**Supplementary Methods II. Methodological and Analytical**

*Study Population:* Clinical, protein, and genetic [biomarker](https://www.sciencedirect.com/topics/neuroscience/biomarkers) samples were from participants of the longitudinal study in aging *Invecchiare in Chianti* (Aging in Chianti, “InCHIANTI Study”), collected at baseline study entry from 1998-2000. This group is a representative sample (n=1,453) of the population of white European origin from two small towns in Tuscany, Italy. The primary aim of the InCHIANTI study was to evaluate physical function and mobility in older community-dwelling individuals. A detailed description of the study design, data collection, and sampling procedure are published elsewhere.1 This secondary study was approved by the ethics committee at *Centre de recherché Clinique du CHUS* and by the Institutional Review Board of the Azienda Sanitaria Area 10 Firenze; all participants consented to participate after having received a full description of the study project #548.

*Predictive Measures:* The International Consensus Group’s (I.A.A.A. /I.A.G.G.) list of potential biomarkers is not meant to be complete, accurate, or exhaustive.2 Our team used a previously published systematic review which identified shared biological markers for physical frailty and cognitive impairment, a total of 289 variables were available in the InCHIANTI database.3

*Genetic and Protein Markers*: After removal of any variables with > 12% missing data, there were 132 putative SNPs and 155 protein biomarkers. To build our model, we used protein markers with implications for clinical research and practice. Genetic risk score estimates (GRS) (i.e. the cumulative genetic risk burden estimated from SNPs of interest), were completed before including the individual single nucleotide polymorphisms (SNPs) in the final models (see GitHub supplementary material table III).

*Clinical Features*: Included age, sex, level of education, anticholinergic burden, and depressive symptoms. Anticholinergic medications were identified in the systematic review as a significant risk factor for cognitive impairment and physical frailty.3 Anticholinergic burden was calculated using the Anticholinergic Cognitive Burden Scale (ACB) and examined as a predictor.4 Current depressive symptomatology was measured using the Center for Epidemiologic Studies Depression Scale (CES-D) self-report scale (0-60), with cutoff points 16 or greater indicating depression.5 Reliability, validity, and factor structure have been similar across a diverse demographic and the scale has been used extensively in epidemiologic studies for depression and physical function.6

*Outcome Measure*

*Cognitive Frailty Measures*: Three instruments were used to measure neuropsychological function of cognitive frailty as determined by Delrieu et al.7 The Mini-Mental State Examination (MMSE) as a test of global mental status and the Trail Making Test, Part A and B (TMT). Attention was assessed using the Trail Making Test (TMT-A) and executive function was assessed using the Trail Making Test (TMT-B) scoring based on time in seconds to completion of a task with a score range of 0 to 300 seconds.8 Individuals who required additional time to complete TMT A and B, but were able to complete the task, were included and assigned a score of 300. Prior research has established precedent for including individuals who complete the TMT over the 300 second time limit to distinguish this group from individuals who cannot complete the task independent of the time limit.9,10 The rationale to include >300 completers in these analyses was made due to the possible presence of slowing without confusion in the target population of individuals with cognitive frailty. TMT, part A and B cut off scores are based on established norms for mild neurocognitive disorders.10 Normative data for time to complete the TMT tests in seconds was stratified by age and education category.10 The InCHIANTI criteria for frailty is defined by Fried et al. as exhaustion, slowness, low physical activity, weakness, and weight loss.11 Additional description of the InCHIANTI data collection and frailty classifications have been previously published.12,13

*Cognitive Frailty Phenotype:* Individuals with evidence of both physical frailty and cognitive impairment without a baseline clinical diagnosis of Alzheimer’s Disease or other dementia were defined as having the cognitive frailty phenotype.7 Phenotypic classification for this study included two models compared with healthy controls.

Healthy controls were defined as robust:

* No physical frailty (≦ 1 criteria) and absence of cognitive impairment (MMSE ≧ 24; Trail A ≦ 78, Trail B ≦ 106).

Model I considers participants with an MMSE ≦ 23 as having cognitive impairment and individuals with one or more of the physical frailty criteria are considered frail.10,11,14

* Physical frailty (≧ 1 criteria) and cognitive impairment (MMSE ≦ 23)

Model II considers participants who completed the MMSE with additional neuropsychiatric testing TMT, Part A and B.9,10 TMT cut off scores for cognitive impairment are based on cut off norms established by Ashendorf et al., 2008 and Strauss et al, 2006.

* Physical frailty (≧ 1 criteria) and cognitive impairment (Trail A ≧ 78, Trail B ≧106)

Numbers of participants with moderate and severe cognitive impairment were insufficient for inclusion as separate categories for pre-frail and frail phenotypes in statistical analyses.

*Statistical Analysis (*Figure 2 shows a summary of our workflow)

We used a cross-sectional (study baseline) data to develop boosted ML models for identification of features associated with cognitive frailty. Then we further qualified the clinical and biological predictors identified in the model by examining their significance by healthy control and cognitive frailty phenotype. Covariates were selected to control for potential confounding effects, including sex, age, education, baseline diagnosis of dementia (n=82), vascular dementia (n=41), depression (n=412), and Parkinson’s disease (n=16).

