



# Mild cognitive impairment and pre-frailty show distinct plasma multi-omic signatures: a cross-sectional study

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**Abstract** Frailty and cognitive impairments frequently co-occur in older adults. It remains unclear whether this overlap reflects shared underlying biological mechanisms or parallel but distinct disease pathways. We hypothesize that physical weakness and cognitive impairment share circulating markers already in an early disease stage. We therefore recruited and analyzed a relatively healthy population with partially overlapping pre-frailty and mild cognitive impairment (MCI), and applied a multi-omics approach to identify shared and distinct molecular signatures in plasma. Community-dwelling older adults were recruited, and frailty status was determined using the Fried frailty criteria. Cognitive function was assessed in all subjects using the Montreal Cognitive

Assessment (MoCA) and MemTrax tests. Blood samples were collected in the fasted state, and plasma was used for multi-omics analyses, including proteomic profiling using the SomaLogic 11 k platform, Nightingale metabolomics, and liquid chromatography–tandem mass spectrometry (LC–MS/MS). A total of 50 community-dwelling older adults ( $79.9 \pm 0.5$  years) were recruited, comprising 29 fit individuals (14 females, 15 males) and 21 pre-frail individuals (9 females, 12 males). Pre-frail individuals scored significantly lower on the MoCA test ( $-12\%$ ,  $P=0.006$ ) and MemTrax accuracy ( $-4\%$ ,  $P=0.03$ ), indicating worse cognitive performance. Proteomics analysis identified 21 biomarkers associated with MCI, including APP, ACHE, and ADGRB1, and 24 biomarkers associated with pre-frailty, including FABP2, KNG1, and MTMR14. Contrary to our hypothesis, no biomarkers were shared between the pre-frailty and

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MCI proteomic signatures. Metabolomics analyses identified two biomarkers associated with pre-frailty and ten associated with MCI, again with no overlap between the two conditions. LC–MS/MS identified C18:0 acylcarnitine as a biomarker for pre-frailty, but not MCI. Despite consistent differences in cognitive performance between fit and pre-frail individuals, pre-frailty and MCI were characterized by distinct proteomic and metabolomic signatures, with no detectable molecular overlap. These findings suggest that the frequently observed clinical co-occurrence of frailty and cognitive impairment arises from parallel but biologically distinct disease processes, at least in early disease stages. If confirmed in a larger cohort, this suggests that both conditions will benefit from disease-specific treatments.

Trial registration: The FITAAL study was registered in the Dutch Trial Register, with registration code NTR6124.

**Keywords** Frailty · Pre-frailty · Mild cognitive impairment · Aging · Multi-omics · Proteomics · Metabolomics · Cognitive performance

## Background

Frailty and cognitive impairment are two significant geriatric syndromes affecting the growing population of older adults [1, 2], and these impairments often co-exist [3]. Both conditions are associated with adverse health outcomes, including functional disability, falls, hospitalization, and mortality [4, 5]. Individuals with cognitive frailty (co-occurrence of frailty and cognitive impairment [6]) face an even higher risk of adverse outcomes [5, 7, 8]. Despite this well documented clinical link, the biological nature of the relationship between frailty and cognitive impairment remains poorly understood [6, 9]. Some overlapping processes have been described, including chronic low-grade inflammation (e.g., IL-6 & TNF- $\alpha$ ), endocrine dysregulation (e.g., IGF-1 & cortisol), metabolic glycaemic and lipid dysregulation (e.g., HbA1c, acylcarnitine and cholesterol composition) and endothelial dysfunction (e.g., ICAM-1 & fibrinogen) [9, 10]. However, studies that have characterized these molecular markers associated with both conditions within the same cohort are lacking.

A major challenge in establishing a relationship between frailty and cognitive impairment stems from the confounding influence of multimorbidity in advanced disease stages. Older adults with established frailty and cognitive decline often present with multiple co-existing chronic conditions [11, 12]. This can mask molecular signals specific to the frailty-cognition relationship.

We hypothesize that early physical weakness and cognitive impairment share circulating markers. To test this hypothesis, we analyzed markers in circulation in a cohort of relatively healthy older adults, approximately half of whom presented with pre-frailty and/or mild cognitive impairment (MCI). By focusing on this population, we aim to minimize the confounding effects of advanced, multi-system disease.

## Methods

### Study design and population

For this study, data from the fit and pre-frail old subjects of the Fitaal study were used. The Fitaal study is a cross-sectional study which was conducted between November 2016 and August 2017 in the Northern part of the Netherlands and has been described in detail elsewhere [13, 14]. This study was approved by the Wageningen University Medical Ethical Committee (NL58289.081.16) and conducted according to Good Clinical Practice guidelines and has been registered in the Dutch Trial Register (NTR6124).

Exclusion criteria included cardiac failure, chronic obstructive pulmonary disease, anemia, cancer, neuromuscular disorders, dementia, contraindications for muscle biopsy, major medical or surgical events within three months prior to study initiation, current participation in another clinical study, use of carnitine supplements, or treatment with medications known to affect metabolism (e.g., corticosteroids or fibrates). After inclusion in the study, older subjects ( $\geq 75$  years) were screened for frailty using the Fried criteria (e.g. weight loss, exhaustion, low physical activity, slowness and weakness) [4]. Fit older subjects were without frailty criteria, while pre-frail older subjects presented one or two frailty criteria. Subjects with more than two frailty criteria were categorized as frail and excluded from the

current analysis. A questionnaire was administered with questions about demographic factors, such as age, years of education (starting at primary school), marital status, medical history, smoking and alcohol consumption. Body composition was measured using a DEXA scan (Hologic Discovery-A, Hologic Inc., Bedford, USA). Cognitive function was assessed and blood samples were collected as well, as explained in more detail below.

