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Plasma Biosignature and Brain Pathology related to Persistent Cognitive Impairment in Late-Life Depression

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Abstract

Cognitive impairment is highly prevalent among individuals with late-life depression (LLD) and tends to persist even after successful treatment. The biological mechanisms underlying cognitive impairment in LLD are complex and likely involve abnormalities in multiple pathways, or "cascades," reflected in specific biomarkers. Our aim was to evaluate peripheral (blood-based) evidence for biological pathways associated with cognitive impairment in older adults with LLD. To this end, we used a data-driven comprehensive proteomic analysis (multiplex immunoassay including 242 proteins), along with measures of structural brain abnormalities (gray matter atrophy and white matter hyperintensity volume via MRI), and brain amyloid-β (Aβ) deposition (PiB-PET). We analyzed data from 80 older adults with remitted major depression (36 with Mild Cognitive Impairment (LLD+MCI) and 44 with normal cognitive function (LLD+NC)). LLD +MCI was associated with differential expression of 24 proteins ($p < 0.05$ and q-value < 0.30) related mainly to the regulation of immune-inflammatory activity, intracellular signaling, cell survival, and protein and lipid homeostasis. Individuals with LLD+MCI also showed greater white matter hyperintensity burden compared with LLD+NC ($p=0.015$). We observed no differences in gray matter volume or brain Aβ deposition between groups. Machine learning analysis showed that a group of three proteins (Apo AI, IL-12, and stem cell factor) yielded accuracy of 81.3%, sensitivity of 75%, and specificity of 86.4% in discriminating participants with MCI from those with normal cognitive function (with an averaged cross-validation accuracy of 76.3%, sensitivity of 69.4% and specificity of 81.8% with nested cross-validation considering the model selection bias). Cognitive impairment in LLD seems to be related to greater cerebrovascular disease along

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with abnormalities in immune-inflammatory control, cell survival, intracellular signaling, protein and lipid homeostasis, and clotting processes. These results suggest that individuals with LLD and cognitive impairment may be more vulnerable to accelerated brain aging and shed light on possible mediators of their elevated risk for progression to dementia.

Keywords

late-life depression; cognition; biomarkers; neurodegeneration; inflammation

Introduction

Major depression is a common psychiatric disorder in older adults, with one-year prevalence rates ranging from 4% to 12% in developed and developing countries[1,2]. Cognitive impairment is common during a depressive episode in older adults and most commonly involves impairments in information processing speed, executive functioning, and episodic memory[3]. When cognitive impairment co-occurs with depression, it tends to persist after remission of the depressive episode and most of these individuals meet diagnostic criteria for mild cognitive impairment[4-6]. In addition, they are at elevated risk for progression to both Alzheimer's Disease (AD) and Vascular Dementia (VaD)[7].

The search for biomarkers related to cognitive impairment in LLD has included a broad spectrum of characteristics that are objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or responses to a therapeutic intervention[8]. For example, there is substantial evidence that executive dysfunction may be related to increased cerebrovascular disease burden and disruption of pre-frontal cortico-subcortical neurocircuitry[9-11]. In addition, individuals with LLD are characterized by higher rates of whole brain and hippocampal atrophy compared to non-depressed individuals that may be related to deficits in episodic memory[12,13]. Individuals with LLD also may have more brain amyloid-β (Aβ) deposition compared to non-depressed individuals, suggesting that cognitive impairment may reflect the emergence of neurodegenerative changes in LLD[14,15].

Additional studies addressing central and peripheral abnormalities in biomarkers suggest that distinct biological pathways or "cascades", are involved in LLD. Individuals with LLD present with pro-inflammatory status[16] that is negatively associated with cognitive performance[17]. Reduced neurotrophic support occurs in both LLD and neurodegenerative disorders[18] and may be an important factor related to cognitive decline in these individuals[19]. Moreover, individuals with LLD have higher levels of oxidative stress markers and activity of glycogen synthase kinase 3β[20,21].

Although these studies have increased understanding of biomarker abnormalities associated with cognitive impairment in LLD, our current knowledge, nonetheless, is fragmented. Indeed, most studies have measured single biomarkers in isolation and thus their results do not provide an integrated view of related biological and molecular processes. Recently, the development of large biomarker panels analyzed by multiplex technology, permitting simultaneous measurement of most relevant biological pathways, has helped to overcome

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some of the current conceptual and methodological limitations of evaluating multiple biomarkers[22,23].

For example, a study of older individuals with subsyndromal depression from the Alzheimer's Disease Neuroimaging Initiative, using a multiplex immunoassay panel with 190 proteins, found significant differences in biomarkers related to glucose metabolism (e.g., insulin), neurotrophic support (e.g., hepatocyte growth factor) and inflammation (e.g., pulmonary and activation-regulated chemokine) compared with participants with no depressive symptoms[24]. Some of these biomarkers had not been previously described in LLD. In addition, multiplex biomarker analyses have also been conducted in older adults with MCI and AD, aiming to develop diagnostic biomarker panels[25,26]. Similarly, blood-based multiplex biomarker analyses now extend to patients with schizophrenia, mid-life major depression and bipolar disorder, each demonstrating differences in multiple biological pathways[27,28].