Model Development: Mechanisms that contribute to the development of cognitive frailty were determined by evaluation of genetic variability, protein and clinical markers as predictors of the development and persistence of cognitive frailty. *Model I* tested prediction of genetic, protein and clinical markers on cognitive frailty with the use of criteria from the MMSE while *Model II* tested prediction of genetic, protein, and clinical markers on cognitive frailty with use of additional neuropsychological testing TMT, Part A and B.9,10

Using a Boosted Tree Approach for Data Pruning: Boosted trees, a machine learning technique for supervised learning, are ensembles of regression trees, similar to decision trees and are used for prediction or classification. The advantage of using a tree boosting approach model for the evaluation of multiple variables simultaneously is that it provides a high predictive value with low bias.15 Additionally, parameters are set to prevent over fitting for the models. Extreme Gradient Boosting (xgboost) in R, statistical software, is an effective method for building a reproducible predictive model for the detection of a complex heterogeneous phenotype such as cognitive frailty with large numbers of predictors. *Xgboost* is based in boosted trees and provides more efficient and accurate predictive modeling with large datasets and a rapid / robust framework for variable selection. Statistical modeling is used to design, test, and validate an accurate method for classifying patients into phenotypic outcomes. The statistical analysis was completed in three steps: 1) analysis of all available variables for feature selection and data reduction, 2) model discovery followed by model validation, and 3) determination of significance in the model features between phenotype and healthy control.

Step 1) The data were randomly divided, two thirds were assigned to the training cohort, and one third was assigned to the validation cohort. One of the features that is central to *xgboost* is its ability to combine multiple trees or “weak predictors” to reach maximum prediction performance while reducing bias. This approach uses large amounts of data from different aspects of clinical, genetic, and biomarker research, strengthening the models’ generalizability and classification power. *Xgboost* iteratively re-weighs the variables, taking a weighted majority; the parameters identified after pruning comprised the final predictive model.16 None of the candidate features in the models are used in the diagnosis of cognitive frailty. This standard technique prevents circularity, overestimation, and over fitting for both the models generated. Parameters for the model include: max depth = “10”, nthread = “12”, nrounds = 5-200, objective = “binary:logistic”, evaluation metric = “auc”, silent =”1”, gamma = default =“0” to control the number of trees, and eta default= “0.3” to prevent over fitting. We used the default setting for all other parameters which can be found in the *xgboost* 0.6 documentation.15 The *xgboost* algorithm iteratively determines the maximum function of a model based on a tree building algorithm (quadratic problem) which creates a node then assigns a prediction point to each leaf; the assigned number is termed “gain” (figure 1). Once the model has reached maximum depth, pruning occurs by taking out the nodes with a negative gain and keeping those with a positive gain. Results from the population predictive model are ranked by gain which is a metric based on each feature’s contribution in the model. When comparing top features to other features in the model, the higher the gain the more important the feature is for prediction of the outcome. *Cover* is a measure of the relative quantity of observations found by one feature and *frequency* is the percentage representing the relative number of time a feature is used in the trees of the model.15 Gain is the most relevant metric to interpreting the rank and importance of each feature.

Step 2. To evaluate the models, we used the evaluation metric area under the curve (AUC). AUC were calculated from each model and used to determine discrimination of participants with cognitive frailty (case) from healthy individuals (control) in the training cohort. An AUC of 0.5 was considered chance, > 0.8 informative, and > 0.9 clinically relevant.

Step 3. Univariate analysis, *t-*tests for continuous and chi-squared tests for binomial traits, were used to determine the significance of the predictor with a Bonferroni correction (to account for multiple comparisons). Of note, no further adjustment for multiple comparison was carried out due to the exploratory, hypothesis-generating nature of this study to protect against type II error (false negative) rather than type I. To evaluate additive effects of SNPs, a positive regression coefficient means that each copy of the allele of interest increases the risk for the cognitive frailty phenotype.17 Our study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health (Bethesda, MD, USA).

Boosted ML models use “ensembling”, a technique that can combine many independent predictors to model interacting systems and determine the top combined predictive factors associated with cognitive frailty.15 At least in theory, the determination of genetic and biological markers that define a clinical group should facilitate a better understanding of the interrelated pathology for cognitive impairment and physical frailty and promote new ideas for understanding complex interacting biological systems. The ML boosted model employs an unbiased logic that does not make assumptions about the relationship among predictors or apply weights in the scoring system. Boosting is an ensembling technique that employs a sequential algorithm which repetitively learns and improves as the model reaches a final prediction. Parameter estimates for each predictive factor and associated descriptive statistics were evaluated to provide biological insight into the underpinnings of the classification algorithm.



Laboratory assay methods

At the baseline survey, most of the participants performed 24-hour urine collection early in the morning mid-stream sample urine for the routine examination. Total urinary polyphenols were measured at the Department of Food Science and Technology, School of Pharmacy, University of Barcelona, Spain. Prior to blood collection all participants consumed a diet free of meat and fish. Participants donated fasting blood samples for routine blood examinations. Blood collection was performed with the standard procedure method to prevent red cell hemolysis. The blood collection included two sets of collection tubes: one for routine tests and second for collecting specimens including serum, plasma, DNA for the biological bank. All routine blood tests, performed in the Laboratory of Clinical Chemistry and Microbiological Assays, Annunziata Hospital in Florence, Italy. Plasma fatty acids (FAs) were measured by the Section of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, Perugia, Italy. The technique used was gas chromatography with a fused silica capillary column to achieve the optimum separation of the different fatty acids.

