

# Plasma sex hormone-binding globulin predicts neurodegeneration and clinical progression in prodromal Alzheimer's disease

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## Research

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# Abstract

**Background:** Sex hormone-binding globulin (SHBG) in plasma has been found to be significantly elevated in subjects with AD. We aimed to investigate whether plasma SHBG was associated with AD biomarkers and could predict neurodegeneration and clinical progression in prodromal AD.

**Methods:** The study tested the cross-sectional relationship between plasma SHBG and CSF AD biomarkers in 707 non-demented adults. Next, the longitudinal influences of plasma SHBG at baseline on dynamic changes of CSF A $\beta$ 42, hippocampus volume, brain metabolism, and cognition were explored in 448 non-demented adults from the Alzheimer's disease Neuroimaging Initiative (ADNI). Finally, the influence of plasma SHBG on the risk of incident AD was explored.

**Results:** This study included 707 participants (mean [SD] age, 62.5 [10.5] years, 416 [58.8%] female) from CABLE and 448 from ADNI-1 (mean [SD] age, 74.8 [7.2] years, 166 [37.5%] female). A positive correlation was found for SHBG levels in plasma and CSF ( $p = 2.12 \times 10^{-10}$ ,  $r = 0.44$ ). Cross-sectional analyses indicated that individuals with higher plasma SHBG had lower levels of CSF A $\beta$ 42 ( $p < 0.005$ ), after adjusting for age, gender, education, APOE4 allele, and cognitive scores. The longitudinal data showed that higher levels of plasma SHBG contribute to accelerated CSF A $\beta$ 42 decrease ( $p < 0.0005$ ), brain metabolism decline ( $p < 0.05$ ), hippocampus atrophy ( $p < 0.01$ ), cognitive decline ( $p < 0.01$ ), and higher risk of AD dementia ( $p < 0.05$ ).

**Conclusions:** Plasma SHBG is associated with CSF A $\beta$ 42 levels and could predict neurodegeneration and clinical progression in prodromal AD. This finding indicates plasma SHBG is a potentially useful, early biomarker for AD.

## 1. Background

Alzheimer's disease (AD) was a disease entity with a continuous course characterized by prodromal changes of cerebrospinal fluid (CSF) pathological proteins (typically lowered  $\beta$ -amyloid<sub>1-42</sub> (A $\beta$ 42) and elevated phosphorylated tau (ptau) protein) followed by brain functional and structural abnormality, and finally devastating cognitive impairment and social disability. Identifying the peripheral biomarkers to predict the disease progression in early stages is of critical importance especially considering that blood test was more readily accessible and economical than other approaches such as CSF or PET imaging. Sex hormone-binding globulin (SHBG) in plasma has been found to be significantly elevated in subjects with AD than their matched controls<sup>1</sup>, suggesting plasma SHBG might be involved in occurrence of AD via however unclear mechanisms. SHBG is one of corticosteroid binding globulins (CBG) that deliver sex hormones in plasma to the outside of cells where some steroids are detached, internalized into the target cells, and bound to the intracellular receptors<sup>2</sup>. It was hypothesized that the breach of equilibrium between the bound and free state might disturb the normal neuroprotective functioning of sex steroids<sup>3 4</sup>.

Till now, it was still controversial for the relationship between plasma SHBG and AD risk. Most researchers focused on their cross-sectional relationships and the conclusions were disputable possibly due to varying sources of biases, such as small sample size and case ascertainment<sup>1 5-8</sup>. Similarly, their longitudinal associations were also inconsistently reported<sup>5 9 10</sup>. Herein, we aimed to investigate whether plasma SHBG could predict neurodegeneration and clinical progression in prodromal AD: 1) we first tested whether plasma SHBG was associated with CSF AD biomarkers; 2) we next explored the values of plasma SHBG in predicting longitudinal changes of CSF AD biomarkers, imaging, and cognition; 3) we finally examined whether plasma SHBG was associated with AD risk.

## **2. Methods**

### **2.1 Study participants**

#### **2.1.1 CABLE participants**

A total of 707 non-demented adults who were northern Han Chinese were derived from Chinese Alzheimer's Biomarker and Lifestyle (CABLE) study. Since 2017, CABLE is an ongoing large-scale study majorly focused on AD's risk factors and biomarkers in Chinese Han population. Individuals were recruited at Qingdao Municipal Hospital, Shandong Province, China. All enrolled participants were Han Chinese aged between 40 to 90 years. The exclusion criteria include: (1) central nervous system infection, head trauma, epilepsy, multiple sclerosis or other major neurological disorders; (2) major psychological disorders; (3) severe systemic diseases (e.g., malignant tumors); (4) family history of genetic diseases. All participants underwent clinical and neuropsychological assessments, biochemical testing, as well as bio-sample (blood and CSF sample) collection. Demographic information and medical history were collected via a structured questionnaire and an electronic medical record system. CABLE was approved by institutional review boards of Qingdao Municipal Hospital and written informed consent was obtained from all participants or their guardians according to the Declaration of Helsinki.

