BACKGROUND

Biomarker changes that occur in the period between normal cognition and the diagnosis of sporadic Alzheimer's disease have not been extensively investigated in longitudinal studies.

METHODS

We conducted a multicenter, nested case–control study of Alzheimer's disease biomarkers in cognitively normal participants who were enrolled in the China Cognition and Aging Study from January 2000 through December 2020. A subgroup of these participants underwent testing of cerebrospinal fluid (CSF), cognitive assessments, and brain imaging at 2-year–to–3-year intervals. A total of

Preclinical Alzheimer's disease has been characterized by the presence of normal cognitive function and abnormal levels of cerebrospinal fluid (CSF) biomarkers.[1] The preclinical stage is typically followed by mild cognitive impairment, which progresses to clinically apparent dementia in some persons. Neuropathologic abnormalities and changes in biomarker levels can begin 15 to 20 years before clinical manifestations of Alzheimer's disease.[2-4]

Changes in CSF biomarkers such as levels of amyloid-beta (Aβ), total tau, phosphorylated tau 181, and neurofilament light chain (NfL) have been indicators in preclinical Alzheimer's disease[5-8] that become abnormal sequentially rather than simultaneously.[9] Some previous studies of the sequential appearance of changes in CSF biomarkers have involved persons with autosomal dominant Alzheimer's disease, which accounts for only a small proportion of Alzheimer's disease cases, and these studies have typically used an estimated number of years before the onset of Alzheimer's disease symptoms to define the timeline of biomarker changes.[10-14]

Determination of the sequence of these changes in sporadic Alzheimer's disease is challenging because a person's clinical course, beginning with normal cognition and progressing to Alzheimer's disease, cannot be predicted.[15] Most studies regarding biomarkers in sporadic Alzheimer's disease have been cross-sectional and may not have reflected alterations of biomarkers over the period from a normal cognitive state to Alzheimer's disease. Longitudinal studies, such as the Alzheimer's Disease Neuroimaging Initiative, have advanced our understanding of preclinical sporadic Alzheimer's disease by exploring these biomarker changes.[16-19]

However, a limitation of these studies has been the underrepresentation of Asian populations, which potentially has limited the generalizability of the results. In addition, the relatively short follow-up periods in previous studies do not reflect the lengthy trajectory over decades of biomarker alterations leading to the onset of Alzheimer's disease. We examined a cohort of participants from one of the nested studies in the China Cognition and Aging Study (COAST) with a goal of estimating the trajectory of changes in several CSF biomarkers in relation to the onset of Alzheimer's disease.

tions, including lumbar puncture, and follow-up. The protocol was approved by the ethics committee of Xuanwu Hospital, Capital Medical University, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Participants were not compensated for participating in the study. The sponsors had no role in the study design; collection, analysis, or interpretation of the data; or the writing of the report.

NESTED CASE–CONTROL APPROACH

Our study required that participants be observed for more than 15 years but not more than 20 years. They would undergo at least three assessments that had to include an initial baseline visit, a visit during which the diagnosis was made, and an intermediate follow-up visit between the two. The overarching COAST study had 52,388 participants in 2000, of whom 32,061 were eligible for and enrolled in the current substudy. Of the participants who were enrolled, 30,272 were excluded (6435 discontinued the study, 3172 were untraceable, 10,470 had died, 2759 were cognitively impaired, 4514 were excluded for [illegible] health [illegible] rtltel3 (t)-40.7 (-537.3 (w)6..5 (-28.2 (0)-2 (r2(n)-208(d d

A	61.3 4.1	61.2 4.1
. (%)		
	328 (50.6)	327 (50.5)
	320 (49.4)	321 (49.5)
, , (%)		
, 6	25 (3.9)	28 (4.3)
, 7 9	28 (4.3)	26 (4.0)
, 10 12	212 (32.7)	210 (32.4)
, 13 17	334 (51.5)	335 (51.7)
, 18 21	49 (7.6)	49 (7.6)
APOE . (%)		
	516 (79.6)	407 (62.8)
	132 (20.4)	241 (37.2)
	121 (18.7)	183 (28.2)
	11 (1.7)	58 (9.0)
	29.5 1.0	29.4 1.2
- B	0	0
	16.8 0.6	16.8 0.7
B		
A_{42} A_{40}	0.1 0.0	0.1 0.0
/	219.2 52.6	214.6 41.2
181 /	48.8 9.9	48.4 7.8
/	633.9 139.3	645.4 140.4
^{3}	7708.3 621.8	7683.0 645.5

