NEUROSCIENCE

Association of naturally occurring antibodies to β-amyloid with cognitive decline and cerebral amyloidosis in Alzheimer's disease

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The pathological relevance of naturally occurring antibodies to β-amyloid (NAbs-Aβ) in Alzheimer's disease (AD) remains unclear. We aimed to investigate their levels and associations with Aβ burden and cognitive decline in AD in a cross-sectional cohort from China and a longitudinal cohort from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study. NAbs-Aβ levels in plasma and cerebrospinal fluid (CSF) were tested according to their epitopes. Levels of NAbs targeting the amino terminus of Aβ increased, and those targeting the mid-domain of Aβ decreased in both CSF and plasma in AD patients. Higher plasma levels of NAbs targeting the amino terminus of Aβ and lower plasma levels of NAbs targeting the mid-domain of Aβ were associated with higher brain amyloidosis at baseline and faster cognitive decline during follow-up. Our findings suggest a dynamic response of the adaptive immune system in the progression of AD and are relevant to current passive immunotherapeutic strategies.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia affecting the elderly. The etiology of AD remains unclear, and disease-modifying therapies are not currently available. β-Amyloid (Aβ) is suggested to play a central role in the pathogenesis of AD (*1*). Recent studies suggest that the adaptive and innate immune systems are involved in the development of AD (*2*). However, the role of humoral immunity in the pathogenesis of AD remains largely unknown.

Naturally occurring antibodies to Aβ (NAbs-Aβ) exist in human blood and cerebral spinal fluid (CSF) (*3*). However, the profiles and pathophysiological significance of NAbs-Aβ in the pathogenesis of AD remain undetermined. NAbs-Aβ may be reduced in patients with AD (*4*) and may aid in Aβ clearance from the brain (*5*). In this regard, intravenous immunoglobulin of class G (IVIG) has been tested as a potential therapeutic agent for AD as it contains NAbs-Aβ (*6*), but a phase 3 clinical trial of IVIG failed to improve the cognitive function of patients with AD (*7*). With one notable exception, active and passive immunotherapy trials have largely failed to reach their primary end points, although some approaches lower the Aβ–positron emission tomography (PET) signal (*8*) and slow the rates of cognitive decline (*9*). Understanding the pathological relevance of NAbs-Aβ in the development of AD may provide important insight for developing effective and safe immunotherapies (*10*).

NAbs-Aβ represents a repertoire that recognize multiple linear epitopes in the Aβ monomer/dimer and conformation-specific epitopes of oligomeric/aggregated Aβ peptides. It remains unknown whether the function of NAbs-Aβ is associated with their respective epitope specificities. In the present study, we aimed to map the epitope specificity of NAbs-Aβ and investigate their epitope-related profile and pathological relevance in AD in two independent cohorts of cognitively normal (CN) subjects and subjects with AD (preclinical and clinical) from China and Australia.

RESULTS

Characteristics of subjects

In the Chongqing cohort, statistical differences were not found for mean age, gender, and education levels between CN and probable AD groups. The frequency of *APOE* ε4 carriers was significantly higher in the AD group in comparison with CN. The mean Mini-Mental State Examination (MMSE) and median Clinical Dementia Rating (CDR) for patients with probable AD were significantly lower than those for participants who were CN (table S1).

In the AIBL cohort, mean age was significantly different among the Aβ-PET$^-$ CN and Aβ-PET$^+$ CN (preclinical AD) and Aβ-PET$^+$ AD (clinical AD) groups. There were no statistical differences in the frequency of males to females among the three groups. Aβ-PET$^+$ CN and Aβ-PET$^+$ AD groups had a significantly higher frequency of *APOE* ε4 carriers as compared with the Aβ-PET$^-$ CN group. MMSE, episodic memory (EM), and AIBL–Preclinical Alzheimer Cognitive Composite (PACC) scores at baseline were all significantly different among the three groups (table S2).

Plasma and CSF profiles of NAbs-Aβ are altered in patients with probable AD

We first tested the profile of NAbs-Aβ in plasma in patients with AD. In the Chongqing cohort, the total plasma levels of NAbs to full-length Aβ1–42 were unchanged in patients with probable AD in comparison with CN participants (fig. S1A). To investigate the composition of NAbs-Aβ repertoire, plasma levels of NAbs targeting different domains of Aβ were investigated. Plasma levels of NAbs-Aβ1–12 and NAbs-Aβ7–18 were higher, while plasma levels

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of NAbs-Aβ19–30 and NAbs-Aβ25–36 were lower in patients with probable AD in comparison with CN participants (Fig. 1, A to D). Statistical differences were not observed in plasma levels of NAbs-Aβ13–24 and NAbs-Aβ31–42 between the two groups (fig. S1, B and C). The profile of NAbs-Aβ in CSF was similar to that in plasma (Fig. 1, E to H, and fig. S1, D to F). Furthermore, plasma levels of NAbs-Aβ were correlated with those in CSF to varying degrees (fig. S2). The above profiles of NAbs-Aβ in AD and CN groups were further confirmed with peptide microarrays, with plasma levels of NAbs determined with peptide microarrays correlated with those detected with enzyme-linked immunosorbent assay (ELISA) (fig. S3).

Plasma profile of NAbs-Aβ is altered in subjects with Aβ deposition

We next tested the profile of NAbs-Aβ in plasma in subjects by Aβ-PET status in the AIBL cohort. The alterations of NAbs-Aβ in the AIBL cohort were similar to those in the Chongqing cohort, as reflected by the higher plasma levels of NAbs-Aβ1–12 and NAbs-Aβ7–18 but lower plasma levels of NAbs-Aβ19–30 and NAbs-Aβ25–36 in Aβ-PET$^+$ subjects when compared to Aβ-PET$^-$ subjects (Fig. 2, A to D). The levels of NAbs-Aβ1–42, NAbs-Aβ13–24, and NAbs-Aβ31–42 were unchanged in Aβ-PET$^+$ subjects in comparison with Aβ-PET$^-$ subjects (fig. S4, A to C).