#### **2.1.2 ADNI participants**

The longitudinal data used in the present study were downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.edu)). As a multicenter study, ADNI is designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. The participants are adults aged 55–90 years with normal cognition (NC), mild cognitive impairment (MCI), or mild Alzheimer's disease (AD). Further information can be found at <http://www.adni-info.org/> and in previous reports<sup>11-13</sup>. For the longitudinal analysis, 800 participants were selected from ADNI-stage 1 (ADNI-1) cohort. Each participant underwent an in-person interview for general health and function at the time of study entry by a standard assessment, including medical history, physical and neurological examination, as well as neuropsychological batteries. Baseline data were collected from 2005–2007 and follow-up data were collected during evaluations at sequential intervals of approximately 12 months. ADNI was approved by institutional review boards of all participating institutions, and written

informed consent was obtained from all participants or their guardians according to the Declaration of Helsinki.

The ADNI sample for the present study was constrained to 448 non-demented individuals who had a blood draw for assessment of SHBG levels at baseline. As for the AD risk cohort, 237 participants had completed follow-up examination during a five-year follow-up.

## **2.2 Measurement of SHBG in plasma and CSF**

For CABLE and ADNI, plasma and CSF sample for each subject was obtained in the morning following an overnight fast. The time from collection to freezing was mostly within two hours. Experimenters were blind to the clinical information of samples. In CABLE, plasma SHBG levels were determined with the Human SHBG ELISA kit (abcam, UK) on the microplate reader (Thermo Scientific™ Multiskan™ MK3). Samples were diluted 100-fold (gradient dilution) and run in duplicate (the mean intra-batch coefficient of variable (CV) of 6.67%). In ADNI, SHBG concentrations were tested using multiplex immunoassay panel on the Luminex xMAP platform by Rules-Based Medicine. The samples were run in singlicate while quality controls were performed in duplicate. The mean inter-assay CV was controlled < 20%.

## **2.3 Measurements of CSF AD biomarkers**

In CABLE, CSF was collected by lumbar puncture in 10 ml polypropylene tubes before sent to the lab within 2 hours. CSF samples were centrifuged at 2000 g for 10 minutes. The thaw/freezing cycle was limited not to surpass 2 times. Baseline CSF A $\beta$ 1–42, tau, and p-tau181 were determined with the ELISA kit (Innotest  $\beta$ -AMYLOID (1–42), hTAU-Ag, and PHOSPHO-TAU (181p); Fujirebio, Ghent, Belgium) on the microplate reader (Thermo Scientific™ Multiskan™ MK3). The within-batch precision values were < 5% (4.8% for A $\beta$ 42, 4.6% for tau, and 2.4% for ptau181). The inter-batch CVs were < 15% (9% for A $\beta$ 42, 12.2% for tau, and 10.9% for ptau181). In ADNI, CSF procedural protocols have been described previously<sup>14</sup>. In brief, baseline CSF A $\beta$ 42 was measured using the INNOBIA AlzBio3 immunoassay (Fujirebio, Belgium). The within-batch precision value was 5.1–7.8% for A $\beta$ 42.

## **2.4 PET imaging**

All ADNI subjects underwent PET scanning procedures to study cerebral glucose metabolism. Subjects were injected with a dose of 18F-FDG in a resting state in a quiet darkened room. All sites performed 3D data acquisition, provided images corrected for Compton scatter, and measured attenuation correction based upon “transmission” and “blank” scans for those systems having rod sources or by CT scan for those sites having a PET/CT scanner.

## **2.5 MRI measurement of hippocampus**

The process of MRI acquisition in ADNI has been described elsewhere<sup>15 16</sup>. In brief, ADNI MRIs were acquired at multiple sites with 1.5T GE, Philips, and Siemens MRI scanners using a magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence. Two high-resolution T1-weighted MRI scans were collected for each participant using a sagittal 3D MP-RAGE sequence with an approximate

TR = 2400 ms, minimum full TE, approximate TI = 1000 ms, and approximate flip angle of 8 degrees. Scans were collected with a 24 cm field of view and an acquisition matrix of  $192 \times 192 \times 166$  (x, y, z dimensions), to yield a standard voxel size of  $1.25 \times 1.25 \times 1.2$  mm. Images were then reconstructed to give a  $256 \times 256 \times 166$  matrix and voxel size of approximately  $1 \times 1 \times 1.2$  mm.

## 2.6 Cognitive measures and AD diagnosis

The Alzheimer's Disease Assessment Scale (ADAS) - cognition section was used to represent the global cognition. The validated composite scores for executive and memory functions were developed by reviewing the neuropsychological batteries to identify items which could be considered indicators of these two domains<sup>17 18</sup>. In brief, the indicators of executive functions include Category Fluency, WAIS-R Digit Symbol, Trails A & B, Digit Span Backwards, and clock drawing. The indicators of memory function include relevant items of the Rey Auditory Verbal Learning Test (RAVLT), ADAS-Cog, Logical Memory, and mini-mental state examination (MMSE). The National Institute of Neurological and Communication Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria<sup>19</sup> was used for the diagnosis of probable AD.

