Preclinical Alzheimer's disease has been characterized by the presence of normal cognitive function and abnormal levels of cerebrospinal fluid (CSF) biomarkers.[1-3] The preclinical stage is typically followed by mild cognitive impairment, which progresses to clinically apparent dementia in some persons.[1] 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 (A), 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 in6.4 (w)12 (t to)6.2 1>459 (i)-7.8 (s) s i

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 these 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 health-related reasons, 1228 had fewer than three assessments, and 1694 were excluded for other reasons), leaving 1789 participants enrolled.

At the last follow-up, 695 participants had received a diagnosis of Alzheimer's disease and 1094 remained cognitively normal (as assessed by testing as described below). After propensity-score matching on the basis of age, sex, and education level, 648 (93.2%) of the participants with Alzheimer's disease were successfully matched in a 1:1 ratio with participants who remained cognitively normal at the last follow-up, and these two groups form the basis for the current report.

Diagnosis of Cognitive Status

The cognitive status of participants was determined at baseline and at each follow-up with the use of three scales. Participants were considered to have no cognitive impairment if they had a score of 27 or higher on the Mini–Mental State Examination (MMSE; range, 0 to 30, with higher scores indicating better performance).[21] Scores of 12 or higher on the Logical Memory Test (LMT), a modification of the episodic memory section of the Wechsler Memory Scale–Revised (scores range from 0 to 25, with higher scores indicating better memory abilities)[22] were considered to indicate normal cognition at baseline. The third scale that was used was the CDR–Sum of Boxes (CDR-SB; range, 0 to 18, with higher scores indicating greater impairment). The scores on these scales and participants' medical records were reviewed by neurologists and taken into account when the clinical diagnosis of Alzheimer's disease was made, in accordance with the National Institute on Aging–Alzheimer's Association criteria.[23] Mild cognitive impairment was diagnosed according to the Petersen criteria.[24]

The same tests were used throughout the period of the study; however, the status of cognitively normal at follow-up was defined as consistent maintenance of a score of 0 on the CDR-SB.[20] CDR-SB scores were independently assessed by physicians who were unaware of other cognitive test results. In cases in which no consensus was reached, a diagnosis was determined by subsequent discussion by a group of neurologists, psychiatrists, and neuropsychologists who were experts in Alzheimer's disease.

Biomarkers

At each follow-up, CSF and blood samples were obtained under morning fasting conditions for *APOE* genotype and routine biochemical tests. Participants were monitored for signs of discomfort for at least 12 hours after undergoing lumbar puncture. Samples were aliquoted and preserved at −80°C until tested. The same tests were consistently used for each participant throughout the study (see the Supplementary Methods section).

The concentrations of the biomarkers were measured with the use of enzyme-linked immunosorbent assay kits (INNOTEST -Amyloid 1–40, INNO thz -Amylod 1'-19.92N16.1 (O)-4.5 ()-5 (t)-NN0,

Table 1. Characteristic of the Participants at Baseline.*

Variable	Cognitively Normal (N=648)	Alzheimer's Disease (N=648)
Age — yr	61.3±4.1	61.2±4.1
Sex — no. (%)		
Male	328 (50.6)	327 (50.5)
Female	320 (49.4)	321 (49.5)
Education, level and total yr — no. of participants (%)		
Primary school, 6 yr	25 (3.9)	28 (4.3)
Middle school, 7–9 yr	28 (4.3)	26 (4.0)
High school, 10–12 yr	212 (32.7)	210 (32.4)
University, 13–17 yr	334 (51.5)	335 (51.7)
Postgraduate, 18–21 yr	49 (7.6)	49 (7.6)
APOE status — no. (%)		
Noncarrier	516 (79.6)	407 (62.8)
Carrier	132 (20.4)	241 (37.2)
Heterozygous	121 (18.7)	183 (28.2)
Homozygous	11 (1.7)	58 (9.0)
Cognitive score†		
MMSE	29.5±1.0	29.4±1.2
CDR-SB	0	0
LMT	16.8±0.6	16.8±0.7
Biomarker values		
Ratio of A_{42} to A_{40}	0.1±0.0	0.1±0.0
Total tau — pg/ml	219.2±52.6	214.6±41.2
Phosphorylated tau 181 — pg/ml	48.8±9.9	48.4±7.8
Neurofilament light chain — pg/ml	633.9±139.3	645.4±140.4
Hippocampal volume — mm^3	7708.3±621.8	7683.0±645.5