### Cognitive function measurements

The MoCA and MTX (MemTrax) tests were administered in random order as described previously. Permission was obtained from the MoCA Institute & Clinique (Quebec, Canada) to use the MoCA for this study. The MoCA assesses seven cognitive functions: execution; naming; attention; language; abstraction; memory/delayed recall; and orientation. The MoCA score is the sum of performance on each cognitive domain and has a maximum of 30 points [15]. MCI was defined as a MoCA score < 24, based on a recent meta-analysis showing that this threshold provides an optimal balance between sensitivity and specificity [16]. The official test instructions were used as a guideline during the administration of the tests. Next to the MoCA, also the computerized MTX test was administered. As a courtesy of MemTrax, LLC (Redwood City, CA, USA), free full versions of the MTX test were provided. In this test, a series of 50 images is shown for up to three seconds each and subjects were instructed to react to repeated images (25 out of the 50 images) as quickly as possible by pressing the spacebar [17]. The MTX test has two outcome measures; the percentage of correct responses (MTXcorrect) and the average reaction time in seconds for repeated images (MTXreaction time), which reflects the time needed to press the spacebar when recognizing a repeated image. The test history of all individual MTX scores and their validity was automatically saved online. The validity of all performed tests was checked, requiring 5 or fewer false positive responses, 10 or more correct recognitions, and average recognition time between 0.4 and 2 s, and only valid tests were included in the analysis. Before the actual MTX test was administered, the

test was explained in detail and a practice test was provided to the subjects.

### Blood sampling and analyses

Venous blood samples were collected in K<sub>2</sub>EDTA-tubes after an overnight fast. Aliquots of plasma were made and stored at −80 °C until further analyses.

Plasma proteomic profiling was performed using the SomaScan 11 K assay (SomaLogic Inc., Boulder, CO, USA), an aptamer-based platform that quantifies approximately 11,000 proteins. EDTA plasma samples were analyzed according to the manufacturer's standard protocols. Protein abundance was reported as relative fluorescence units (RFUs). Quality control and normalization were performed by SomaLogic using their standard procedures. Normalized RFU values were used for statistical analysis.

Plasma samples were measured using the standardized, targeted high-throughput proton NMR (<sup>1</sup>H NMR) metabolomics from Nightingale Health (Nightingale Health Ltd., Helsinki, Finland, [nightingalehealth.com/biomarkers](https://nightingalehealth.com/biomarkers)). This platform provides simultaneous quantification of 162 individual metabolites and 87 metabolite ratios or sizes. All 249 parameters were used in subsequent statistical analyses.

Plasma levels of 26 acylcarnitine derivatives (list available as supplementary file 1) were quantified using targeted liquid chromatography-tandem mass spectrometry profiling (UltiMate3000 RSLC, Thermo Scientific, Sunnyvale, CA, USA; QTRAP 5500, AB-Sciex, Framingham, MA, USA) and MassChrom Amino Acids and Acylcarnitines kit (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany). Quantification was performed using stable isotope-labeled internal standards.

### Statistical analysis

All omics data were log-transformed prior to analysis to approximate normal distributions. Differential expression analyses were performed in R (version 2024.12.1) using the *limma* package. Linear models were fitted to compare (i) pre-frail versus fit participants and (ii) cognitively normal versus MCI participants within the same cohort. As frailty and cognitive status were not

mutually exclusive, individuals could contribute to both comparisons. For the SomaScan 11 K dataset, statistical significance was defined as  $-\log_{10}(p\text{-value}) > 2$ . For Nightingale metabolomics and LC–MS/MS datasets, statistical significance was defined as  $-\log_{10}(p\text{-value}) > 1.3$ . In case of continuous data, comparisons between two independent groups for clinical characteristics (e.g., age, BMI) were conducted using independent-samples *t*-tests in SPSS (version 30.0, IBM Corp., Armonk, NY, USA). In case of categorical data, comparisons between two independent groups for lifestyle behavior (e.g., smoking/alcohol intake) were conducted using a Chi-square test. Data visualization and graphical representations were generated using GraphPad Prism (version 10.5, GraphPad Software, San Diego, CA, USA). Values are displayed as average  $\pm$  SEM. Two-sided *P*-values were used and if lower than 0.05 were considered significant.

## Results

### Population characteristics

Age, BMI and body composition did not significantly differ between fit and pre-frail groups, or between mild cognitively impaired and not impaired groups. In addition, marital status, living conditions, education level, smoking status and alcohol intake did not differ between the groups. In contrast, the fit groups performed better in tests related to physical function, such as grip strength, 400 m walk test, time to perform five chairstands and total SPPB score ( $P < 0.05$ , Table 1).

### Pre-frailty is associated with lower global cognition and memory accuracy

Pre-frail participants had significantly lower MoCA scores compared to fit participants ( $P < 0.01$ , Fig. 1A). Memory accuracy on the Memtrax task was also significantly lower in the pre-frail compared to the fit group ( $P < 0.05$ , Fig. 1B). Reaction time did not differ significantly between groups, although a trend toward slower responses in the pre-frail group was observed ( $P = 0.09$ , Fig. 1C). The distribution of participants across frailty and cognitive status categories is provided as Supplementary File 2.