Software for analyses

All statistical analyses were carried out using R V. 3.2.1. R is a free, open-source software that provides many statistical and graphic techniques. R packages used included ‘glm2’-Fitting Generalized Linear Models, ‘Ordinal’-Regression Models for Ordinal Data, and ‘xgboost’-Extreme Gradient Boosting15,18,19. The software package PLINK, an analysis toolset was used for the management of genotype data and basic associating testing17,20.

Model generation

The predictive genetic and laboratory biomarkers were identified in a comprehensive systematic review and analyzed using an Extreme Gradient Boosting (xgboost) in R15. While boosting was initially developed for machine learning, ‘xgboost’ in R is based in boosted trees. Xgboost is an open source tool and a variant of the gradient boosting machine and uses a tree based model. Xgboost is used in this study for a supervised learning problem where the variables identified from the systematic review are used to predict three phenotypes cognitive decline, physical frailty, and cognitive frailty.

Evaluation of the model

With the use of any predictive model in machine learning there is a chance for inflated risk of capitalizing on chance features (overfitting) in the data. Overfitting of the integrative model was mitigated in two ways: 1) having a distinct training and validation process for the model and 2) using xgb in R which has a built-in parameter settings for selection to reduce poor predictive performance. *Internal validation:* A randomly assigned training subset was used to validate the model within the InCHIANTI cohort *in silico* (via simulation).

Calibration of the model

Parameter estimates for each predictive factor and associated descriptive statistics was evaluated to provide biological insight into the underpinnings of the classification algorithm. We first evaluated the calibration by partitioning the data into 5, 10, 20, 30, 40, 50, 75, 100 and 200 groups and then ran the calibration test. Next, we repeated tests for all possible values between 5-200 groups and evaluated the distribution of the test statistic. The best prediction thresholds were determined using AUC, 87.7% for Model I and 86.4% for Model II. Population predictive features by phenotype ranked by gain for Model I are presented in Tables 4-6 and Model II Tables 7-9.

Genetic Data

Genotypic data was generated at the National Institute on Aging’s Laboratory of Neurogenics. Samples of genomic DNA extracted from leukocytes1. Genotypic data used for the model were extracted out of the binary Plink files from the InCHIANTI database. SNPs which could not be identified in the binary files were extracted from genotype imputed files, genotype imputation was completed with Minimac (V2). The SNPs included meet the following standard: per variant and per sample missingness < 5%, European ancestry, MAF < 0.001 and a rsq < 0.3. Additionally, Samples were filtered for 95% or greater genotyping call rate, no ancestry outliers, and no sex discrepancies.

Biomarker Selection

Laboratory and genetic biomarkers were selected based on the results of a systematic review of the literature performed using the following online databases: PubMed, Embase, Scopus, Web of Science, LILACS, Gene Indexer, and GWAS Central. The systematic review findings and results tables are available in a previous publication.3

