Anxiety adds the risk of cognitive progression and is associated with axon/synapse degeneration among cognitively unimpaired older adults

Lin Sun,[a,***,f] Wei Li,[a,f] Qi Qiu,[a,f] Yang Hu,[d] Zhi Yang,[b,c,**] and Shifu Xiao,[a,*] for the Alzheimer's Disease Neuroimaging Initiative[e]

[a]Department of Psychiatry, Alzheimer's Disease and Related Disorders Center, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China
[b]Beijing Key Laboratory of Mental Disorders, National Clinical Research Center for Mental Disorders & National Center for Mental Disorders, Beijing Anding Hospital, Capital Medical University, Beijing, PR China
[c]Advanced Innovation Center for Human Brain Protection, Capital Medical University, Beijing, PR China
[d]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Institute of Psychological and Behavioural Science, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China

eBioMedicine
2023;94: 104703
Published Online 8 July 2023
https://doi.org/10.1016/j.ebiom.2023.104703

Summary

Background Mental symptoms have been shown to be associated with dementia. As the most common neuropsychiatric disorder, it is unclear whether and why anxiety increases the risk of cognitive progression in elderly.

Methods The aim of this study was to investigate the longitudinal effects of anxiety on cognitive impairment in non-dementia elderly and to explore the underlying biological processes using multi-omics including microarray-based transcriptomics, mass spectrometry-based proteomics and metabolomics, cerebrospinal fluid (CSF) biochemical markers, and brain diffusion tensor imaging (DTI). The Alzheimer's Disease Neuroimaging Initiative (ADNI), Chinese Longitudinal Healthy Longevity Survey (CLHLS) and Shanghai Mental Health Centre (SMHC) cohorts were included.

Findings Anxiety was found to increase the risk of subsequent cognitive progression in the ADNI, and a similar result was observed in the CLHLS cohort. Enrichment analysis indicated activated axon/synapse pathways and suppressed mitochondrial pathways in anxiety, the former confirmed by deviations in frontolimbic tract morphology and altered levels of axon/synapse markers, and the latter supported by decreased levels of carnitine metabolites. Mediation analysis revealed that anxiety's effect on the longitudinal cognition was mediated by brain tau burden. Correlations of mitochondria-related expressed genes with axon/synapse proteins, carnitine metabolites, and cognitive changes were found.

Interpretation This study provides cross-validated epidemiological and biological evidence that anxiety is a risk factor for cognitive progression in non-dementia elderly, and that axon/synapse damage in the context of energy metabolism imbalance may contribute to this phenomenon.

Funding The National Natural Science Foundation of China (82271607, 81971682, and 81830059) for data analysis and data collection.

Keywords: Anxiety; Cognitive progression; Axon/synapse damage; Mitochondrial function; Energy metabolism

Introduction

Dementia is a clinical syndrome with progressive deterioration of cognition and impairment of daily functioning. Alzheimer's disease (AD) is the most common form of dementia in the elderly, characterised by extracellular amyloid beta (Aβ) deposition and

*Corresponding author. No. 600 South Wanping Road, Xuhui Distinct, Shanghai, PR China.
**Corresponding author. No. 5 Ankang Hutong, Xicheng Distinct, Beijing, PR China.
***Corresponding author. No. 600 South Wanping Road, Xuhui Distinct, Shanghai, PR China.
E-mail addresses: xiaoshifu@msn.com (S. Xiao), yangz@mail.ccmu.edu.cn (Z. Yang), xiaosuan2004@126.com (L. Sun).
[e]The data used to prepare this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). ADNI investigators contributed to the design and conduct of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete list of ADNI investigators is available at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.
[f]These authors have contributed equally to this work.

Research in context

Evidence before this study

Little is known about the associations of cognitive function, cerebrospinal fluid (CSF)-derived biomarkers of Alzheimer's disease (AD), transcriptomics, and brain imaging, with anxiety. Most previous studies suffered from small sample sizes and cross-sectional designs, leading to ambiguous results. Evidence for an independent relationship between anxiety and subsequent cognitive progression and the underlying mechanism remains limited.

Added value of this study

The present study established the association between anxiety and cognitive progression in non-dementia elderly based on comprehensive analysis of largely longitudinal cohorts. Multi-omics analyses, including microarray-based transcriptomics, mass spectrometry (MS)-based proteomics and metabolomics, DTI-derived metrics, and axon-synapse biomarkers, identified a link between anxiety and pathology of axon-synapse injury and energy metabolism imbalance.

Implications of all the available evidence

Our results demonstrated an independent relationship between anxiety and subsequent cognitive progression in non-dementia elderly, as well as a potential underlying mechanism. The findings have clinical implications suggesting that treating anxiety and targeting mitochondrial dysfunction may be effective in preventing dementia.

hyperphosphorylated tau (p-tau) protein aggregation. A comprehensive understanding of early AD risk factors is crucial for dementia prevention, to help individuals while they are still in the preclinical stages of disease. Neuropsychiatric symptoms of dementia have gained interest among researchers and clinicians because of their significant involvement in preclinical AD.[1] Previous studies have shown an association between depression and the risk of all-cause dementia.[2–4] However, as depression often co-occurs with anxiety, depression scales such as the Hamilton Rating Scale for Depression (HAMD) and the Geriatric Depression Scale (GDS) commonly include an assessment of anxiety. Thus, it is unclear whether anxiety is an independent risk factor for dementia compared to depression alone. Anxiety subsyndromes are more prevalent in older adults, and some studies suggest that anxiety has long-term effects on the central nervous system,[5,6] even when depression is controlled.[7] A meta-analysis of six cohorts found that anxiety has an increased risk of dementia,[8] and may be a prodromal sign of dementia in community samples.[9] Biological studies have associated anxiety with increased Aβ burden in non-dementia elderly,[10–13] and with abnormal cerebrospinal fluid (CSF) total tau (t-tau) levels in patients with mild cognitive impairment (MCI),[14] suggesting a pathological connection between anxiety and AD. Meanwhile, anxiety has also been linked to reduced volumes of hippocampal subfields[15] and cortical thickness of temporal lobes,[16] which suggests that anxiety may serve as an early marker of brain structure in AD.

Previous evidence non the relationship between anxiety and cognitive impairment in non-dementia older people is limited to small sample observations or cross-sectional study, leaving the underlying physiopathology unknown. The present study aimed to address this gap by using large longitudinal cohorts and multi-omics analyses. Longitudinal effects of anxiety on cognitive impairment were examined in two longitudinal cohorts (n = 1080 and 2196). We then analysed microarray-based transcriptomics, MS-based proteomics and metabolomics, and CSF biochemical markers to identify possible biological pathways linking anxiety to later cognitive impairment. We also examined microstructural measures of brain white matter using two datasets (n = 347 and 99) to characterise the macroscopic effects of anxiety on the brain in elderly.

Methods

Study design and samples

The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (http://adni.loni.usc.edu/),[17,18] is a multisite dataset designed to investigate the clinical symptoms, imaging, genetic, and biochemical biomarkers of AD, launched in 2003. Data collection and sharing in ADNI was approved by the institutional review boards of all participating institutions, and all participants or their guardians provided written informed consent in accordance with the Declaration of Helsinki. Participants in this study were older adults aged 55–90 years. Each participant had an in-person interview for health and neuropsychological assessments at baseline and the annual follow-up.

Part of the ADNI (n = 2272) cohort is involved in our analysis. After applying inclusion and exclusion criteria (Fig. 1), a total of 1070 non-dementia elderly were included in this study. Subjects were assessed with the Neuropsychiatric Inventory (NPI)-anxiety subscale,[19] and divided into normal and anxiety groups. Participants reporting no anxiety symptoms before cognitive progression were selected for the normal group (n = 810), while those reporting anxiety symptoms before cognitive progression for the anxiety group (n = 260). All subjects underwent cognitive assessment, including Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog), Functional Activities Questionnaire (FAQ), and ADNI

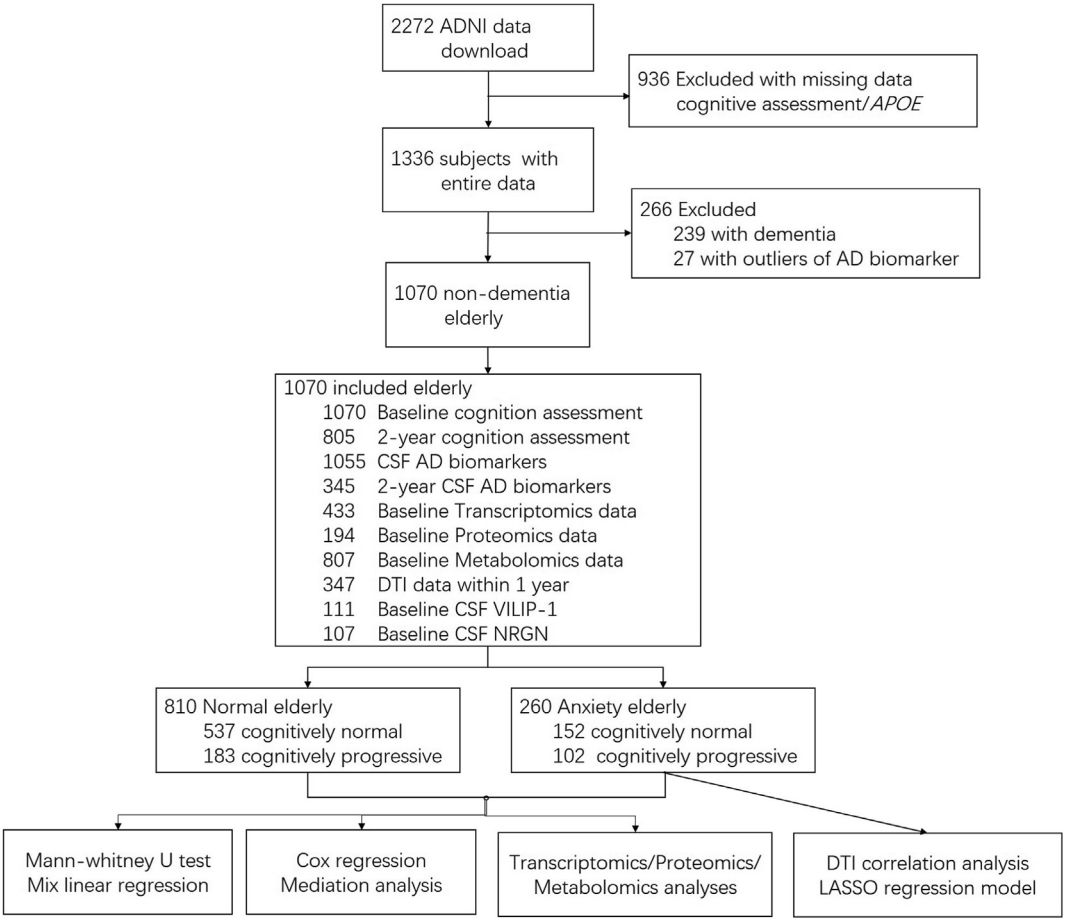

Fig. 1: Trial identification, inclusion, and exclusion in ADNI cohort.

