

Carotid Atherosclerosis Associated with Tau Pathology and Cognitive Function in Cognitively Intact Adults: The Chinese Alzheimer's Biomarker and Lifestyle (CABLE) Study

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ABSTRACT

Background: Carotid atherosclerosis has been implicated in cognitive decline, but the evidence from current studies is insufficient and the detailed mechanism remains unclear.

Objective: This study aimed to explore the association of carotid atherosclerosis with cognitive function and Cerebrospinal Fluid (CSF) Alzheimer's Disease (AD) biomarkers, as well as attempted to investigate the underlying mechanisms.

Methods: This study included 365 participants with objective normal cognition from the Chinese Alzheimer's Biomarker and Lifestyle (CABLE) database. Multiple linear regression models were utilized to assess the associations of carotid atherosclerosis Carotid Intima-Media Roughness (CIMR), Carotid Intima-Media Thickness (CIMT), carotid plaque and CIMT level with CSF AD biomarkers and cognitive function. The mediation analyses were used to explore whether CSF AD biomarkers mediated the carotid atherosclerosis and cognitive function.

Result: We found that CIMR, carotid plaque and CIMT level were significantly associated with tau pathology (T-tau (Total Tau) and P-tau (Phosphorylated Tau), $p < 0.05$); all markers of carotid atherosclerosis were associated with cognitive function (CM-MMSE (China-Modied Mini-Mental State Examination) and MoCA (Montreal Cognitive Assessment), $p < 0.05$). Furthermore, mediation analyses revealed that the effect of carotid plaque on cognitive decline was partially mediated by tau pathology (proportion of mediation=19.7%, $p = 0.012$).

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Conclusion: This study indicated that carotid atherosclerosis was associated with tau pathology and cognitive function and tau pathology partially mediated the association between carotid atherosclerosis and cognitive function.

Keywords: Alzheimer's disease; Biomarkers; Carotid atherosclerosis; Cerebrospinal fluid; Cognitive function

INTRODUCTION

Dementia is a prominent cause of death and disability worldwide, imposing a significant burden on health and care systems around the world [1]. Alzheimer's Disease (AD), as the most common form of dementia, is characterized pathologically by aberrant amyloid deposition, total tau and phosphorylated tau (indicated by A β _{42/40}, T-tau and P-tau, respectively) and clinically by cognitive and behavioral impairments [2]. Currently, there is no effective treatment that can cure AD or slow down its onset and progression [3]. Significantly, recent studies found that differences in Cerebrospinal Fluid (CSF) biomarkers between the AD and cognitively normal groups existed several years before the onset of AD [4,5]. Therefore, it is important to identify the potentially modifiable risk factors associated with AD to facilitate the early prevention of AD.

Carotid atherosclerosis is an important contributor in the onset and development of dementia and AD [6-9]. Increasing research suggested that markers reflecting the burden of carotid atherosclerosis including Carotid Intima-Media Roughness (CIMR), Carotid Intima-Media Thickness (CMT) and carotid plaque, were associated with cognitive functioning impairment in individuals at risk of AD development [10-12]. Furthermore, a long-term longitudinal cohort study demonstrated that carotid plaque exacerbated cognitive impairment related to AD [13]. In this context, several researchers proposed that carotid atherosclerosis could be considered as a modifiable risk factor for AD [14-16]. Nevertheless, the precise mechanisms underlying the association between carotid atherosclerosis and AD remain unknown. Few studies have investigated the bio-mechanisms underlying the association of carotid atherosclerosis with cognitive dysfunction, as well as whether pathological AD biomarkers influenced the relationship between carotid atherosclerosis and cognition. Thus, the primary objectives of this study were to (1) investigate the associations between carotid atherosclerosis and cognitive function; (2) explore the associations between carotid atherosclerosis and CSF AD biomarkers; and (3) examine whether the impact of carotid atherosclerosis on cognitive performance was mediated by AD pathology in 365 participants.

MATERIALS AND METHODS

The Chinese Alzheimer's Biomarker and Lifestyle (CABLE) database

The Chinese Alzheimer's Biomarker and Lifestyle (CABLE) study, initiated in 2017, is an ongoing large-scale independent cohort study primarily focusing on risk factors and biomarkers for AD, aiming to facilitate early diagnosis of AD among the Chinese Han population [17]. All enrolled participants in the CABLE were recruited from Qingdao Municipal Hospital, Shandong Province, China. The CABLE database was conducted in compliance with the Declaration of Helsinki. The study has been approved by the Institutional Review Board of Qingdao Municipal Hospital. Written informed consent was obtained from all the participants.