### Plasma proteomics signatures of pre-frailty and MCI are distinct

Twenty-four differentially expressed plasma proteins in pre-frail vs. fit older adults were identified (Fig. 2A), of which none were overlapping (Fig. 2B) with the 21 differentially expressed proteins in MCI versus cognitively normal older adults (Fig. 2C). Top differentially expressed proteins contained, fatty acid-binding protein 2 (FABP2), kininogen-1 (KNG1), and myotubularin-related protein 14 (MTMR14), which were significantly altered across pre-frail vs. fit groups (all  $P < 0.01$ ), but not in MCI vs. cognitively normal older adults (Fig. 2D). Similarly, amyloid beta precursor protein (APP), acetylcholinesterase (ACHE), and brain-specific angiogenesis inhibitor 1 (ADGRB1), which were significantly differently expressed in MCI vs. cognitively normal older adults (all  $P < 0.01$ ), but not in pre-frail vs. fit older adults (Fig. 2E). In support of these observations, principal component analyses demonstrated no separation of pre-frail vs. fit older adults based on the 21 MCI biomarkers (Fig. 2F), as well as no separation of the MCI vs. cognitively normal older adults based on the 24 pre-frailty biomarkers (Fig. 2G).

### Plasma metabolomics signatures of pre-frailty and MCI are distinct

One metabolite in pre-frail vs. fit older adults was differentially expressed (Fig. 3A), which was not shared (Fig. 3B) with the 10 metabolites significantly differentially expressed in MCI vs. cognitively normal older adults (Fig. 3C). In pre-frail vs. fit groups the phospholipids to total lipids ratio in very small VLDL particles was significantly different ( $P < 0.05$ ), but not in MCI vs. cognitively normal older adults (Fig. 3D). The ratio of apolipoprotein B to apolipoprotein A1 differed between MCI vs. cognitively normal groups, as did VLDL cholesterol and cholesteryl esters in VLDL ( $P < 0.05$ ), but not in pre-frail vs. fit groups (Fig. 3E).

### One acylcarnitine was differentially expressed in pre-frail vs. Fit older adults

One of the examined plasma carnitine derivatives was significantly altered in pre-frail versus fit older adults, while no significant differences were detected between MCI and cognitively normal older

**Table 1** Participant characteristics stratified by pre-frailty status (fit vs. pre-frail) and cognitive status (not impaired vs. impaired). Values are presented as mean  $\pm$  SEM. The presence

of different letters (a–b) across a row indicates significant differences among the two respective groups

| Parameter                      | Pre-frailty status           |                               | Cognitive status              |                           |
|--------------------------------|------------------------------|-------------------------------|-------------------------------|---------------------------|
|                                | Fit ( <i>n</i> = 29)         | Pre-frail ( <i>n</i> = 21)    | Not impaired ( <i>n</i> = 14) | Impaired ( <i>n</i> = 36) |
| Age                            | 79.4 $\pm$ 0.5               | 80.5 $\pm$ 0.8                | 79.2 $\pm$ 0.7                | 80.1 $\pm$ 0.6            |
| BMI                            | 25.5 $\pm$ 0.6               | 27.1 $\pm$ 0.8                | 25.7 $\pm$ 0.7                | 26.3 $\pm$ 0.7            |
| Body fat (%)                   | 28.9 $\pm$ 1.3               | 30.8 $\pm$ 1.3                | 30.7 $\pm$ 1.9                | 29.3 $\pm$ 1.0            |
| Lean mass (%)                  | 67.7 $\pm$ 1.2               | 66.1 $\pm$ 1.2                | 66.3 $\pm$ 1.8                | 67.3 $\pm$ 1.0            |
| BMC (%)                        | 3.2 $\pm$ 0.1                | 3.0 $\pm$ 0.1                 | 2.9 $\pm$ 0.1                 | 3.2 $\pm$ 0.1             |
| Handgrip strength (kg)         | 31.4 $\pm$ 1.6 <sup>a</sup>  | 24.0 $\pm$ 1.4 <sup>b</sup>   | 26.5 $\pm$ 2.1                | 29.0 $\pm$ 1.4            |
| 400 m walk test (s)            | 319.5 $\pm$ 5.5 <sup>a</sup> | 355.6 $\pm$ 12.8 <sup>b</sup> | 324.4 $\pm$ 11.1              | 338.7 $\pm$ 8.2           |
| Time five stands (s)           | 12.7 $\pm$ 0.6 <sup>a</sup>  | 15.2 $\pm$ 1.1 <sup>b</sup>   | 13.3 $\pm$ 0.9                | 13.9 $\pm$ 0.7            |
| SPPB (points)                  | 10.2 $\pm$ 0.3 <sup>a</sup>  | 9.0 $\pm$ 0.5 <sup>b</sup>    | 10.3 $\pm$ 0.5                | 9.5 $\pm$ 0.3             |
| Marital status, <i>n</i> (%)   |                              |                               |                               |                           |
| Married                        | 18 (62)                      | 11 (52)                       | 8 (57)                        | 21 (58)                   |
| Widowed                        | 4 (14)                       | 9 (43)                        | 4 (29)                        | 9 (25)                    |
| Divorced                       | 3 (10)                       | 1 (5)                         | 1 (7)                         | 3 (8)                     |
| Never married                  | 4 (14)                       | 0 (0)                         | 1 (7)                         | 3 (8)                     |
| Living situation, <i>n</i> (%) |                              |                               |                               |                           |
| Independent                    | 28 (97)                      | 21 (100)                      | 14 (100)                      | 35 (97)                   |
| Service flat                   | 1 (3)                        | 0 (0)                         | 0 (0)                         | 1 (3)                     |
| Education, <i>n</i> (%)        |                              |                               |                               |                           |
| Unknown                        | 0 (0)                        | 1 (5)                         | 0 (0)                         | 1 (3)                     |
| Primary education              | 3 (10)                       | 4 (19)                        | 1 (7)                         | 6 (17)                    |
| Secondary education            | 4 (14)                       | 4 (19)                        | 2 (14)                        | 6 (17)                    |
| Lower vocational education     | 3 (10)                       | 2 (10)                        | 1 (7)                         | 4 (11)                    |
| Secondary vocational education | 8 (28)                       | 4 (19)                        | 3 (21)                        | 9 (25)                    |
| University of applied sciences | 8 (28)                       | 5 (24)                        | 7 (50)                        | 6 (17)                    |
| Research University            | 3 (10)                       | 1 (5)                         | 0 (0)                         | 4 (11)                    |
| Smoking, <i>n</i> (%)          |                              |                               |                               |                           |
| Currently smoking              | 2 (7)                        | 3 (14)                        | 3 (21)                        | 2 (6)                     |
| Smoked in the past             | 19 (66)                      | 14 (67)                       | 6 (43)                        | 27 (75)                   |
| Never smoked                   | 8 (28)                       | 4 (19)                        | 5 (36)                        | 7 (19)                    |
| Alcohol (glasses/week)         |                              |                               |                               |                           |
| None                           | 3 (10)                       | 3 (14)                        | 3 (21)                        | 3 (8)                     |
| 0–1                            | 10 (34)                      | 6 (29)                        | 3 (21)                        | 13 (36)                   |
| 1–7                            | 11 (38)                      | 8 (38)                        | 4 (29)                        | 15 (42)                   |
| 8–14                           | 4 (14)                       | 3 (14)                        | 3 (21)                        | 4 (11)                    |
| 15–21                          | 1 (3)                        | 0 (0)                         | 1 (7)                         | 0 (0)                     |
| More than 21 glasses           | 0 (0)                        | 1 (5)                         | 0 (0)                         | 1 (3)                     |