**Supplementary Data Table I: Laboratory values as they appear in the InCHIANTI** **Datasets by Clinical Category**

|  |  |  |
| --- | --- | --- |
| **Inflammatory/Immunity** | **Nutrient Biomarker** | **Lipid Metabolism** |
| BL Uric acid (mg/dL) | BL Omega-3 fatty acids as % of total fatty acid area | BL Lipids: total cholesterol (mg/dL) |
| BL Urinary cortisol (µg/mL) | BL Omega-3 plasma fatty acid weight (mg/L) | BL Lipids: HDL cholesterol (mg/dL) |
| BL 24-hour urinary cortisol (µg/24 hours) | BL Omega-3 fatty acids as % of total fatty acid weight | BL Lipids: triglycerides (mg/dL) |
| BL C-reactive protein - low sensitivity (µg/mL) | BL Omega-3 fatty acids as % of total fatty acid mols | BL Lipids: LDL cholesterol (mg/dL) |
| BL C-reactive protein - high sensitivity (µg/mL) | BL Omega-6 fatty acids as % of total fatty acid area | BL Lipoprotein(a) (mg/dL) |
| BL Interleukin-6 via ELISA ultrasensitive (pg/mL) | BL Omega-6 plasma fatty acid weight (mg/L) |  |
| BL IL-6 high-sensitivity ELISA calculated from ELISA ultrasensitive (pg/mL) | BL Omega-6 fatty acids as % of total fatty acid weight | **Metabolomics(plasma lipids)** |
| BL Soluble IL-6 receptor via ELISA (ng/mL) | BL Omega-6 fatty acids as % of total fatty acid mols | BL Fatty acid C16:0 (palmitiA91:A116c) area |
| BL Interleukin-10 via ELISA (pg/mL) | BL Ratio of Omega-6:Omega-3 as % of total fatty acid area | BL Fatty acid C16:0 (palmitic) area |
| BL Interleukin-1 receptor antagonist via ELISA ultrasensitive (pg/mL) | BL Ratio of Omega-6:Omega-3 as % of total fatty acid weight | BL Fatty acid C16:0 as % of total fatty acid area |
| BL Interleukin-1B via ELISA (pg/mL) | BL Ratio of Omega-6:Omega-3 as % of total fatty acid mols | BL Fatty acid C16:0 weight (mg/L) |
| BL Interleukin-18 via ELISA ultrasensitive using plasma (pg/mL) | BL Vitamin B6 via high performance liquid chromatography (ng/mL) | BL Fatty acid C16:0 as % of total fatty acid weight |
| BL Transforming growth factor-B1 (pg/mL) | BL Vitamin B6 via high performance liquid chromatography (nmol/L) | BL Fatty acid C16:0 (µmol/L) |
| BL Tumor necrosis factor-a via multiplex technology (pg/mL) | BL Vitamin E gamma tocopherol, high performance liquid chromatography (µmol/L) | BL Fatty acid C16:0 as % of total fatty acid mols |
| BL Soluble TNF-a receptor I via quantitative sandwich EIA (pg/mL) | BL Vitamin E alpha tocopherol, high performance liquid chromatography (µmol/L) | BL Fatty acid C20:0 (arachidic) area |
| BL Soluble TNF-a receptor II via quantitative sandwich EIA (pg/mL) | BL Vitamin E gamma tocopherol, high performance liquid chromatography, assay #2 (µmol/L) | BL Fatty acid C20:0 as % of total fatty acid area |
| BL TNF-related apoptosis-inducing ligand (pg/mL) | BL Vitamin E alpha tocopherol, high performance liquid chromatography, assay #2 (µmol/L) | BL Fatty acid C20:0 weight (mg/L) |
| BL Interleukin-8 via Bio-Plex (pg/mL) | BL Beta-carotene via high performance liquid chromatography (µmol/L) | BL Fatty acid C20:0 as % of total fatty acid weight |
| BL Interleukin-12 via Bio-Plex (pg/mL) | BL Lycopene via high performance liquid chromatography (µmol/L) | BL Fatty acid C20:0 (µmol/L) |
| BL Monocyte chemoattractant protein-1 via Bio-Plex (pg/mL) | BL Total proteins (g/dL) | BL Fatty acid C20:0 as % of total fatty acid mols |
| BL Macrophage inflammatory protein-1b via Bio-Plex (pg/mL) | BL Albumin (%) | BL Fatty acid C20:5 n-3 cis (eicosapentaenoic, EPA) area |
| BL Serum cortisol (µg/dL) |  | BL Fatty acid C20:5 n-3 as % of total fatty acid area |
| BL Serum cortisol (nmol/L) |  | BL Fatty acid C20:5 n-3 weight (mg/L) |
| BL Dehydroepiandrosterone sulfate (µg/dL) |  | BL Fatty acid C20:5 n-3 as % of total fatty acid weight |