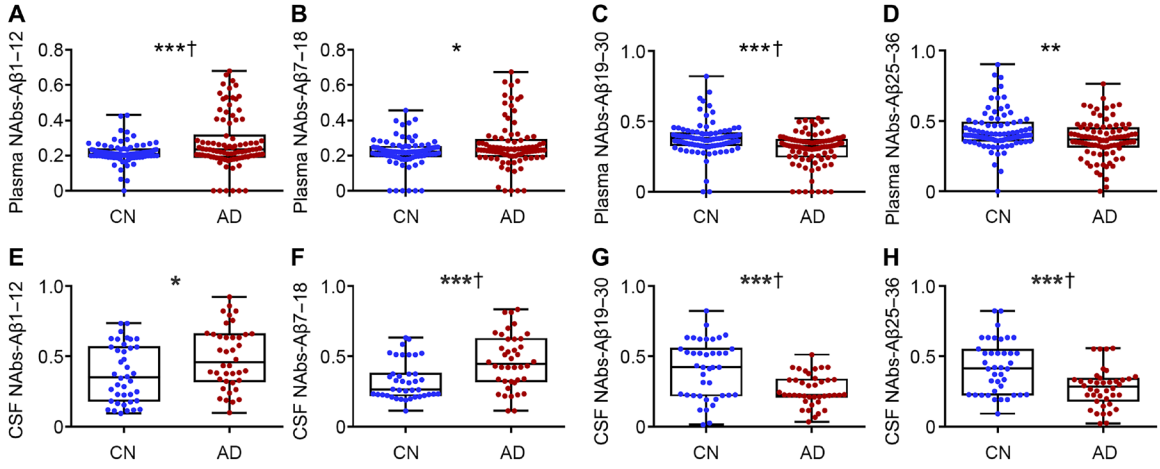

Fig. 1. Plasma and CSF levels of NAbs targeting different domains of Aβ in the Chongqing cohort. (**A** to **D**) Comparison of plasma levels of (A) NAbs-Aβ1–12, (B) NAbs-Aβ7–18, (C) NAbs-Aβ19–30, and (D) NAbs-Aβ25–36 between CN ($n = 91$) and AD ($n = 91$). Unpaired t test. (**E** to **H**) Comparison of CSF levels of (E) NAbs-Aβ1–12, (F) NAbs-Aβ7–18, (G) NAbs-Aβ19–30, and (H) NAbs-Aβ25–36 between CN ($n = 40$) and AD ($n = 40$). Unpaired t test. * denotes nominal significance only at $P < 0.05$. ** denotes nominal significance only at $P < 0.01$. *** denotes significance at the Bonferroni correction at $P < 0.001$. † denotes less than Bonferroni-corrected α.

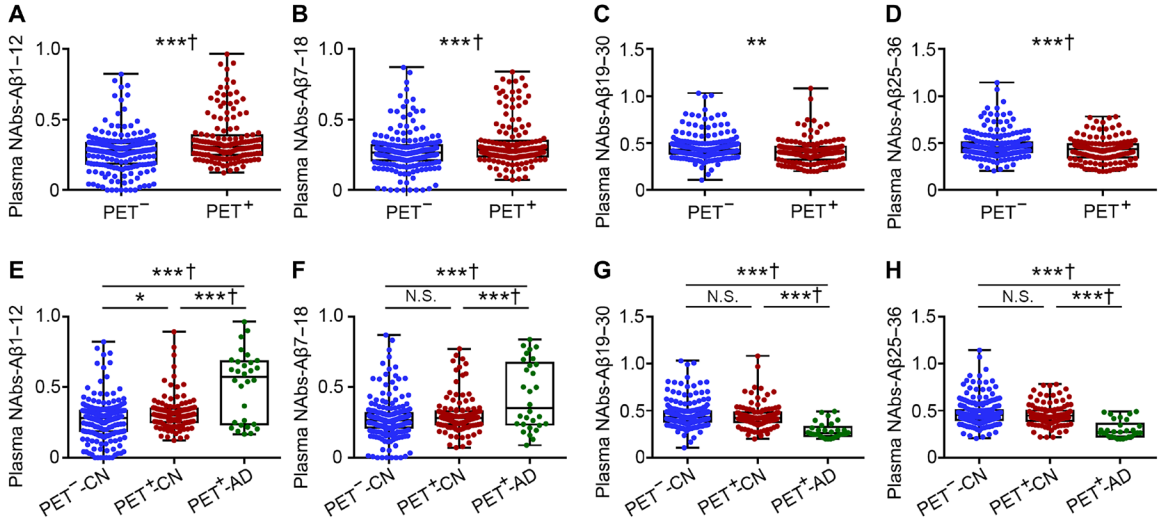

Fig. 2. Plasma levels of NAbs targeting different domains of Aβ in the AIBL cohort. (**A** to **D**) Comparison of plasma levels of (A) NAbs-Aβ1–12, (B) NAbs-Aβ7–18, (C) NAbs-Aβ19–30, and (D) NAbs-Aβ25–36 between the Aβ-PET$^-$ ($n = 210$) and Aβ-PET$^+$ ($n = 150$) subjects. Unpaired t test. (**E** to **H**) Comparison of plasma levels of (E) NAbs-Aβ1–12, (F) NAbs-Aβ7–18, (G) NAbs-Aβ19–30, and (H) NAbs-Aβ25–36 among the Aβ-PET$^-$ CN ($n = 210$), Aβ-PET$^+$ CN ($n = 120$), and Aβ-PET$^+$ AD ($n = 30$) groups. One-way analysis of variance (ANOVA). N.S. denotes no statistical difference. * denotes nominal significance only at $P < 0.05$. ** denotes nominal significance only at $P < 0.01$. *** denotes significance after Bonferroni correction at $P < 0.001$. † denotes less than Bonferroni-corrected α.