Memory test (ADNI-MEM). The study involved 5415 person-times over a follow-up period of up to 168 months with annual assessments.

Cognitively normal subjects had MMSE scores between 24 and 30, a Clinical Dementia Rating (CDR) of 0, and no memory complaints. Mild cognitive impairment (MCI) was diagnosed using Petersen's criteria, which included an MMSE score between 24 and 30, a CDR of 0.5, objective evidence of memory loss, and preserved functional performance preventing a diagnosis of AD-dementia.[20] Dementia was diagnosed with an MMSE score of <26, a CDR of ≥0.5, and meeting NINCDS/ADRDA criteria for dementia.[21] During follow-up, cognitively stable individuals included those with stable normal cognitive function, stable MCI, or MCI reversing to cognitively normal. Cognitively progressive individuals included those with MCI progressing to dementia, or cognitively normal progressing to MCI or dementia. The normal group consisted of 537 cognitively normal and 183 cognitively progressive elderly at follow-up, while the anxiety group included 152 cognitively normal and 102 cognitively progressive elderly.

The Chinese Longitudinal Healthy Longevity Survey (CLHLS 1998–2018, n = 44,620) (http://opendata.pku.edu.cn/)[22] uses a multistage, stratified cluster sampling and recruited participants from half of the counties and cities in 22 of the 31 provinces of China between 1998 and 2018 among the oldest-old. The vital status of the study participants was ascertained at follow-up waves, and all surviving participants were re-interviewed.

The validation dataset for cognition was from CLHLS cohort. A screening question was used to assess anxiety symptoms at baseline, and 737 participants who reported feeling fearful or anxious "always" or "often" were included in the anxiety group, conversely, 5652 participants who reported feeling anxious "sometimes", "rarely", or "never" were included in the normal group, while 71 with missing data or who answered "could not answer" were excluded. Propensity score-matching (PSM) was used to reduce selection bias between the normal and anxiety groups. A 1:2 case-to-control ratio was used, with a caliper of 0.25, using the MatchIt package in R. After matching, 732 anxiety elderly and 1464 normal elderly were selected (Fig. 2). The study

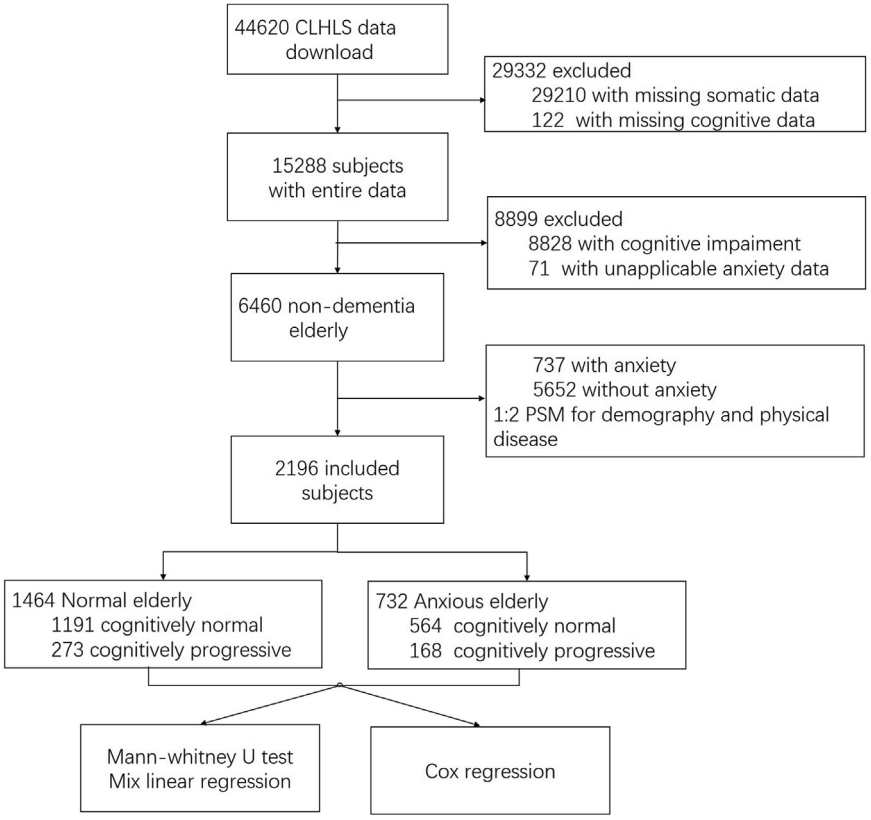

Fig. 2: Trial identification, inclusion, and exclusion in CLHLS cohort.

used the Chinese version of the Mini-Mental State Examination (CMMSE) to assess cognitive impairment, with a score below 18 points indicating moderate-severe cognitive impairment.[23] A total of 4404 person-times over a follow-up period of up to 204 months, were included. Cognitively stable individuals were defined as stable normal, and cognitively progressive individuals were defined as cognitively normal progressing to cognitive impairment. In the CLHLS dataset, the normal group consisted of 1191 cognitively normal and 273 cognitively progressive elderly, while the anxiety group included 564 cognitively normal and 168 cognitively progressive elderly.

The validation dataset for neuroimaging was from the Shanghai Mental Health Centre (SMHC 2012–2021, n = 99) cohort.[24] All subjects were excluded for dementia according to the criteria from the Diagnostic and Statistical Manual for Mental Disorders IV (DSM IV). All were given the interview for anxiety screening by psychiatrist, asking "Whether you often worry about the future", "whether you often feel restless and upset", "whether you often worry that something bad will happen to you", and more. Finally, 37 anxious subjects and 62 normal subjects were included.

Sex information was collected according to the self-reports of the study participants.

Analyses of CSF proteomics, blood transcriptomics and metabolomics

A total of 141 CSF peptides using Multi Reaction Monitoring (MRM) targeted MS (http://adni.loni.usc.edu/data-samples/biospecimen-data/) were measured in 138 normal and 56 anxious subjects, while 19,456 expressed genes using the Affymetrix Human Genome U219 Array (https://ida.loni.usc.edu/pages/access/geneticData.jsp/) were tested in 322 normal and 111 anxious subjects. Technical details were described in the "Biomarkers Consortium CSF Proteomics MRM dataset" and "Microarray Gene Expression Profile Methods", respectively.[25,26] Blood metabolomics containing 138 carnitine metabolites using AD Metabolomics Consortium Duke Biocrates P180 Kit Flow injection analysis (http://adni.loni.usc.edu/data-samples/biospecimen-data/), were used for differential analysis in 569 normal and 238 anxious subjects.

The Limma package[27] in R was performed to analyze the fold changes (FC) and differential levels of multi-omics data between groups. Gene Set Enrichment

Analysis (GSEA)[28] in R was used to compare the biological process (BP) between groups using the c5.go.bp.v7.5.symbols.gmt from the Molecular Signatures Database (MSigDB),[28] and the disease pathway using the Disease-Perturbations-from-GEO-up.txt from the Enrichr database.[29] All genes or proteins were subjected to GSEA analysis[28] and significantly different demographics were included as covariates. Differentially expressed genes by Limma analysis between groups and all proteins were included in a bidirectional orthogonal partial least squares (O2PLS) model[30] in R, to identify the most influential variables from transcriptomics and proteomics (n = 89). Adjusted $p < 0.05$ was considered significant.

Measurement of CSF-based AD biomarkers

CSF biomarkers were obtained from ADNI (http://adni.loni.usc.edu/data-samples/%20biospecimen-data/) using Roche Elecsys immunoassay, including $A\beta_{42}$, p-tau, and t-tau, in 1055 participants at baseline and 2038 person-times at follow-up. CSF neurogranin (NGRN) and visinin-like protein 1 (VILIP1) were also tested using the Erenna® immunoassay system in independent laboratories and batches, with 111 subjects tested for NGRN and 107 subjects for VILIP1. Before analysis, all concentrations were normalized to Z-score, and outliers beyond ±3 were excluded.