adults (Fig. 4A–C). Specifically, C18:0 acylcarnitine differed significantly between fit and pre-frail participants ( $P < 0.05$ , Fig. 4D), whereas no significant differences were observed between cognitively normal and MCI older adults.

## Discussion

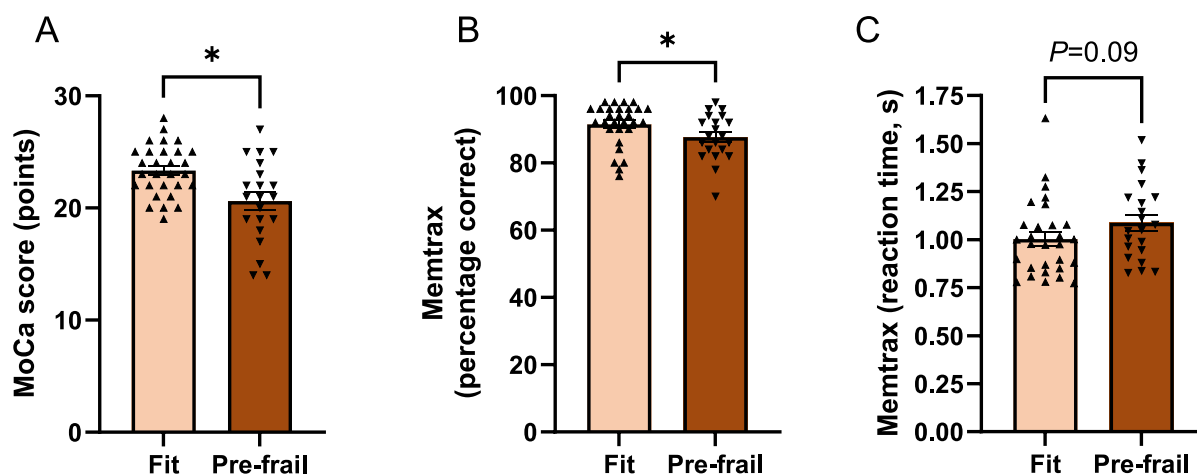
The aim of this study was to disentangle the biological relationship between pre-frailty and MCI in relatively healthy, community-dwelling older adults using

an extensive multi-omics approach. Although pre-frail individuals exhibited significantly lower global cognitive performance and memory accuracy compared to fit individuals, we observed no overlap in proteomic, metabolomic, or acylcarnitine signatures between pre-frailty and MCI. These findings suggest that, at least in early disease stages, the frequently observed clinical co-occurrence of frailty and cognitive impairment does not appear to be driven by shared systemic molecular mechanisms detectable in plasma.