We also analyzed the differences in plasma levels of NAbs-Aβ by both Aβ-PET status and AD diagnosis (preclinical versus clinical). This subgroup analysis indicated that Aβ-PET$^+$ patients with AD had the highest plasma levels of NAbs-Aβ1–12 and NAbs-Aβ7–18 but the lowest plasma levels of NAbs-Aβ19–30 and NAbs-Aβ25–36 in comparison with preclinical AD (Aβ-PET$^+$ CN) and Aβ-PET$^-$ CN subjects (Fig. 2, E to H). Plasma levels of NAbs-Aβ1–12 were also higher in Aβ-PET$^+$ CN than those in Aβ-PET$^-$ CN (Fig. 2E). No differences were observed in the plasma levels of NAbs-Aβ1–42, NAbs-Aβ13–24, and NAbs-Aβ31–42 among Aβ-PET$^-$ CN, Aβ-PET$^+$ CN, and Aβ-PET$^+$ AD subjects (fig. S4, D to F). These findings indicate that the levels of NAbs targeting the N terminus of Aβ are increased, while NAbs targeting the mid-domain of Aβ are decreased in subjects with probable or confirmed AD compared with subjects with preclinical AD and non-AD CN controls.

Correlations of NAbs-Aβ with cerebral Aβ deposition as a continuous variable

We examined the association between NAbs-Aβ levels and cerebral Aβ deposition as a continuous variable. Plasma levels of NAbs-Aβ1–12 and NAbs-Aβ7–18 were positively correlated with the amyloid load in the brain expressed as centiloid (Fig. 3, A and B). However, plasma levels of NAbs-Aβ19–30 (only at a nominally significant level) and NAbs-Aβ25–36 were negatively associated with the centiloid score (Fig. 3, C and D). Plasma levels of NAbs-Aβ1–42, NAbs-Aβ13–24, and NAbs-Aβ31–42 were not correlated with centiloid score (fig. S5).

Correlations of NAbs-Aβ with baseline cognitive status

We further analyzed the correlations between NAbs-Aβ levels targeting different domains of Aβ and the cognitive function in the AIBL cohort at baseline in Aβ-PET groups. Higher plasma levels of NAbs-Aβ1–12 and NAbs-Aβ7–18 were correlated with lower PACC scores (worse cognition) in the Aβ-PET$^+$ group, but not in the Aβ-PET$^-$ group (Fig. 4, A and B). Lower plasma levels of NAbs-Aβ19–30 and NAbs-Aβ25–36 were correlated with lower PACC scores (worse cognition) in the Aβ-PET$^+$ group, but not in the Aβ-PET$^-$ group (Fig. 4, C and D). Lower plasma levels of NAbs-Aβ31–42 were correlated with lower PACC scores (worse cognition) in the Aβ-PET$^-$ group, but not in the Aβ-PET$^+$ group (fig. S6C). Plasma levels of NAbs-Aβ1–42 and NAbs-Aβ13–24 were not correlated with PACC scores even at the nominal significance level in either group (fig. S6, A and B). The associations of NAbs-Aβ levels with EM were similar to those with PACC (Fig. 4, E to H, and fig. S6, D to F).

Associations between baseline plasma NAbs-Aβ and the rates of subsequent cognitive decline

In the total AIBL cohort, linear mixed models showed that higher levels of the N-terminal autoantibody NAbs-Aβ1–12 and lower levels of mid-domain autoantibodies NAbs-Aβ19–30 and NAbs-Aβ25–36 were associated with lower PACC scores (Table 1, biomarker rows 1, 4, and 5). Assessing the interaction between each of the NAbs-Aβ and time with PACC, the N-terminal autoantibodies NAbs-Aβ1–12 and NAbs-Aβ7–18 and the mid-domain autoantibody NAbs-Aβ25–36

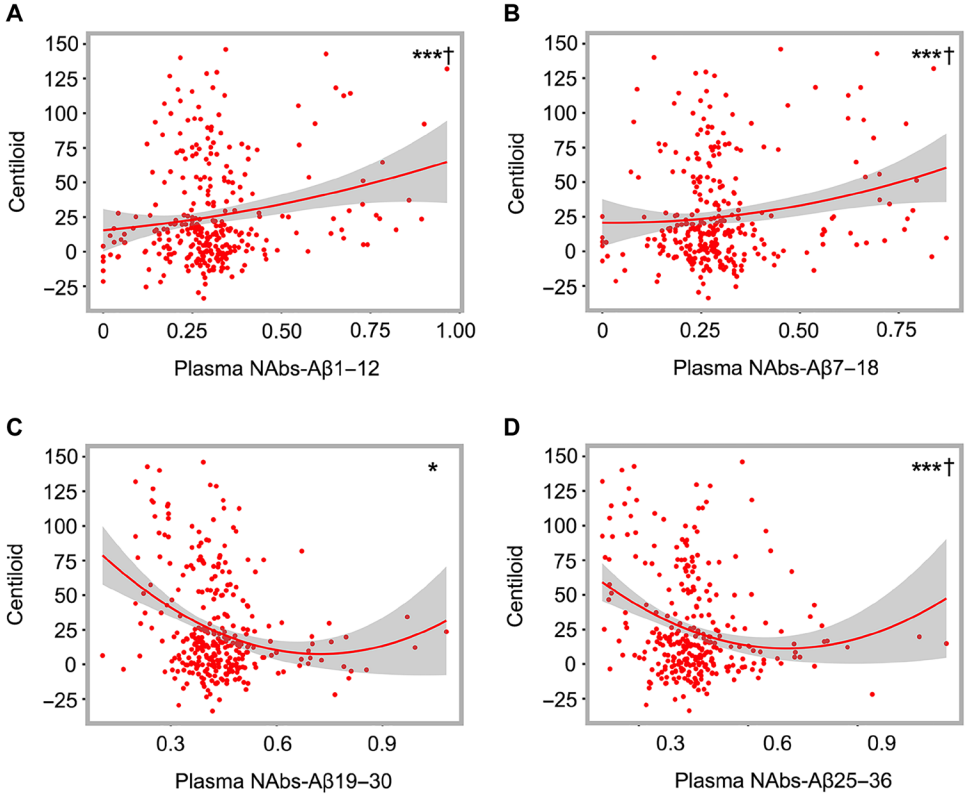

Fig. 3. Correlations between plasma levels of NAbs targeting different domains of Aβ and load of Aβ deposition in the brain at baseline in the AIBL cohort. Fit lines are shown for the correlations between plasma levels of (**A**) NAbs-Aβ1–12, (**B**) NAbs-Aβ7–18, (**C**) NAbs-Aβ19–30, and (**D**) NAbs-Aβ25–36 and the centiloid score. The shaded areas represent the 95% confidence interval. Spearman correlation analysis. * denotes nominal significance only at $P < 0.05$. *** denotes significance after Bonferroni correction at $P < 0.001$. † denotes less than Bonferroni-corrected α.