* Plus–minus values are means ±SD. A denotes amyloid-beta, and APOE apolipoprotein E gene.
† Scores on the Mini–Mental State Examination (MMSE) range from 0 to 30, with higher scores indicating better-performance. Totals for the Clinical Dementia Rating–Sum of Boxes (CDR-SB) range from 0 to 18, with higher values indicating greater cognitive impairment. Scores for the Logical Memory Test (LMT) range from 0 to 25, with higher scores indicating better memory abilities.

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.[35]

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.

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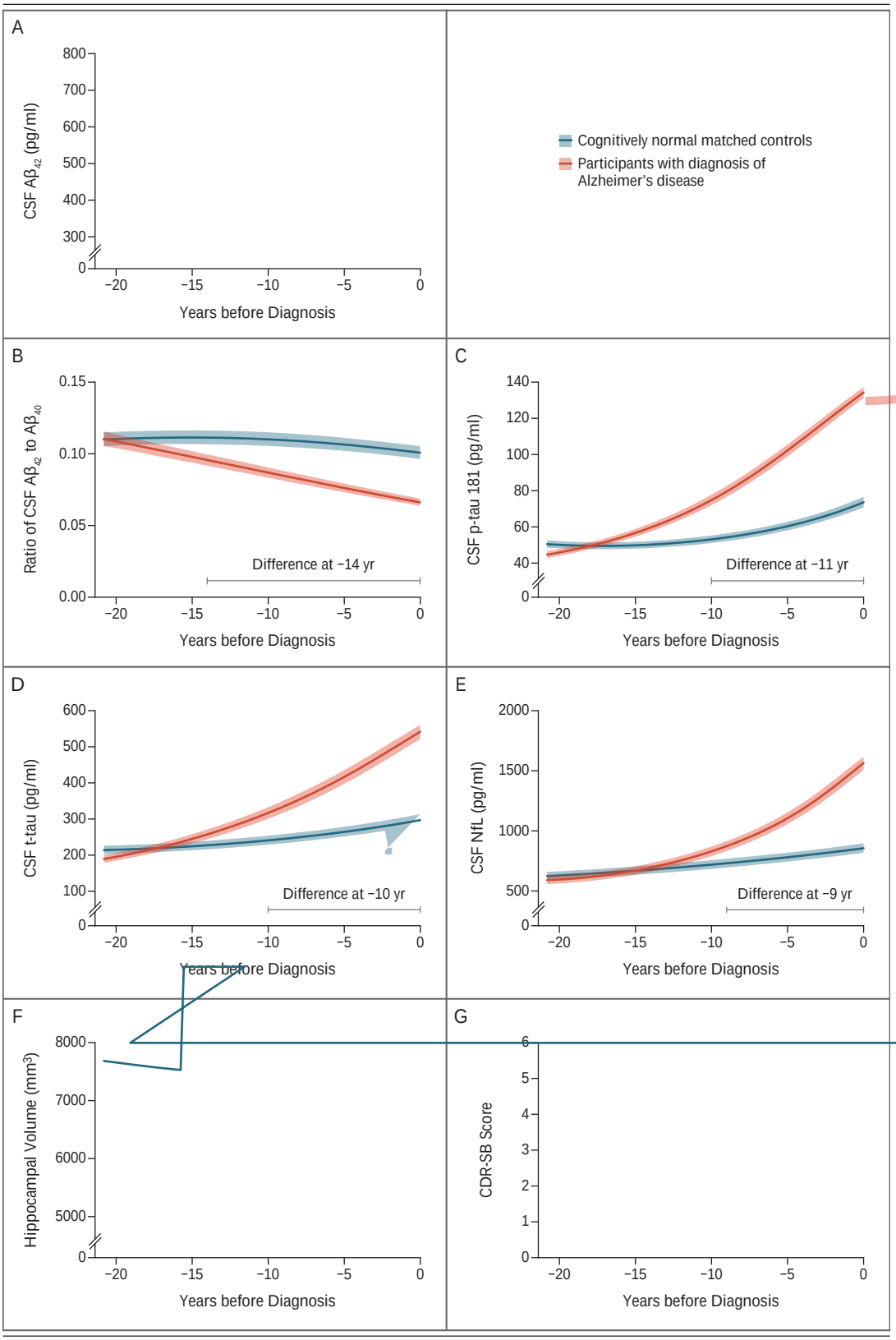
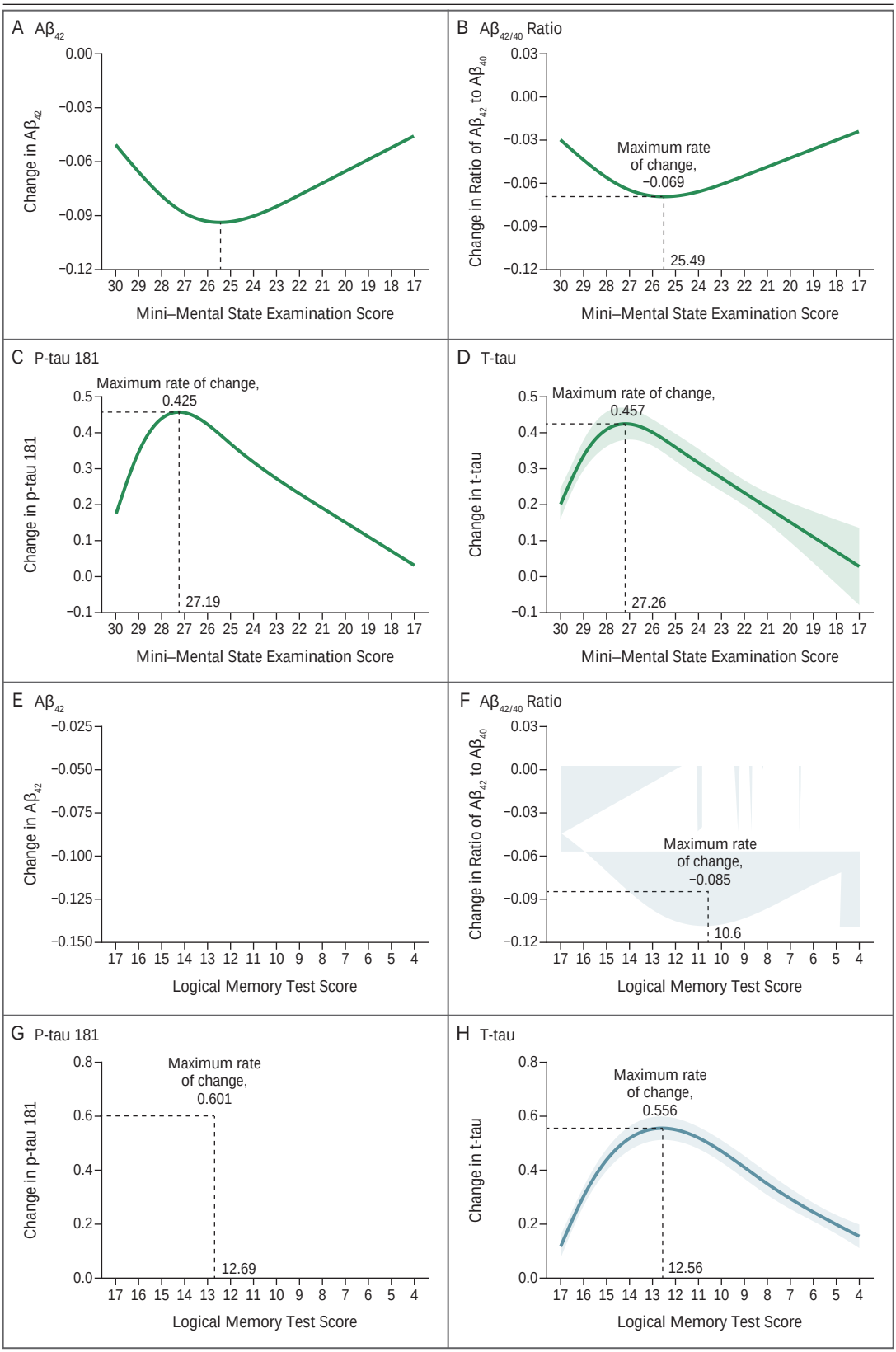