Participants

Participants included in the CABLE were Han Chinese aged between 40 and 90 years old. The main exclusion criteria were: (1) central nervous system infections, multiple sclerosis, head trauma, neurodegenerative diseases other than AD (e.g., epilepsy, Parkinson's disease), or other major neurological disorders; (2) significant psychological disorders; (3) severe systemic diseases (e.g., malignancy tumors); (4) family history of genetic disorders. Demographic information and medical history were collected through structured questionnaires supplemented by an electronic medical record system. All diagnoses were assessed by two medical professionals with standardized training in cognitive disorders by means of complete performance on neuropsychological tests, combined with CSF biomarkers and Magnetic Resonance Imaging (MRI) examinations. The baseline cognitive status of participants was determined using the China Modified Mini-Mental State Examination (CM-MMSE) and the Montreal Cognitive Assessment (MoCA). Objective cognitive impairment was defined as having a CM-MMSE score ≤ 17 for those without education, a CM-MMSE score ≤ 20 for those with no more than 6 years of education, a CM-MMSE score ≤ 24 for those with more than 6 years of education. A total of 2334 participants were enrolled in the CABLE study, out of which 1959 participants who did not undergo carotid ultrasound examination or did not provide CSF biomarkers data were excluded. Ultimately, our study included 365 individuals.

Markers of carotid atherosclerosis

The carotid arteries were assessed by certified and well-trained medical sonographers using Philips color Doppler ultrasound machines. All participants underwent the examinations in a supine position with their head tilted back to expose their neck. The lengths of bilateral common carotid artery, carotid bifurcation and internal carotid arteries were examined transversely first and then longitudinally in grayscale and color flow modes. First, the length of the common carotid arteries, the carotid bifurcation as well as the internal and external carotid arteries beginning were examined transversally; then longitudinally in lumen-diameter, grayscale and color flow. Ultrasound variables (dynamic range, depth range, power, reject, edge, grey scale and smoothness) were constant during all the examinations. Our markers of interest include: (1) the presence of CIMR, defined as granular echogenicity of deep, normally anechoic intimal-medial layer^[18,19]; (2) the presence of CIMT, indicated by CIMT ≥ 0.9 mm in one or both bilateral carotid arteries^[20]; (3) the presence of carotid plaque (plaque), indicated by CIMT ≥ 1.5 mm in one or both bilateral carotid arteries^[21]; (4) Graded based on CIMT level (level): 0 mm ≤ 1.0 mm, 1=1.0 mm-1.2 mm, 2=1.2 mm-1.4 mm, 3 ≥ 1.4 mm^[22,23].

CSF AD biomarkers

CSF samples were obtained via lumbar puncture by a qualified physician in 10 ml polypropylene tubes and promptly transported to the laboratory for processing within 2 h. After centrifugation at $2000 \times g$ for 10 min, the samples were divided and stored in an enzyme-free Eppendorf (EP) tubes (AXYGEN; PCR-02-C) at -80°C . The freeze-thawing cycle was limited to two times or less. CSF levels of $\text{A}\beta_{42}$, $\text{A}\beta_{40}$, P-tau and T-tau levels were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) kits (Innotest β -AMYLOID (1-42) (catalog number: 81583); β -AMYLOID (1-40) (catalog number: 81585); PHOSPHO-TAU (181p) (catalog number: 81581); hTAU-Ag (catalog number: 81579); Fujirebio, Ghent, Belgium. All measurements were performed in duplicate by professional technicians blinded to clinical information. Within-batch Coefficients of Variations (CVs) were $<5\%$ (4.8% for $\text{A}\beta_{42}$, 3.6% for $\text{A}\beta_{40}$, 2.4% for P-tau and 4.6% for T-tau). Inter-batch CVs were $<20\%$ (9% for $\text{A}\beta_{42}$, 3.6% for $\text{A}\beta_{40}$, 10.9% for P-tau and 12.2% for T-tau). Among these CSF AD biomarkers, we utilized the $\text{A}\beta_{42/40}$ ratio instead of the absolute value of $\text{A}\beta_{42}$ since the ratio could better represent amyloid deposition and diagnostic accuracy^[24-26].

Angiogenesis

The angiogenesis experiment followed Sigma Aldrich's kit protocol. HUVECs were gathered, suspended in media and placed on ECMatrix™ at 5×10^4 cells per well. In each well of a 96-well plate, 3,000 cells were initially seeded. The cells were then exposed to various treatments involving Rigemed D, which included the following conditions: A negative control, a positive control with 50 μ M of hydrogen peroxide and groups with different concentrations (1%, 1.5% and 2% v/v) of Rigemed D. Treatments were introduced within a sterile, ventilated fume hood. The gel and cells on the plate were incubated at 37 °C for 6 h. After incubation, cell observation occurred using an inverted light microscope at 40X magnification.

APOE- ϵ 4 genotyping

The QIAamp® DNA Blood Mini Kit (250) was utilized for extracting DNA from fasting blood samples. Subsequently, the DNA was isolated and stored in an enzyme-free EP tube at -80 °C until completion of APOE genotyping for the study. APOE- ϵ 4 status was determined by genotyping rs7412 and rs429358 using a restriction fragment-length polymorphisms technique. Participants were categorized as either APOE- ϵ 4 non-carriers or APOE- ϵ 4 carriers (with at least one copy of the APOE- ϵ 4 gene).

