	symptomatic mutation carrier
MMSE	Mini-Mental State Exam
MRI	magnetic resonance imaging
NC	mutation non-carrier
NCRAD	National Cell Repository for Alzheimer's Disease
PET	positron emission tomography
PIB	Pittsburgh Compound B
PSEN1	presenilin-1
PSEN2	presenilin-2
Ptau	phosphorylated tau
QC	quality control
SUVR	standardized uptake value ratio
VILIP-1	visinin-like protein 1

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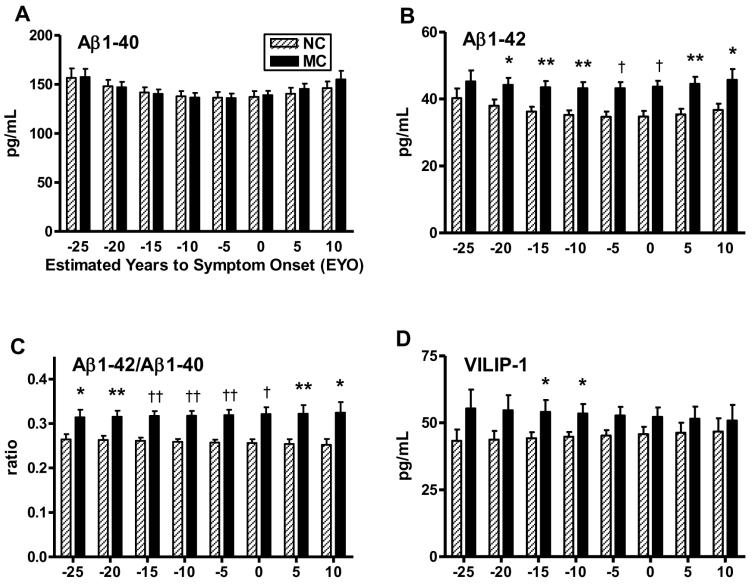

Figure 1.

Histograms of predicted mean plasma biomarker concentrations in mutation non-carriers and carriers at select levels of estimated years to symptom onset (EYO) as determined by general linear mixed models, accounting for within-family dependencies. Estimated trajectories of plasma (**A**) Aβ1-40, (**B**) Aβ1-42, (**C**) the ratio of Aβ1-42/1-40 and (**D**) VILIP-1 are shown for mutation non-carriers (NC, hatched) and mutation carriers (MC, solid). Values represent mean ± SE. MC significantly different from NC at a given EYO at *p<0.05, **p<0.01, †p<0.001, ††p<0.0001.

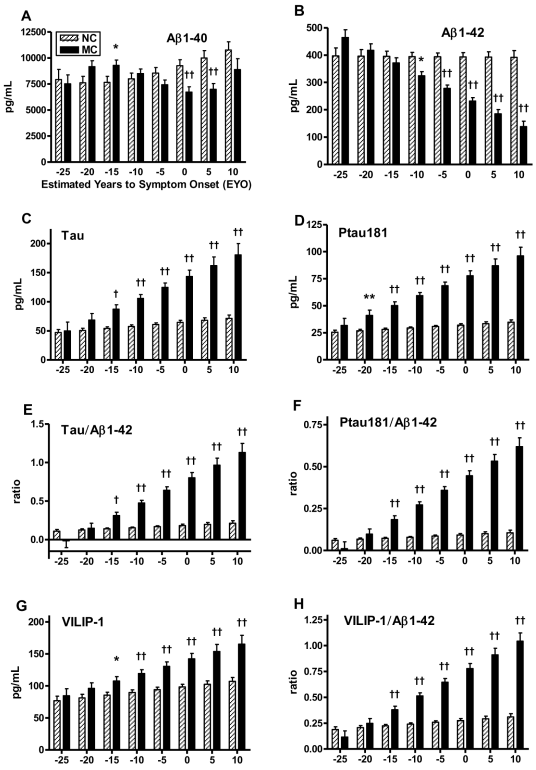

Figure 2.