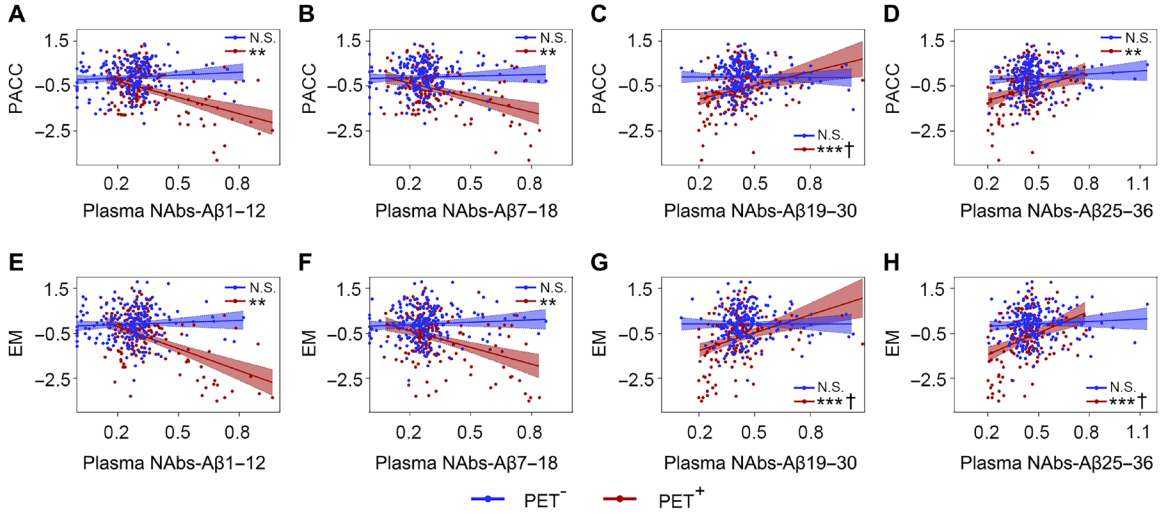

Fig. 4. Correlations between plasma levels of NAbs targeting different domains of Aβ and cognitive function at baseline in the AIBL cohort. (**A** to **D**) Fit lines are shown for the correlations between plasma levels of (A) NAbs-Aβ1–12, (B) NAbs-Aβ7–18, (C) NAbs-Aβ19–30, and (D) NAbs-Aβ25–36 and PACC in different subgroups. (**E** to **H**) Fit lines are shown for the correlations between plasma levels of (E) NAbs-Aβ1–12, (F) NAbs-Aβ7–18, (G) NAbs-Aβ19–30, and (H) NAbs-Aβ25–36 and EM in different subgroups. The shaded areas represent the 95% confidence interval. Spearman correlation analysis. N.S. denotes no statistical difference. ** denotes nominal significance only at $P < 0.01$. *** denotes significance after Bonferroni correction at $P < 0.001$. † denotes less than Bonferroni-corrected α.

were associated with changes of PACC over time in both unadjusted and adjusted models (adjusted for age, gender, diagnosis, and *APOE* ε4 allele status), albeit at the nominal significance level only (Table 1). These relations were more prominent in subjects with *APOE* ε4 allele carriers, with differential relationships between carriers and non-carriers (Fig. 5 and fig.S7).

In the Aβ-PET$^+$ group, the above relations were similar to those of the total cohort; however, only the N-terminal autoantibody NAbs-Aβ7–18 and the mid-domain autoantibody NAbs-Aβ19–30 remained associated with PACC changes over time after adjusting for age, gender, and *APOE* ε4 allele status (Table 1). In contrast with the total cohort, the overall fragment autoantibody NAbs-Aβ1–42 was strongly associated with the change in PACC score over time (Table 1). Similarly, the association between biomarker levels and EM retained significance in the Aβ-PET$^+$ group. However, in the total cohort, the association between biomarker and EM remained significant in main effect only (table S3). These findings indicate that higher levels of NAbs targeting the N terminus of Aβ and lower NAb targeting the mid-domain of Aβ are correlated with faster rates of cognitive decline from the PACC score only, indicating that maybe the association is more prominent in the earlier changes in cognition.

DISCUSSION

In the present study, we found that there was an epitope-specific alteration pattern of NAbs-Aβ in plasma and CSF in both patients with probable AD and those with confirmed AD. Moreover, the associations of NAbs-Aβ with the Aβ burden and cognition were discordant with respect to the N-terminal and mid-domain epitopes of Aβ. The N-terminal NAbs-Aβ increase and the mid-domain NAbs-Aβ decrease as AD progresses from preclinical to clinical stages.

NAbs-Aβ ubiquitously exist in the blood and CSF of both normal subjects and subjects with AD. The alteration of the NAbs-Aβ levels in AD relative to CN controls has not been consistent in previous studies (*11*). For example, Britschgi *et al.* (*12*) identified no statistical difference of NAbs-Aβ levels between patients with AD and controls. Qu *et al.* (*13*) reported reduced circulating levels of NAbs-Aβ in patients with AD. In contrast, increased circulating NAbs-Aβ in patients with AD were also detected in previous studies (*14*, *15*). This discrepancy could be explained by differences in age, gender, disease stage, and assay methods among those studies (*16*). We found that the alteration of NAbs-Aβ differed by their epitopes of Aβ, suggesting that NAbs-Aβ should not be regarded as an integral pool, which would mask the specific changes of different NAbs-Aβ.