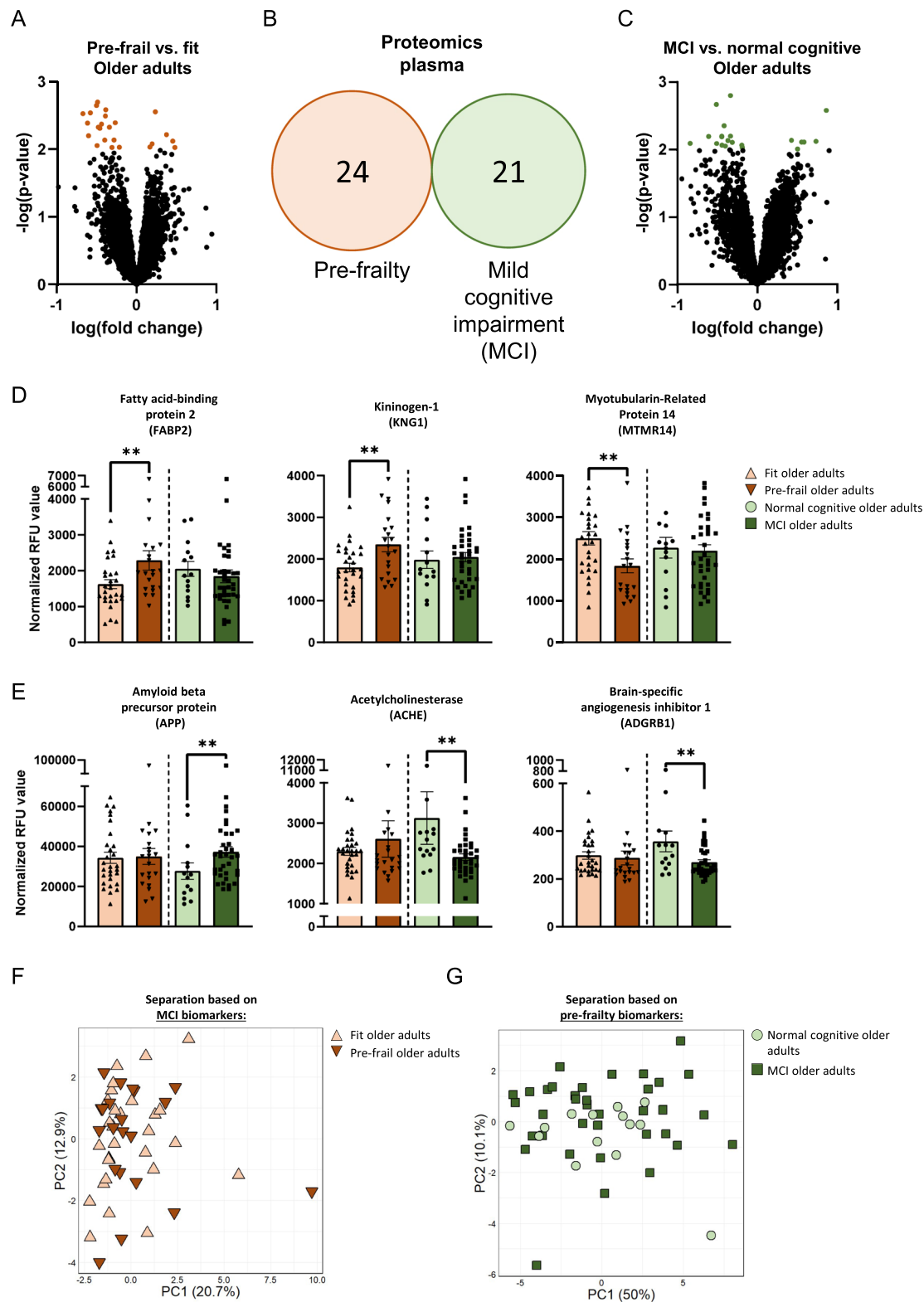
Proteomic profiling revealed 24 differentially expressed proteins associated with pre-frailty and 21 associated with MCI, with no shared biomarkers (Fig. 2). The MCI-associated proteins (e.g., an increased APP, decreased ACHE and decreased ADGRB1) are biologically plausible in the context of synaptic integrity, amyloid processing, and cholinergic signaling, reinforcing that the identified signature likely reflects early neurobiological alterations [18–20]. For example, APP is a transmembrane protein involved in neuronal growth, synaptic plasticity, and tissue repair, and dysregulated APP processing contributes to amyloid- $\beta$  accumulation and the synaptic dysfunction associated with mild cognitive impairment and Alzheimer's disease. Circulating APP levels have been investigated as accessible biomarkers of early cognitive decline and neurodegenerative disease [18, 21, 22]. Moreover, ACHE plays a central role in cholinergic neurotransmission and cognitive function. Altered ACHE levels and activity have been reported in MCI [23] and have been proposed

**Fig. 2** Plasma proteomics analysis using the SomaScan 11 k platform. **A** Volcano plot showing differential protein abundance in pre-frail vs. fit older adults. Colored dots indicate significance. **B** Venn diagram depicting the number of significantly altered protein levels in pre-frail vs. fit older adults and in older adults with mild cognitive impairment (MCI) vs. normal cognitive function. **C** Volcano plot showing differential protein abundance in older adults with MCI vs. normal cognitive function. Colored dots indicate significance. **D** Normalized relative fluorescence units (RFU) values of the top proteins associated with pre-frailty, including fatty acid-binding protein 2 (FABP2), kininogen-1 (KNG1), and myotubularin-related protein 14 (MTMR14). **E** Normalized RFU values of the top proteins associated with MCI, including amyloid beta precursor protein (APP), acetylcholinesterase (ACHE), and brain-specific angiogenesis inhibitor 1 (BAI1/ADGRB1). **F** Principal component analysis (PCA) based on the 21 proteins associated with MCI, visualizing fit and pre-frail older adults. **G** PCA based on the 24 proteins associated with pre-frailty, visualizing older adults with MCI and normal cognitive function. Data are shown as mean  $\pm$  SEM; \*\*\* $P < 0.01$

as biomarkers reflecting early cholinergic dysfunction during cognitive decline [19]. A similar pattern was observed for the pre-frailty-associated proteins, which are part of pathways relevant to muscle function, vascular biology, and systemic inflammation. For example, MTMR14 is a muscle-specific inositol phosphatase, and reduced levels have previously been linked to age-related decline in muscle function, consistent with our findings [24]. KNG1, a component of the coagulation cascade, can exert pro-inflammatory effects depending on its splice variant [25] and elevated levels have also been reported as a biomarker of