Histograms of predicted mean CSF biomarker concentrations in mutation non-carriers and carriers at select levels of estimated years to symptom onset (EYO) as determined by general linear mixed models, accounting for within-family dependencies. Estimated trajectories of CSF (**A**) Aβ1-40, (**B**) Aβ1-42, (**C**) tau, (**D**) ptau181, (**E**) tau/Aβ1-42 ratio, (**F**) ptau181/Aβ1-42 ratio, (**G**) VILIP-1 and (**H**) VILIP-1/ Aβ1-42 ratio are shown for mutation non-carriers (NC, hatched) and mutation carriers (MC, solid). Values for Aβ1-42 were obtained with AlzBio3. Values represent mean ± SE. MC significantly different from NC at a given EYO at *p<0.05, **p<0.01, †p<0.001, ††p<0.0001.

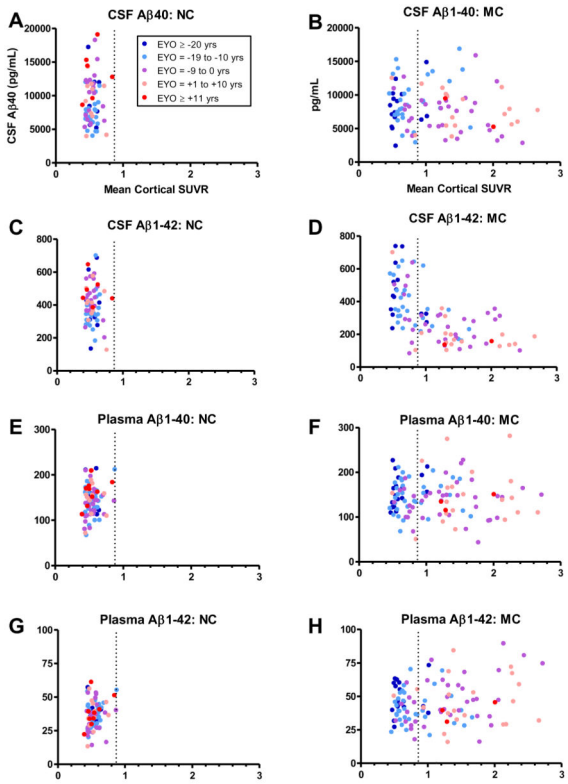

Figure 3.

Association between fluid Aβ measures and mean cortical PIB retention in mutation non-carriers and carriers. Concentrations of CSF (**A, B**) Aβ1-40 and (**C, D**) Aβ1-42, and concentrations of plasma (**E, F**) Aβ1-40 and (**G, H**) Aβ1-42 are shown for mutation non-carriers (NC, left column) and carriers (MC, right column). Units on the Y axes are pg/mL. Units on the X-axes are mean cortical PIB standardized uptake value ratio (SUVR) calculated from prefrontal cortex, gyrus rectus, lateral temporal and precuneus regions using a brainstem (pons) grey matter reference following application of partial volume correction. Cortical PIB-positivity in this ADAD cohort is defined as SUVR ≥0.85 (vertical dashed line) based on a *k*-means clustering algorithm implemented in R (see Materials and Methods section). Symbol colors identify groupings of the estimated years to symptom onset (EYO) in the participant groups, with blue-to-red gradations extending from dark blue (EYO earlier than −20 years) to dark red (EYO later than +11 years).

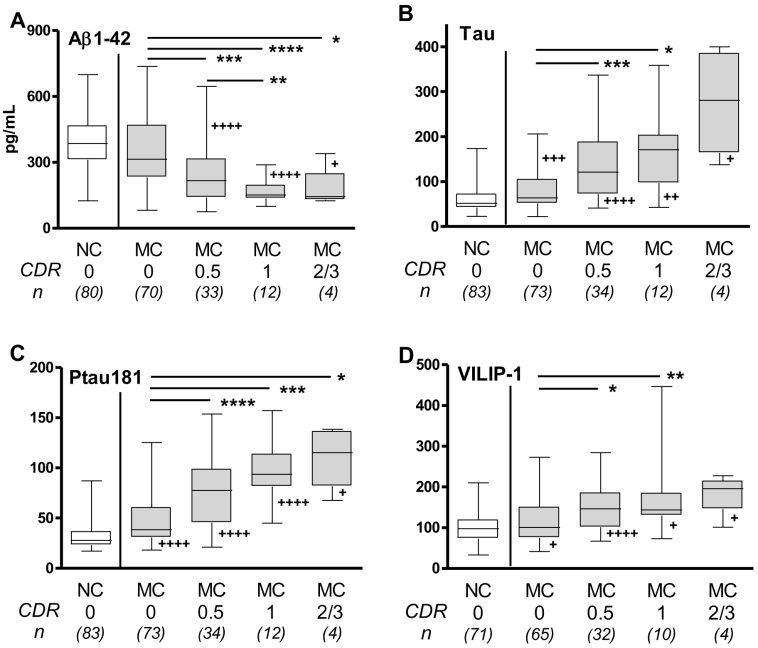

Figure 4.