The pathogenic relevance of NAbs-Aβ in AD remains largely unexplored. Our findings suggest that NAbs-Aβ may function differently in the pathogenesis of AD with respect to their epitopes. It has been suggested that the N terminus of Aβ is the dominant epitope and is expressed on the surface of aggregated fibrillary deposits (*17*). Antibodies to the N terminus of Aβ are considered competent in clearing Aβ deposits, as reflected by the fact that the most effective monoclonal antibodies to Aβ, which lower the Aβ-PET signal, are thought to target the N terminus of Aβ, including gantenerumab (nearly 100% reduction) (*18*), aducanumab (70% reduction) (*8*), BAN2401 (70% reduction) (*19*), donanemab (70% reduction) (*20*), and bapineuzumab (20% reduction) (*21*). The mid-domain epitope of Aβ has long been considered to represent the fold that drives oligomerization and toxicity [e.g., solanezumab (*9*) and crenezumab (*22*)]; the C terminus is usually considered to be buried and inaccessible in the fibrillary aggregates [e.g., ponezumab (*23*)]. Therefore, antibodies to the N terminus of Aβ are expected to promote clearance of Aβ aggregates, and those to the mid-domain epitopes are expected to abrogate the toxicity of oligomers. Our findings indicate that levels of NAbs to the N terminus of Aβ in increasing rank were Aβ-PET$^-$-CN, Aβ-PET$^+$-CN (preclinical AD), and Aβ-PET$^+$-AD (clinical AD), suggesting that NAbs to the N terminus of Aβ increase, while NAbs to the mid-domain of Aβ decrease with the progression of AD. These changes most likely indicate the failure to clear Aβ and/or inability to inhibit Aβ toxicity in the synapse by NAbs-Aβ during the development of AD. As AD progresses, the amount of fibrillar Aβ increases, which, in

Table 1. Correlations of NAbs-Aβ with the rate of PACC decline in the total cohort and the Aβ-PET$^+$ subgroup. Note: Linear mixed models with adjustment of age, gender, education level, and *APOE* ε4 genotype.

	Parameter	β (SE) unadjusted	*P* values (unadjusted)	β (SE) adjusted	*P* values (adjusted)
Total cohort					
NAbs-Aβ1–12	Biomarker	−1.52 (0.36)	**3.23×10^{-5}***	−0.903 (0.325)	**0.00576**
NAbs-Aβ7–18	Biomarker	−0.834 (0.378)	**0.0279**	−0.369 (0.332)	0.267
NAbs-Aβ13–24	Biomarker	0.355 (0.269)	0.188	0.558 (0.232)	**0.0166**
NAbs-Aβ19–30	Biomarker	2.32 (0.393)	**8.14×10^{-9}***	1.89 (0.345)	**8.07×10^{-8}***
NAbs-Aβ25–36	Biomarker	2.62 (0.394)	**1.17×10^{-10}***	2.28 (0.343)	**1.19×10^{-10}***
NAbs-Aβ31–42	Biomarker	0.288 (0.606)	0.635	1.02 (0.523)	0.051
NAbs-Aβ1–42	Biomarker	−0.245 (0.179)	0.172	−0.117 (0.155)	0.451
NAbs-Aβ1–12	Time	0.03 (0.019)	0.116	0.0227 (0.019)	0.234
NAbs-Aβ7–18	Time	0.016 (0.0187)	0.394	0.0167 (0.0187)	0.373
NAbs-Aβ13–24	Time	−0.00526 (0.0167)	0.752	2.51×10^{-5} (0.017)	0.999
NAbs-Aβ19–30	Time	−0.0256 (0.0311)	0.412	−0.0053 (0.0312)	0.866
NAbs-Aβ25–36	Time	0.0291 (0.0316)	0.358	0.0504 (0.0316)	0.113
NAbs-Aβ31–42	Time	−0.0147 (0.011)	0.18	−0.00896 (0.011)	0.415
NAbs-Aβ1–42	Time	−0.0136 (0.0192)	0.479	−0.0084 (0.0193)	0.663
NAbs-Aβ1–12	Biomarker × Time	−0.181 (0.059)	**0.00248**	−0.139 (0.059)	**0.0198**
NAbs-Aβ7–18	Biomarker × Time	−0.13 (0.0573)	**0.0249**	−0.114 (0.0573)	**0.0484**
NAbs-Aβ13–24	Biomarker × Time	−0.0481 (0.0415)	0.248	−0.0474 (0.0415)	0.254
NAbs-Aβ19–30	Biomarker × Time	0.00985 (0.0645)	0.879	−0.0248 (0.0647)	0.702
NAbs-Aβ25–36	Biomarker × Time	−0.109 (0.0642)	0.0916	−0.143 (0.0642)	**0.0269**
NAbs-Aβ31–42	Biomarker × Time	−0.0875 (0.0855)	0.307	−0.0943 (0.0856)	0.272
NAbs-Aβ1–42	Biomarker × Time	−0.0112 (0.0251)	0.656	−0.0109 (0.0252)	0.667
Aβ-PET$^+$ group					
NAbs-Aβ1–12	Biomarker	−1.96 (0.659)	0.00341	−1.71 (0.603)	**0.00514**
NAbs-Aβ7–18	Biomarker	−1.21 (0.707)	0.088	−0.694 (0.643)	0.282
NAbs-Aβ13–24	Biomarker	0.831 (0.497)	0.0966	1.18 (0.439)	**0.00801**
NAbs-Aβ19–30	Biomarker	3.7 (0.747)	**1.65×10^{-6}***	3.02 (0.694)	**2.32×10^{-5}***
NAbs-Aβ25–36	Biomarker	3.77 (0.765)	**1.82×10^{-6}***	3.38 (0.694)	**2.41×10^{-6}***
NAbs-Aβ31–42	Biomarker	−0.28 (1.23)	0.821	0.211 (1.1)	0.848
NAbs-Aβ1–42	Biomarker	−0.259 (0.372)	0.487	−0.112 (0.333)	0.736
NAbs-Aβ1–12	Time	−0.0098 (0.0582)	0.867	−0.0183 (0.0576)	0.751
NAbs-Aβ7–18	Time	0.0199 (0.0537)	0.711	0.0177 (0.0533)	0.74
NAbs-Aβ13–24	Time	−0.11 (0.042)	**0.00985**	−0.102 (0.0416)	**0.0159**
NAbs-Aβ19–30	Time	−0.287 (0.0877)	**0.00128***	−0.273 (0.0864)	**0.00187***
NAbs-Aβ25–36	Time	0.0209 (0.0887)	0.814	0.0237 (0.0872)	0.786
NAbs-Aβ31–42	Time	−0.0929 (0.0298)	**0.00225**	−0.0895 (0.0296)	**0.00295**
NAbs-Aβ1–42	Time	0.0578 (0.047)	0.221	0.0557 (0.0466)	0.233
NAbs-Aβ1–12	Biomarker × Time	−0.282 (0.177)	0.114	−0.248 (0.175)	0.159
NAbs-Aβ7–18	Biomarker × Time	−0.362 (0.155)	**0.0212**	−0.35 (0.154)	**0.0248**