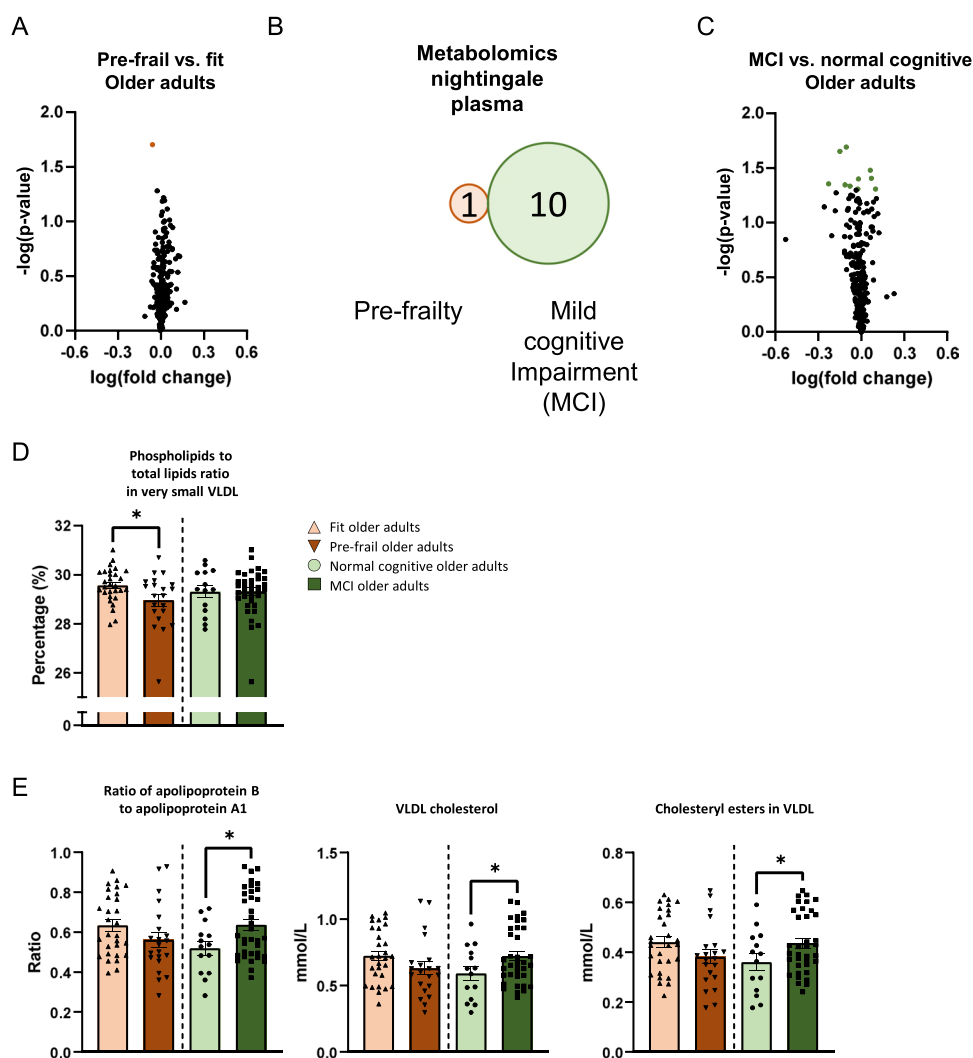


**Fig. 1** Cognitive performance in fit ( $n=29$ ) and pre-frail ( $n=21$ ) older adults. (A) Montreal Cognitive Assessment (MoCA) score, (B) MemTrax accuracy (% correct), and (C) MemTrax reaction time (s). Data are shown as mean  $\pm$  SEM; \* $P < 0.05$



frailty in a previous proteomics study [26]. Increased levels of FABP2 are associated with impaired gut barrier function and systemic inflammation [27], but have not previously been reported in relation to pre-frailty and may represent a novel marker of early physical vulnerability. Although these biomarkers for MCI and pre-frailty are not novel, their replication provides internal validation of our analytical approach by confirming involvement of previously

established biological pathways. The main contribution of this study therefore does not lie in the identification of new individual biomarkers, but rather in the absence of overlap between the proteomic signatures of early pre-frailty and MCI. This negative, yet informative finding suggests that these conditions may represent biologically distinct processes at the circulating molecular level when assessed within the same well-characterized cohort.