Analyses of DTI metrics

The DTI data from ADNI were available (https://ida.loni.usc.edu/pages/access/studyData.jsp? categoryId=14&subCategoryId=30), and detailed processing was described in "Microsoft Word- DTI-ADNI_Methods-Thompson-Oct2012.docx". Anatomical images were linearly aligned to a version of the Colins27 brain template[31] using FSL's flirt[32] with 6 degrees of freedom. The mean of all voxels from each region of interest from the atlas was obtained from fractional anisotropy (FA) and mean diffusivity (MD) of 40 tracts (detailed tracts shown in Supplementary Table S1) from 347 subjects in ADNI and 99 subjects in SMHC.

The DTI data from SMHC was acquired using an echo planar imaging sequence with 64 non-collinear directions and 3 diffusion weightings, including $b1$-value = 0, $b2$-value = 1000 s/mm^2, and $b3$-value = 2000s/mm^2. TR = 5620 ms, TE = 106 ms, FOV = 256 mm, slice number = 74, thickness = 2 mm and voxel size = 2.0 × 2.0 × 2.0 mm^3. For diffusion tensor modelling, only volumes with b = 0 and b = 1000 s/mm^2 were used.

The Least Absolute Shrinkage and Selection Operator (LASSO) regression was applied to select the best features for anxiety from DTI data. In the ADNI dataset, a randomly assigned 70% of all subjects were used as the training set, and the remaining 30% as the test set. The SMHC cohort was used as the validation set. After feature selection, an internal 10-fold cross-validation was performed on the training data using binary classification between groups and the glmnet package in R.[33] Discriminative power was estimated by quantifying the area under the curve (AUC) of the receiver-operator characteristic (ROC). DSI Studio software was used to visualize the significant DTI tracts. The relationship between anxiety-susceptible regions and cognition was investigated in all subjects.

Statistical analyses

The Kolmogorov–Smirnov test was used to assess the normality of continuous data. The Mann–Whitney U test for continuous data with a non-normal distribution, and the chi-squared test for categorical variables, were used to test differences of variables between groups in ADNI and CLHLS datasets. Based on data from similar studies,[34,35] the minimum sample sizes required to detect differences in CSF tau levels between groups, were calculated using G-Powere software as 187 and 93, respectively. The sample size in our analysis meets the minimum sample size requirement. Statistical power was calculated using PASS, assuming an alternative hypothesis of two unequal means with a simulation of 10,000. Effect size was calculated using η2, which is derived from the statistical values of Mann–Whitney U or Chi-square and the sample sizes of two groups. The Spearman correlation with false discovery rate (FDR) correction was used as a measure of correlation in non-parametric statistics.

Linear mixed-effect model was performed to explore the longitudinal effects of anxiety on differences in clinical outcomes, and multivariate Cox regression analysis was used to predict risk factors for cognitive progression. Age, education, sex, *APOE*, hypertension, diabetes, and baseline MMSE were included as covariates to adjust for their potential confounding effects. The annual rate of change in cognition was determined using linear mixed regression models with MMSE, ADAS-cog, FAQ, and ADNI-MEM as dependent variables and time (years from baseline) as the independent variable to control for random intercept and slope. A slope representing the annual rate of change was then calculated for each subject. Longitudinal analyses were restricted to subjects with at least 3 time points.

Mediation analysis was used to examine whether brain tau burden mediated the pathway from anxiety to cognitive function. X was designated as anxiety, mediator was CSF t-tau or p-tau levels at baseline, and Y was the outcome (2-year cognitive function or annual rate of change in cognitive function). Age, education, sex, and *APOE* were included as covariates. Model 4 of the PROCESS macro from the bruceR package in R was used with bootstrapping of 1000 iterations, to estimate the total effect, direct effect, and indirect effect.

Statistical significance was set at p value < 0.05. All analyses were performed using SPSS 17.0 or R version 4.2.1.

Ethics

For the ADNI and CLHLS data, all participants provided written informed consent approved by the institutional review board of each participating institution. Data collection in the SMHC cohort was conducted in accordance with the recommendations of the Shanghai Mental Health Center Ethical Standards Committee on Human Experimentation (reference number: 2012–19). Written informed consent was obtained from all participants or their legal guardians in accordance with the Declaration of Helsinki.

Role of funding sources

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Results

Participant characteristics

Table 1 summarises the characteristics of the participants in the ADNI dataset. Participants with anxiety were more likely to be male (58.8%) and *APOE* ε4 carriers (54.2%), and had more impaired cognitive function and quality of life (Mann–Whitney test, $p < 0.05$). Table 2 depicts demographics and clinical measures in the CLHLS dataset. Participants with anxiety had a more impaired cognitive function compared to normal elderly (Mann–Whitney test, $p < 0.05$). Sex-disaggregated data for ADNI and CLHLS datasets is available in Supplementary Table S2.

Longitudinal effects of anxiety on cognitive function and cognitive progression risk

We found that the anxiety group had significant longitudinal impairments in cognition and quality of life

Characteristics	Normal elderly	Anxious elderly	U or $\chi 2$	p value (statistical power) (η^2)
Baseline				
N	810	260		
Age (years)	72.900 ± 6.936	72.110 ± 7.330	9.888×10^4	0.138
Sex (male ratio) (male/female)	0.505	0.588	5.506	0.019 (0.654) (0.005)
Education (years)	16.390 ± 2.568	16.150 ± 2.762	1.010×10^5	0.311
Hypertension (ratio) (Yes/No)	0.411	0.446	0.992	0.319
Diabetes (ratio) (Yes/No)	0.600	0.400	0.191	0.662
APOE (ε4 ratio) (ε4/non ε4)	0.422	0.542	11.461	0.001 (0.923) (0.011)
MMSE	28.460 ± 1.667	27.830 ± 1.895	8.439×10^4	<0.001 (1.000) (0.022)
ADAS-cog	13.500 ± 6.521	16.000 ± 7.255	8.382×10^4	<0.001 (0.999) (0.023)
FAQ	1.450 ± 3.012	3.690 ± 4.524	6.399×10^4	<0.001 (1.000) (0.085)
ADNI-MEM	0.640 ± 0.748	0.259 ± 0.711	7.542×10^4	<0.001 (1.000) (0.044)
CSF biomarker (ng/ml)				
N	799	256		
Aβ42	1.037 ± 0.470	0.895 ± 0.427	8.300×10^4	<0.001 (0.986) (0.020)
t-tau	0.252 ± 0.105	0.278 ± 0.120	8.927×10^4	0.002 (0.928) (0.009)
p-tau	0.024 ± 0.012	0.272 ± 0.014	8.761×10^4	0.001 (0.974) (0.011)
VILIP1	0.148 ± 0.045 (N = 78)	0.183 ± 0.074 (N = 33)	0.949×10^3	0.029 (0.587) (0.043)
NRGN	2.146 ± 0.821 (N = 76)	2.871 ± 1.313(N = 31)	0.764×10^3	0.004 (0.704) (0.076)
Cognitive conversion				
N	720	254		
Cognitive progression (ratio)	0.254	0.402	19.711	<0.001 (0.989) (0.020)
2-year follow-up				
N	584	221		
MMSE	27.690 ± 3.013	26.670 ± 2.978	4.850×10^4	<0.001 (0.986) (0.037)
ADAS-cog	13.920 ± 9.126	18.320 ± 9.744	4.603×10^4	<0.001 (1.000) (0.049)
FAQ	2.900 ± 5.866	6.570 ± 7.256	3.897×10^4	<0.001 (1.000) (0.094)
ADNI-MEM	0.616 ± 0.965 (n = 494)	0.154 ± 0.896 (n = 204)	3.557×10^4	<0.001 (1.000) (0.054)
CSF biomarker (ng/ml)				
N	237	108		
Aβ42	0.977 ± 0.476	0.877 ± 0.423	1.111×10^4	0.049 (0.418) (0.011)
t-tau	0.261 ± 0.110	0.317 ± 0.139	9.602×10^3	<0.001 (0.992) (0.04)
p-tau	0.024 ± 12.206	0.031 ± 0.016	9.653×10^3	<0.001 (0.994) (0.039)

Table 1: **Characteristics of participants in the ADNI dataset.**

Characteristics	Normal elderly	Anxious elderly	U or $\chi 2$	p value (statistical power) (η^2)
N	1464	732		
Age (years)	90.310 ± 7.565	90.530 ± 7.648	5.275×10^5	0.552
Sex (male ratio)	0.350	0.346	0.036	0.849
Education (years)	1.350 ± 2.807	1.460 ± 2.871	5.229×10^5	0.253
Hypertension (ratio) (Yes/No/Unknow)	0.136	0.156	1.586	0.452
Diabetes (ratio) (Yes/No/Unknow)	0.700	0.500	0.488	0.784
CMMSE	25.930 ± 3.260	25.650 ± 3.252	5.063×10^5	0.034 (0.473) (0.002)
Cognitive progression (ratio)	0.186	0.230	5.631	0.018 (0.658) (0.003)

Table 2: **Characteristics of participants in the CLHLS dataset.**

compared to the normal group (Fig. 3a and c) (Supplementary Table S3). The proportion of participants with cognitive progression was higher in the anxiety group, with relative risks of 1.580 (95% CI: 1.327, 1.881) in the ADNI and 1.231 (95% CI: 1.038, 1.460) in the CLHLS. Individuals with anxiety showed an increased risk of cognitive progression through Cox regression, with age, education, sex, *APOE*, hypertension, diabetes, baseline MMSE score, and other psychiatric symptoms as covariates in the ADNI (Fig. 3b), and similar results occurred in the CLHLS (Fig. 3d).

Axon/synapse pathways and psychiatric/cognitive disorder-related pathways in anxiety

For proteomics, participant characteristics and FC data are presented in Supplementary Tables S4 and S5, respectively. GO biological process (BP) analysis of anxiety revealed activated pathways related to the synaptic signaling, synapse organisation, axon development, and transport regulation through MSigDB (Fig. 4a1–a3). Disease pathway analysis revealed that pathways relevant to psychiatric/cognitive disorder, such as schizophrenia, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), were significantly enriched for anxiety using the Enrichr database (Supplementary Fig. S1).