(*continued on next page*)

	Parameter	β (SE) unadjusted	*P* values (unadjusted)	β (SE) adjusted	*P* values (adjusted)
NAbs-Aβ13–24	Biomarker × Time	0.0435 (0.0967)	0.654	0.0246 (0.0961)	0.799
NAbs-Aβ19–30	Biomarker × Time	0.433 (0.195)	**0.0273**	0.402 (0.191)	**0.0369**
NAbs-Aβ25–36	Biomarker × Time	−0.259 (0.196)	0.187	−0.262 (0.192)	0.175
NAbs-Aβ31–42	Biomarker × Time	−0.00977 (0.266)	0.971	−0.034 (0.264)	0.898
NAbs-Aβ1–42	Biomarker × Time	−0.205 (0.0561)	**0.000357***	−0.201 (0.0558)	**0.000426***

*Less than Bonferroni-corrected α. (α = 0.002). $P < 0.05$ were expressed as boldface.

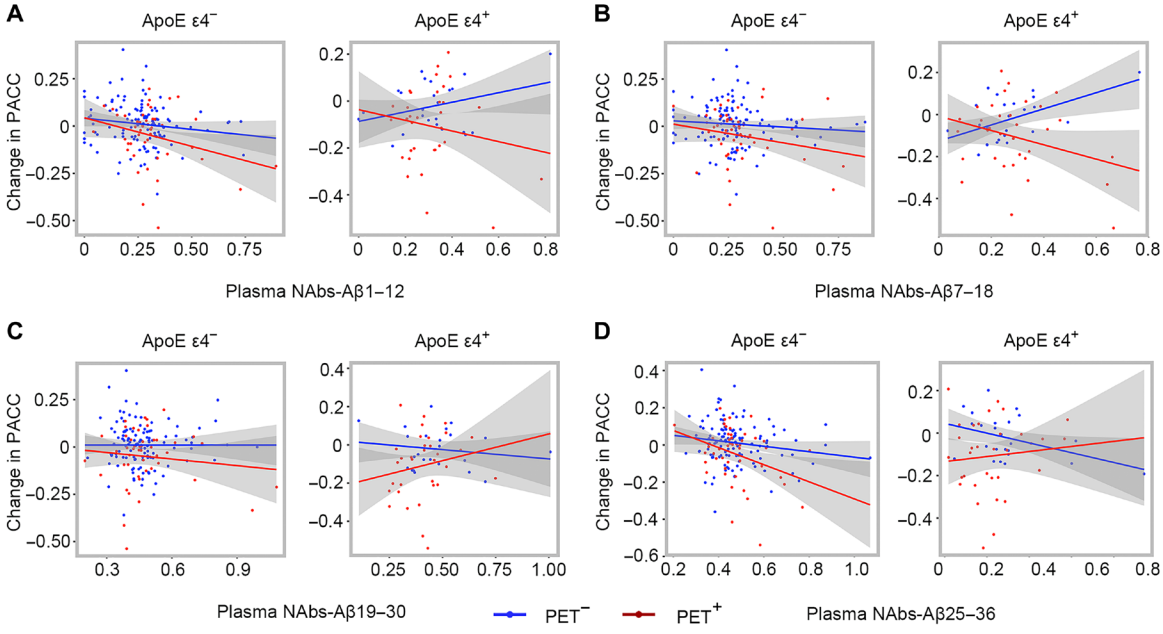

Fig. 5. Linear mixed-effects model plots for plasma levels of NAbs targeting different domains of Aβ and speed of cognitive decline in the AIBL cohort. The plots have been separated for *APOE* ε4 allele status given its effect on both PACC score and the biomarkers. Fit lines are shown for the relationships between plasma levels of (**A**) NAbs-Aβ1–12, (**B**) NAbs-Aβ7–18, (**C**) NAbs-Aβ19–30, and (**D**) NAbs-Aβ25–36 and speed of PACC decline in different subgroups.

turn, may cause the innate and adaptive immune reactive systems to increase their immune responses to the N terminus of Aβ. However, the increase of NAbs-Aβ is not able to adequately remove the accumulation of Aβ fibrils. At the same time, the total amount of the mid-domain Aβ epitope may decrease as the equilibrium moves away from oligomeric/protofibrillar Aβ toward the N-terminal exposed fibrillar Aβ. It is also possible that these autoantibodies might further accelerate AD progress, as antibodies to the N terminus of Aβ are suggested to be able to cause neuronal toxicity (*24*) and amyloid-related imaging abnormalities (ARIA) (*25*) and even promote Aβ production (*26*).