| BL Dehydroepiandrosterone sulfate (nmol/L) |  | BL Fatty acid C20:5 n-3 (µmol/L) |
| BL Cortisol:DHEAS ratio (based on nmols) |  | BL Fatty acid C20:5 n-3 as % of total fatty acid mols |
| BL Soluble CD14 via ELISA (ng/mL) |  | BL Fatty acid C22:0 (behenic) area |
| BL Fibrinogen (mg/dL) |  | BL Fatty acid C22:0 as % of total fatty acid area |
| BL Erythrocyte sedimentation rate (ESR) (mm/hour) |  | BL Fatty acid C22:0 weight (mg/L) |
| BL Homocysteine via FPIA analysis (µmol/L) |  | BL Fatty acid C22:0 as % of total fatty acid weight |
| BL Resistin via EIA (ng/mL)- |  | BL Fatty acid C22:0 (µmol/L) |
| BL Adiponectin via RIA (µg/mL)-(metabolic function) |  | BL Fatty acid C22:0 as % of total fatty acid mols |
| BL Advanced glycation endproduct (AGE): Carboxymethyl-lysine (ng/mL) |  | BL Fatty acid C24:0 (lignoceric) area |
| BL Alpha-1 globulin (%) |  | BL Fatty acid C24:0 as % of total fatty acid area |
| BL Alpha-2 globulin (%) |  | BL Fatty acid C24:0 weight (mg/L) |
| BL Alpha-2-macroglobulin (mg/dL) |  | BL Fatty acid C24:0 as % of total fatty acid weight |
| BL Beta globulins (%) |  | BL Fatty acid C24:0 (µmol/L) |
| BL Endogenous secretory receptor for AGEs (ng/mL) |  | BL Fatty acid C24:0 as % of total fatty acid mols |
| **Renal/Electrolyte** | **Hematology/Liver** | **Endocrine/Hormones** |
| BL Na+ (mEq/L) | BL White blood cells (WBC) (n, K/µL) | BL Blood glucose (mg/dL) |
| BL Ca++ (mg/dL) | BL Neutrophils (n, K/µL) | BL 25(OH)-D (25-hydroxyvitamin D) via RIA (nmol/L) |
| BL Urinary creatinine (mg/dL) | BL Lymphocytes (n, K/µL) | BL Parathyroid hormone, two-site immunoradiometric assay (pg/mL) |
| BL 24-hour urinary creatinine (mg/24 hours) | BL Monocytes (n, K/µL) | BL Thyroid stimulating hormone, TSH (mIU/L) |
| BL Creatinine clearance, 24-hr urine (mL/minute) | BL Neutrophils (%) | BL Free thyroxine, fT4 (ng/dL) |
| BL Urinary Ca (mmol/L) | BL Lymphocytes (%) | BL Plasma insulin via RIA (mIU/L) |
| BL Urinary Na (mmol/L) | BL Monocytes (%) | BL Total testosterone (ng/mL) |
| BL Urine glucose (mg/dL) | BL Red blood cells (RBC) (n, millions/µL) | BL Total testosterone (nmol/L) |
| BL Urine proteins (mg/dL) | BL Hemoglobin (g/dL) | BL Free testosterone (ng/dL), Vermeulen |
| BL Urine hemoglobin (mg/dL) | BL Hematocrit (%) | BL Free testosterone (nmol/L), Vermeulen |
| BL Urine ketones (mg/dL) | BL Mean corpuscular volume (MCV) (fL) | BL Estradiol via radioimmunoassay (pg/mL) |
| BL Urine bilirubin (mg/dL) | BL Mean corpuscular hemoglobin (MCH) (pg) | BL Estradiol via radioimmunoassay (nmol/L) |
| BL Urine urobilinogen (mg/dL) | BL MCH concentration (MCHC) (g/dL) | BL C-terminal telopeptide of type-1 collagen (ng/mL) |
| BL Urine nitrites | BL Red cell distribution width (RDW) (%) | BL Total insulin-like growth factor-1, serum, immunoradiometric assay (ng/mL)-(IGFBP1) |
| BL Serum creatinine (mg/dL) | BL Mean platelet volume (MPV) (fL) | BL IGF binding protein-3, serum, immunoradiometric assay (ng/mL) \*\*\*corrected\*\*\* |
| BL Blood urea nitrogen (mg/dL) | BL Ferritin (ng/mL) | BL IGF binding protein-3, serum, immunoradiometric assay (nmol/L) |
| BL Creatine phosphokinase (U/L) | BL Folate via RIA (ng/mL) |  |
| BL Cystatin C (mg/L) | BL Folate via RIA (nmol/L) |  |
|  | BL Vitamin B12 via RIA (pg/mL) |  |
|  | BL Vitamin B12 via RIA (pmol/L) |  |
|  | BL Methylmalonic acid(methylmalonic aciduria), MMA (µmol/L) |  |
|  | BL Soluble transferrin receptor (nmol/L) |  |
|  | BL Soluble transferrin receptor (mg/L) |  |
|  | BL GOT (also known as AST) (U/L) |  |
|  | BL GPT (also known as ALT) (U/L) |  |
|  | BL Gamma glutamyl transferase (U/L) |  |
|  | BL Retinol via high performance liquid chromatography (µmol/L) |  |