For transcriptomics, participant characteristics and FC data are presented in Supplementary Tables S6 and S7, respectively. GO BP analysis revealed activated pathways relevant to axon/synapse function, including synaptic plasticity, actin filament movement, neurotransmitter transport for anxiety through MSigDB (Fig. 4b1–b3). Disease pathway analysis revealed the pathways relevant to psychiatric/cognitive disorders, such as HD, Parkinson's disease (PD), intellectual and developmental disability, schizophrenia, bipolar disorder for anxiety using the Enrichr database (Supplementary Fig. S1)

Changes in CSF axon/synapse biomarkers in anxiety

Compared to the normal group, the anxiety group had higher levels of CSF t-tau, p-tau (Table 1, Fig. 4c1 and c2), VILIP1, and NGRN (Table 1, Fig. 4c3 and c4). NGRN and VILIP1 showed strong correlations with both CSF t-tau and p-tau levels in all subjects (Fig. 4d1–d4). Significant correlations were observed between CSF biomarkers and annual changes in cognitive function, including MMSE, MEM, FAQ, and ADAS-cog (Spearman correlation, adjusted $p < 0.05$) (Fig. 4e).

Predictive DTI-derived metrics for anxiety

Participant characteristics are presented in Supplementary Table S8. After feature selection by LASSO regression (Fig. 5a1 and a2), 8 DTI-derived metrics were identified with non-zero regression coefficients (Fig. 5a3), and the value of lambda. min was 0.025. ROC curve analysis showed that AUC of the model based on 8 DTI-derived metrics was 0.799 in the training set (Fig. 5b1), 0.724 in the test set (Fig. 5b2) in the ADNI, 0.707 in the validation set (Fig. 5b3) in the SMHC to discriminate anxious from normal elderly. The Spearman correlation between 8 DTI-derived metrics and clinical characteristics was plotted as a heatmap (Fig. 5c). Furthermore, 4 DTI-derived MD measures, including left cingulate hippocampus (CGH-L), right cingulate hippocampus (CGH-R), right tapetum (TAP-R), and left uncinate fasciculus (UNC-L), were significantly correlated with cognitive function and brain tau burden in all subjects (Fig. 5d).

The mediation effect of brain tau burden on the association between anxiety and cognitive function

In the ADNI, and found that CSF t-tau and p-tau mediated the association of anxiety with cognitive function, including 2-year cognition (Fig. 6a1–b4) and annual rate of change in cognition (Fig. 6c1–d4). The mediation effect was considered to be partial mediation, with the proportion ranging from 11.1% to 33.3%.

Energy metabolism imbalance in anxiety

In ADNI, GO BP analysis revealed suppressed pathways relevant to energy metabolism, such as mitochondrial respiratory chain complex assembly, aerobic respiration, oxidative phosphorylation (Fig. 7a1–a3), and

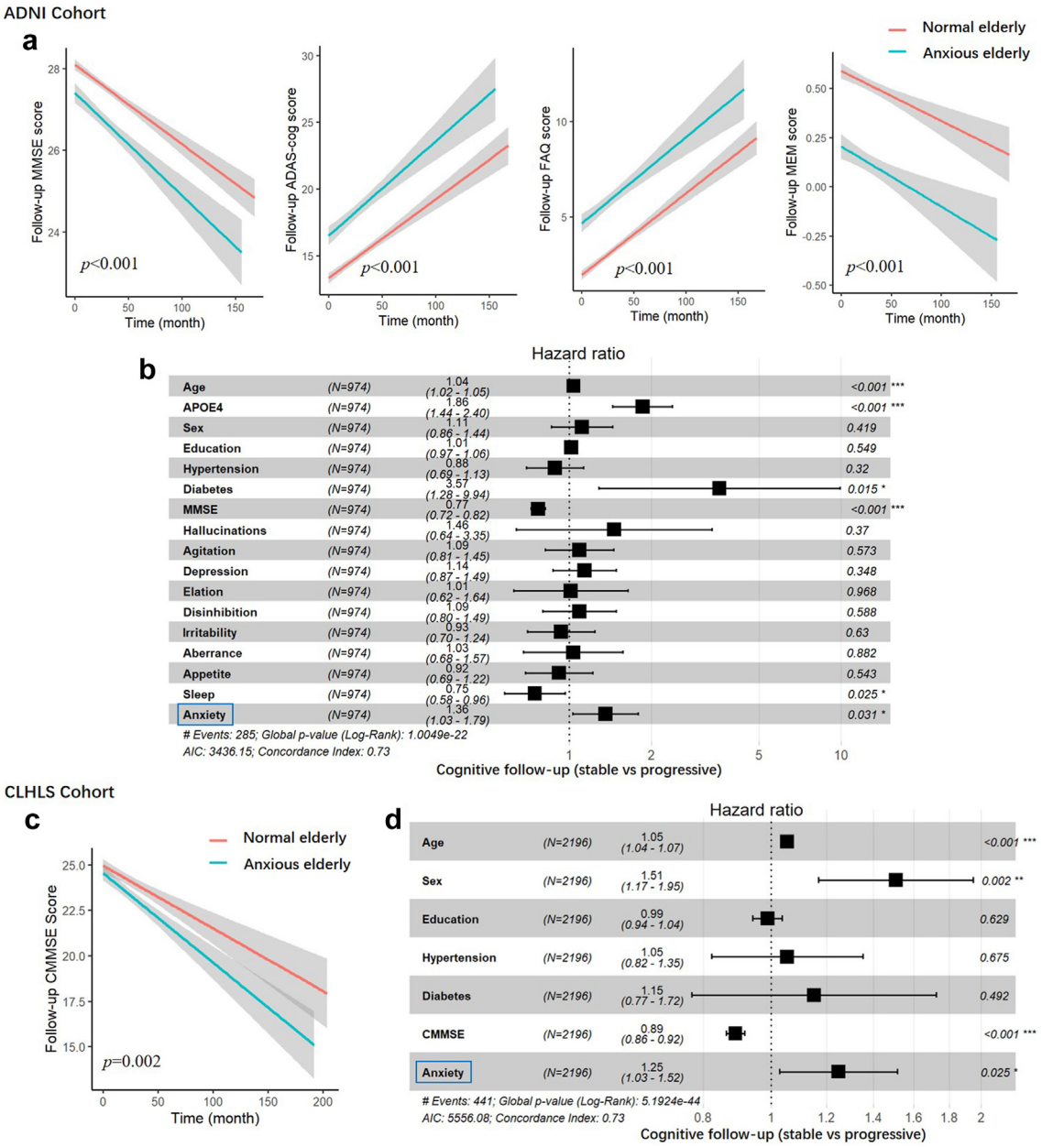

Fig. 3: Anxiety may significantly increase the risk of cognitive progression, as evidenced by different changes in follow-up MMSE, ADAS-cog, FAQ, and ADNI-MEM between normal and anxious groups based on the linear mixed-effects model in the ADNI (baseline n = 1070, follow-up person-times n = 5415) (a). The Cox proportional hazards model estimated the cognitive progression risk of anxiety after adjusting for age, education years, sex, *APOE*, hypertension, diabetes, baseline MMSE score, and multiple psychiatric symptoms (n = 974) (b). Different change in follow-up CMMSE between normal and anxious groups in the CLHLS (baseline n = 2196, follow-up person-times n = 4404) (c). Cox model estimated the cognitive conversion risk of anxiety after adjustment for age, years of education, sex, hypertension, diabetes, and baseline CMMSE score (d).

mitochondrial ribosome, such as ribosome biogenesis, mitochondrial RNA processing, mitochondrial translation (Fig. 7b1–b3).

Participant characteristics and FC data for metabolomics are presented in Supplementary Tables S9 and S10, respectively. The anxiety group exhibited decreased levels of carnitine metabolites, such as glutarylcarnitine, tetradecenoylcarnitine, and hydroxypropionylcarnitine (Limma analysis, adjusted $p < 0.05$) (Fig. 7c1). Multi-omics correlation analysis demonstrated significant