Statistical analyses

In order to describe baseline characteristics of the participants, categorical variables were presented as numbers and percentages, while continuous variables were expressed as means with Standard Deviations (SD). The characteristics were compared using Chi-square tests (categorical variables) and Mann-Whitney U test (continuous variables). CSF AD biomarker measurements underwent normalization through the box-cox transformation and were standardized via Z-scale in the case of skewed distributions.

Multiple linear regression models were used to explore the associations of carotid atherosclerosis with CSF AD biomarkers and cognitive function after adjusting for age (continuous), sex (categorical), education (continuous), APOE- ϵ 4 status (categorical). Moreover, sensitivity analyses were conducted by adding further covariates, including self-report histories of high blood pressure (yes or no), diabetes mellitus (yes or no), hyperlipidemia (yes or no) and cerebrovascular disease (yes or no).

Furthermore, subgroup analyses stratified by age, sex and APOE- ϵ 4 status were performed. Next, mediation analyses based on Baron and Kenny's method were conducted to examine whether the associations between carotid atherosclerosis and cognition were mediated by AD pathology. Mediation effects would be established if all the criteria are simultaneously satisfied: (1) significant associations between carotid atherosclerosis and CSF AD biomarkers; (2) significant associations between carotid atherosclerosis and cognitive measures; (3) significant associations between CSF AD biomarkers and cognitive measures and 4) attenuated associations between carotid atherosclerosis and cognitive measures when adding CSF AD biomarkers (the mediators) in the regression model. Additionally, the indirect effect or attenuation was estimated, with significance determined through 10,000 bootstrapped iterations. Each path of the mediator model was corrected for age, sex, education and APOE- ϵ 4 status. All statistical analyses and visualization were performed using IBM SPSS Statistics 26 software and R-Studio software (version 4.2.0). The significance threshold was set at a $p < 0.05$ (two-tailed).

Participant characteristics

The demographical and clinical characteristics of the participants are summarized in Table 1. This study enrolled 365 participants from the CABLE database. The study sample had an age range of 40 to 90 years (mean $\pm$ SD=65.82 $\pm$ 10.04), an average education level of 9.92 $\pm$ 4.59 years, a female percentage of 46.30% and an APOE- ϵ 4 carriers proportion of 15.89%. In terms of cognitive scores, the study sample had an average CM-MMSE score of 26.63 $\pm$ 3.87 and an average MoCA score of 21.84 $\pm$ 5.52.

Table 1. Characteristics of participants from CABLE database.

Variable	Descriptive statistics total (N=365)
Age (y) mean (SD)	65.82 (10.04)
Sex (female) N (%)	169 (46.30%)
Education (y) mean (SD)	9.92 (4.59)
APOE- ϵ 4 carriers N (%)	47 (15.89%)
A β _{42/40} mean (SD)	0.07 (0.76)
P-tau (pg/ml) mean (SD)	42.29 (17.15)
T-tau (pg/ml) mean (SD)	233.92 (140.62)
CM-MMSE mean (SD)	26.63 (3.87)
MoCA mean (SD)	21.84 (5.52)
Note: CN: Cognitively Normal Participants; SCD: Participants with Subjective Cognitive Decline; APOE- ϵ 4: Apolipoprotein E ϵ 4 gene; A β : Amyloid- β ; P-tau: Phosphorylated Tau protein; T-tau: Total Tau protein; CM-MMSE: China-Modified Mini-Mental State Examination; N: Number; Y: Years; SD: Standard Deviation.	

Difference in CSF, AD biomarkers and cognitive function between participants with and without carotid atherosclerosis significant differences were observed in CSF levels of P-tau ($p=0.039$) and T-tau ($p=0.028$) (Figures 1A and 1B), as well as scores of CM-MMSE ($p=0.002$) and MoCA ($p<0.001$) (Figures 2A and 2B) between participants with and without CIMR. Similarly, significant differences were found in CSF levels of P-tau ($p=0.018$) and T-tau ($p=0.045$) (Figures 1C and 1D), as well as scores of CM-MMSE ($p=0.045$) and MoCA ($p=0.005$) (Figures 2C and 2D) between participants with and without CIMT.

Furthermore, significant differences were observed in CSF levels of P-tau ($p=0.048$) and T-tau ($p=0.028$) (Figures 1E and 1F), as well as scores of CM-MMSE ($p=0.026$) and MoCA ($p<0.001$) (Figures 2E and 2F) between participants with and without carotid plaque. As the CIMT level increased, differences in CSF levels of P-tau ($p=0.012$) and T-tau ($p=0.019$) increased (Figures 1G and 1H), as well as the CM-MMSE ($p=0.029$) and MoCA score ($p=0.003$) decreased (Figures 2G and 2H) as shown in Figures 1 and 2. However, in terms of these carotid atherosclerosis, no intergroup differences were found in CSF A β _{42/40} (CIMR: A β _{42/40}, $p=0.072$; CIMT: A β _{42/40}, $p=0.243$; carotid plaque: A β _{42/40}, $p=0.655$; CIMT level increased: A β _{42/40}, $p=0.324$).