Evaluation of CSF biomarker change over disease progression in mutation non-carriers and carriers as estimated from cross-sectional baseline measures from individuals at different stages of dementia severity. Box and whisker plots (median ± 25th/75th percentiles) of CSF (**A**) Aβ1-42, (**B**) tau, (**C**) ptau181 and (**D**) VILIP-1 obtained at baseline are shown for mutation non-carriers (NC, white box, as the normal reference group) and mutation carriers (MC, grey boxes) with the indicated Clinical Dementia Rating scores (CDR 0=cognitively normal; 0.5=very mild; 1=mild; 2=moderate; 3=severe). The number of participants in each group is shown in italics in parentheses. Units on Y axes are pg/mL. Horizontal lines indicate statistical significance between the groups as determined by general linear mixed models, accounting for within-family dependencies: * P<0.05, ** p<0.01, *** p<0.001, **** p<0.0001; Different from NC: + P<0.05, ++ p<0.01, +++ p<0.001, ++++ p<0.0001.

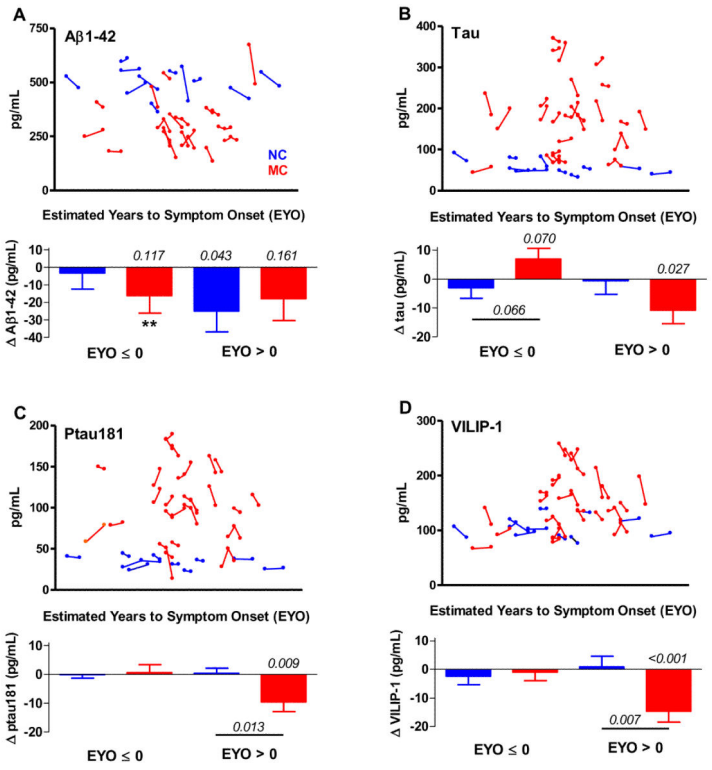

Figure 5.

Evaluation of CSF biomarker change over time in mutation non-carriers (NC) and mutation carriers (MC) in the longitudinal cohort. Concentrations of CSF (**A**) Aβ1-42, (**B**) tau, (**C**) ptau181 and (**D**) VILIP-1 are shown as a function of the estimated years to symptom onset (EYO) at the time of sample collection. In order to maintain participant and investigator blinding to mutation status, only the estimated age at symptom onset (vertical dashed line, EYO=0) is shown. Top(s), spaghetti plots show individual data points, Bottom(s), histograms show the associated estimated mean (SE) annual within-individual rate of change in biomarker concentrations in the two genetic groups in the period prior to their estimated age at symptom onset (EAO, EYO≤0) or the period after their EAO (EYO>0). General linear mixed models were utilized that allowed for different slopes in the genetic groups, as well as based on where participants fell with respect to their estimated years to symptom onset at baseline (EYO≤0 vs EYO>0). In order to account for the within-individual and within-family correlations, the models incorporated random intercepts/slopes at the individual level and a random intercept at the family level. Blue, NC; red, MC. Italicized numbers above the mean group slopes correspond to the significant or near significant p values comparing the slopes to zero. Italicized numbers above the horizontal lines correspond to the significant p values comparing the slopes between the NC and MC groups within the indicated EYO range (EYO≤0 vs EYO>0).