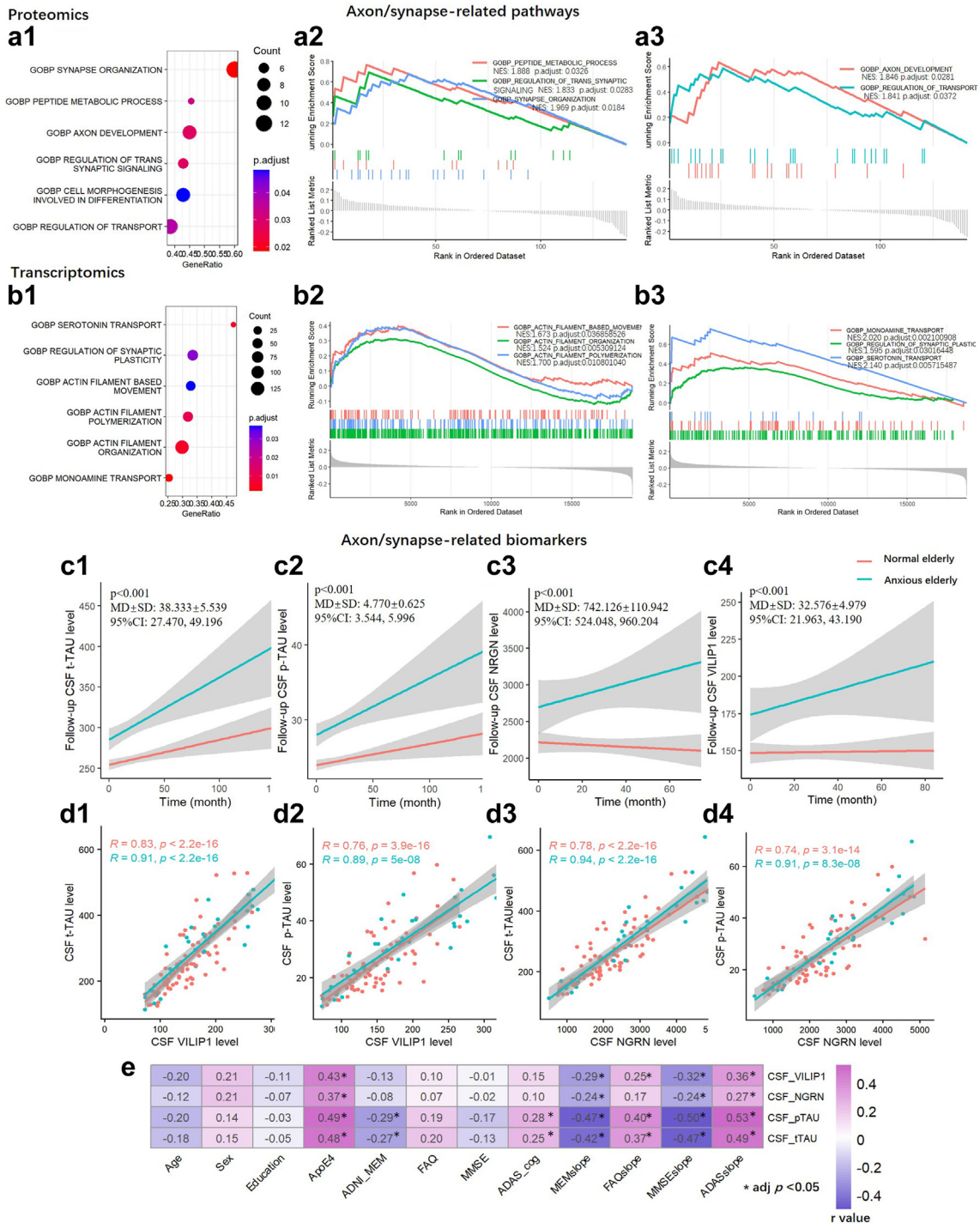

Fig. 4: Axon/synapse-related pathways and biomarkers for anxiety. GO BP analysis showed activated pathways relevant to synapse organisation, synaptic signaling, axon development, synaptic plasticity, serotonin transport in proteomics (n = 194) (a1–a3) and transcriptomics (n = 433) (b1–b3) for anxiety (GSEA analysis, adjusted $p < 0.05$). Significant differences of CSF follow-up axon/synapse markers, including t-tau, p-tau (baseline n = 1055, follow-up person-times n = 2038), NRGN (baseline n = 107, follow-up person-times n = 434) and VILIP1 (baseline n = 111, follow-up person-times n = 441), between normal and anxiety groups (c1–c4). Significant correlations between NRGN (n = 107) and VILIP1 (n = 111) with levels of t-tau (d1, d3) and p-tau (d2, d4). A heatmap displaying the correlations between CSF axon/synapse biomarkers and various factors, including demographics, baseline cognitive function, and annual changes in cognitive function. The value presented within each box represents the correlation coefficient (r), with an asterisk (*) denoting adjusted $p < 0.05$ (Spearman correlation) (e).

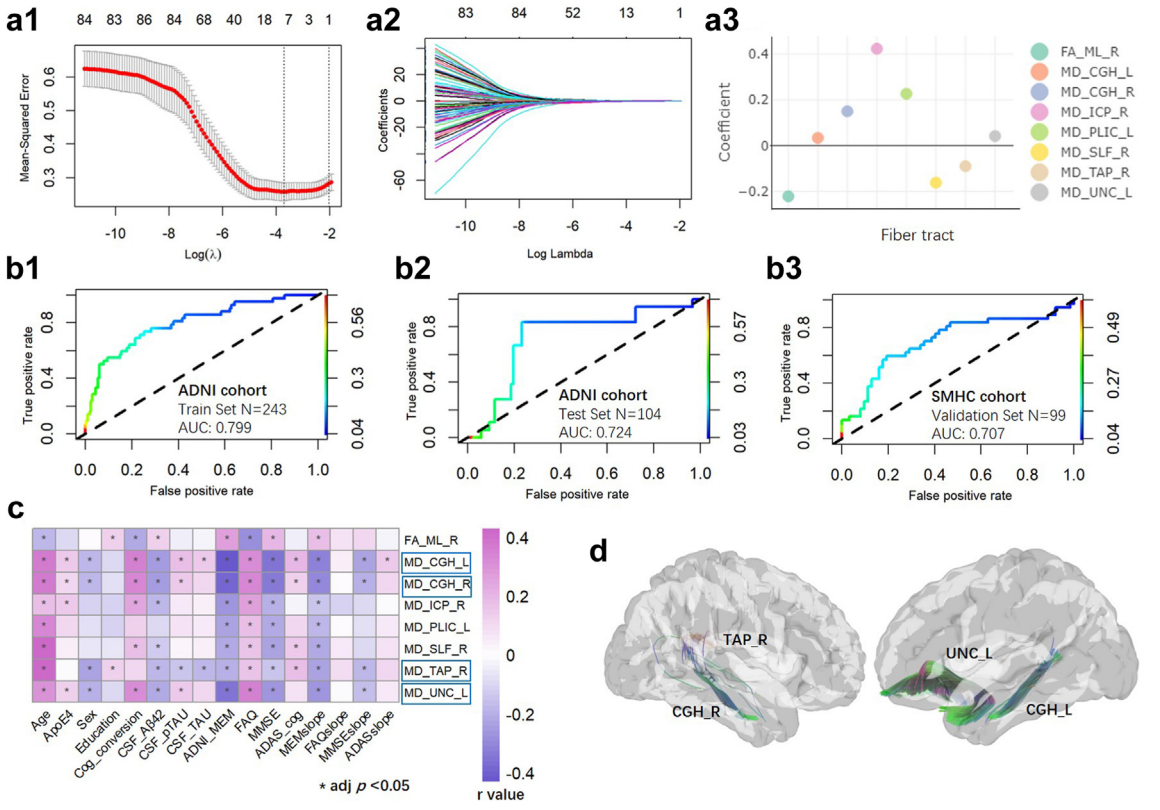

Fig. 5: DTI-derived metrics for anxiety. 40 FA and MD indices of DTI tracts were selected to construct the LASSO model (a1 and a2), and 8 DTI-derived metrics were identified to discriminate between anxious and normal elderly (a3). ROC curves analysis of the training set (n = 243) (b1), test set (n = 104) (b2) and validation set (n = 99) (b3). Correlation between the 8 DTI-derived metrics and clinical characteristics is shown as a heatmap (Spearman correlation, adjusted $p < 0.05$) (c). The 4 DTI-derived metrics associated with tau burden were plotted using the DSI studio program (d).

correlations among mitochondria/ribosome-related expressed genes, axon/synapse proteins, carnitine metabolites, and cognition (Spearman correlation, adjusted $p < 0.05$) (Supplementary Fig. S2).

The joint analysis of transcriptomics and proteomics identified the most influential variables, including the *ATP6V1G2* gene, CYP1A2 and APOB proteins involved in the energy metabolism, and the *MYNN, WARS2,* and *CAMP* genes, BASP1 and NGF proteins involved in brain function (Fig. 7c2–c4).

Discussion

This study has demonstrated a longitudinal contribution of anxiety and late-life cognitive progression among non-dementia older adults, and proposed axon/synapse degeneration in the context of mitochondrial dysfunction as a potential biological mechanism contributing to this association. Transcriptomic and proteomic analyses linked anxiety to the axon/synapse pathway and mitochondrial energy metabolism pathway. The former pathway was further validated by deviations in frontolimbic tract morphology and altered levels of CSF axon/synapse biomarkers, and the latter pathway was supported by the abnormal blood carnitine metabolites. The connections of the results from various analyses were shown in Fig. 8.

Our clinical findings were based on the ADNI (aged population) and the CLHLS (advanced aged population) cohorts with long-term follow-up, in which the association between anxiety and subsequent cognitive progression remained significant after adjustment for multiple psychiatric symptoms, including depression, hallucination, elation, agitation, disinhibition, irritability, aberrance, and appetite. These findings extend previous studies reporting the vulnerability of anxiety to cognitive progression[5,6,8,36] by revealing a temporal predictive risk of anxiety for cognitive progression. Although anxiety and depressive symptoms are closely related and often co-occur, research has suggested that their effects on cognitive progression in elderly may differ. Depression has been suggested to interfere with neurodegeneration and thus to play a more important role at a later clinical stage of AD.[37] Anxiety tends to occur earlier in the