The neurobiologic mechanisms of cognitive impairment are very complex and probably involve interplay of changes in brain structure, neuropathology and systemic neurochemical abnormalities. Within this context, the current study sought to evaluate blood-based biomarkers and structural and molecular brain changes related to cognitive impairment in older adults with LLD. We used a data-driven, comprehensive multiplex proteomic analysis, along with measures of structural brain abnormalities (gray matter atrophy), cerebrovascular burden (white matter hyperintensity volume), and brain Aβ deposition [measured via positron emission tomography (PET) using Pittsburgh Compound-B (PiB)[29]]. We also sought to elucidate biological pathways and molecular processes related to these peripheral biomarkers. Although we had no *a priori* hypotheses, given the intentionally data-driven design of the study, we expected to uncover novel circulating peripheral biomarkers related to cognitive impairment and to brain pathological measures (gray matter atrophy, cerebrovascular disease and Aβ deposition). We anticipate that observations from such an approach will inform subsequent confirmatory studies.

Methods

Subject recruitment and cognitive assessment

Eighty older adults age 65 years with remitted LLD were included in this analysis (36 with MCI and 44 with normal cognitive function). All of the participants were enrolled in a research clinic based in the University of Pittsburgh's NIMH-sponsored Advanced Center for Intervention and Services Research for Late-Life Mood Disorders. All had previously met DSM-IV criteria for current unipolar Major Depressive Disorder without psychotic features and were successfully treated to response (i.e., Hamilton Depression Rating of 10 or less for two consecutive weeks) in pharmacotherapy and/or interpersonal psychotherapy intervention trials.

Exclusion criteria encompassed substance abuse within the past year, unstable medical illness (precluding participation in clinical trials for depression), history of psychosis, bipolar disorder, neurologic disorder (including dementia) or significant head trauma (defined as loss of consciousness > 30 minutes). Written informed consent was provided

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prior to entering the neuroimaging study. The study was approved by the University of Pittsburgh Institutional Review Board and the Radioactive Drug Research Committee.

Following successful remission of mood symptoms, participants underwent structural MRI and PiB-PET imaging as detailed below, along with a detailed neuropsychiatric evaluation. The evaluation included the 17-item Hamilton Depression Rating Scale, neurologic examination, the Clinical Dementia Rating, the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), medical history, and medication review. The University of Pittsburgh Alzheimer Disease Research Center (ADRC) comprehensive neuropsychological assessment was performed by trained examiners supervised by a senior neuropsychologist (MAB). The neuropsychological battery included at least two tests per domain, assessing language, visuoconstructional/visuospatial ability, attention/information processing speed, episodic delayed memory, and executive functions (**See** supplementary Table 1 **for the list of tests included in the battery**). Performance on each test in the neuropsychological battery was classified as normal or abnormal (>1.5 SDs below individuals of comparable age and education level) based on normative data collected from the University of Pittsburgh ADRC. For this study, we then created a standardized score for each neuropsychological domain assessed in addition to global score (represented by the mean score) based on the individual tests' Z-score to allow the comparison of the results across distinct cognitive domains.

All relevant clinical, neuropsychological, and MRI data (but not Pib-PET results) were reviewed in a consensus diagnostic adjudication conference by faculty members of the University of Pittsburgh ADRC. Participants were classified as having MCI if they met the following criteria: (1) informant reported a decline in cognitive function on the IQCODE; (2) there were no neurological, psychiatric (except for previous history of major depressive disorder), or systemic illnesses that could explain the presence of cognitive deficits; and (3) there was at least one abnormal neuropsychological test score. MCI diagnoses were further classified according to the modified Petersen criteria[30]. If at least one episodic memory test score was abnormal, participants were classified as "amnestic MCI". If no memory test scores were abnormal, participants were classified as "non-amnestic MCI".

Proteomic analysis

After cognitive assessment, whole blood samples were withdrawn with EDTA tubes by antecubital venous puncture. Plasma samples were separated, aliquoted, and stored at −80 °C. Plasma samples (750μL) were sent to the Myriad RBM® laboratory (Austin, TX, USA) for biomarker measurements. We used the Human DiscoveryMAP® 250+ v2.0 assay, which simultaneously assesses 242 different protein analytes with a multiplex immunoassay panel (rbm.myriad.com). This multiplex assay aims to provide a comprehensive analysis of biomarker abnormalities in individual medical conditions as well as for drug development. We chose this assay due to its *a priori* coverage of biomarkers related to different biological cascades that are of relevance to our studies (e.g., vascular, degenerative, inflammation, trophic factors, adhesion molecules) since they have been previously reported to be altered in several conditions, including cancer, cardiovascular diseases, and metabolic disorders as well as psychiatric and neurodegenerative disorders. Myriad RBM® has attempted to

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validate each of the 242 analytes up to Clinical Laboratory Improvement Amendment (CLIA) standards, but the Human DiscoveryMAP® v2.0 assay is not yet CLIA approved. Each analyte has an individual standard curve with between 6 and 8 reference standards and each plate is run with three levels of quality control measures (low, medium and high dilutions).

Structural neuroimaging

Magnetic Resonance (MR) acquisition—MR scanning was performed at 1.5 Tesla (n=16) or 3.0 Tesla (n=64) using a GE Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI) or a Siemens MAGNETOM Trio 3 Tesla Scanner (Siemens Medical Solutions USA, Malverne, PA), respectively.