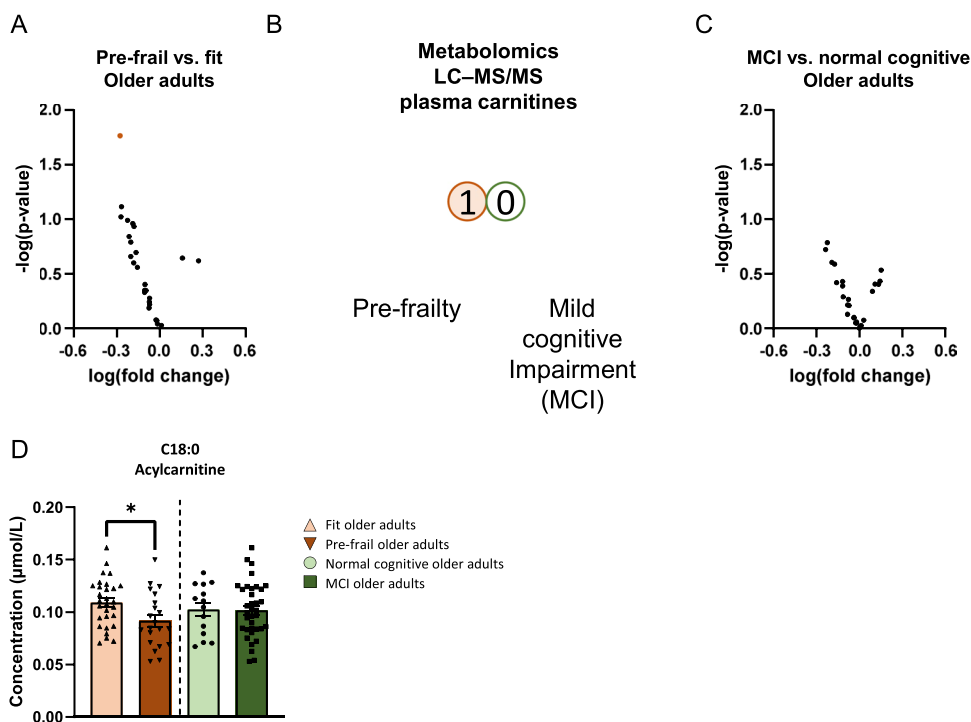
**Fig. 3** Plasma metabolomics analysis using the Nightingale platform. **A** Volcano plot showing differential metabolite levels in pre-frail vs. fit older adults. The colored dot indicates significance. **B** Venn diagram depicting the number of significantly altered metabolites in pre-frail vs. fit older adults and in older adults with mild cognitive impairment (MCI) vs. normal cognitive function. **C** Volcano plot showing differential metabolite levels in older adults with MCI vs. normal cognitive function.

Colored dots indicate significance. **D** Normalized values of the top metabolite associated with pre-frailty, namely the phospholipids-to-total lipids ratio in very small VLDL. **E** Normalized values of the top metabolites associated with MCI, including the ratio of apolipoprotein B to apolipoprotein A1, VLDL cholesterol, and cholesteryl esters in VLDL. Data are shown as mean  $\pm$  SEM; \* $P < 0.05$

Metabolomics analyses reinforced this divergence. MCI, but not pre-frailty, was characterized by elevations in lipid-related parameters, including the apolipoprotein B/A1 ratio and VLDL-associated lipids. Together, these changes point towards a shift in lipoprotein metabolism in the direction of a more pro-atherogenic profile, reflecting an increased contribution of ApoB-containing lipoproteins and triglyceride-rich particles. This pattern is consistent with emerging evidence linking dyslipidemia (particularly elevated VLDL and ApoB-driven lipid transport) to cognitive decline and neurodegenerative risk [28, 29]. Notably, these lipid subclass biomarkers are more consistently and repeatedly linked to MCI and neurodegenerative risk than to pre-frailty. In contrast, we only found one read-out to be significantly related to pre-frailty, which was the phospholipids to total lipids ratio in very small VLDL (Fig. 3D). The observed decrease in this ratio in pre-frailty may reflect alterations in the surface composition or remodeling of very small VLDL particles. Although a lower phospholipids to

total lipids ratio is often associated with increased particle size, VLDL particle size itself was not significantly associated with pre-frailty in our analysis ( $P=0.75$ ). This suggests that subtle changes in lipoprotein composition or turnover, rather than overt size differences, may characterize pre-frailty. Collectively, these findings emphasize that MCI is associated with a coherent, pro-atherogenic lipid signature, whereas pre-frailty is characterized by an increased particle size in very small VLDL. These findings further underscore the absence of shared metabolic signatures between MCI and pre-frailty.

Only one acylcarnitine (C18:0) was decreased in pre-frailty vs. fit, but not in MCI vs. normal cognitive function (Fig. 4). Circulating acylcarnitines may reflect perturbations in mitochondrial fatty acid oxidation, suggesting subtle mitochondrial or lipid utilization changes in early physical vulnerability. Indeed, plasma acylcarnitines have previously been reported as biomarkers of declining physical function in older adults [30, 31], and disturbances in



**Fig. 4** Plasma acylcarnitine profiling by LC-MS/MS. **A** Volcano plot showing differential plasma acylcarnitine levels in pre-frail vs. fit older adults. The colored dot indicates significance. **B** Venn diagram depicting the number of significantly altered acylcarnitines in pre-frail vs. fit older adults and in older adults with mild cognitive impairment (MCI) vs. normal

cognitive function. **C** Volcano plot showing differential plasma acylcarnitine levels in older adults with MCI vs. normal cognitive function. **D** Plasma concentration of C18:0 acylcarnitine in fit and pre-frail older adults, and in older adults with normal cognitive function and MCI. Data are shown as mean  $\pm$  SEM;  $*P < 0.05$

acylcarnitine metabolism (including C18:0) have also been described in MCI and Alzheimer's disease [32, 33]. However, the lower C18:0 levels observed in pre-frail subjects are somewhat unexpected, since a reduced metabolic function is usually associated with not lower but higher circulating levels of acylcarnitines (reflecting incomplete fatty acid oxidation) [34]. Interestingly, a similar decrease in C18:0 levels has been reported in myalgic encephalomyelitis/chronic fatigue syndrome patients as well, although the underlying mechanism remains unclear [35, 36]. This apparent discrepancy suggests that C18:0 may in fact not follow the canonical accumulation pattern of acylcarnitines during mitochondrial dysfunction. In addition, the absence of acylcarnitine alterations in our MCI stratification may reflect differences in disease stage, lifestyle factors, or the relatively healthy nature of our cohort. Importantly, our findings suggest that mitochondrial lipid metabolism may be more closely aligned with early physical decline than with early cognitive impairment in this population. This would associate with the dominant fuels used in brain (glucose) and skeletal muscle (fatty acids).