A
CSF Aβ42 (pg/ml)
800
700
600
500
400
300
0
−20
−15
−10
−5
0
Years before Diagnosis
Cognitively normal matched controls
Participants with diagnosis of Alzheimer's disease
B
Ratio of CSF Aβ42 to Aβ40
0.15
0.10
0.05
0.00
Difference at −14 yr
Years before Diagnosis
C
CSF p-tau 181 (pg/ml)
140
120
100
80
60
40
0
Difference at −11 yr
Years before Diagnosis
D
CSF t-tau (pg/ml)
600
500
400
300
200
100
0
Difference at −10 yr
Years before Diagnosis
E
CSF NfL (pg/ml)
2000
1500
1000
500
0
Difference at −9 yr
Years before Diagnosis
F
Hippocampal Volume (mm3)
8000
7000
6000
5000
0
Years before Diagnosis
G
CDR-SB Score
6
5
4
3
2
1
0
Years before Diagnosis

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


A Aβ42
Change in Aβ42
0.00
−0.03
−0.06
−0.09
−0.12
30 29 28 27 26 25 24 23 22 21 20 19 18 17
Mini–Mental State Examination Score
B Aβ42/40 Ratio
Change in Ratio of Aβ42 to Aβ40
0.03
0.00
−0.03
−0.06
−0.09
−0.12
Maximum rate of change, −0.069
25.49
30 29 28 27 26 25 24 23 22 21 20 19 18 17
Mini–Mental State Examination Score
C P-tau 181
Change in p-tau 181
Maximum rate of change, 0.425
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
27.19
30 29 28 27 26 25 24 23 22 21 20 19 18 17
Mini–Mental State Examination Score
D T-tau
Change in t-tau
Maximum rate of change, 0.457
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
27.26
30 29 28 27 26 25 24 23 22 21 20 19 18 17
Mini–Mental State Examination Score
E Aβ42
Change in Aβ42
−0.025
−0.050
−0.075
−0.100
−0.125
−0.150
17 16 15 14 13 12 11 10 9 8 7 6 5 4
Logical Memory Test Score
F Aβ42/40 Ratio
Change in Ratio of Aβ42 to Aβ40
0.03
0.00
−0.03
−0.06
−0.09
−0.12
Maximum rate of change, −0.085
10.6
17 16 15 14 13 12 11 10 9 8 7 6 5 4
Logical Memory Test Score
G P-tau 181
Change in p-tau 181
Maximum rate of change, 0.601
0.8
0.6
0.4
0.2
0.0
12.69
17 16 15 14 13 12 11 10 9 8 7 6 5 4
Logical Memory Test Score
H T-tau
Change in t-tau
Maximum rate of change, 0.556
0.8
0.6
0.4
0.2
0.0
12.56
17 16 15 14 13 12 11 10 9 8 7 6 5 4
Logical Memory Test Score

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 representative

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

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Disclosure forms provided by the authors are availablrcien03030uu toY(1)--.1-046 Tw

33. Kalaria RN, Maestre GE, Arizaga R, et al. Alzheimer's disease and vascular-dementia in developing countries: prevalence, management, and risk factors. Lancet Neurol 2008;7:812-26.
34. Gao F, Lv X, Dai L, et al. A combination model of AD biomarkers revealed by machine learning precisely predicts Alzheimer's dementia: China Aging and Neurodegenerative Initiative (CANDI) study. Alzheimers Dement 2022 June 6 (Epub ahead of print).
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