The protocol on the 1.5T scanner: 3D structural MR images were acquired at a coronal orientation using 3D Spoiled Gradient Recalled Echo (TR/TE = 5/25 ms; flip angle = 40°; FOV = 24×18cm, 124 slices, slice thickness = 1.5mm, in-plane resolution 0.9375 mm × 0.9375 mm). The T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) was acquired in the axial orientation (TR/TE 9004/172 ms; TI = 2200 ms, 24 slices, in plane resolution 0.78 mm × 0.78 mm); section thickness was 5mm with a 1-mm intersection gap, a 24cm field of view and a 192 × 256 pixel matrix.

The protocol on the 3T scanner: 3D structural MR images were acquired at a sagittal orientation using 3D magnetization-prepared rapid acquisition with gradient echo (MPRAGE). MPRAGE (TR/TE=2300/2.98 ms, 256 slices, slice thickness 1.2 mm, flip angle = 9, in-plane resolution 1 mm × 1 mm). The T2-weighted FLAIR was acquired in the axial orientation (TR=9160 ms, TE=90 ms, TI =2500 ms, 48 slices, in-plane resolution 1mm × 1mm); section thickness was 3mm with no intersection gap, a 24cm field of view and a 192 × 256 pixel matrix.

Analysis of MR Imaging—We used a fully automated method for localizing and quantifying voxels as white matter hyperintensity (WHM) on the FLAIR images, and then converted these values to a volume (1 voxel = 4.2mm3)[31]. Individual regions of white matter changes were summed to create a variable representing total white matter hyperintensity burden for each participant, and then were expressed as the ratio of total white matter hyperintensity volume (WHMV) by total white matter volume. Because at higher field strength there is greater sensitivity for detecting white matter hyperintensities, we calculated a factor to convert the white matter hyperintensity burden measured at 1.5T to the estimated white matter hyperintensity burden at 3.0T (3T = 1.5T*1.4649 – 0.000592, R2 = 0.91, p<0.0001), using data from an independent group of 7 cognitively normal individuals who were scanned on both MR scanners.

Normalized whole brain gray matter volume was calculated as the ratio of gray matter volume over intracranial volume. Gray matter volume and intracranial volume were estimated from the SPGR and MPRAGE images using standard processing streams; total intracranial volume was computed as the volume contained within the ‘inner skull’ using the brain extraction tool (BET) with an advanced option (–A) and gray matter volume was

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estimated using FAST (FMRIB's Automated Segmentation Tool) with a 3-tissue model (Gray, White, and CSF).

Brain Aβ imaging

PiB-PET Acquisition: PiB was synthesized based on published methods developed by our group and details of the PET acquisition can be found in previous reports[32]. Participant preparation included immobilization of the head using a thermoplastic mask to minimize head motion. The PiB-PET data were acquired on a Siemens/CTI ECAT HR+ scanner (with Neuro-insert) in 3D imaging mode (63 parallel planes; axial field-of-view: 15.2 cm). Transmission scanning (10-15 min) was performed in order to correct the PET emission data for photon attenuation, using rotating 68Ge/68Ga rods with electronic windowing to minimize acceptance of scatter and noise. PiB was administered to participants via intravenous injection (>500 Ci/mmole, 14.8 ± 1.6 mCi). All participants were scanned over 40-70 min post-injection interval. PET data were reconstructed using filtered back-projection. Data was corrected for photon attenuation, scatter, and radioactive decay. The final reconstructed PET image resolution was ~ 6 mm (transverse and axial).

Analysis of PiB-PET Images: Co-registration of the PET and MR datasets were accomplished using automated image registration methods. If inter-frame subject motion was evident in the PET data, this motion was first corrected using a more extensive frame-by-frame registration procedure, prior to PET and MR co-registration. The region of interest (ROI) delineation was performed as previously described[33]. Twenty-five ROIs were hand-drawn on the co-registered anatomical MR images using guidelines established within the laboratory that have proven to yield high inter-rater reliability (intra-class correlation coefficient 0.90) (supplementary neuroimaging information). Thirty ROIs were generated on multiple consecutive MR images on which the structure was visualized, and included: anterior cingulate cortex, frontal cortex, lateral temporal cortex, parietal cortex, precuneus and anteroventral striatum. To generate a global PiB-PET measure, an arithmetic mean value (G6PIB) was calculated across these 6 regions. This mean is negatively biased relative to a voxel-weighted mean. Additional ROIs were generated in subcortical white matter to assess nonspecific white matter PiB retention and in cerebellar gray matter to estimate nonspecific grey matter PiB retention (i.e., reference region)[34]. The ROIs were then applied to the co-registered PET images and right and left hemisphere regions were averaged to minimize the impact of noise and motion.

The standardized uptake value (SUV) was determined for each ROI as an average rate of PiB uptake over the 50-70 minute post-injection period, and this value was scaled by the individual's injected dose and body mass. The regional SUV measures were normalized to the cerebellar reference region SUV to generate SUV tissue ratio (SUVR) measures of PiB retention. The SUV and SUVR values were corrected for atrophy-related cerebrospinal fluid dilution (CSF) using a 2-component MR-based correction routinely used in our laboratory[35]. A voxel average CSF correction was computed that varies from 0 to 1 (where 0 is CSF only) and this was applied to each regional or global SUVR value.