**Supplementary Data Table II: Variants included in the Genomic Risk Score GRS calculations and individual effect estimates of single variates for predictive modeling. Phenotype association is based on the findings from the systematic review and the relationship found between variant and disease outcome.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variant Name-Allele | Allele Frequency  (%) | Gene/Closest RefSeq Gene | Variant Detail-dbSNP | Phenotype Association |
| rs1048945\_C | 1.3 | APEX1 | rs1048945 C/G Ancestral: G Minor: C | Cognition |
| rs1052133\_G | 20.6 | OGG1 | rs1052133 C/G Ancestral: C Minor: G | Cognition |
| rs1064039\_T | 19.0 | CST3 | rs1064039 A/G Ancestral: G Minor: T | Cognition |
| rs10793294\_C | 21.7 | GAB2 | rs10793294 A/C Ancestral: G Minor: C | Cognition |
| rs10883631\_G | 48.4 | BTRC | rs10883631 A/G Ancestral: G Minor: A | Frail |
| rs10883642\_G | 48.4 | BTRC | rs10883642 A/G Ancestral: A Minor: A | Frail |
| rs11225434\_C | 47.9 | WTAPP1 | rs11225434 C/T Ancestral: T Minor: C | Cognition |
| rs113263161\_A | 10.4 | **CCRL2**/LOC102724297 | rs113263161 A/G Ancestral: G Minor: A | Cognition |
| rs1133174\_A | 41.0 | SORL1 | rs1133174 A/G Ancestral: G Minor: A | Cognition |
| rs11574428\_A | 10.2 | CCRL2 | rs11574428 A/T Ancestral: T Minor: A | Cognition |
| rs11575821\_A | 11.4 | **CCRL2**/LOC102724297 | rs11575821 A/G Ancestral: G Minor: A | Cognition |
| rs1207568\_A | 19.4 | KLOTHO | rs1207568 C/T Ancestral: C Minor: A | Cognition |
| rs13113697\_T | 27.2 | **HS3ST1**/LOC107986178 | rs13113697 G/T Ancestral: G Minor: T | Cognition |
| rs1468063\_T | 12.4 | FAS | rs1468063 A/G Ancestral: G Minor: T | Cognition |
| rs1566728\_C | 14.1 | PTPRJ | rs1566728 A/G Ancestral: G Minor: C | Frail |
| rs16944\_A | 33.4 | IL1B | rs16944 A/G Ancestral: A Minor: A | Cognition |
| rs1799990\_G | 30.9 | PRNP | rs1799990 A/G Ancestral: A Minor: G | Cognition |
| rs1800629\_A | 12.3 | TNF | rs1800629 A/G Ancestral: G Minor: A | Cog/Frail |
| rs1800764\_C | 47.6 | ACE | rs1800764 C/T Ancestral: C Minor: T | Cognition |
| rs1800796\_C | 5.0 | IL6 | rs1800796 C/G Ancestral: G Minor: C | Cog/Frail |
| rs1801394\_G | 43.9 | MTRR | rs1801394 A/G Ancestral: A Minor: G | Frail |
| rs2047812\_A | 14.8 | PTPRJ | rs2047812 C/T Ancestral: C Minor: A | Frail |
| rs2227729\_G | 7.5 | VTN | rs2227729 C/T Ancestral: C Minor: G | Frail |
| rs2228145\_C | 38.0 | IL6-R | rs2228145 A/C/T Ancestral: A Minor: C | Cognition |
| rs2228467\_C | 8.2 | CCL4 | rs2228467 C/T Ancestral: T Minor: C | Cognition |
| rs2229238\_T | 16.9 | IL6-R | rs2229238 C/T Ancestral: C Minor: T | Cognition |
| rs2267163\_T | 36.5 | TCN2 | rs2267163 C/T Ancestral: C Minor: T | Frail |
| rs2283368\_C | 12.3 | KLOTHO | rs2283368 C/T Ancestral: T Minor: C | Cognition |
| rs2465481\_A | 47.0 | GNAI1 | rs2465481 C/T Ancestral: C Minor: A | Cognition |
| rs2714465\_G | 45.0 | GNAI1 | rs2714465 A/G Ancestral: A Minor: G | Cognition |
| rs3092960\_A | 10.7 | CCR2 | rs3092960 A/G Ancestral: G Minor: A | Cognition |
| rs3131609\_C | 32.8 | USP50 | rs3131609 A/G Ancestral: A Minor: C | Cognition |
| rs360722\_A | 16.9 | IL18 | rs360722 C/T Ancestral: T Minor: A | Frail |
| rs3865444\_A | 27.1 | CD33 | rs3865444 G/T Ancestral: G Minor: A | Cognition |
| rs4147929\_A | 19.3 | ABCA7 | rs4147929 A/G Ancestral: G Minor: A | Cognition |
| rs429358\_C | 6.9 | APOE | rs429358 C/T Ancestral: C Minor: C | Cognition |
| rs4316\_T | 38.1 | ACE | rs4316 C/T Ancestral: C Minor: T | Cognition |
| rs4845622\_C | 38.6 | IL6R | rs4845622 A/C Ancestral: A Minor: C | Cognition |
| rs4968782\_G | 41.0 | **ACE** | rs4968782 A/G Ancestral: G Minor: G | Cognition |
| rs55636820\_A | 6.0 | BIN1 | rs55636820 A/G Ancestral: G Minor: A | Cognition |
| rs562020\_A | 34.6 | KLOTHO | rs562020 C/T Ancestral: T Minor: A | Cognition |
| rs573521\_A | 47.2 | MMP3 | rs573521 C/T Ancestral: C Minor: A | Cognition |