Figure 1. Difference in CSF AD biomarkers between participants with and without carotid atherosclerosis were examined by Mann-Whitney U test or Kruskal-Wallis test. **Note:** P-tau: Phosphorylated Tau Protein; T-tau: Total Tau Protein; CIMR: Carotid Intima-Media Roughness, 0=no, 1=yes; CIMT: Carotid Intima-Media Thickness, 0=no, 1=yes; plaque, CIMT<1.0 mm, 1=1.0 mm-1.2 mm, 2=1.2 mm-1.4 mm, 3 $\geq$ 1.4 mm.

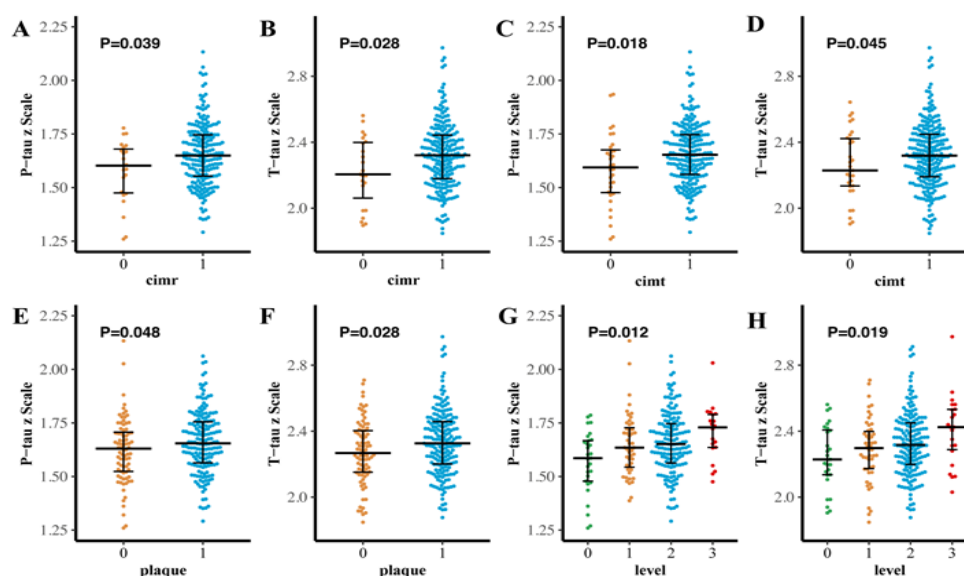
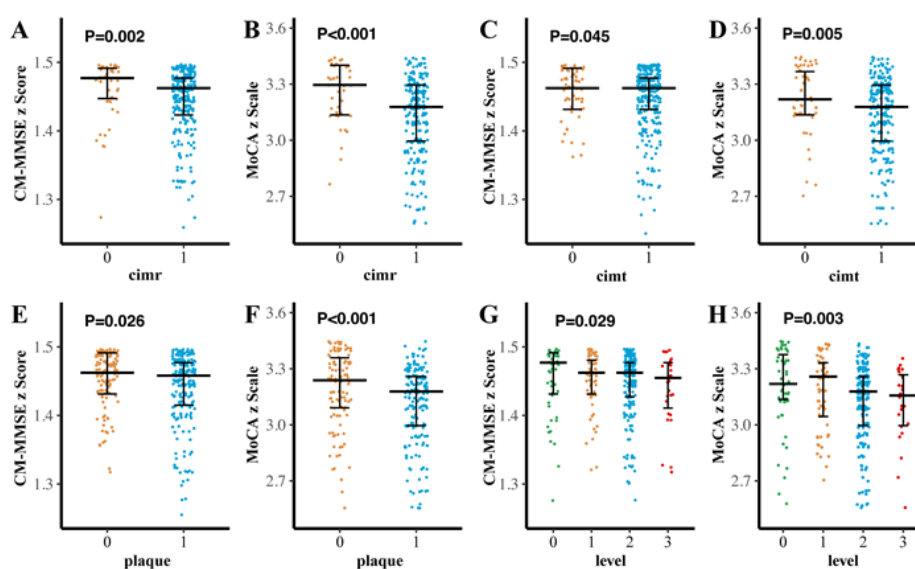


Figure 2. Difference in cognitive function between participants with and without carotid atherosclerosis were examined by Mann-Whitney U test or Kruskal-Wallis test. **Note:** CM-MMSE: China-Modied Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; CIMR: Carotid Intima-Media Roughness, 0=no, 1=yes; CIMT: Carotid Intima-Media Thickness, 0=no, 1=yes; plaque, the carotid plaque, 0=no, 1=yes; level, CIMT level, 0 =CIMT<1.0 mm, 1=1.0 mm-1.2 mm, 2 mm-1.2 mm-1.4 mm, 3 mm $\geq$ 1.4 mm.