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Statistical analysis

Differences in socio-demographic, cognitive and neuroimaging data were evaluated by independent t-tests (continuous variables) or chi-square test (dichotomous variables).

Differential expression analysis—Prior to statistical analysis, we carried out a quality assessment of the protein multiplex assay. Proteins with more than 20% of missing values, due to measurement error and/or more than 60% of measures below the lower detection limit of the assay (as specified by MyriadRBM) were excluded from all analyses. In case an analysate had 59% or fewer measurements below the lower detection limit, we imputed its value using the k-nearest neighbors method. Thus, we excluded 55 of 242 proteins measured in the multiplex assay, leaving 187 proteins in the differential expression analysis (Supplementary Figure 1 shows the distribution of missing values and measures below the lower detection limit of the assay).

A random intercept model (RIM) with variable selection[36] was applied to detect main effects of each comparison: Cognitive status, measures of structural brain abnormalities (gray matter atrophy), cerebrovascular burden (white matter hyperintensity volume), and brain Aβ deposition to detect differentially expressed proteins, while accounting for potential confounding variables at the same time. Sex and age were cofactors in all analyses; and for the gray matter analysis we included the scanner type as an additional cofactor. Linear models were fitted using 0~3 selected cofounding variables combined with the main factor. Variable selection was achieved through Bayesian Information Criterion. The obtained p-values were adjusted by random permutation of sample labels (B=1,000 times), and the false discovery rate (FDR) was controlled by the Benjamini-Hochberg procedure (see additional details of statitistical methods in supplementary statistical material). For the main factors, LLD-MCI was coded as a binary variable and brain Aβ deposition, whole brain gray matter volume, and white matter hyperintensity volume were coded as continuous variables. A liberal FDR cutoff at 0.3 was used to select differentially expressed biomarkers in this exploratory analysis.

Pathway enrichment analysis—We applied pathway enrichment analysis to identify enriched functional annotation of the identified differentially expressed proteins. Two-thousand-eleven pathways were downloaded and parsed from the MsigDB database from GO, KEGG and BIOCARTA. Pathways associated with more than 200 genes were excluded to avoid general terms. The pathway enrichment analysis was applied on the differentially expressed proteins associated with the main factors LLC-MCI, whole brain gray matter volume and white matter hyperintensity volume, respectively. Detailed description of the pathway enrichment analysis is available in the supplementary material.

Adaptive weighted analysis on the common differentially expressed proteins —We carried out an adaptively weighted meta-analysis[37] of p-values to further investigate the differentially expressed proteins across the three main factors (whole brain gray matter volume, white matter hyperintensity volume and brain Aβ deposition). This method searches all combinations of groups with the strongest signal and adjusts for the degrees of freedom in the inference. The meta-analysis results generated a 0-1 weight vector that reveals

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homogeneity and heterogeneity evidence across the three main factors. For example, a differentially expressed marker with (1,1,1) weight means that the marker is differentially expressed in all three factors while a weight vector of (1,0,0) means that the protein is differentially expressed across measures of whole brain gray matter volume but not across measures of white matter hyperintensity volume or brain Aβ deposition.

Predicting LLC-MCI with machine learning technique—We constructed a predictive model with a machine learning method using support vector machines (with linear kernel) to predict the classification of LLD+MCI and LLC+NC. Proteins to be included in the model were selected based on top statistical significance (i.e., small p-value) with the requirement of large effect sizes (>20% log-scale fold change average group expression (log2 scale) difference greater than 0.2)[38]. Prediction models were tested with protein numbers ranging from 2 to 20.

Results

Table 1 shows the socio-demographic, clinical, cognitive and neuroimaging characteristics of the LLD participants, classified by cognitive status. Among LLD+MCI participants, 20 were classified as amnestic MCI and 16 as non-amnestic MCI. Due to the small sample size in each MCI subgroup we analyzed all LLD+MCI as a single group. Participants with MCI had fewer years of education and greater white matter hyperintensity burden compared to those with normal cognitive function.

Twenty-four proteins were significantly associated with cognitive impairment in LLD (p-value <0.05 and q-value <0.3). Specifically, we observed differences in biomarkers related to: (1) inflammatory cascades (e.g., higher levels of CCL13 - MCP-4, CXCL11 - interferon inducible T cell alpha chemoattractant, CCL18 - PARC, and lower levels of interleukin-12-P40); (2) trophic factors (e.g., reduced levels of stem cell factor), and (3) nutrient sensing and insulin signaling cascades (reduced levels of IGFBP3 and IGFBP5). We also found differences in biomarkers related to pathways not previously reported in the literature: (4) lipid transportation (Apo AI, Apo AII), (5) adhesion molecules (MMP9), and (5) clotting processes (tPA, vitamin K dependent protein) (Table 2). Using a more stringent criteria for significance (q-value<0.05) revealed only 3 proteins to be reliably associated with LLD +MCI: Interleukin 12 (p=0.00002, q-value=0.005), stem cell factor/kit ligand (p=0.0001, q-value=0.01), and alpha1-antichymotrypsin (p=0.0005, q-value=0.03).