## 2.8 Statistical analyses

R version 3.5.1 and GraphPad Prism 7.00 software were used for statistical analyses and figure preparation.  $p < 0.05$  was considered significant except where specifically noted. Chi-square tests (for categorical variables), one-way ANOVA (for continuous variables with normal distributions), and Kruskal-Wallis test (for continuous variables with skewed distributions) were used to compare the baseline demographic, clinical, and diagnostic characteristics.

In case of skewed distribution (Shapiro-Wilk test  $> 0.05$ ) for dependent variable, transformation was performed to approximate a normal distribution via "car" package of R software. As for CABLE, linear regression was used to explore the cross-sectional relationship between SHBG (high vs. low with cutoff = 46.74 nmol/L) and CSF A $\beta$ 42 or tau levels after adjusting for age, gender, education, APOE4 status, and MMSE score at baseline. Interaction terms for gender (male vs. female) and APOE4 status (positive vs. negative) were used to explore whether strata effect existed. In case of any potential interaction ( $p < 0.1$ ), subgroup analysis was further performed. As for ADNI data, the longitudinal influences of plasma SHBG at baseline on CSF AD biomarkers, cognition, hippocampus volume, and brain metabolism were explored using linear mixed effects models adjusting for age, gender, education, APOE4 status, and clinical diagnosis at baseline. First, the adjusted change rate of AD biomarkers and cognition during follow-up was extracted and Spearman correlation analyses were performed to test the relationship between baseline plasma SHBG and change rate of these indexes over follow-up. Meanwhile, The linear mixed effects models had random intercepts and slopes for time and an unstructured covariance matrix for the random effects and included the interaction between time (continuous) and plasma SHBG (high vs low) as predictor. All outcome variables in linear mixed-effects models were standardized to z scores to facilitate comparisons between modalities. The "lm", "nlme", "ggplot2", and "car" packages in R 3.4.3 software were used to perform the above analyses.

Finally, the influence of plasma SHBG on the risk of incident AD was explored using the time-dependent Cox proportional hazard models. The adjusted risk, expressed as hazard ratio (HR) and 95% confidence interval (CI), were estimated for the association between incident AD and plasma SHBG levels at baseline. Individuals who did not develop AD or who were lost were censored at the time of their last evaluation. The dependent measure was time from entry into the cohort to AD diagnosis. To estimate the influence of those lost, the baseline characteristics of those lost to follow-up and those who completed the follow-up were compared. In the Cox proportional hazard models, three models were employed with or without covariates: 1) analyses were conducted without adjusted covariates; 2) we adjusted for age, gender, education, APOE4 status, and diagnosis; 3) we added diabetes mellitus type 2 (DM2), current depression, body mass index (BMI), hypertension, hyperlipidemia, sleep disorder, stroke history, cardiovascular disease (CVD), alcohol abuse, hearing loss, current smoker, and cancer as covariates.

Multiple subgroup and sensitivity analyses were conducted. First, given the gender differences in factors regulating SHBG levels<sup>20</sup>, we re-ran all analyses in men and women separately. Second, we tested the robustness of the results by excluding those who developed dementia within one year since baseline. Third, we repeated all analyses after further adjusting for plasma levels of total testosterone (TT) or free testosterone index (FTI) which were calculated by dividing TT by SHBG.

## 3. Results

### 3.1 Characteristics of participants

A total of 707 non-demented individuals were included in CABLE. The mean (SD) age of the study sample was 62.5 (SD = 10.5) years old and female accounted for roughly 59%. Participants who have higher plasma SHBG levels tend to be older ( $p < 0.0005$ ) and achieved less MMSE scores ( $p < 0.05$ ). As for ADNI, 450 non-demented individuals were included at baseline. The study sample was older ( $74.8 \pm 7.2$  years) and comprised less female (37%). Participants who have higher plasma SHBG levels tend to be older ( $p = 0.03$ ) and female ( $p < 0.0001$ ) (Table 1)

### 3.2 Correlation analysis of SHBG levels in plasma and CSF

Data of SHBG levels both in plasma and in CSF were available for 188 subjects from ADNI-1. The correlation analysis showed that SHBG levels in plasma and CSF were highly related for both sexes ( $p = 2.12 \times 10^{-10}$ ,  $r = 0.44$ ) (Figure 1A).

### 3.3 Plasma SHBG was associated with lower CSF A $\beta$ 42

CSF biomarker abnormality is deemed as the earliest change during the AD course. The cross-sectional analyses revealed that plasma SHBG was negatively correlated with CSF levels of CSF A $\beta$ 42 ( $r = -0.17$ ,  $p = 0.0001$ ). After adjusting age, gender, education, APOE4 status, and MMSE at baseline, significant associations were found between plasma SHBG and CSF A $\beta$ 42 ( $p < 0.005$ ) (Figure 1B). No interactions with gender or APOE4 status were found, though the subgroup analyses indicated that the association

was only significant in *APOE4* non-carriers ( $p < 0.005$ ). Longitudinally, higher plasma SHBG was associated with faster decline of CSF A $\beta$ 42 (Figure 1C), after adjusting for age, gender, education, *APOE4* status, and clinical diagnosis. No significant associations were revealed for CSF tau proteins (Table e-1) though potential interactions with *APOE4* status were found (Table e-2).