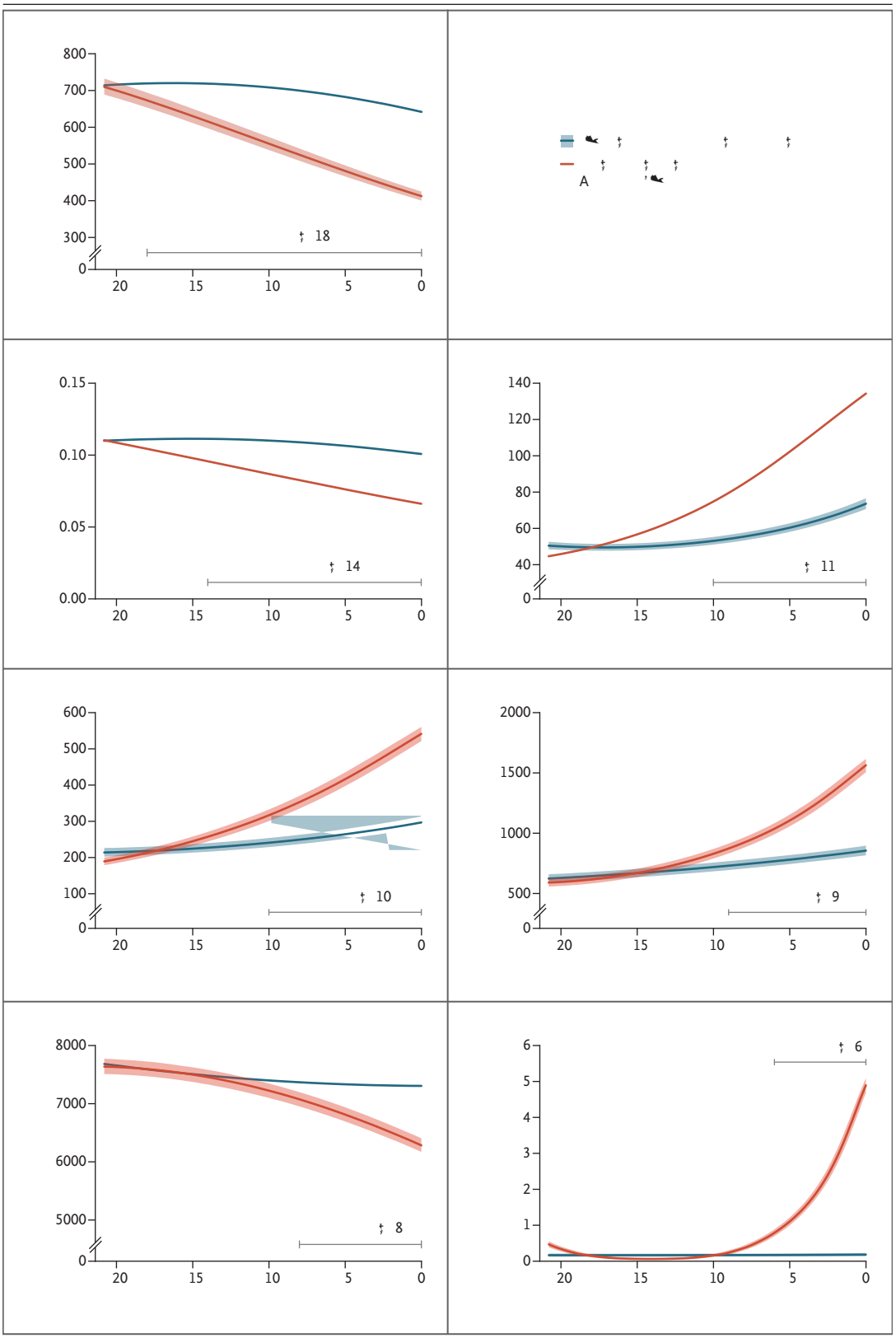
* . A - , *APOE* . ( ) 0 30, B (- B) 0 18, ( ) 0 25, .