Table 1

Characteristics of the cross-sectional DIAN cohort at baseline

	NC N=96	MC-AS N=84	MC-S N=62
Age, y (SD)	40.3 (10.1)	34.6 (8.7)*	45.2 (10.2)*†
Parental AAO, y (SD)	46.8 (7.0)	47.3 (6.2)	45.3 (8.7)
Participant EYO, y (SD)	−6.5 (12.4)	−12.7 (8.2)*	−0.1 (7.8)*†
Female, n (%)	58 (60.4)	49 (58.3)	36 (58.1)
APOE ε4+, n (%)	24 (25.3)	20 (24.1)	16 (26.2)
Family mutations, n (%)			
PSEN1	59 (61.5)	59 (70.2)	52 (83.9)
PSEN2	11 (11.5)	10 (11.9)	2 (3.2)
APPT	26 (27.0)	15 (17.9)	8 (12.9)
CDR, n (%)			
0	96 (100)	84 (100)	–
0.5	–	–	40 (64.5)
1	–	–	17 (27.5)
2	–	–	3 (4.8)
3	–	–	2 (3.2)
CDR-SB (SD)	0.06 (0.20)	0.02 (0.11)	3.71 (3.78) *†
MMSE (SD)	29.0 (1.3)	29.1 (1.3)	22.9 (6.9)*†
Plasma			
Aβ1-40	144.2 (34.1)	144.6 (36.4)	145.0 (48.4)
Aβ1-42	36.6 (10.5)	43.1 (12.7)*	46.3 (17.8)*
Aβ1-42/1-40	0.258 (0.065)	0.304 (0.075)*	0.336 (0.149)*
Aβx-40	148.9 (36.4)	152.6 (40.1)	153.4 (49.4)
Aβx-42	27.7 (7.4)	33.5 (9.6)*	37.4 (15.9)*
Aβx-42/x-40	0.195 (0.064)	0.230 (0.075)*	0.269 (0.188)*
VILIP-1	45.3 (17.3)	53.4 (34.9)	51.6 (25.0)
CSF			
Aβ1-40	9076.9 (3429.6)	8587.2 (3868.1)	7375.6 (2831.4)*
INNO Aβ1-42	712.4 (288.7)	649.7 (335.0)*	384.0 (215.8)*†
Aβ1-42	392.6 (121.9)	357.3 (167.2)	220.7 (116.7)*†
Tau	59.8 (27.6)	81.4 (44.4)*	157.0 (92.5)*†
Ptau181	30.3 (10.9)	46.4 (22.8)*	83.0 (35.4)*†
Tau/Aβ1-42	0.163 (0.098)	0.314 (0.266)*	0.883 (0.604)*†
Ptau181/Aβ1-42	0.085 (0.051)	0.181 (0.154)*	0.481 (0.260)*†
VILIP-1	93.7 (32.0)	106.8 (45.2)	148.9 (63.7)*†
VILIP-1/Aβ1-42	0.257 (0.123)	0.388 (0.261)*	0.819 (0.416)*†

Demographic variables correspond to mean (SD) or n (%) as indicated. Biomarker variables correspond to mean (SD). Continuous demographic variables were compared with general linear mixed models, gender and APOE4 status were compared with mixed-effects logistic regression models, and family mutations were compared with a mixed-effects multinomial logistic regression model. Pairwise testing was conducted only after an omnibus test suggested significant ($p<0.05$) differences between groups.

* $p< 0.05$ compared with NC ,

† $p< 0.05$ compared with MC-AS. Units for single biomarker analytes are pg/mL. Analyte combinations are represented as ratios.