### *3.4 Plasma SHBG accelerates hippocampus atrophy and brain metabolism decline*

In the prodromal stage of AD, CSF biomarker change was followed by the structural atrophy as well as brain metabolic decline. We found individuals with higher plasma SHBG showed faster hippocampus atrophy ( $p = 0.028$ , Figure 2A) and brain metabolism decline ( $p = 0.025$ , Figure 2B). No significant interaction terms were found and sensitivity analyses barely change the results.

### *3.5 Plasma SHBG contributes to cognitive decline*

With the accumulation of biomarker and imaging abnormalities, cognitive impairments become the core manifestations with the disease progress. We identified that higher plasma SHBG levels conferred faster decline in general cognition (Figure 2C and 2D), memory function (Figure 2E), and executive function (Figure 2F). Sensitivity analyses excluding those who developed AD within one year since baseline or adding TT and FTI as covariates barely influenced the results.

### *3.6 Plasma SHBG increases AD dementia risk*

In the cohort for incident AD dementia, 199 subjects were lost and 237 were finally included (with a follow-up duration of  $3.2 \pm 2.4$  years), among whom 164 subjects (36%) developed AD dementia. No significant differences in age, sex, education, and plasma SHBG at baseline were found between those who completed the follow-up and those who were lost. Compared with the lowest tertile (T1), subjects with higher plasma SHBG were associated with an average of 50% (T2) and 60% (T3) increased risk of developing AD dementia, independent of age, sex, education, *APOE* genotype, and diagnosis. The significance barely changed after further adjusting lifestyle factors and vascular risk factors. (Figure 3) Stratified analyses indicated that the influences of plasma SHBG levels on the risk of incident AD dementia were stronger in the male group and those with advanced age. (Table 2) Sensitivity analyses excluding those who developed AD dementia within one year since baseline or adding TT and FTI as covariates did not change the results.

## **4. Discussion**

The present study provided multiple lines of evidence supporting that plasma SHBG was a promising biomarker for disease progression in prodromal AD. We found that higher levels of plasma SHBG were associated with CSF A $\beta$ 42 decrease, brain metabolism decline, hippocampus atrophy, cognitive decline, and increased AD risk.

Our findings that SHBG was a significant risk factor might be explained by the “free hormone hypothesis”<sup>4</sup> that SHBG could contribute to AD via modulating the bioavailability of steroids by keeping them from having their biological activity. The SHBG-bound fraction of hormone is considered to be non-bioactive and is prevented from playing roles in neuronal functioning, such as synaptic formation, turnover, and transmission. The loss of these neuroprotective processes might finally contribute to structural and functional abnormalities of brain, and finally the occurrence of AD or cognitive disorders. It was found that the physiological response of mice to corticosteroid was decreased when the CBG expression was genetically suppressed<sup>21</sup>, indicating that SHBG might also modulate the physiologic effects of its steroid cargos. Thus, it could be postulated that plasma SHBG might passively and actively influence neuroprotective functioning of steroids and further the neurodegeneration and clinical progression of prodromal AD<sup>22 23</sup>.

The strata effect in relation to the association of plasma SHBG and AD is complex, for which the evidence set seemed far less robust and needs further investigated. Although we did not find influences of age strata on the effect sizes, subjects in the ADNI study are majorly constrained to adults at late-life stage. A recent imaging study indicated that higher plasma SHBG levels were also significantly associated with lower total and parietal GM volumes in middle-aged men<sup>24</sup>. Future studies should explore whether the associations found here can also be generalized to middle-aged adults. In accordance with previous findings<sup>25 26</sup>, the levels of plasma SHBG were higher in females. As for the stratified effect from gender, no interaction effects were found although some statistical significance might be compromised by the decreased sample size in female.

In the past decades, the preventive effects of hormone replacement therapy against cognitive decline or dementia have been widely studied and unfortunately ended up with little success. It is also noteworthy that previous randomized controlled trials (RCTs) tended to purely focus on the hormone per se and hardly considered the hormone transporter (such as SHBG). More efforts are thus needed in the future to understand pathophysiological roles of SHBG in AD occurrence, which might provide new clues for future trials to prevent AD.

Several limitations exist. First, the conclusion from some subgroup analyses, such as analysis of gender strata, is less robust due to the constrained sample size, which thus should be further validated in larger studies. Second, we only included androgens as covariates, but cannot perform further analyses adjusted for other sex steroid hormones transported by SHBG (such as estrogen and progesterone). Third, the attrition bias might influence the credibility of the longitudinal analyses. Therefore, high-quality cohort studies are warranted in the future to further confirm these findings. Fourth, different platforms to measure SHBG in both cohorts constrained us from comparison.