Over the past two decades, active and passive immunotherapies targeting Aβ, as proofs of concept, have shown efficacy in reducing (but not completely eliminating) aggregated Aβ deposits (*8*, *27*, *28*). Little is known about their effect on soluble oligomeric or protofibrillar Aβ species. Failure to halt or reverse the cognitive decline in prodromal and early clinical AD remains unexplained (*29*), but up to 30% slowing of decline has been reported (*30*). Our study indicates that the balance between the autoantibodies against N-terminal and mid-domain epitopes of Aβ is altered as AD progresses, and this may inform the design of immunotherapeutic strategies. While we have not addressed the contribution of neo-epitopes arising from post-translational modification (N-terminal truncations, pyro-glutamylation, isomerization, oligomeric covalent cross-linking, etc.), the decrease in mid-domain NAbs-Aβ with disease progression suggests that attempts to reverse this might be therapeutically useful, particularly if this helps to neutralize the toxicity of the smaller oligomeric species (*31*, *32*). The recent encouraging results on aducanumab suggest that naturally occurring autoantibodies to the N terminus may have therapeutic utility (*33*). It remains to be seen whether a similar approach in commercially exploiting autoantibodies to the mid-domain will prove equally efficacious.

MATERIALS AND METHODS

Study subjects

Chongqing cohort

A total of 91 patients with sporadic AD were consecutively recruited from Daping Hospital in Chongqing, China. The same number of CN controls was randomly recruited from the health examination center of Daping Hospital. Exclusion criteria included (i) concomitant neurologic disorders potentially affecting cognitive function; (ii) severe cardiac, pulmonary, hepatic, renal, or neoplastic disorders; (iii) autoimmune diseases; and (iv) refusal to participate in the study. Among these subjects, 40 patients with AD and 40 CN received lumbar puncture, and CSF was collected. Written consents were obtained from all participants or their legal representatives.

The neuropsychological evaluation was conducted following our previous protocol (*34*). In brief, the cognitive and functional status was assessed using the MMSE and Activities of Daily Living (ADL). The subjects who were abnormal in MMSE assessment were further administered a battery of neuropsychological tests, including CDR, Field Object Memory Evaluation for detecting extensive cognitive dysfunction mainly composed of memory, Rapid Verbal Retrieve for detecting the function of semantic memory, Wechsler Adult Intelligence Scale (Digit Span and Block Design subtests) for evaluating immediate memory and function of graphical recognition, Pfeiffer Outpatient Disability Questionnaire for assessing ability of social activities, Hamilton Depression Rating Scale for measuring emotional status, and Hachinski Ischemic Score (HIS) to determine the presence of significant cerebrovascular disease.

The diagnosis of AD dementia was made following the protocol described in our previous studies (*35*). In brief, dementia was diagnosed on the basis of criteria modified from the *DSM-IV* (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). The subjects with dementia were further subjected to brain computed tomography or magnetic resonance imaging. Diagnosis of probable AD dementia was made according to the criteria of the National Institute of Neurological and Communicative Diseases and Stroke/AD and Related Disorders Association. The study was approved by the Institutional Review Board of Daping Hospital and registered in the Chinese Clinical Trial Registry (no. ChiCTR-OCC-12002212).

AIBL subjects

The Australian Imaging, Biomarkers and Lifestyle (AIBL) study is a longitudinal study of aging, neuroimaging, biomarkers, lifestyle, and clinical and neuropsychological analysis, with a focus on early detection and lifestyle risk factors (www.aibl.csiro.au). Subjects in the AIBL study were followed up for 72 months with visits at baseline and 18-month intervals (visits at 18, 36, 54, and 72 months). Specifics regarding participant recruitment, study design, and clinical assessments were previously described (*36*). Using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) international criteria for AD diagnosis, a clinical review panel determined disease classifications at each assessment time point to ensure accurate and consistent diagnoses among the participants. As most of the subjects recruited in the present study are CN preclinical subjects, the AIBL-PACC (*37*) was used as a measure of cognitive change over time. The PACC consists of the MMSE, Wechsler Adult Intelligence Scale–Revised Digit Symbol Coding, Wechsler Memory Scale–Revised Logical Memory delayed recall, and the Free and Cued Selective Reminding Test (free recall plus total recall). EM, which is suggested to decline 4 to 8 years before executive function and 7 to10 years before other cognitive domains, was chosen as another measure of cognitive change over time (*38*). The institutional ethics committees of Austin Health, St. Vincent's Health, Hollywood Private Hospital, and Edith Cowan University granted ethics approval for the AIBL study. All volunteers gave written informed consent before participating in the study.

In the present study, 360 AIBL participants, comprising 210 Aβ-PET$^-$ CN subjects, 120 Aβ-PET$^+$ CN subjects, and 30 Aβ-PET$^+$ AD patients with baseline Aβ-PET imaging, were selected. For baseline cross-sectional analysis, three Aβ-PET tracers were used (see below). For longitudinal studies, only ^{11}C Pittsburgh Compound B (^{11}C-PiB) was used.

Neuroimaging

Aβ-PET imaging was conducted using the ^{11}C-PiB, ^{18}F-florbetapir, or ^{18}F-flutemetamol radioligands. Briefly, a 30-min acquisition was started 40 min after PiB injection, and 20-min acquisitions were performed 50 min after florbetapir injection and 90 min after flutemetamol injection. All studies were transformed into centiloids using the prescribed standard centiloid cortical mask and the standard centiloid whole cerebellum mask (*39*). A centiloid threshold of at least 20 was used to classify Aβ positivity.

Epitope mapping of NAbs-Aβ

Biotinylated Aβ1–42 and peptides with partial sequences of Aβ1–42 were synthesized as 12–amino acid peptides by GL Biochem Ltd. (Shanghai, China), including peptides corresponding to Aβ1–12, Aβ7–18, Aβ13–24, Aβ19–31, Aβ25–36, and Aβ31–42. To reduce steric hindrance and provide maximum binding capacity of the antibodies, Aβ1–42, Aβ1–12, Aβ7–18, and Aβ13–24 were synthesized with a GGK linker on the C terminus and biotinylated on the terminal lysine. The Aβ19–31, Aβ25–36, and Aβ31–42 peptides were synthesized with a GGK linker on the N terminus and biotinylated on the terminal lysine. These peptides were used for the testing of NAb targeting the corresponding domains of Aβ.