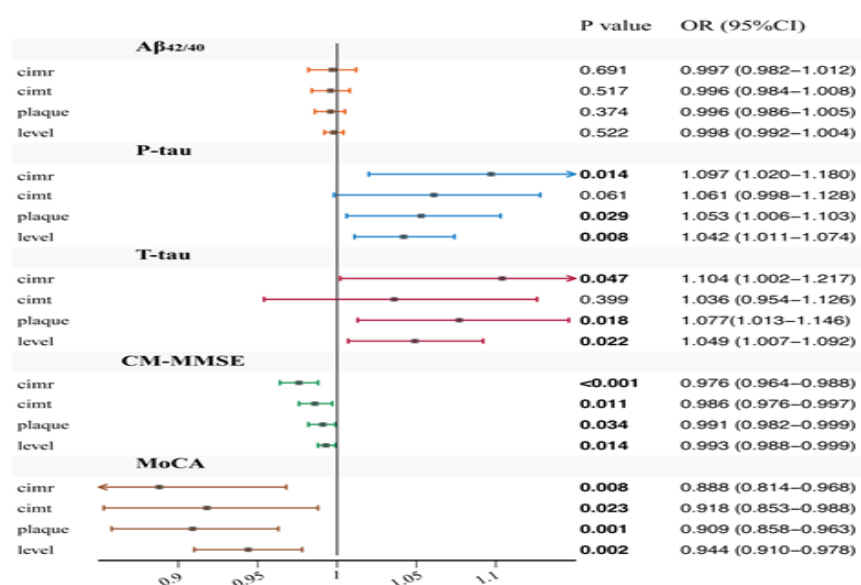


Associations of carotid atherosclerosis with CSF AD biomarkers and cognitive function

In the whole sample of subjects, the presence of CIMR was significantly associated with higher levels of P-tau ($\beta=0.092$, $p=0.014$) and T-tau ($\beta=0.099$, $p=0.047$), as well as lower scores on CM-MMSE ($\beta=-1.499$, $p<0.001$) and MoCA ($\beta=-2.519$, $p=0.008$) (Figure 3). Besides, CIMT was significantly associated with lower scores on both CM-MMSE ($\beta=-0.826$, $p=0.011$) and MoCA ($\beta=-1.631$, $p=0.023$) (Figure 3). Moreover, carotid plaque was significantly associated with higher levels of P-tau ($\beta=0.052$, $p=0.029$) and T-tau ($\beta=0.075$, $p=0.018$), as well as lower scores on CM-MMSE ($\beta=-0.597$, $p=0.034$) and MoCA ($\beta=-1.907$, $p=0.001$) (Figure 3). Furthermore, the CIMT level was also

significantly associated with higher levels of P-tau ($\beta=0.041$, $p=0.008$) and T-tau ($\beta=0.048$, $p=0.022$), as well as lower scores on CM-MMSE ($\beta=-0.405$, $p=0.014$) and MoCA ($\beta=-1.159$, $p=0.002$). However, the association between CIMR, CIMT, carotid plaque and CIMT level with $A\beta_{42/40}$ level ($p>0.05$) was not observed, as well as no significant association between CIMT and tau pathology (P-tau, $\beta=0.059$, $p=0.061$; T-tau, $\beta=0.036$, $p=0.399$).

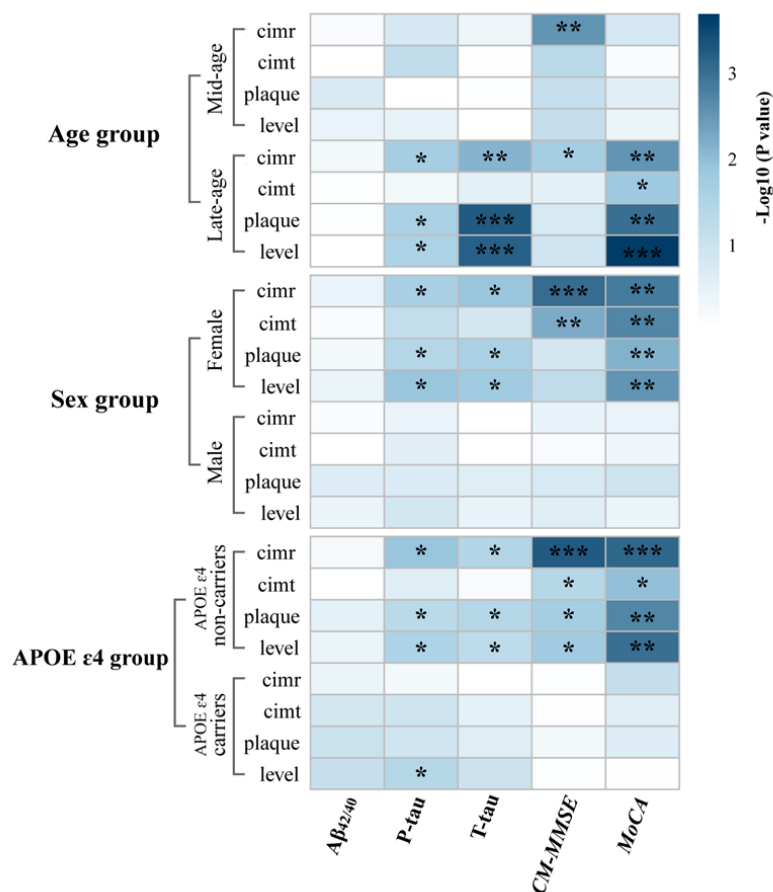
Figure 3. Associations between carotid atherosclerosis with cognition and CSF AD biomarkers. Analyses were adjusted for age, sex, education and APOE- $\epsilon 4$ status. **Note:** CM-MMSE: China-Modied Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; CIMR: Carotid Intima-Media Roughness, 0=no, 1=yes; CIMT: Carotid Intima-Media Thickness, 0=no, 1=yes; plaque, the carotid plaque, 0=no, 1=yes; level, CIMT level, 0=CIMT<1.0 mm, 1=1.0 mm-1.2 mm, 2=1.2 mm-1.4 mm, 3 $\geq$ 1.4 mm.