## 5. Conclusions

Plasma SHBG might serve as a promising predictor of disease progression in early stage of AD course. The values of plasma SHBG in prediction and prevention of AD are still to be addressed, especially in

larger prospective studies in the future.

## **Declarations**

### **Ethics declarations**

#### **Ethics approval and consent to participate**

CABLE was approved by institutional review boards of Qingdao Municipal Hospital and written informed consent was obtained from all participants or their guardians according to the Declaration of Helsinki. ADNI was approved by institutional review boards of all participating institutions, and written informed consent was obtained from all participants or their guardians according to the Declaration of Helsinki.

#### **Consent for publication**

Not applicable.

#### **Availability of data and materials**

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

#### **Competing interests**

The authors declare that they have no competing interests.

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#### **Authors' contributions**

Dr. Wei Xu: conceptualization of the study, collection and analysis of the data, drafting and revision of the manuscript, and prepared all the figures. Dr Chen-Chen Tan: collection and analysis of the data, and revision of the manuscript. Dr Bing-Jie Su: measurement of plasma SHBG in CABLE. Dr Xue-Ning Shen: revision of the manuscript. Ms Xi-Peng Cao: revision of the language. Prof. Yan-Lin Bi: collection of the data and revision of the manuscript. Prof Jin-Tai Yu, Prof Qiang Dong, and Prof. Lan Tan: conceptualization and design of the study and revision of the manuscript.

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## Abbreviations

AD: Alzheimer's Disease

CSF: Cerebrospinal fluid

A $\beta$ 42:  $\beta$ -amyloid<sub>1-42</sub>

SHBG: Sex hormone-binding globulin

CBG: Corticosteroid binding globulins

CABLE: Chinese Alzheimer's Biomarker and Lifestyle

ADNI: Alzheimer's Disease Neuroimaging Initiative

NC: Normal cognition

MCI: Mild cognitive impairment

CV: Coefficient of variable

MP-RAGE: Magnetization prepared rapid acquisition gradient echo

ADAS: Alzheimer's Disease Assessment Scale

RAVLT: Rey Auditory Verbal Learning Test

MMSE: Mini-mental state examination

NINCDS-ADRDA: National Institute of Neurological and Communication Disorders/Alzheimer's Disease and Related Disorders Association

HR: Hazard ratio

CI: Confidence interval

DM2: Diabetes mellitus type 2

BMI: Body mass index

CVD: Cardiovascular disease

TT = Total testosterone

FTI: free testosterone index;

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## Tables

**Table 1 Baseline characteristics of participants**

Cohort *	CABLE				ADNI			
	Total	Low	High	<i>p</i> value	Total	Low	High	<i>p</i> value
N.	707	353	354	...	448	224	224	...
Age (mean ± SD, year)	62.5 ± 10.5	61.1 ± 10.4	64.0 ± 10.4	<b>0.0003</b>	74.8 ± 7.2	74.1 ± 7.2	75.6 ± 7.2	<b>0.038</b>
Gender (M/F)	291/416	133/220	158/196	<b>0.06</b>	282/166	168/56	114/110	<b>&lt;0.0001</b>
Education (mean ± SD, year)	9.7 ± 5.5	9.9 ± 4.4	9.6 ± 6.4	0.10	15.6 ± 3.0	15.6 ± 3.1	15.7 ± 2.9	0.93
<i>APOE</i> ε4 carrier status (%)	16.5% (117)	15.9% (56)	17.2% (61)	0.62	47.5% (213)	48.2% (108)	46.9% (105)	0.78
MMSE (mean ± SD)	27.1 ± 3.2	27.3 ± 3.2	27.0 ± 3.1	<b>0.046</b>	27.3 ± 1.8	27.4 ± 1.8	27.1 ± 1.8	0.11
SHBG (mean ± SD, nmol/L)	52.9 ± 31.3	29.8 ± 10.2	75.9 ± 28.1	<b>&lt; 0.0001</b>	61.7 ± 27.2	41.2 ± 10.2	82.3 ± 23.0	<b>&lt; 0.0001</b>

MMSE: mini-mental state examination; HC: healthy control; MCI: mild cognitive impairment

\*cutoff is defined as median level of plasma SHBG: > 46.74 nmol/L for CABLE and > 56.23 nmol/L for ADNI

**Table 2 Hazard ratios (HR) with corresponding 95% confidence intervals (95% CI) of the association of SHBG levels with risk of Alzheimer's disease according to strata of age, sex, and BMI**