The ELISA was conducted following the protocol validated by a previous study (*40*). Nunc 96-well ELISA (Covance, USA) plates precoated with 150 μl per well of streptavidin (Sigma-Aldrich, USA) solution were coated with biotinylated peptides (10 μg/ml) at 4°C overnight (150 μl per well). Phosphate-buffered saline (PBS) was used as a negative control. The plates were blocked with 1% gelatin (w/v; Sigma-Aldrich, USA) at 37°C for 1 hour. Plasma or CSF samples (100 μl) were added to each well by a 1:100 dilution with 1% bovine serum albumin in Phosphate Buffered Saline with 0.2% Tween-20 (PBST) and incubated overnight at 4°C. For detection, a horseradish peroxidase–conjugated goat anti-human immunoglobulin G (IgG) (H+L) antibody (Pierce, USA) and 3,3,5,5-tetramethylbenzidine (TMB; Sigma-Aldrich, USA) as enzymatic substrate were used. Absorbance was measured at a wavelength of 450 nm with a plate reader (Thermo Fisher Scientific, USA). Monoclonal antibodies 6E10 (recognizes amino acids 1 to 16; Sigma-Aldrich, USA), 4G8 (recognizes amino acids 17 to 25; BioLegend, USA), and 8G7 (recognizes the C terminus of Aβ1–42; Enzo Life Sciences, USA) at a concentration of 1 μg/ml were used as control antibodies to validate peptide coating and epitope availability. The antibody titers were measured in duplicate, and the means of each measure were used for statistical analysis.

Peptide microarray

The synthesized Aβ proteins, along with negative [bovine serum albumin (BSA)] and positive control (anti-human IgG antibody), were printed

on PATH substrate slide (Grace Bio-Labs, USA) to generate autoantibody arrays using Super Marathon printer (Arrayjet, UK). Protein arrays were stored at −80°C until use. A rubber gasket was mounted onto each slide to create individual chambers for the subarrays.

For determination of plasma NAbs-Aβ by peptide microarray, the arrays stored at −80°C were warmed to room temperature, followed by incubation in blocking buffer (3% BSA in PBS buffer with 0.1% Tween 20) for 3 hours. Plasma samples were diluted 1:100 in PBS containing 0.1% Tween 20. A total of 200 μl of diluted plasma or buffer was incubated with each subarray overnight at 4°C. The arrays were washed with PBST, and bound autoantibodies were detected by incubating with Cy3-conjugated goat anti-human IgG (Jackson ImmunoResearch, PA, USA). The antibodies were diluted 1:1000 in PBST and incubated at room temperature for 1 hour. The microarrays were then washed with PBST and dried by centrifugation at room temperature and scanned by LuxScan 10K-A (CapitalBio Corporation, China) with the parameters set as 95% laser power/PMT 480. The fluorescence intensity data were extracted by GenePix Pro 6.0 software (Molecular Devices, CA, USA).

Statistical analysis

Baseline characteristics were summarized using descriptive statistics, with continuous variables described as median/mean where appropriate and categorical data were summarized as absolute frequencies and percentages. Demographic or clinical factors that differed between groups were entered as covariates in subsequent statistical models. Comparative group *P* values were determined via independent samples *t* test and one-way analysis of variance (ANOVA). For three group analyses, post hoc *P* values were determined using Tukey's adjustment for multiple comparisons. Given the number of overall comparisons of marginal means presented here (seven biomarkers), *P* values are compared against a Bonferroni-adjusted α given the number of tests per hypothesis: one for testing biomarkers against a clinical diagnosis [α = 0.05/number of tests (7), 0.007; Figs. 1 and 2], one for testing biomarkers against AD pathology (α = 0.05/8, 0.006; Fig. 2), and another for longitudinal analyses of NAbs-Aβ with the AIBL PACC and EM score (α = 0.05/14, 0.004; Table 1 and table S3).

Spearman rank correlation analyses were conducted to examine the correlation of NAbs-Aβ with centiloid values (Fig. 3 and fig. S5) and with baseline cognitive function (slope shown per Aβ-PET group; Fig. 4 and fig. S6). *P* values are marked with variations of * for each test, with significance for comparison against a Bonferroni-adjusted α (Fig. 3: NAbs versus centiloid, α = 0.05/7, 0.007; Fig. 4: NAbs versus cognition, α = 0.05/28, 0.002).

Linear mixed models (LMMs) were used to assess the association of plasma levels of NAbs-Aβ with the rate of cognitive decline, as reflected by the time-related change of EM and PACC, with adjustment for age, gender, and *APOE* genotype. Models were performed for both the complete sample and the Aβ-PET$^+$ group only. For graphical purposes, LMMs were computed using biomarker values split by the median to assess participants with either low or high levels of each biomarker with respect to the relationship between *APOE* ε4 allele status, cognition, and time. *P* values are compared against a Bonferroni-adjusted α for seven biomarkers and two cognitive composite scores across both complete and PET-Aβ$^+$ groups (α = 0.05/28, 0.002; Table 1 and table S3).

Apart from the post hoc comparisons where Tukey's post hoc *P* values are presented, all *P* values presented are unadjusted, and results talk to significance when the *P* values are less than the corrected α, or nominal significance where specified. Statistical analyses were performed using SPSS software (version 18.0) and the R statistical environment (version 4.0).

For the data analysis of peptide microarray, the autoantibody level was expressed as signal-to-noise ratio. Comparison of autoantibody level between AD and CN groups was conducted using the independent samples *t* test. Correlations of autoantibody level determined by ELISA versus the same values determined by peptide microarrays were assessed by Spearman correlation analysis.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/1/eabb0457/DC1

View/request a protocol for this paper from *Bio-protocol*.

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