BRAIN VOLUMETRIC IMAGING

Structural magnetic resonance imaging of the brain was performed with 3.0-T scanners (Siemens) with a 20-channel phased-array head–neck coil. The absolute volume of each region of interest was determined with the use of FreeSurfer software, version 5.3.0 (Table S2). The relative region-of-interest volume (the absolute region-of-interest volume as a percentage of the intracranial volume) was calculated to correct for differences in brain size among the participants. Left and right hippocampal volumes were summed to assess the degree of brain atrophy.[25]

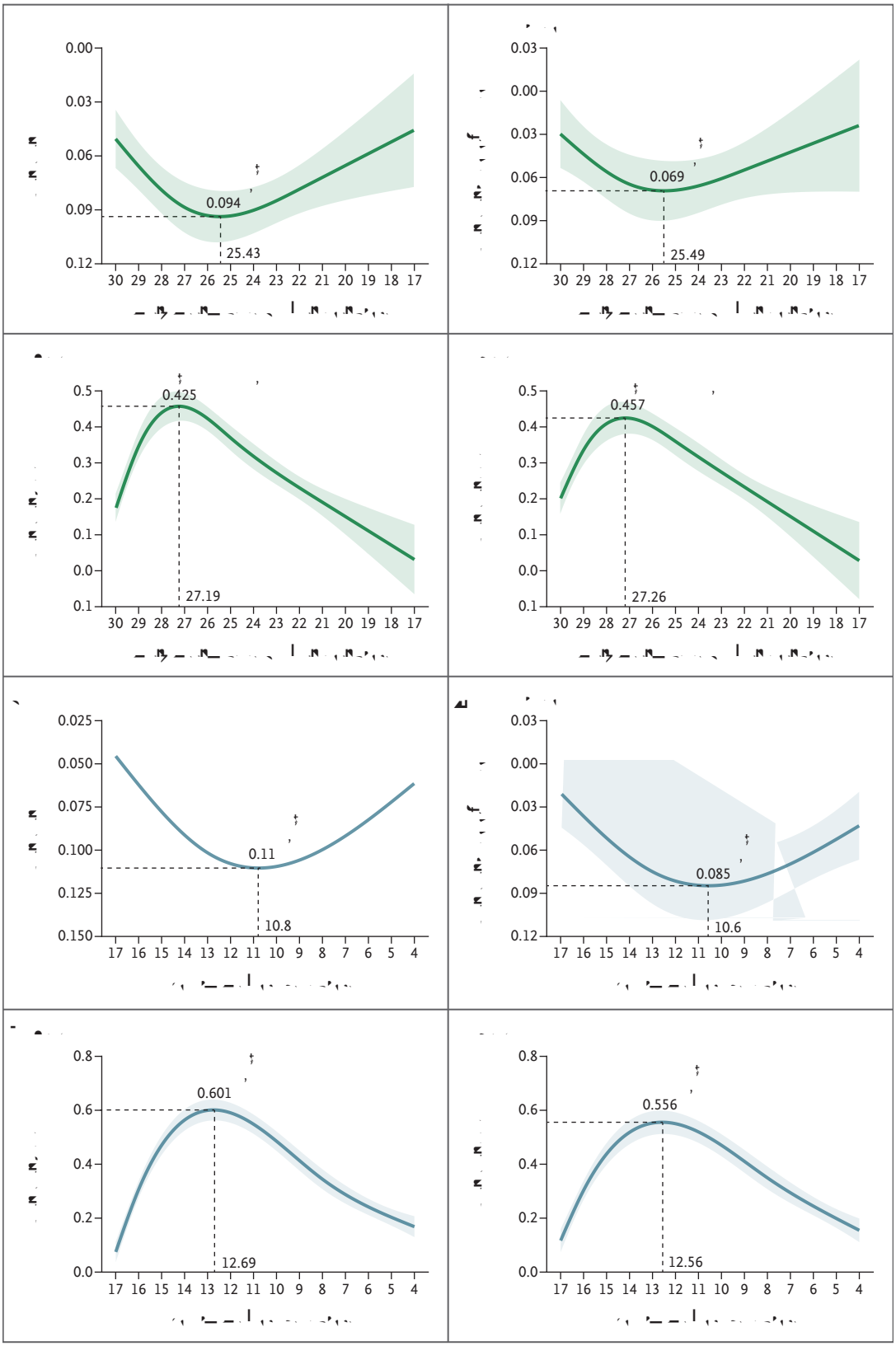
STATISTICAL ANALYSIS

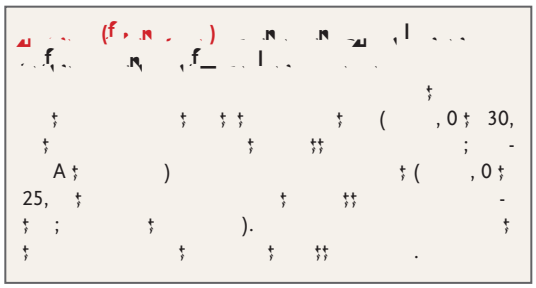
Biomarker trajectories were assessed with the use of a backward timescale. In analyses of participants in whom Alzheimer's disease was diagnosed, year 0 was used to denote the year of diagnosis. In analyses of cognitively normal participants, year 0 corresponded to the end of follow-up.



We used the R software, version 4.3.1, lcmm package (R Foundation for Statistical Computing) to establish latent-class mixed models for the estimation of the trajectories of each biomarker over time (see the Supplementary Methods section).[26,27] These models incorporated quadratic functions of retrospective time and were adjusted for case–control status, covariates (e.g., age, sex, education level, and *APOE* status), and their interactions with time and time squared. Within-participant correlations were accounted for by correlated random intercepts and slopes of time and time squares. Spline functions were integrated into the models to capture potential variations in biomarker trajectories over time. The final models with the optimal number of knots were determined with the use of the Akaike and Bayesian information criteria.[28]

Using the R software mvtnorm package for Wald tests, we evaluated the differences in biomarkers between participants with Alzheimer's disease and cognitively normal participants for each year up to year 0; a negative value dehe R st3309 (r)-10 ( )]TJETEMC9 (a)-29.1 (l)7.1 (d t)-20.4 (e)-10. 0 10 62 261 T7