	Tertile*	N (case/total)	Model 1 <sup>a</sup>		Model 2 <sup>b</sup>		Model 3 <sup>c</sup>	
			HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Total (n = 237)	T1 (reference)	50/89	1	...	1	...	1	...
	T2	55/77	1.52 (1.04-2.24)	0.03	1.50 (1.01-2.21)	0.04	1.71 (1.08-2.72)	0.02
	T3	57/71	1.91 (1.31-2.80)	0.001	1.63 (1.07-2.47)	0.02	1.48 (0.99-2.23)	0.06
Male (n = 157)	T1 (reference)	36/70	1	...	1	...	1	...
	T2	40/56	1.71 (1.09-2.68)	0.02	1.65 (1.04-2.61)	0.03	1.63 (1.01-2.64)	0.04
	T3	25/31	2.31 (1.38-3.86)	0.001	2.10 (1.23-3.59)	0.01	2.05 (1.15-3.66)	0.02
Female (n = 80)	T1 (reference)	14/19	1	...	1	...	1	...
	T2	15/21	1.04 (0.50-2.26)	0.92	1.28 (0.59-2.79)	0.54	0.83 (0.31-2.22)	0.72
	T3	32/40	1.18 (0.63-2.22)	0.60	1.19 (0.62-2.31)	0.60	1.86 (0.83-4.17)	0.13
Age < 75y (n = 127)	T1 (reference)	31/52	1	...	1	...	1	...
	T2	24/37	1.09 (0.64-1.86)	0.75	1.09 (0.63-1.88)	0.76	0.95 (0.52-1.74)	0.87
	T3	28/38	1.48 (0.89-2.47)	0.13	1.48 (0.81-2.68)	0.20	2.03 (0.91-4.52)	0.08
Age ≥ 75y (n = 110)	T1 (reference)	19/37	1	...	1	...	1	...
	T2	31/40	2.13 (1.20-3.79)	0.01	2.28 (1.23-4.22)	0.01	2.28 (1.19-4.34)	0.01
	T3	29/33	2.65 (1.47-4.77)	0.001	2.05 (1.08-3.88)	0.03	1.88 (0.94-3.78)	0.07
BMI < 26 kg/m <sup>2</sup> (n = 122)	T1 (reference)	24/38	1	...	1	...	1	...
	T2	26/36	1.27 (0.73-2.21)	0.40	1.52 (0.84-2.75)	0.17	1.58 (0.81-3.06)	0.18
	T3	41/48	1.79 (1.08-2.96)	0.03	1.75 (0.96-3.17)	0.07	1.87 (0.99-3.56)	0.06
BMI ≥ 26 kg/m <sup>2</sup> (n = 115)	T1 (reference)	26/51	1	...	1	...	1	...
	T2	29/41	1.76 (1.03-2.99)	0.04	1.86 (1.05-3.31)	0.04	1.84 (0.96-3.53)	0.07
	T3	16/23	1.71 (0.92-3.19)	0.09	1.52 (0.77-3.02)	0.23	2.08 (0.79-5.44)	0.14

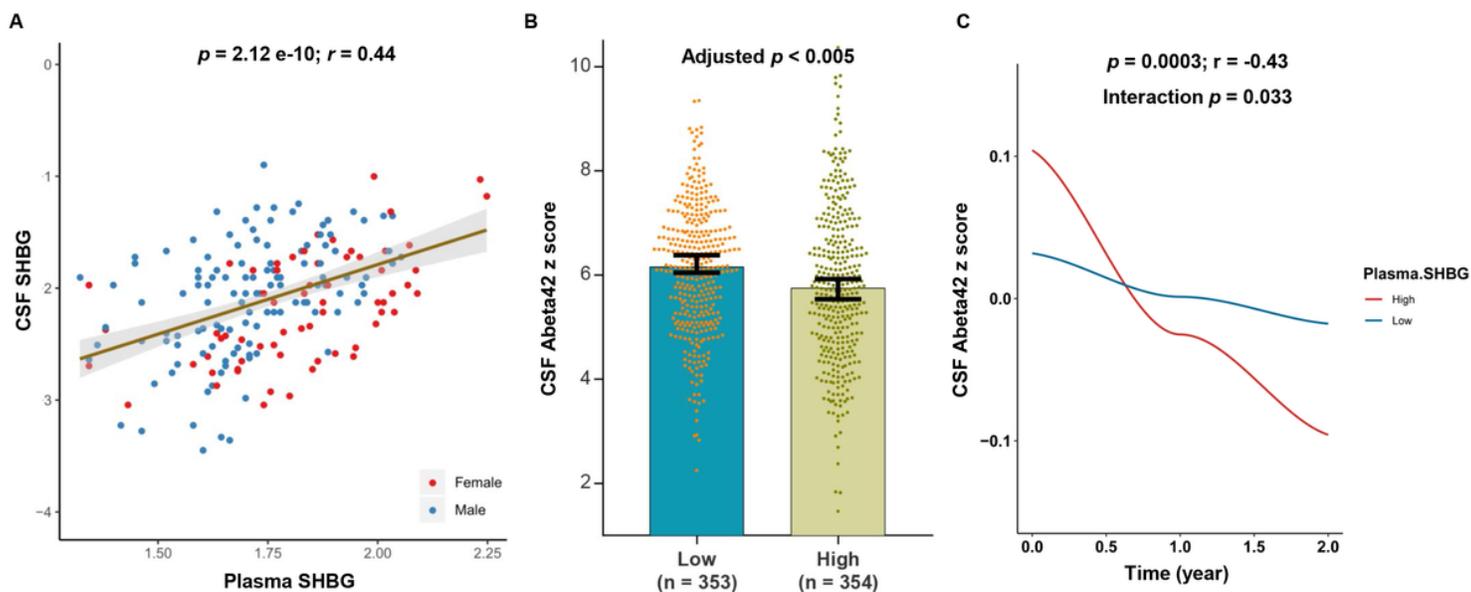
\*SHBG levels (nmol/L) were log10-transformed: T1 <1.67; T2 = 1.67-1.84; T3>1.84

<sup>a</sup>model 1 - crude HR with no covariates adjusted

<sup>b</sup>model2 - HR adjusted for age, gender, education, *APOE4* status, and diagnosis at baseline.