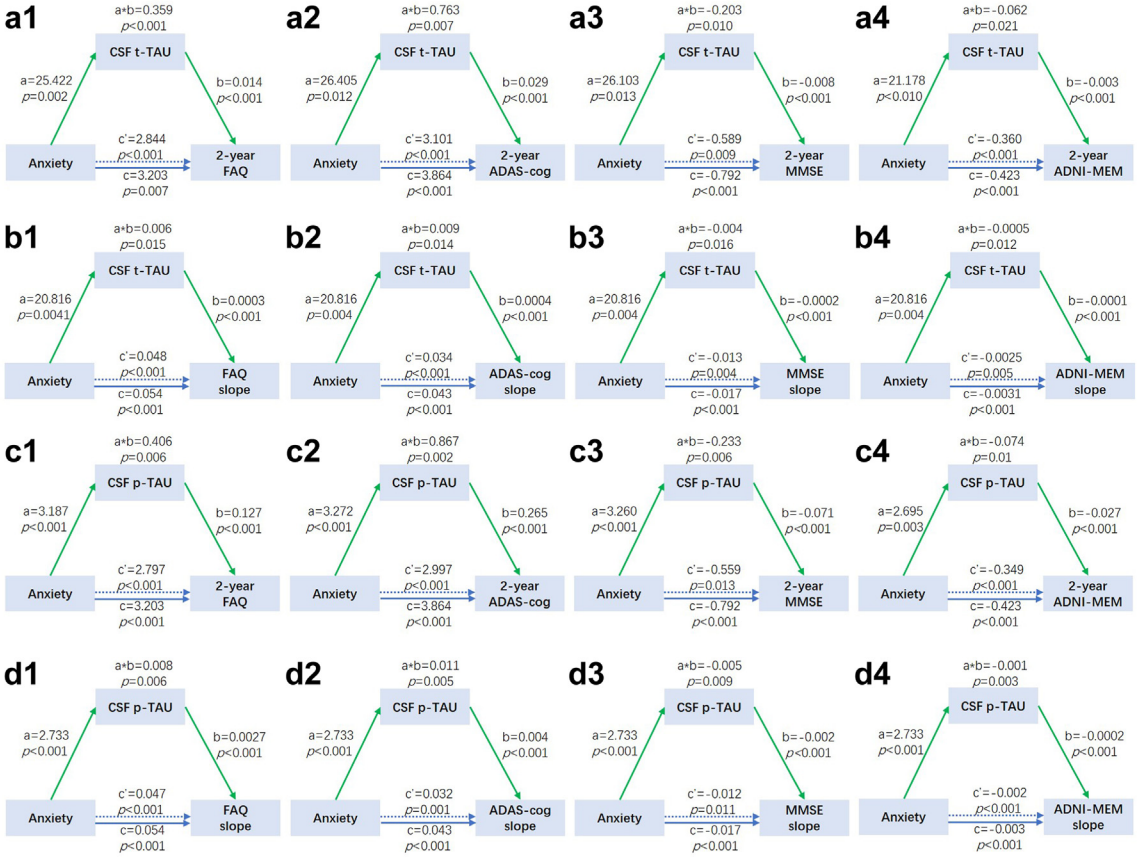

Fig. 6: Mediation analyses about the relationship among anxiety, brain tau burden and longitudinal cognitive function in non-dementia elderly. CSF t-tau and p-tau as statistical mediators. The total effect of anxiety on longitudinal cognitive function, measured with 2-year (n = 796) (a1–a4, c1–c4) and annual change rates (n = 696) (b1–b4, d1–d4) of FAQ, ADAS-cog, MMSE and MEM, is designated as c. The mediated effect of brain tau burden is designated as a*b. The direct effect of the mediators on the cognitive function, is denoted as c'. Models were adjusted for age, sex, education and *APOE*. Statistical significance was set at p value < 0.05 (Mediation analysis).

preclinical phase of AD, even before cognition changes.[37] As anxiety is more widespread than depression in elderly,[9] and commonly used GDS scale often include measures of anxiety,[38] it is difficult to exclude the effects of anxiety in depression-based studies. In contrast, the present study controlled for the effects of multiple psychiatric symptoms, and thus demonstrated the predictive risk of anxiety, independent of depression, for subsequent cognitive progression. In addition, *APOE* ε4, as a risk factor for cognitive progression, showed a significant difference between groups in our study. Previous studies have suggested that the *APOE* ε4 may be more common in individuals with anxiety disorders,[39,40] but further research is needed to confirm this relationship. When controlling for variables including *APOE*, anxiety remained a risk factor for cognitive progression, as supported by the results of linear mixed regression and Cox regression in the CLHLS.

To date, the pathways and pathologies associated with anxiety remain unclear. Our functional enrichment analyses based on transcriptomics and proteomics linked anxiety to the axon/synapse pathways that are an essential cause of cognitive progression. A zebrafish model with a highly anxious phenotype showed an altered pathway related to serotonin neurotransmission,[41] similar to one of the biological processes selected in our analysis. Previous research on CSF biomarkers has reported higher Aβ burden in elderly with anxiety,[10–12] while other studies have found that higher tau burden may be relevant to anxiety.[14] Our analysis revealed higher levels of both Aβ and tau burden in individuals with anxiety, but a significant interaction was observed between anxiety and brain tau burden, rather than Aβ pathology. Similarly, subcortical neurofibrillary tangle accumulation has been found associated with anxiety in patients with dementia.[42] Our mediation analysis showed that anxiety partially (11–33%) conveys its effects on cognitive progression through an indirect effect of brain tau pathology, consistent with animal findings that tau can exacerbate anxiety-related stress

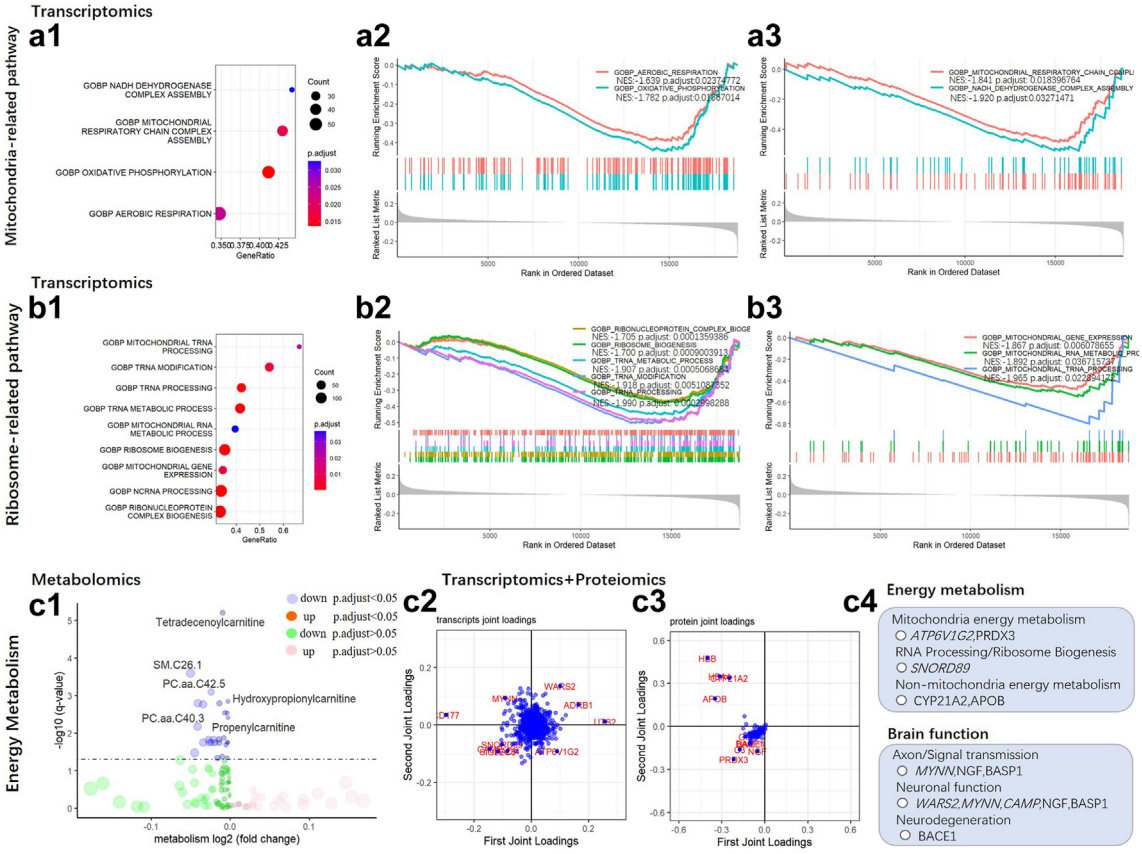

Fig. 7: Energy metabolism imbalance in anxiety through multi-omics approaches. Transcriptomics showed suppressed pathways relevant to mitochondrial respiratory chain complex assembly, aerobic respiration, ATP synthesis coupled electron transport, and others as identified by GO BP analysis (n = 433) (GSEA analysis, adjusted $p < 0.05$) (a1–a3). Transcriptomics revealed suppressed pathways relevant to ribosome biogenesis, mitochondria RNA processing, mitochondria translation, and more (n = 433) (GSEA analysis, adjusted $p < 0.05$) (b1–b3). Metabolomics analysis demonstrated significantly decreased carnitine metabolites such as propenylcarnitine, butyrylcarnitine, isovalerylcarnitine, *etc.* in anxiety using volcano plot (n = 807) (Limma analysis, adjusted $p < 0.05$) (c1). Focusing on the joint analysis of transcriptomics and proteomics, an O2PLS model (n = 89) was built to identify the top ten most influential expressed genes (c2) and proteins (c3), highlighting the underlying pathological pathways (c4).

responses.[43] VILIP1, like t-tau, is a non-specific marker of neuron-axon injury, and the postsynaptic NRGN, is a marker of synaptic damage.[38] NRGN was also a promising biomarker for early diagnosis of AD and was biochemically associated with VILIP1.[44] Consistent with the changes in tau burden, our analysis found that CSF concentrations of VILIP1 and NRGN increased longitudinally in anxious elderly, and both were associated with tau burden and annual changes in cognitive function. These results point to axonal and synaptic dysfunction in anxiety from a neuropathological perspective, similar to the findings that synaptic level loss[43,45] and axon pathfinding defects[46] are associated with anxiety-like behavior in rats and zebrafish. Additionally, disease pathway analysis revealed links between anxiety and psychiatric/cognitive disorder pathways, demonstrating the partially shared pathogenic pathways of anxiety with psychiatric disorders such as schizophrenia and bipolar disorder. The analysis also showed an association between anxiety and neurodegenerative diseases such as HD, PD, and ALS.