White matter hyperintensity volume, a proxy pathological marker of cerebrovascular disease, was associated with differential expression of 32 proteins (p-value <0.05 and q-value <0.3) (Supplementary Table 2). These proteins are markers associated with lipid metabolism (e.g., Apo AI, Lpa), clotting process and vascular reactivity (e.g., tPA, EGF, THBS1), immune-inflammatory control (e.g., IL-8, TNFα R2), adhesion molecules (TIMP1), markers of white matter damage (NSE), cell survival and apoptosis (FAS ligand receptor).

Whole brain gray matter volume (lower volume is associated with more atrophy in older adults), a marker of structural damage and the emergence of neurodegenerative changes in

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the central nervous system, was associated with the differential expression of 30 proteins (p-value <0.05 and q-value <0.3). It is worth noting that higher cortisol level was associated with lower gray matter volume. This finding is in agreement with the literature, in which the pathophysiology of depression is related to dysfunction of the HPA-axis which leads to a hypercortisolemic state which in turn may lead to brain atrophy[39]. In addition, higher adhesion molecule expression (e.g., VCAM) was associated with lower gray matter volume, which builds on previous findings in post-mortem brain tissue[40]. We also found protein markers associated with immune-inflammatory activity and angiogenesis control (e.g., YKL-40, IL6Rβ, IL12p40, IP-10), lipid transportation (ApoAI, APOCIII) and cell growth and trophic support (e.g., HGF, ErbB3) (Supplementary Table 3).

Interestingly, we did not find any significant association between brain Aβ burden (using continuous or dicothomous measures) and protein expression in these participants.

Machine learning prediction model for cognitive impairment

For LLD+MCI predicton, the model generating the smallest leave-one-out cross validation error rate (81.3% accuracy, 75% sensitivity and 86.4% specificity) included 3 proteins, with Apo AI, IL-12, and stem cell factor being selected in every cross-validation loop. Since the performance evaluation is optimistically estimated from selection bias, a nested cross-validation was applied to correct for the selection bias from different numbers of proteins[41,42] (Supplementary Figure 2). Here, in addition to calculating the accuracy from each model, we separated the cohorts for the model selection step and iteratively tested the predictive value on left-out samples. Results indicate on average a nested cross-validation of 76.3% overall accuracy (with 69.4% sensitivity and 81.8% specificity) in predicting LLD-MCI vs. LLD-NC. The 3-protein model displayed the highest individual non-corrected prediction accuracy 81.3% (75% sensitivity and 86.4% specificity) (Supplementary Table 4).

Annotated functional pathway analysis

Pathway analysis can provide comprehensive information on the primary biological functions and processes related to the differentially expressed proteins. The main biological processes and molecular functions related to cognitive impairment were the regulation of immune-inflammatory activity, intracellular signaling, cell survival, and protein and lipid homeostasis (Table 3). The main biological processes and molecular functions related to white matter hyperintensity burden were the regulation of clotting processes, cell survival, intracellular signaling, and protein metabolism (Supplementary Table 5). The primary biologic processes and molecular functions related to whole brain gray matter volume was the regulation of immune-inflammatory processes (Supplementary Table 6).

Adapted weighted meta-analysis

The meta-analysis identified 12 proteins as significantly associated with cerebrovascular disease, gray matter atrophy, or brain Aβ deposition (p-value <0.05 and q-value < 0.3) (Table 4). The adaptive weights of the 11 proteins are all (1,1,0), related to differential expression in whole brain gray matter volume and white matter hyperintensity volume, but not in brain Aβ deposition. Annotated functional pathway analysis revealed that the main

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biologic processes and molecular functions common to the brain pathological measures were clotting process, organ morphogenesis, PPARα pathways and intracellular signaling (Supplementary Table 7).

Discussion

This is the first data-driven, comprehensive analysis of peripheral circulating proteins and their related pathways, along with measures of brain pathology associated with cognitive impairment in LLD. This study represents a significant advance in its attempt to provide an integrated view of abnormalities related to cognitive impairment in LLD using multiple biomarker modalities (i.e., structural and molecular brain imaging, and circulating biomarkers). We acknowledge that this integrated perspective is correlative at this point and that subsequent studies, informed by the current results, will need to test for functional integration. We found that cognitive impairment in LLD was associated with increased white matter hyperintensity volume, but no differences in gray matter volume or brain Aβ burden. This is in contrast with what is generally found in individuals with AD or MCI due to AD[43] and suggests that MCI in LLD may be driven by non-AD, vascular related-changes[44]. In addition, we confirmed that peripheral changes in well-known biological cascades, such as inflammation, neurotrophic markers, and the presence of hypercorticosolemia, are related to both cognitive impairment and brain pathology in LLD. It is worth noting that the protein which showed the strongest effect was the cytokine IL-12 P40. The Il-12 P40 has a long-half life and is released by activated macrophages, dendritic cells, and B-cells[45]. It is a master regulator of adaptive type 1, cell-mediated immunity, the critical pathway involved in protection against neoplasia and also has important anti-angiogenic effects[46]. The low levels of this cytokine, as well as significant differences in others inflammatory markers, reinforces the evidence of major dysregulation of the immune-inflammatory control in older adults with a history of major depression and cognitive impairment.

Peripheral biomarkers findings

Besides finding differences in biomarkers that are consistent with the literature, we uncovered abnormalities in other biomarkers and biological pathways related to these clinical and pathological characteristics of LLD, such as apoptosis, abnormal regulation of protein and lipid homeostasis, and clotting processes. Surprisingly, brain Aβ burden was not associated with differential expression of proteins in these participants. A meta-analysis revealed that 11 distinct proteins are commonly associated with cerebrovascular disease and gray matter volume; these proteins mostly relate to the regulation of clotting process, organ morphogenesis, PPARα pathways and intracellular signaling.