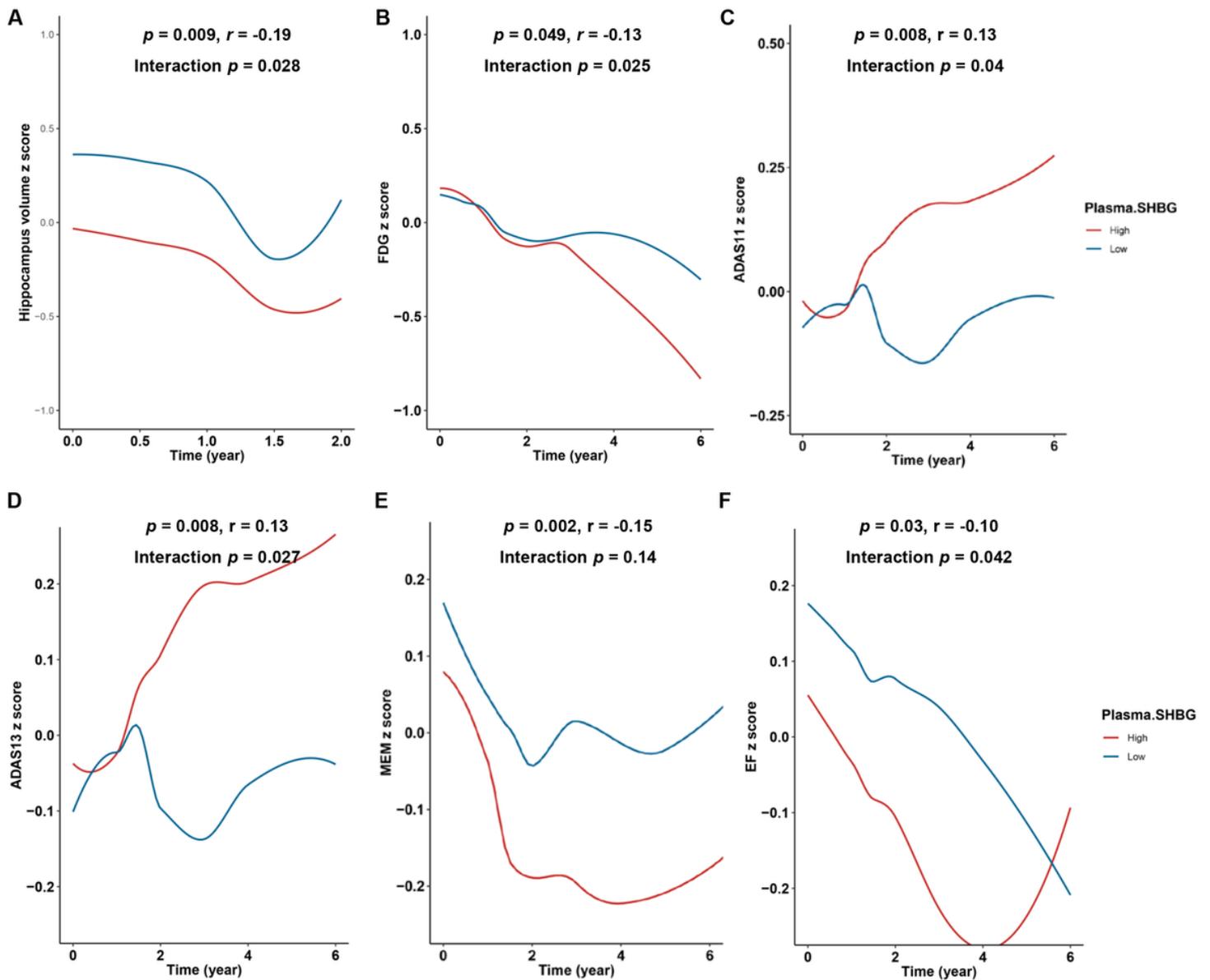
<sup>c</sup>model 3 -HR adjusted for model 1 + diabetes mellitus type 2, depression, body mass index, hypertension, hyperlipidemia, sleep disorder, stroke history, cardiovascular disease, alcohol abuse, hearing loss, current smoking, and cancer.