Currently, it remains unclear which brain structures are vulnerable to anxiety. Anxiety may have a direct negative effect on the brain through the hypothalamic–pituitary axis.[37] In this study, the DTI technique was adopted to investigate tau-related white matter degeneration, characterised by increased mean water diffusivity as probed by MD, indicating lower axonal packing density. Hyperphosphorylation of tau leads to reduced microtubule binding and deterioration of intra-axonal cytoskeletal integrity.[47] The 8-DTI-metrics model based on LASSO regression was constructed to discriminate anxious from normal elderly, which was highly associated with brain tau burden and cognitive function. The

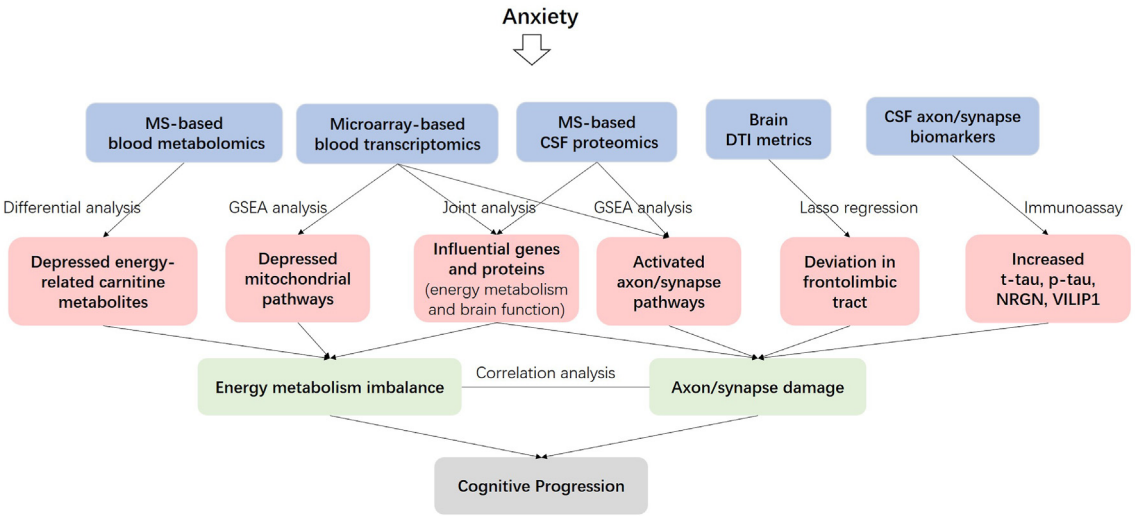

Fig. 8: Evidence from multi-omics studies.

DTI indices were mainly concentrated along the corticolimbic pathway connecting the cingulum and hippocampus (CGH), the orbitofrontal cortex and hippocampus or amygdala (UNC), and the corpus callosum and dorsal lateral cortex of the occipital-temporal lobes (TAP). Abnormalities in these regions have been reported among individuals with anxiety, including changes in the corticolimbic circuit featuring the amygdala and the medial prefrontal cortex (PFC),[48] lower FA in the right frontal region, specifically the UNC,[49] asymmetry in the morphology of bilateral amygdala-ventral PFC tracts,[50] and increased adult hippocampal neurogenesis.[51] Our research also showed that irregular diffusivity in the frontolimbic circuit, which dominantly connects the hippocampus, amygdala, and frontal lobe, plays an important role in anxiety. Meanwhile, the hippocampus and frontal lobe are major regions of neuronal loss and atrophy in neurodegenerative diseases and are closely related to cognitive function.[52] How does anxiety stress affect axon/synapse function? The brain mitochondria are considered a critical mediator of energy homeostasis, adapting to anxiety stress through complex interactions that influence cellular function.[53] The theory of bidirectional interplay between anxiety and mitochondrial function has emerged recently.[54] Our transcriptomic analysis showed that mitochondrial pathways responsible for energy production, and mitochondrial ribosome pathways responsible for mitochondria translation, were both suppressed in anxiety, suggesting impaired mitochondrial biogenesis and energy metabolism. Meanwhile, our metabolomics differential analysis revealed decreased levels of carnitine metabolites in anxiety. Carnitine derivatives are essential for the transport of fatty acids into mitochondria for β oxidation,[55] and decreased levels of carnitine during ageing contribute to the decline in mitochondrial function.[56] Joint analysis of transcriptomics and proteomics identified the most influential genes and proteins, many of which are involved in energy metabolism and brain function. We found that mitochondria-related genes were correlated with axon/synapse proteins, carnitine metabolites, and cognition, supporting the close relationship between mitochondrial energy metabolism and brain function in anxiety in anxiety. Similarly, reduced mitochondrial biogenesis in highly anxious outbred rats[57] and mitochondrial dysfunction in mice model of anxiety-like behavior[58] have been reported. The possible hypothesis is that mitochondrial dysfunction in anxious elderly disrupts axon/synapse energy homeostasis, ultimately leading to cognitive progression.

This study has several limitations. The lack of tau positron emission computed tomography (PET) limited our ability to investigate the regional tau pathology underlying anxiety. The lack of CSF mitochondrial markers prevented us from directly assessing mitochondrial energy metabolism in the brain. Including both cognitively normal and MCI subjects as non-dementia subjects may have influenced the heterogeneity of the population. The small number of detected protein species in the proteomics may limit the averages of our findings. Future studies with larger sample sizes and more comprehensive proteomic analyses are needed. The lack of molecular biological experiments weakens the support for our hypothesis about the pathological processes of the brain under anxiety. Incorporating molecular biological experiments in future studies could directly elucidate the underlying mechanisms. Although we used two large cohorts in our study, there may still be underlying differences between our cohorts and the broader population. Therefore, future multi-center studies with larger sample sizes

would be beneficial to enhance the generalizability of our results.

In summary, our study using comprehensive multi-omics analyses of large longitudinal cohorts, revealed that anxiety increases the risk of subsequent cognitive progression through axon/synapse dysfunction in the context of energy metabolism imbalance in non-dementia elderly. Treating anxiety and targeting mitochondrial dysfunction may be an effective way to prevent dementia.

Contributors

Lin Sun designed and drafted the manuscript. Lin Sun, Qi Qiu, and We Li analyzed multi-omics data. Yang Hu analysed the DTI data. Lin Sun and Zhi Yang has accessed and verified the data, and revised the manuscript. Shifu Xiao supervised the experiment. Lin Sun made the decision to submit the manuscript. All authors approved the final version of the manuscript for submission.

Data sharing statement

ADNI data are available at: https://ida.loni.usc.edu/pages/access/studyData.jsp?project=ADNI. CLHLS data are available at: http://opendata.pku.edu.cn/. SMHC raw data and all R codes can be requested via correspondence email.

Declaration of interests

The authors declare no competing interests. The authors were not precluded from accessing data in the study, and they accept responsibility to submit for publication.

Acknowledgements

Data collection and sharing for the ADNI data section was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Data collection and sharing for the CLHLS data section was organized by Research Center for Healthy Aging and Development of Peking University and National Development Research Institute. This study was supported by the National Natural Science Foundation of China (82271607, 81971682, and 81830059) for data analysis and data collection.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104703.