The changes we found in several peripheral circulating biomarkers reflect abnormalities in various biological processes, but commonly involve abnormalities in immune-inflammatory control, cell survival, nutrient sensing, intracellular signaling, protein and lipid homeostasis, endothelial function, and clotting processes. These are essential biological processes for the maintenance of systemic and brain health, and abnormalities in these cascades suggest a severe disruption in homeostatic control related to cognitive impairment and brain

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pathological changes in LLD. Such changes, if persistent, could lead to a maladaptive homeostatic state (through an increased allostatic load) and to accelerated aging processes[47-49]. In turn, this sequence of events could increase the risk of developing age-related disorders, such as AD and other dementias, greater clinical comorbidities, and increased risk of death[49]. Longitudinal follow-up of these participants is underway to confirm this hypothesis.

Previous studies have used a similar approach for identifying biomarker panels in individuals with AD and MCI. Ray and colleagues[25] found that 18 proteins could distinguish AD participants from those with other dementias and could predict the risk of AD in MCI participants with overall accuracy > 80%. Not only were some of the proteins identified in Ray's study also found in the present analysis, e.g., Epithelial Growth Factor and angiogenin, but we also found similar biological process, e.g., regulation of immune response and apoptosis. More recently, Hu and colleagues[26], using a similar approach to ours, found 22 proteins that were related to MCI and AD. Likewise, we found similar proteins to be associated with MCI in our study (e.g., Apo AI, Apo H, IL-12 P40, and stem cell factor). Altogether, these findings suggest that cognitive impairment may represent a final common pathway of abnormalities in several biological cascades, in particular those related to immune/inflammatory response, neurotrophic support, and lipid metabolism, which occur in many neuropsychiatric disorders and may not be specific to any one disorder.

The present study demonstrates the feasibility of a targeted proteomic approach to simultaneously evaluate large panels of peripheral biomarkers in LLD. It is worth noting that we found some of the same proteins that were differentially expressed in older adults with subsyndromal depression in the Alzheimer's Disease Neuroimaging Initiative cohort, in particular CXCL18/PARC and CXCL11/ITAC[24]. Given the large number of biomarkers covered by assays, our findings suggest the involvement of multiple biological processes and pathways in the pathophysiology of cognitive impairment and related brain pathologic changes in LLD. In addition, our findings suggest that LLD is a highly heterogeneous condition, in which changes in different biological cascades may each contribute to its pathophysiology.

Neuroimaging findings

Our finding of higher cerebrovascular related biomarkers in participants with LLD+MCI is in line with the literature[51]. Our data suggest that white matter hyperintensity volume (a measure of cerebrovascular burden) is associated with abnormalities in multiple biological pathways, mainly endothelial function, platelet activation and vascular reactivity, immune-inflammatory control, lipid and protein homeostasis. Such abnormalities may indicate the occurance or an increased risk of developing vascular-related clinical and cognitive disorders, including cardiovascular diseases and VaD, which are commonly observed outcomes in LLD[7].

We did not find a significant difference in total gray matter volume in participants with LLD +MCI. This is in contrast to previous studies in the literature[52,53]. Nonetheless, our findings are in agreement with a study that evaluated both gray matter volume and white matter hyperintensities and showed that cognitive impairment in LLD was associated with the

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severity of white matter hyperintensities and not with whole brain gray matter atrophy[54]. Finally, the peripheral biomarkers that were associated with changes in total gray matter volume were mainly related to abnormalities in immune-inflammatory control. Altogether, these findings highlight the relevance of vascular-related abnormalities to neurodegenerative disorders (e.g., AD) and may contribute to the increased risk for this disorder in individuals with LLD.

We did not find a significant difference in the cerebral amyloid burden in LLD+MCI participants. This is in agreement with neuropathological studies that have not found a significant increase in Aβ plaques in older adults with depression[54]. On the other hand, this finding is in contrast to previous studies with older adults with LLD[14,15] and non-depressed subjects with MCI that showed increased cerebral Aβ burden[55]. These discrepant findings sugest that the neurobiological mechanisms of cognitive impairment in LLD are highly heterogeneous and probably do not involve overt abnormalities in brain Aβ metabolism. In addition, we can hypothesize that the increased risk of AD in LLD may not be due to significant abnormalities in Aβ metabolism in the brain, but due to reduced brain reserve secondary to neurobiologic abnormalities that take place in older adults with depression. This in turn may render the brain more vulnerable to the downstream toxic effect of Aβ, leading to the emergence of dementia after a depressive episode in older adults. Longitudinal follow-up of these participants is underway to confirm this hypothesis.