## Figures



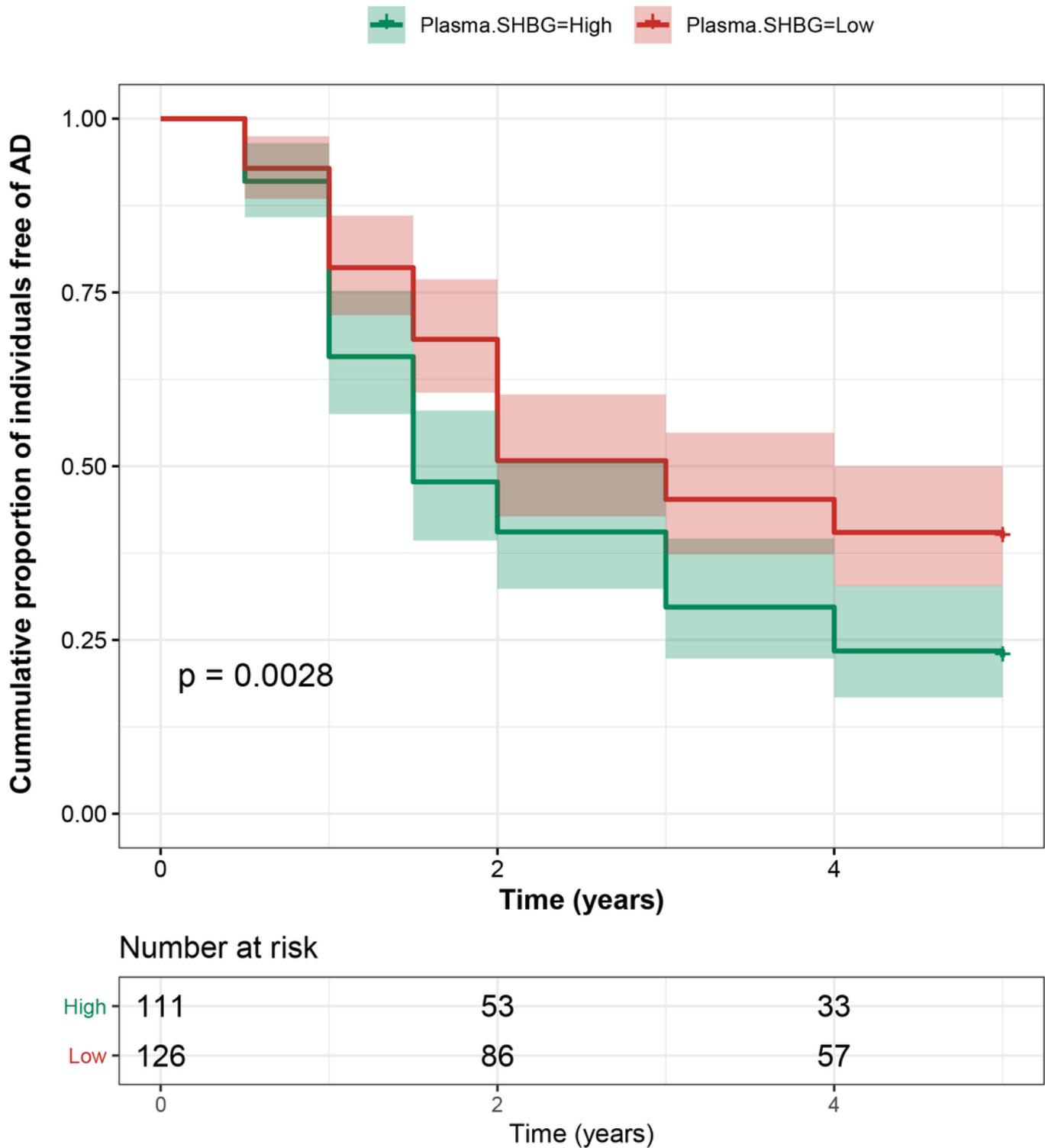
**Figure 1**

Relationships of plasma SHBG with CSF SHBG and CSF Aβ42. Plasma and CSF levels of SHBG were highly correlated (1A). Higher levels of plasma SHBG was associated with lower levels of CSF Aβ42 after adjusting for age, gender, education, *APOE4* status, and MMSE at baseline (1B); Higher levels of plasma SHBG was associated with faster decline of CSF Aβ42 after adjusting for age, gender, education, *APOE4* status, and diagnosis at baseline (1C).



**Figure 2**

Relationships of plasma SHBG with hippocampus atrophy, brain metabolism decline, and cognitive decline. Individuals with higher SHBG showed faster rate of hippocampus atrophy (2A) and brain metabolism decline (2B). Individuals with higher plasma SHBG levels exhibited faster decline in general cognition (2C and 2D), memory function (2E), and executive function (2F). All models were adjusted for age, sex, education, APOE4 status, and baseline diagnosis. Abbreviations: ADAS = Alzheimer’s Disease Assessment Scale; MEM = Memory function; EF = Executive function. The first p value with index (r) indicates the relationship between baseline plasma SHBG and change rate of the above phenotypes over follow-up. The second p value represents association results from the linear mixed effects models.



**Figure 3**

Association between plasma SHBG and AD risk. Subjects with higher plasma SHBG were associated with an increased risk of developing AD, independent of age, sex, education, APOE genotype, and diagnosis.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tablee2.docx](#)
- [Tablee1.docx](#)