References

1 Ng KP, Pascoal TA, Mathotaarachchi S, et al. Neuropsychiatric symptoms predict hypometabolism in preclinical Alzheimer disease. *Neurology*. 2017;88(19):1814–1821.
2 da Silva J, Goncalves-Pereira M, Xavier M, Mukaetova-Ladinska EB. Affective disorders and risk of developing dementia: systematic review. *Br J Psychiatry*. 2013;202(3):177–186.
3 Beck A, Burdett M, Lewis H. The association between waiting for psychological therapy and therapy outcomes as measured by the CORE-OM. *Br J Clin Psychol*. 2015;54(2):233–248.
4 Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry*. 2013;202(5):329–335.
5 Kosten TA, Kim JJ, Lee HJ. Early life manipulations alter learning and memory in rats. *Neurosci Biobehav Rev*. 2012;36(9):1985–2006.
6 Lahdepuro A, Savolainen K, Lahti-Pulkkinen M, et al. The impact of early life stress on anxiety symptoms in late adulthood. *Sci Rep*. 2019;9(1):4395.
7 Starkstein SE, Jorge R, Petracca G, Robinson RG. The construct of generalized anxiety disorder in Alzheimer disease. *Am J Geriatr Psychiatry*. 2007;15(1):42–49.
8 Santabarbara J, Lipnicki DM, Villagrasa B, Lobo E, Lopez-Anton R. Anxiety and risk of dementia: systematic review and meta-analysis of prospective cohort studies. *Maturitas*. 2019;119:14–20.
9 Subramanyam AA, Kedare J, Singh OP, Pinto C. Clinical practice guidelines for geriatric anxiety disorders. *Indian J Psychiatry*. 2018;60(Suppl 3):S371–S382.
10 Cai WJ, Tian Y, Ma YH, et al. Associations of anxiety with amyloid, tau, and neurodegeneration in older adults without dementia: a longitudinal study. *J Alzheimers Dis*. 2021;82(1):273–283.
11 Pichet Binette A, Vachon-Presseau E, Morris J, et al. Amyloid and tau pathology associations with personality traits, neuropsychiatric symptoms, and cognitive lifestyle in the preclinical phases of sporadic and autosomal dominant alzheimer's disease. *Biol Psychiatry*. 2021;89(8):776–785.
12 Donovan NJ, Locascio JJ, Marshall GA, et al. Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults. *Am J Psychiatry*. 2018;175(6):530–537.
13 Krell-Roesch J, Lowe VJ, Neureiter J, et al. Depressive and anxiety symptoms and cortical amyloid deposition among cognitively normal elderly persons: the Mayo Clinic Study of Aging. *Int Psychogeriatr*. 2018;30(2):245–251.
14 Ramakers IHGB, Verhey FRJ, Scheltens P, et al. Anxiety is related to Alzheimer cerebrospinal fluid markers in subjects with mild cognitive impairment. *Psychol Med*. 2013;43(5):911–920.
15 Ahmed-Leitao F, Rosenstein D, Marx M, Young S, Korte K, Seedat S. Posttraumatic stress disorder, social anxiety disorder and childhood trauma: differences in hippocampal subfield volume. *Psychiatry Res Neuroimaging*. 2019;284:45–52.
16 Zhang X, Suo X, Yang X, et al. Structural and functional deficits and couplings in the cortico-striato-thalamo-cerebellar circuitry in social anxiety disorder. *Transl Psychiatry*. 2022;12(1):26.
17 Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's disease neuroimaging initiative (ADNI): clinical characterization. *Neurology*. 2010;74(3):201–209.
18 Trojanowski JQ, Vandeerstichele H, Korecka M, et al. Update on the biomarker core of the alzheimer's disease neuroimaging initiative subjects. *Alzheimers Dement*. 2010;6(3):230–238.
19 Bubu OM, Williams ET, Umasabor-Bubu OQ, et al. Interactive associations of neuropsychiatry inventory-questionnaire assessed sleep disturbance and vascular risk on alzheimer's disease stage progression in clinically normal older adults. *Front Aging Neurosci*. 2021;13:763264.
20 Pugh EA, Kemp EC, van Dyck CH, Mecca AP, Sharp ES. Alzheimer's disease neuroimaging I. Effects of normative adjustments to the montreal cognitive assessment. *Am J Geriatr Psychiatry*. 2018;26(12):1258–1267.
21 Sun L, Li W, Li G, Xiao S, Alzheimer's Disease Neuroimaging I. Prefrontal Abeta pathology influencing the pathway from apathy to cognitive decline in non-dementia elderly. *Transl Psychiatry*. 2021;11(1):534.
22 Zeng Y. Towards deeper research and better policy for Healthy aging –Using the unique data of Chinese longitudinal healthy longevity Survey. *China Economic J*. 2012;5(2-3):131–149.
23 Gao M, Kuang W, Qiu P, Wang H, Lv X, Yang M. The time trends of cognitive impairment incidence among older Chinese people in

the community: based on the CLHLS cohorts from 1998 to 2014. *Age Ageing*. 2017;46(5):787–793.
24 Sun L, Guo C, Song Y, Sheng J, Xiao S. Alzheimer's disease neuroimaging I. Blood BMP6 associated with cognitive performance and alzheimer's disease diagnosis: a longitudinal study of elders. *J Alzheimers Dis*. 2022;88(2):641–651.
25 Spellman DS, Wildsmith KR, Honigberg LA, et al. Development and evaluation of a multiplexed mass spectrometry based assay for measuring candidate peptide biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF. *Proteomics Clin Appl*. 2015;9(7-8):715–731.
26 Park YH, Hodges A, Risacher SL, et al. Dysregulated Fc gamma receptor-mediated phagocytosis pathway in Alzheimer's disease: network-based gene expression analysis. *Neurobiol Aging*. 2020;88:24–32.
27 Leek J, Johnson W, Parker H, Jaffe A, Storey JJB. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*. 2012;28(6):882–883.
28 Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102(43):15545–15550.
29 Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. 2016;44(W1):W90–W97.
30 Bouhaddani SE, Houwing-Duistermaat J, Salo P, Perola M, Jongbloed G, Uh HW. Evaluation of O2PLS in Omics data integration. *BMC Bioinformatics*. 2016;17 Suppl 2(Suppl 2):11.
31 Holmes CJ, Hoge R, Collins L, Woods R, Toga AW, Evans AC. Enhancement of MR images using registration for signal averaging. *J Comput Assist Tomo*. 1998;22(2):324–333.
32 Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*. 2002;17(2):825–841.
33 Simon N, Friedman J, Hastie T, Tibshirani R. Regularization paths for cox's proportional hazards model via coordinate descent. *J Stat Softw*. 2011;39(5):1–13.
34 Loureiro JC, Stella F, Pais MV, et al. Cognitive impairment in remitted late-life depression is not associated with Alzheimer's disease-related CSF biomarkers. *J Affect Disord*. 2020;272:409–416.
35 Gonzales MM, Insel PS, Nelson C, et al. Chronic depressive symptomatology and CSF amyloid beta and tau levels in mild cognitive impairment. *Int J Geriatr Psychiatry*. 2018;33(10):1305–1311.
36 Pietrzak RH, Lim YY, Neumeister A, et al. Amyloid-beta, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. *JAMA Psychiatry*. 2015;72(3):284–291.
37 Pink A, Krell-Roesch J, Syrjanen JA, et al. A longitudinal investigation of Abeta, anxiety, depression, and mild cognitive impairment. *Alzheimers Dement*. 2022;18(10):1824–1831.
38 Day GS, Yarbrough MY, Kortvelyessy P, et al. Prospective quantification of CSF biomarkers in antibody-mediated encephalitis. *Neurology*. 2021;96(20):e2546–e2557.
39 Baekken PM, Skorpen F, Stordal E, Zwart JA, Hagen K. Depression and anxiety in relation to catechol-O-methyltransferase Val158Met genotype in the general population: the Nord-Trondelag Health Study (HUNT). *BMC Psychiatry*. 2008;8:48.
40 Lee RS, Hermens DF, Porter MA, Redoblado-Hodge MA. A meta-analysis of cognitive deficits in first-episode Major Depressive Disorder. *J Affect Disord*. 2012;140(2):113–124.
41 Horzmann KA, Lin LF, Taslakjian B, Yuan C, Freeman JL. Anxiety-related behavior and associated brain transcriptome and epigenome alterations in adult female zebrafish exposed to atrazine during embryogenesis. *Chemosphere*. 2022;308(Pt 3):136431.
42 Ehrenberg AJ, Suemoto CK, Franca Resende EP, et al. Neuropathologic correlates of psychiatric symptoms in alzheimer's disease. *J Alzheimers Dis*. 2018;66(1):115–126.
43 Liu H, Yang Z, Yu C, et al. Tau aggravates stress-induced anxiety by inhibiting adult ventral hippocampal neurogenesis in mice. *Cereb Cortex*. 2022;33(7):3853–3865.
44 Dulewicz M, Kulczynska-Przybik A, Borawska R, Slowik A, Mroczko B. Evaluation of synaptic and axonal dysfunction biomarkers in alzheimer's disease and mild cognitive impairment based on CSF and bioinformatic analysis. *Int J Mol Sci*. 2022;23(18):10867.
45 Roy A, Sharma S, Nag TC, Katyal J, Gupta YK, Jain S. Cognitive dysfunction and anxiety resulting from synaptic downscaling, hippocampal atrophy, and ventricular enlargement with intracerebroventricular streptozotocin injection in male wistar rats. *Neurotox Res*. 2022;40(6):2179–2202.
46 Koh A, Tao S, Goh YJ, et al. A Neurexin2aa deficiency results in axon pathfinding defects and increased anxiety in zebrafish. *Hum Mol Genet*. 2021;29(23):3765–3780.
47 Wen Q, Risacher SL, Xie L, et al. Tau-related white-matter alterations along spatially selective pathways. *Neuroimage*. 2021;226:117560.
48 Fox AS, Shackman AJ. The central extended amygdala in fear and anxiety: closing the gap between mechanistic and neuroimaging research. *Neurosci Lett*. 2019;693:58–67.
49 Li H, Lin X, Liu L, et al. Disruption of the structural and functional connectivity of the frontoparietal network underlies symptomatic anxiety in late-life depression. *Neuroimage Clin*. 2020;28:102398.
50 Kim W, Kim MJ. Morphological similarity of amygdala-ventral prefrontal pathways represents trait anxiety in younger and older adults. *Proc Natl Acad Sci U S A*. 2022;119(42):e2205162119.
51 Anacker C, Luna VM, Stevens GS, et al. Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*. 2018;559(7712):98–102.
52 Baazaoui N, Iqbal K. Alzheimer's disease: challenges and a therapeutic opportunity to treat it with a neurotrophic compound. *Biomolecules*. 2022;12(10):1409.
53 Morella IM, Brambilla R, More L. Emerging roles of brain metabolism in cognitive impairment and neuropsychiatric disorders. *Neurosci Biobehav Rev*. 2022;142:104892.
54 Filiou MD, Sandi C. Anxiety and brain mitochondria: a bidirectional crosstalk. *Trends Neurosci*. 2019;42(9):573–588.
55 Naidu GS, Lee IY, Cho OK, Park YH. Conversion of gamma-butyrobetaine to L-carnitine by Achromobacter cycloclast. *J Ind Microbiol Biotechnol*. 2001;26(5):309–315.
56 Liu D, Zeng X, Li L, Ou ZL. Carnitine promotes recovery from oxidative stress and extends lifespan in C. elegans. *Aging (Albany NY)*. 2020;13(1):813–830.
57 Chen F, Sun J, Chen C, et al. Quercetin mitigates methamphetamine-induced anxiety-like behavior through ameliorating mitochondrial dysfunction and neuroinflammation. *Front Mol Neurosci*. 2022;15:829886.
58 Gebara E, Zanoletti O, Ghosal S, et al. Mitofusin-2 in the nucleus accumbens regulates anxiety and depression-like behaviors through mitochondrial and neuronal actions. *Biol Psychiatry*. 2021;89(11):1033–1044.