matched cognitively normal group. The study included only Han Chinese persons. Within both groups, men slightly outnumbered women. Baseline CSF biomarker levels, cognitive scores, and hippocampal volumes were similar in the two groups. Participants in whom Alzheimer's disease ultimately developed were more likely than their matched controls to be carriers of the *APOE* ε4 allele (37.2% vs. 20.4%). Overall, the participants had a level of education that slightly surpassed the educational norms for the general population of older adults in China. The representativeness and other characteristics in the study population as compared with the Chinese general population are shown in Table S3.

The median follow-up was 19.9 years (interquartile range, 19.5 to 20.2). The number of participants whose follow-up times differed from the prespecified schedule and the proportion with missing data at each follow-up are shown in Tables S4 and S5. In general, biomarker and clinical data were missing at one or more visits for less than 16% of the participants in each group.

BIOMARKER CHANGES

Figure 1 shows the modeled estimated biomarker trajectories for each group, and Figure S1 shows spaghetti plots of biomarker changes in each participant. As compared with the level of CSF $A\beta_{42}$ in cognitively normal controls, the level in participants in whom Alzheimer's disease developed differed an estimated 18 years before diagnosis; the difference in mean values at that time (negative values indicate the biomarker was lower in participants with Alzheimer's disease than in normal controls) was −59.13 pg per milliliter (95% confidence interval [CI], −108.08 to −10.18) (Tables S6 and S15). A difference in the ratio of CSF $A\beta_{42}$ to $A\beta_{40}$ between the two groups appeared an estimated 14 years before the diagnosis of Alzheimer's disease (difference in mean values, −0.01 pg per milliliter; 95% CI, −0.02 to −0.001) (Tables S7 and S16). Differences between the two groups in CSF phosphorylated tau 181 and total tau concentrations occurred an estimated 11 years and 10 years before diagnosis, respectively; at those times, the mean differences in phosphorylated tau 181 and total tau concentrations were 7.10 pg per milliliter (95% CI, 1.10 to 13.10) and 87.10 pg per milliliter (95% CI, 45.10 to 129.10), respectively (Tables S8, S9, S17, and S18).