Abbreviations: AAO, age at symptom onset; Aβ, amyloid-β; *APOE* ε4+, presence of at least one ε4 allele of apolipoprotein E; *APP*, amyloid precursor protein; CDR, Clinical Dementia Rating score (0=cognitively normal, 0.5=very mild; 1=mild; 2=moderate; 3=severe); CDR-SB, CDR Sum of Boxes (range 0-18, with 0 indicating no impairment); EYO, estimated years to symptom onset; INNO, INNOTEST ELISA; MC-AS, asymptomatic mutation carrier (CDR 0); MC-S, symptomatic mutation carrier (CDR >0); MMSE, Mini-Mental State Exam score (range 0-30, with 30 as perfect score); NC, mutation non-carrier; *PSEN1*, presenilin 1; *PSEN2*, presenilin 2; ptau, phosphorylated tau; VILIP-1, visinin-like protein 1.

Table 2

Baseline characteristics of the longitudinal DIAN sub-cohort

	NC N=11	All MC N=26	MC-AS N=9	MC-S N=17
Age, y (SD)	40.0 (8.3)	42.1 (8.3)	37.7 (7.7)	44.4 (7.9)
Female, n (%)	5 (46%)	13 (50%)	4 (44%)	9 (53%)
APOE ε4+, n (%)	2 (18%)	11 (42%)	7 (78%)*	4 (24%)†
MMSE (SD)	29.4 (0.9)	25.0 (4.6)*	28.9 (1.4)	22.9 (4.3)*†
CDR, n (%)				
0	11 (100)	9 (34.6)	9 (100)	-
0.5	-	11 (42.3)	-	11 (64.7)
1	-	6 (23.1)	-	6 (35.3)
LP interval, mo (SD)	23.8 (12.1)	13.7 (4.9)	15.5 (7.9)	12.8 (1.8)
CSF Aβ1-42	517.6 (57.3)	316.4 (116.3)*	344.4 (103.6)*	299.5 (123.5)*
CSF Tau	58.2 (18.1)	183.1 (88.7)*	164.8 (116.4)*	192.8 (72.4)*
CSF Ptau181	33.4 (7.5)	108.4 (42.9)*	105.7 (52.0)*	109.8 (38.9)*†
CSF VILIP-1	115.0 (19.4)	164.2 (59.5)	156.3 (65.9)	168.4 (57.6)*
EYO≤0				
Aβ1-42	514.3 (67.1)	320.7 (100.1)*	344.4 (103.6)*	278.0 (86.8)*
Tau	64.2 (19.2)	175.2 (101.6)*	164.8 (116.4)	190.8 (82.1)*
Ptau181	34.9 (7.7)	109.4 (47.6)*	105.7 (52.0)*	115.0 (44.0)*
VILIP-1	115.0 (18.5)	155.2 (66.6)	156.3 (65.9)	153.5 (74.0)
EYO>0				
Aβ1-42	523.5 (43.5)	310.3 (141.4)*	-	310.3 (141.4)*
Tau	47.8 (11.2)	193.9 (70.8)*	-	193.9 (70.8)*
Ptau181	30.6 (7.3)	107.0 (37.8)*	-	107.0 (37.8)*
VILIP-1	114.9 (23.9)	176.5 (48.6)	-	176.5 (48.6)

Demographic variables correspond to mean (SD) or n (%) as indicated. Biomarker variables correspond to mean (SD). Continuous demographic variables were compared with general linear mixed models, while gender and APOE4 status were compared with mixed-effects logistic regression models. Pairwise testing was conducted only after an omnibus test suggested significant ($p<0.05$) differences between groups.

* $p< 0.05$ compared with NC ,

† $p< 0.05$ compared with MC-AS. Units for biomarker analytes are pg/mL.

Abbreviations: Aβ, amyloid-β; *APOE* ε4+, presence of at least one ε4 allele of apolipoprotein E; CDR, Clinical Dementia Rating score (0=cognitively normal, 0.5=very mild; 1=mild); CDR-SB, CDR Sum of Boxes (range 0-18, with 0 indicating no impairment); EYO, estimated years to symptom onset; LP, lumbar puncture; MC, mutation carrier; MC-AS, asymptomatic mutation carrier (CDR 0); MC-S, symptomatic mutation carrier (CDR >0); MMSE, Mini-Mental State Exam score (range 0-30, with 30 as perfect score); NC, mutation non-carrier; ptau, phosphorylated tau; VILIP-1, visinin-like protein 1.