A major strength of this study is the integrated multi-omics approach within a single, well-characterized cohort of relatively healthy older adults. By assessing frailty and cognition within the same individuals, we avoided cross-cohort comparisons that may confound biological interpretation. In addition, by focusing on pre-frailty rather than full frailty and excluding major comorbidities, we minimized confounding effects likely induced by advanced systemic disease. Furthermore, the cohort was highly homogeneous, covering a small age and body composition range highly similar between all groups analyzed (fit vs prefrail, cognitive healthy vs impaired, Table 1). Besides these strengths, several limitations should be acknowledged. As this study has a cross-sectional design, temporal relationships cannot be established, thereby limiting causal inference and preventing conclusions about whether the observed molecular differences precede or result from pre-frailty or mild cognitive impairment. In addition, the relatively modest sample size may have limited power to detect subtle overlapping effects. Consequently, we cannot exclude the possibility that shared molecular features between pre-frailty and MCI exist but remained undetected in the present study. Larger, adequately powered cohorts will be required to determine whether subtle overlapping biomarkers emerge when statistical power is increased.

Moreover, given the exploratory nature of this study and the large number of proteins analyzed, the identified molecular signatures should be considered hypothesis-generating and require validation in independent cohorts. Furthermore, although plasma is an accessible and clinically relevant matrix, it may not accurately reflect tissue-specific biology, particularly brain-derived molecular alterations relevant to MCI or muscle-specific processes relevant to frailty. Future longitudinal studies with larger cohorts and integration of imaging and/or cerebrospinal fluid biomarkers are needed to determine whether indeed the initial are distinct and convergence of biological processes occurs with disease progression. In addition, correlations between individual frailty components and cognitive function would be of interest, as these analyses may provide further insight into the relationship between physical and cognitive impairment; however, larger studies are needed to robustly evaluate such associations.

In conclusion, our results challenge the assumption (and our initial hypothesis) that cognitive decline observed in pre-frail individuals necessarily reflects a shared disease process. Instead, these data suggest that in relatively healthy older adults, pre-frailty and mild cognitive impairment are clinically associated but biologically distinct at the circulating proteomic and metabolomic level. The absence of shared proteomic and metabolomic signatures suggests that the frequently observed clinical co-occurrence of frailty and cognitive impairment may arise from parallel but biologically distinct disease processes, at least in early disease stages. If confirmed in larger independent cohorts, this suggests that both conditions will benefit from targeted disease specific treatments.

**Abbreviations** *ACHE*: Acetylcholinesterase; *ADGRB1*: Adhesion G protein-coupled receptor B1; *ApoA1*: Apolipoprotein A1; *ApoB*: Apolipoprotein B; *APP*: Amyloid beta precursor protein; *BMI*: Body mass index; *BMC*: Bone mineral content; *DEXA*: Dual-energy X-ray absorptiometry; *EDTA*: Ethylenediaminetetraacetic acid; *FABP2*: Fatty acid-binding protein 2; *HbA1c*: Glycated hemoglobin; *ICAM-1*: Intercellular adhesion molecule 1; *IGF-1*: Insulin-like growth factor 1; *IL-6*: Interleukin 6; *KNG1*: Kininogen-1; *LC-MS/MS*: Liquid chromatography–tandem mass spectrometry; *MCI*: Mild cognitive impairment; *MoCA*: Montreal Cognitive Assessment; *MTMR14*: Myotubularin-related protein 14; *MTX*: MemTrax

test; *MTXcorrect*: Percentage of correct responses on the MemTrax test; *MTXspeed*: Reaction speed on the MemTrax test (inverse of reaction time); *NMR*: Nuclear magnetic resonance; *NTR*: Dutch Trial Register; *PCA*: Principal component analysis; *RFU*: Relative fluorescence units; *SEM*: Standard error of the mean; *SPPB*: Short Physical Performance Battery; *SPSS*: Statistical Package for the Social Sciences; *TNF- $\alpha$* : Tumor necrosis factor alpha; *VLDL*: Very low-density lipoprotein

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**Author contribution** MDvdH, AGN, FRvdL, and JK designed, conducted, or supervised the human trial and were responsible for participant recruitment, clinic visit, and sample collection. JCBCdJ, KWWK, and LV analyzed the data. LC–MS/MS experiments were conducted by NJGMV and OK. JCBCdJ and AGN drafted the manuscript. All authors contributed to the interpretation of data, critically revised the article and approved the final version.

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**Data availability** All data are available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate** The study was conducted according to the declaration of Helsinki, was approved by the medical ethical committee of Wageningen University (METC nr. 16/20), and is registered in the Dutch Trial Register (NTR6124). All participants provided written informed consent prior to enrollment.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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