Limitations

The present results should be viewed in light of several limitations. Because the LLD sample size is relatively small and we conducted a large number of analyses related to peripheral biomarkers, the risk of both false positive and false negative results should be noted. As an exploratory analysis, we used a liberal FDR rate (q-value < 0.3), which might have yielded a significant number of false positive results. Although we used a well-validated machine learning technique (leave-one-out cross-validation) to evaluate the pool of biomarkers that best differentiates LLD+MCI from LLD+NC and we also took the model selection bias into consideration by calculating an averaged performance using nested cross validation, our results should be replicated in independent samples. Furthermore, the annotated functional pathway analysis relies on databases that, despite providing comprehensive coverage of known biological processes and molecular functions, are under continuous updating as novel biological processes and molecular functions of proteins are identified and reported. The results of annotated functional analysis represent the current state of the knowledge related to the proteins differentially expressed, but may change as novel knowledge emerges. In addition, the proteins were measured in the plasma and it is not clear to what extent the changes observed in the periphery reflect central nervous system biological changes. Although the work has revealed very informative proteomic information related to cognitive function in LLD, we cannot make statements specific to depression in older adults as we did not have a non-depressed comparison group. The time elapsed between depressive episode and neuropsychological assessment is relatively large and may have influenced the final results. However, these results are consistent with previous studies that showed that cognitive impairment following remissison is persistent and may endure for up to 4 years[4,6]. Since this is an exploratory study, the present observations need to be replicated in other

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independent samples, preferably including non-depressed elderly control subjects. Finally, although the current findings broaden our view of the neurobiological abnormalities related to cognitive impairment in LLD, their implications for prevention and treatment are not clear at this time. Intervention studies should address the extent to which these abnormalities represent permanent “damage” or may be partly or completely reversible.

Conclusion

The present study provides a more comprehensive and integrated view than previous studies, of the neurobiological changes related to cognitive impairment in LLD. We found that cognitive impairment in LLD is associated with markers of cerebrovascular disease and abnormalities in multiple biological cascades. Better understanding of the neurobiology of cognitive impairment in LLD can provide novel targets for the development of more specific interventions not only for its prevention and treatment, but also for its down-stream negative outcomes, including the development of dementia and related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical, cognitive and neuroimaging data

	Diagnosis				
	No Cognitive Disorder	Mild Cognitive Impairment	Statistics	df	p-value
Clinical characteristics					
Gender (W / M)	38 / 6	24 / 12	$\chi^2 = 4.40$	1	0.036
Age (years)	72.4 ± 6.0	73.4 ± 6.0	t = 0.69	78	0.49
Education (years)	14.9 ± 2.6	13.5 ± 2.8	t = 2.32	78	0.023
HDRS-17	4.4 ± 3.0	5.2 ± 3.4	t = 1,16	78	0.25
Time Since Remission (days)	755 [160-2117]	401 [57-1270]	Z = 1.6	78	0.9
Cognitive performance					
Language (z-score)	0.2 ± 0.6	−0.3 ± 0.6	t = 3.71	78	<0.001
Visuospatial (z-score)	−0.3 ± 0.7	−0.7 ± 0.6	t = 2.60	78	0.009
Attention/Speed (z-score)	0.1 ± 0.7	−0.6 ± 1.1	t = 3.50	78	0.001
Memory (z-score)	0.6 ± 0.6	−0.4 ± 0.7	t = 6.30	78	<0.001
Executive (z-score)	0.2 ± 0.9	−0.3 ± 0.6	t = 2.54	78	0.013
Global (z-score)	0.1 ± 0.5	−0.4 ± 0.4	t = 5.42	78	<0.001
Neuroimaging data					
White Matter Hyperintensity Volume	0.006 ± .0078	0.0201 ± 0.0372	t = 2.49	78	0.015
Whole Brain gray matter volume	0.32 ± 0.04	0.36 ± 0.05	t = 1.45	78	0.15
PiB Continuous (Global 6)	1.61 ± 0.43	1.59 ± 0.40	t = 0.15	78	0.87
PiB (%positive)	23%	26%	χ^2=0.10	1	0.4

HDRS-17: Hamilton Depression Rating scale – 17 items; PIB: Pittsburgh Compound-B.

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Table 2

Proteins related to Mild Cognitive Impairment in LLD subjects

protein	Disease effect	p-value (permuted)	q-value (permuted)
Interleukin 12 P40 (IL-12 P40)	−0.323	0.00002	0.005
Stem Cell Factor (SCF)	−0.353	0.0001	0.01
Alpha-1 Antichymotrypsin (AACT)	0.340	0.0005	0.03
Apolipoprotein AI (ApoAI)	−0.354	0.001	0.07
Apolipoprotein AII (ApoAII)	−0.333	0.002	0.07
Insulin like Growth Factor Binding Protein 5 (IGFBP-5)	−0.322	0.002	0.07
Tissue type Plasminogen activator (tPA)	0.313	0.002	0.07
Mesothelin (MSLN)	−0.475	0.003	0.08
Angiogenin	0.260	0.004	0.08
Transthyretin (TTR)	−0.278	0.006	0.11
Apolipoprotein C I (ApoCI)	−0.216	0.01	0.17
Apolipoprotein A IV (ApoAIV)	−0.629	0.01	0.20
Pulmonary and Activation Regulated Chemokine (PARC)	0.295	0.01	0.20
Interferon inducible T cell alpha chemoattractant (ITAC)	0.581	0.01	0.20
Aldose Reductase	−0.225	0.01	0.21
Epidermal Growth Factor Receptor (EGFR)	0.232	0.01	0.22
Vitamin K Dependent ProteinS (VKDPS)	−0.128	0.02	0.23
Insulin like Growth Factor Binding Protein 3 (IGFBP-3)	−0.478	0.02	0.25
Growth Regulated alpha protein (GROalpha)	0.500	0.02	0.26
Apolipoprotein H (ApoH)	−0.178	0.02	0.27
Human Epidermal Growth Factor Receptor 2 (HER2)	0.160	0.03	0.27
Matrix Metalloproteinase 9 (MMP-9)	0.326	0.03	0.27
Monocyte Chemotactic Protein 4 (MCP-4)	0.305	0.03	0.27
Thrombospondin-1	−0.480	0.03	0.27

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Table 3

Biological processes and molecular functions related to mild cognitive impairment in LLD (q-value < 0.1).