Visual inspection of the curves of concentrations of each CSF marker showed that these differences continued to widen over time. A difference between the groups in CSF NfL was observed 9 years before diagnosis, with visual inspection of the curves showing its trajectory progressively deviating from the concentrations observed in cognitively normal groups at that time to a final mean difference in NfL level of 228.29 pg per milliliter (95% CI, 122.42 to 334.16) (Tables S10 and S19). The combined bilateral hippocampal volume decreased with age in both groups; however, the decrease began to differ between the two groups 8 years before diagnosis, at which time there was a mean difference in volume of −358.94 mm^3 (95% CI, −613.20 to −104.69) in the group with Alzheimer's disease as compared with the control group (Tables S11 and S20).

The Alzheimer's disease group differed from the control group in terms of mean CDR-SB scores at an estimated 6 years before diagnosis (Tables S12 and S21). After exclusion of participants with one or more missing biomarker values across follow-up visits, the times of divergence between groups were similar to those in the main analysis (Table S13). When we included participants who had data only from baseline and the year of diagnosis (80 with Alzheimer's disease and 15 who were cognitively normal), the findings remained similar (Table S14).

We placed each biomarker trajectory on a scale from −1 to 1 by using the standardization of fitted values, anchoring the first time point that showed a group difference to −1 to generate superimposed trajectories (Fig. 2). On visual inspection, an initial increase followed by a decrease was apparent in the rate of change in CSF biomarkers in participants with Alzheimer's dis-

ease; in the control group, the rate of change appeared to have flatter trajectories (Fig. S5).

In individual participants with Alzheimer's disease, the progression of CSF $A\beta_{42}$ concentration, CSF $A\beta_{42/40}$ ratio, total tau concentration, and phosphorylated tau 181 concentration in relation to cognitive decline appeared to initially accelerate and, on visual inspection, peaked at an MMSE score of approximately 25 and an LMT score of approximately 11 (Fig. 3A, 3B, 3E, and 3F). Subsequently, despite further decline in cognitive scores, the rate of change appeared to slow. The rate of change in total tau concentration increased until it reached an MMSE score of 27.26 and an LMT score of 12.56 (Fig. 3D and 3H) and thereafter appeared to slow. The annual rate of change for phosphorylated tau 181 concentration peaked at an MMSE score of 27.19 and an LMT score of 12.69 (Fig. 4C and 4G).

DISCUSSION

In this study assessing change in CSF biomarkers in 648 persons who ultimately received a diagnosis of Alzheimer's disease and the same number of matched persons who remained cognitively normal, the times before Alzheimer's disease diagnosis at which biomarkers diverged between groups ranged from 18 years for CSF $A\beta_{42}$ concentration to 6 years for cognitive decline as measured on the CDR-SB, a scale that has been widely used in clinical trials. The results with regard to changes in the biomarkers in sporadic Alzheimer's disease are similar in most respects to the temporal sequence of the appearance of differences of biomarkers in studies of autosomal dominant Alzheimer's disease, although the alterations in $A\beta_{42}$ concentration became evident nearly a decade later in our study.[10-12] Therefore, the timing of the appearance of changes in biomarkers may differ between sporadic and autosomal dominant Alzheimer's disease.

Consistent with results from previous studies of sporadic Alzheimer's disease,[2,9,15,29-31] the results of our study show an apparent accelerated change in concentrations of CSF biomarkers followed by a slowing of this change up to the time of diagnosis of Alzheimer's disease. We explored associations between the rates of biomarker changes with cognitive function and found that the most rapid change in rate occurs in persons who have MMSE scores in the range of 25 to 27.

The strengths of this study include its prospective and multicenter nature, relatively large sample, long follow-up time, and repeated CSF and imaging assessments. However, our trial has some weaknesses. The participants were Han Chi-

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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