| rs5744256\_G | 18.3 | IL18 | rs5744256 C/T Ancestral: T Minor: G | Frail |
| rs603050\_T | 31.3 | WTAPP1 | rs603050 A/G Ancestral: G Minor: T | Cognition |
| rs611646\_T | 48.6 | ATM | rs611646 A/T Ancestral: A Minor: A | Frail |
| rs61812598\_A | 37.9 | IL6-R | rs61812598 A/G Ancestral: G Minor: A | Cognition |
| rs6441977\_A | 10.2 | CCRL2 | rs6441977 A/G Ancestral: G Minor: A | Cognition |
| rs650108\_A | 30.1 | MMP3 | rs650108 A/G Ancestral: G Minor: A | Cognition |
| rs6762266\_C | 10.4 | **CCRL2** | rs6762266 C/T Ancestral: T Minor: C | Cognition |
| rs679620\_T | 46.7 | MMP3 | rs679620 A/G Ancestral: G Minor: T | Cognition |
| rs6808835\_T | 10.5 | CCRL2 | rs6808835 G/T Ancestral: T Minor: T | Cognition |
| rs7110631\_C | 31.2 | **PICALM** | rs7110631 C/G Ancestral: G Minor: C | Cognition |
| rs7396366\_C | 36.0 | AP2A2 | rs7396366 G/T Ancestral: T Minor: C | Cognition |
| rs7412\_T | 6.6 | APOE | rs7412 C/T Ancestral: C Minor: T | Cognition |
| rs7497104\_T | 28.6 | MYO9A | rs7497104 C/T Ancestral: T Minor: T | Cognition |
| rs7926920\_A | 46.9 | WTAPP1 | rs7926920 A/G Ancestral: G Minor: A | Cognition |
| rs9267487\_C | 6.5 | DDX39B | rs9267487 C/T Ancestral: T Minor: C | Frail |
| rs9349407\_C | 24.5 | CD2AP | rs9349407 C/G Ancestral: G Minor: C | Cognition |
| rs948399\_C | 26.9 | MMP3 | rs948399 C/T Ancestral: T Minor: C | Cognition |
| rs9527025\_C | 14.8 | KLOTHO | rs9527025 C/G Ancestral: C Minor: C | Cognition |
| rs3219484\_T | 3.8 | MUTYH | rs3219484\_ A/G Ancestral: G Minor: T | Cognition |
| rs12752888\_C | 26.8 | **ACOT11**/LOC105378734 | rs12752888 C/T Ancestral: T Minor: C | Cognition |
| rs1539053\_A | 45.6 | DAB1 | rs1539053 C/T Ancestral: T Minor: G | Cognition |
| rs3811448\_A | 19.3 | TDRD10 | rs3811448 A/G Ancestral: A Minor: A | Cognition |
| rs4129267\_T | 37.9 | IL6-R | rs4129267 C/T Ancestral: C Minor: T | Cognition |
| rs915179\_G | 36.0 | LMNA | rs915179 A/G Ancestral: G Minor: A | Cognition |
| rs9919256\_A | 13.7 | LMNA | rs9919256 A/G Ancestral: A Minor: A | Cognition |
| rs6131\_T | 19.4 | SELP | rs6131 A/G Ancestral: A Minor: T | Frail |
| rs3818361\_A | 19.5 | CR1 | rs3818361 C/T Ancestral: C Minor: A | Cognition |
| rs1260326\_C | 46.3 | GCKR | rs1260326 C/T Ancestral: C Minor: T | Frail |
| rs744373\_G | 28.2 | **BIN1** | rs744373 C/T Ancestral: T Minor: G | Cognition |
| rs7561528\_A | 31.2 | **BIN1**/LOC105373605 | rs7561528 A/G Ancestral: A Minor: A | Cognition |
| rs11894266\_C | 43.5 | **SSB** | rs11894266 C/T Ancestral: C Minor: T | Cognition |
| rs6747918\_A | 49.2 | CASP8 | rs6747918 A/G Ancestral: A Minor: A | Frail |
| rs2929408\_A | 22.4 | KAT2B | rs2929408 G/T Ancestral: G Minor: A | Frail |
| rs737267\_T | 25.6 | SLC2A9 | rs737267 A/G/T Ancestral: G Minor: T | Frail |
| rs9461448\_G | 4.7 | PGBD1 | rs9461448 G/T Ancestral: T Minor: G | Cognition |
| rs9446432\_C | 8.2 | **C6orf155** | rs9446432 C/T Ancestral: T Minor: C | Cognition |
| rs9384428\_C | 32.5 | **MIR1202**/LOC101928923 | rs9384428 C/T Ancestral: T Minor: C | Cognition |
| rs4646450\_A | 16.4 | CYP3A5 | rs4646450 C/T Ancestral: T Minor: A | Frail |
| rs11767557\_C | 16.8 | EPHA1-AS1 | rs11767557 C/T Ancestral: T Minor: C | Cognition |
| rs11771145\_A | 32.9 | EPHA1-AS1 | rs11771145 A/G Ancestral: A Minor: A | Cognition |
| rs11136000\_T | 39.0 | CLU | rs11136000 C/T Ancestral: T Minor: T | Cognition |
| rs1157242\_T | 16.2 | **KCNU1** | rs1157242 A/G Ancestral: G Minor: T | Cognition |
| rs7840202\_C | 29.9 | UBR5 | rs7840202 A/C Ancestral: C Minor: C | Cognition |
| rs7920721\_G | 39.4 | ECHDC3 | rs7920721 A/G Ancestral: A Minor: G | Cognition |
| rs7905675\_A | 34.9 | **TFAM** | rs7905675 A/G Ancestral: A Minor: G | Cognition |
| rs17117126\_G | 9.5 | **CH25H** | rs17117126 A/G Ancestral: G Minor: G | Cognition |
| rs6265\_T | 21.6 | BDNF | rs6265 A/G Ancestral: G Minor: T | Cognition |
| rs1566729\_T | 14.1 | PTPRJ | rs1566729 A/G Ancestral: G Minor: T | Frail |