Subgroup analyses

According to age, sex and APOE- $\epsilon 4$ status, subgroups analyses were used to identify specific vulnerable population. We found that the aforementioned associations of carotid atherosclerosis with CSF AD biomarkers and cognitive function remained statistically significant: (1) CIMR: In mid-age (CM-MMSE, $\beta=-1.358$, $p=0.003$), in late-age (P-tau, $\beta=0.131$, $p=0.021$; T-tau, $\beta=0.311$, $p=0.008$; CM-MMSE, $\beta=-1.962$, $p=0.021$; MoCA, $\beta=-5.523$, $p=0.003$), in women (P-tau, $\beta=0.117$, $p=0.023$; T-tau, $\beta=0.168$, $p=0.013$; CM-MMSE, $\beta=-1.707$, $p<0.001$; MoCA, $\beta=-3.479$, $p=0.001$) and in APOE- $\epsilon 4$ non-carriers (P-tau, $\beta=0.108$, $p=0.013$; T-tau, $\beta=0.118$, $p=0.035$; CM-MMSE, $\beta=-1.460$, $p<0.001$; MoCA, $\beta=-3.388$, $p<0.001$) (Figure 4); (2) CIMT: In late-age (MoCA, $\beta=-3.394$, $p=0.015$), in women (CM-MMSE, $\beta=-1.331$, $p=0.006$; MoCA, $\beta=-3.147$, $p=0.002$) and in APOE- $\epsilon 4$ non-carriers (CM-MMSE, $\beta=-0.739$, $p=0.043$; MoCA, $\beta=-2.164$, $p=0.011$) (Figure 4); (3) carotid plaque: In late-age adults (P-tau, $\beta=0.073$, $p=0.025$; T-tau, $\beta=0.157$, $p<0.001$; MoCA, $\beta=-2.834$, $p=0.001$), in women (P-tau, $\beta=0.072$, $p=0.038$; T-tau, $\beta=0.106$, $p=0.026$; MoCA, $\beta=-2.314$, $p=0.007$) and in APOE- $\epsilon 4$ non-carriers (P-tau, $\beta=0.039$, $p=0.050$; T-tau, $\beta=0.072$, $p=0.040$; CM-MMSE, $\beta=-0.678$, $p=0.021$; MoCA, $\beta=-2.065$, $p=0.002$); (4) CIMT level: In late-age (P-tau, $\beta=0.054$, $p=0.027$; T-tau, $\beta=0.117$, $p<0.001$; MoCA, $\beta=-2.327$, $p<0.001$), in women (P-tau, $\beta=0.053$, $p=0.013$; T-tau, $\beta=0.071$, $p=0.016$; MoCA, $\beta=-1.454$, $p=0.003$), in APOE- $\epsilon 4$ carriers (P-tau, $\beta=0.076$, $p=0.039$) and in APOE- $\epsilon 4$ non-carriers (P-tau, $\beta=0.038$, $p=0.029$; CM-MMSE, $\beta=-0.444$, $p=0.016$; MoCA, $\beta=-1.393$, $p=0.001$) (Figure 4).

Figure 4. Associations between carotid atherosclerosis with cognition and CSF AD biomarkers in subgroups stratified by age, sex and APOE- ϵ 4 status. Analyses were adjusted for: (1) In age subgroup, sex, education and APOE ϵ 4 status; (2) In sex subgroup, age, education and APOE- ϵ 4 status; (3) In APOE- ϵ 4 status subgroup, age, sex and education. **Note:** *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, APOE- ϵ 4, Apolipoprotein E4 gene; CSF, cerebrospinal fluid; A β : Amyloid- β ; P-tau: Phosphorylated Tau Protein; T-tau: Total Tau Protein; CM-MMSE: China-Modied Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; CIMR: Carotid Intima-Media Roughness, 0=no, 1=yes; CIMT: Carotid Intima Media Thickness, 0=no, 1=yes; plaque, the carotid plaque, 0=no, 1=yes; level, CIMT level, 0=CIMT < 1.0 mm, 1=1.0-1.2 mm, 2=1.2-1.4 mm, 3 ≥ 1.4 mm.