Biological pathways and molecular processes	match gene	p-value (permuted)	q-value (permute)
GO MF ENZYME ACTIVATOR ACTIVITY	APOA1 /APOA2 /APOA4 /IGFBP3 /CXCL1 /	0.001	0.027
GO BP LIPOPROTEIN METABOLIC PROCESS	APOA1 /APOA2 /APOA4 /	0.002	0.027
GO BP REGULATION OF HYDROLASE ACTIVITY	APOA2 /ANG /EGFR /	0.002	0.027
GO BP REGULATION OF PHOSPHORYLATION	ANG /EGFR /IGFBP3 /	0.004	0.040
GO BP LIPID HOMEOSTASIS	APOA1 /APOA2 /APOA4 /	0.008	0.044
GO MF PROTEIN HETERODIMERIZATION ACTIVITY	IL12B /APOA2 /EGFR /ERBB2 /	0.008	0.044
GO MF PROTEIN DIMERIZATION ACTIVITY	IL12B /APOA2 /APOA4 /EGFR /ERBB2 /	0.008	0.044
GO BP REGULATION OF CELLULAR PROTEIN METABOLIC PROCESS	IL12B /APOA2 /EGFR /IGFBP3 /	0.010	0.044
GO BP ESTABLISHMENT OF PROTEIN LOCALIZATION	APOA1 /APOA2 /ANG /EGFR /	0.010	0.044
KEGG BLADDER CANCER	EGFR /ERBB2 /MMP9 /THBS1 /	0.016	0.053
GO BP REGULATION OF PROTEIN SECRETION	APOA1 /APOA2 /ANG /	0.020	0.053
GO BP LIPID TRANSPORT	APOA1 /APOA2 /APOA4 /	0.019	0.053
GO BP REGULATION OF SECRETION	APOA1 /APOA2 /ANG /	0.020	0.053
GO BP PHOSPHOLIPASE C ACTIVATION	ANG /EGFR /	0.019	0.053
GO BP POSITIVE REGULATION OF TRANSFERASE ACTIVITY	ANG /EGFR /ERBB2 /	0.017	0.053
GO BP IMMUNE EFFECTOR PROCESS	IL12B /APOA1 /APOA2 /	0.027	0.054
GO BP POSITIVE REGULATION OF EPITHELIAL CELL PROLIFERATION	EGFR /ERBB2 /	0.025	0.054
GO BP NEGATIVE REGULATION OF SECRETION	APOA1 /APOA2 /	0.025	0.054
GO BP REGULATION OF PROTEIN METABOLIC PROCESS	IL12B /APOA2 /EGFR /IGFBP3 /	0.027	0.054
GO BP REGULATION OF PROTEIN STABILITY	APOA1 /APOA2 /	0.025	0.054
GO BP PROTEIN SECRETION	APOA1 /APOA2 /ANG /	0.032	0.058
GO MF COPPER ION BINDING	ANG /APOA4 /	0.032	0.058
REACTOME CHYLOMICRON MEDIATED LIPID TRANSPORT	APOA1 /APOA2 /APOA4 /	0.034	0.059
REACTOME LIPOPROTEIN METABOLISM	APOA1 /APOA2 /APOA4 /	0.051	0.082
GO CC ENDOCYTIC VESICLE	APOA1 /EGFR /	0.051	0.082
GO BP CYTOKINE PRODUCTION	IL12B /APOA1 /APOA2 /	0.064	0.098

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Table 4

Meta-analysis of protein related to white matter hyperintensities, whole brain gray matter atrophy and brain amyloid deposition

	p-value (permuted)	q-value (permute)	white matter hyperintensities	whole brain gray matter atropy	Brain amyloid deposition
Apolipoprotein A I (ApoA I)	0.00053	0.09	1	1	0
Macrophage Colony Stimulating Factor 1 (M-CSF)	0.00291	0.2	1	1	0
Neutrophil Gelatinase Associated Lipocalin (NGAL)	0.00393	0.2	1	1	0
YKL 40	0.00423	0.2	1	1	0
Vitronectin	0.01031	0.24	1	1	0
Glutathione S Transferase alpha (GST alpha)	0.01813	0.26	1	1	0
Myeloperoxidase (MPO)	0.01711	0.26	1	1	0
N terminal prohormone of brain natriuretic peptide (NT pro-BNP)	0.01983	0.26	1	1	0
Receptor tyrosine protein kinase erbB 3 (ErbB3)	0.01439	0.26	1	1	0
FASLG Receptor (FAS)	0.02964	0.27	1	1	0
Luteinizing Hormone (LH)	0.0257	0.27	1	1	0
Neuropilin 1	0.03007	0.27	1	1	0