Sensitivity analyses

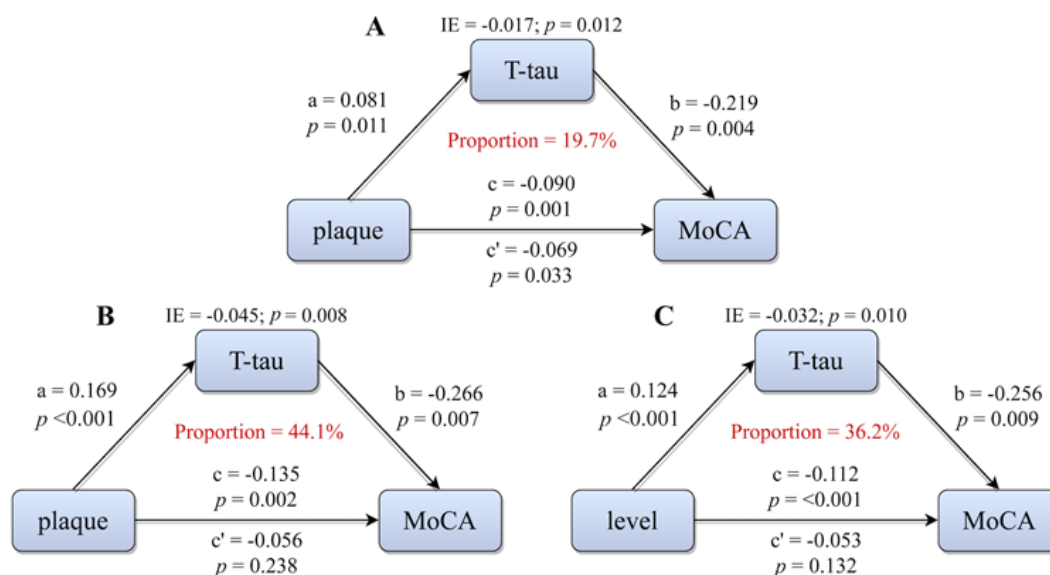
In order to be results more credible, additional sensitivity analyses were performed. The above results remained consistent after adjusting for various covariates (Model 1: Age, sex, education, APOE ϵ 4 status and high blood pressure; Model 2: Age, sex, education, APOE- ϵ 4 status and diabetes mellitus; Model 3: Age, sex, education, APOE- ϵ 4 status and hyperlipidemia; Model 4: Age, sex, education, APOE- ϵ 4 status and cerebrovascular disease; $p < 0.05$).

Causal mediation analyses

These above findings indicated that carotid atherosclerosis was associated with tau pathology and cognitive impairment, so causal mediation analysis was used to explore the underlying mechanisms of cognitive impairment. In the total participants, the associations between carotid plaque and MoCA scores was partially mediated via T-tau (proportion of mediation=19.7%, $p=0.012$). Furthermore, in women, the above associations remained significant

(proportion of mediation=44.1%, $p=0.008$), as well as we found that T-tau (proportion of mediation=36.2%, $p=0.010$) mediated the association between CIMT level and MoCA scores (Figure 5).

Figure 5. CSF AD biomarkers mediation analyses of the associations between carotid atherosclerosis and cognition. Analyses were adjusted for age, sex, education and APOE- $\epsilon 4$ status. **Note:** T-tau: Total Tau protein; MoCA: Montreal Cognitive Assessment.



DISCUSSION

The present study systematically investigates the interrelationships of carotid atherosclerosis (assessment by CIMR, CIMT, carotid plaque and CIMT level), CSF AD biomarkers and cognitive impairment among participants without objective cognitive impairment. Our findings suggested that: (a) there was a statistically significant association between carotid atherosclerosis and cognitive impairment; (b) carotid atherosclerosis exhibited a significant association with tau pathology; (c) tau pathology partially mediated the effects of carotid atherosclerosis on cognitive dysfunction. These results confirmed the associations between carotid atherosclerosis and cognitive impairment and also supported the hypothesis that carotid atherosclerosis could be a potentially modifiable risk factor for AD.

Previous evidence has demonstrated that carotid atherosclerosis preceded the onset of later cognitive impairment and was associated with AD dementia [16]. Subclinical carotid atherosclerosis manifests as the presence of Carotid Intima Media Roughness (CIMR), Carotid Intima Media Thickness (CIMT) and carotid plaque [27,28]. In order to refine our study, we categorized CIMT into different level. Our results are consistent with previous research findings from cross-sectionally and longitudinally cohort studies linking carotid atherosclerosis to cognitive functioning [29]. The Baltimore Longitudinal Study of Aging found that CIMT was longitudinally associated with an increased risk of accelerated cognitive decline [29]. Additionally, a Northern Manhattan Study found that carotid plaque was associated with worse cognition, while another study with a follow-up period of 7 years found lower cognitive measurements in participants with carotid plaque [30]. Furthermore, the Cardiovascular Health Study recruited 4006 stroke-free individuals of old age and observed a positive correlation between CIMT level and cognitive impairment, particularly in those with high-grade (>75% narrowing of diameter) stenosis. The Sternstunden der Gesundheit study suggested

that the presence of CIMR may serve as a predictor for identifying individuals at risk for developing carotid atherosclerosis [12]. The Kaohsiung Atherosclerosis Longitudinal Study indicated that indicators related to carotid artery could enhance predictive ability for assessing cognitive dysfunction. In this study, we not only demonstrated a significant association between carotid atherosclerosis and cognitive impairment, but also identified the mediating effect of tau pathology.

Cortical amyloid deposition and tau pathology, which can identify prodromal AD in the MCI (Mild Cognitive Impairment) stage, have been established as CSF core biomarkers. To further explore the mechanism by which carotid atherosclerosis affects cognition remains unclear, this study incorporated AD biomarkers. Our findings revealed that carotid plaque and CIMT level were significantly associated with T-tau and P-tau levels, but no correlation was observed between CIMT and tau pathology. The Malmö Diet and Cancer Study demonstrated that both carotid plaque and heavy CIMT were significantly associated with AD pathology in cognitively unimpaired individuals [8]. Moreover, the Framingham Heart Study indicated that vascular risk factors may exacerbate tau burden, which is more pronounced in the elderly. This negative result may be interpreted as the severity of CIMT or confounding effects from other variables. Additionally, it was found that there was a connection between CIMR and the levels of tau-related biomarkers. No association was found between carotid atherosclerosis and amyloid level. The pathological mechanism of AD is still unknown and one of the suggested is the hypothesis of cerebral hypoperfusion.

Cerebrovascular disease promotes the increase of tau protein levels, which may be caused by increased cerebrovascular resistance and chronic hypoperfusion of the corresponding brain region, resulting in impaired clearance of tau pathological protein. Furthermore, various animal models suggested that chronic cerebral hypoperfusion leads to greater tau burden and its hyperphosphorylation. However, the relationship between longstanding cerebral hypoperfusion and AD pathology remains controversial based on a cross-sectional PET imaging study. Previous studies have indicated that tau tangles may precede amyloidosis in specific cortical regions related with cognition; alternatively, it can also be argued that the formation of amyloid plaque is a process over an extended period.

Our study identified several subgroup effects that carotid atherosclerosis was closely linked with tau pathology and cognitive impairment in women, old-aged and *APOE-ε4* non-carriers. In further mediation analysis, we found that the effect of carotid atherosclerosis on cognition was partly mediated by tau pathology and this finding was also present in the female cohort. The average age of the population in this study was 66 years and most of the women were postmenopausal. Previous studies have shown that postmenopausal women have decreased levels of sex hormones, leading to an increased risk of atherosclerosis. The Baltimore Longitudinal Study of Aging suggested that a greater relevance between carotid atherosclerosis and regional cerebral blood flow was exhibited in the female subgroup compared to male [30]. This may explain downstream tau deposition and cognitive dysfunction, consistent with the cerebral hypo perfusion hypothesis. Due to its inexpensive and non-invasive characteristics, ultrasound is widely used in the preliminary screening of high-risk population of carotid atherosclerosis. At present, the drugs and treatments that can significantly improve this disease have been proven in large clinical trials, such as statins, ezetimibe and carotid artery revascularization. With the exploration of potential risk factors for AD and significant advances in its cerebrospinal fluid biomarkers in the preclinical stage, primary and secondary prevention is increasingly possible.

CONCLUSION

In conclusion, carotid atherosclerosis was associated with tau pathology and cognitive function, as well as might contribute to AD-related cognitive impairment *via* affecting tau pathology. Therefore, early management and intervention of carotid atherosclerosis may help prevent or delay the occurrence of AD.

Our study had several strengths. The primary strength of our study is the study population with preclinical AD, which makes the study more clinically relevant. Additionally, our study is one of the few to explore the interaction of carotid atherosclerosis, AD biomarkers and cognition. There are also limitations that should be acknowledged in this study. This was a retrospective and observational study, thus it's impossible to clarify cause and effect. Furthermore, although we found associations of carotid atherosclerosis with tau pathology and cognitive function, the exact underlying mechanism is unclear. Future studies are needed to detect the interventions effects of carotid atherosclerosis management on clinical endpoints such as MCI or AD and explore the mechanisms.

DECLARATIONS

Ethics approval and consent to participate

The CABLE study was conducted in accordance with the Declaration of Helsinki and the protocol for this study was approved by the Institutional Ethics Committee of Qingdao Municipal Hospital. Written informed consent was obtained from all study participants directly or from their caregivers.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

None of the authors have financial disclosures and conflicts of interest.

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AUTHORS' CONTRIBUTIONS

LT and MST conceptualized the study and revised the manuscript. MZZ and CKS analyzed and interpreted the data, drafting and revision of the manuscript and prepared the figures. YMC, QH, ZQZ and FG participated in the interpretation of the data and revision of the manuscript. All authors contributed to the writing and revisions of the paper. All authors read and approved the final manuscript.

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