| rs583791\_C | 49.5 | MS4A6A | rs583791 A/G Ancestral: G Minor: C | Cognition |
| rs610932\_T | 48.5 | MS4A6A | rs610932 A/C Ancestral: A Minor: T | Cognition |
| rs662196\_C | 49.6 | MS4A6A | rs662196 A/G Ancestral: G Minor: C | Cognition |
| rs670139\_T | 31.2 | MS4A4E | rs670139 A/C/T Ancestral: C Minor: T | Cognition |
| rs676309\_C | 31.1 | MS4A4E | rs676309 A/G Ancestral: A Minor: C | Cognition |
| rs11827375\_A | 10.5 | **C11orf30** | rs11827375 A/G Ancestral: G Minor: A | Cognition |
| rs3851179\_T | 36.0 | **PICALM** | rs3851179 A/G Ancestral: G Minor: T | Cognition |
| rs541458\_C | 31.6 | **PICALM** | rs541458 C/T Ancestral: T Minor: C | Cognition |
| rs10501927\_G | 23.6 | CNTN5 | rs10501927 G/T Ancestral: T Minor: G | Cognition |
| rs495366\_A | 30.1 | WTAPP1 | rs495366 A/G Ancestral: G Minor: A | Cognition |
| rs645419\_A | 46.7 | MMP3 | rs645419 A/G Ancestral: G Minor: A | Cognition |
| rs10502262\_T | 27.7 | SORL1 | rs10502262 A/G Ancestral: G Minor: T | Cognition |
| rs1614735\_G | 47.6 | SORL1 | rs1614735 G/T Ancestral: T Minor: G | Cognition |
| rs2298813\_A | 4.0 | SORL1 | rs2298813 A/G Ancestral: G Minor: A | Cognition |
| rs3781835\_A | 2.3 | SORL1 | rs3781835 A/G Ancestral: G Minor: A | Cognition |
| rs4935774\_C | 20.5 | SORL1 | rs4935774 C/T Ancestral: C Minor: C | Cognition |
| rs4363657\_C | 15.2 | SLCO1B1 | rs4363657 C/T Ancestral: T Minor: C | Frail |
| rs1799986\_T | 17.4 | LRP1 | rs1799986 A/C/T Ancestral: C Minor: T | Frail |
| rs398655\_C | 45.0 | KLOTHO | rs398655 G/T Ancestral: G Minor: A | Cognition |
| rs648202\_T | 13.7 | KLOTHO | rs648202 C/T Ancestral: C Minor: T | Cognition |
| rs9526984\_G | 7.4 | KLOTHO | rs9526984 A/G Ancestral: A Minor: G | Cognition |
| rs9527024\_A | 14.8 | KLOTHO | rs9527024 A/G Ancestral: A Minor: A | Cognition |
| rs9536314\_G | 14.7 | KLOTHO | rs9536314 A/G/T Ancestral: T Minor: G | Cognition |
| rs2287396\_T | 17.7 | GSTZ1 | rs2287396 C/T Ancestral: C Minor: T | Frail |
| rs7175373\_C | 29.1 | **MYO9A** | rs7175373 A/C/G Ancestral: C Minor: C | Cognition |
| rs129968\_A | 39.8 | CREBBP | rs129968 A/G Ancestral: A Minor: G | Frail |
| rs3785880\_G | 39.8 | MAPT | rs3785880 G/T Ancestral: T Minor: G | Cognition |
| rs2526378\_G | 46.8 | TSPOAP1 | rs2526378 C/T Ancestral: C Minor: A | Cognition |
| rs4343\_A | 40.1 | ACE | rs4343 A/G Ancestral: A Minor: G | Cognition |
| rs4459609\_C | 40.9 | **ACE** | rs4459609 A/C Ancestral: A Minor: C | Cognition |
| rs3764650\_G | 11.8 | ABCA7 | rs3764650 G/T Ancestral: T Minor: G | Cognition |
| rs157580\_G | 39.1 | TOMM40 | rs157580 A/G Ancestral: G Minor: G | Cognition |
| rs2075650\_G | 7.5 | TOMM40 | rs2075650 A/G Ancestral: G Minor: G | Cognition |
| rs405509\_T | 42.8 | APOE | rs405509 A/C Ancestral: C Minor: T | Cognition |
| rs597668\_C | 12.0 | **EXOC3L2** | rs597668 C/T Ancestral: C Minor: C | Cognition |
| rs6859\_A | 38.8 | NECTIN2 | rs6859 A/G Ancestral: G Minor: A | Cognition |
| rs8106922\_G | 44.8 | TOMM40 | rs8106922 A/G Ancestral: A Minor: G | Cognition |
| rs17411904\_C | 7.7 | **PCK1** | rs17411904 C/T Ancestral: T Minor: C | Cognition |
| rs2833383\_T | 27.9 | TIAM1 | rs2833383 C/T Ancestral: C Minor: T | Frail |
| rs4646316\_T | 27.7 | COMT | rs4646316 C/T Ancestral: C Minor: T | Frail |
| rs4680\_A | 46.4 | COMT | rs4680 C/T Ancestral: G Minor: A | Cognition |
| rs740234\_G | 24.2 | TCN2 | rs740234 C/T Ancestral: T Minor: G | Frail |

*Notes:* \*Proxy SNP, Cog/Frail – variant was found for both phenotypes in the systematic review, bold text indicates the closest gene

**Supplementary Data Table III. Laboratory Biomarkers found to be statistically significantly different between healthy controls and individuals with cognitive frailty based on MMSE ranked by importance (gain) from Model I**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cognitive Frailty Features** | **Gain** | **Cover** | **Frequency** | **Importance** | **Control Mean** | **SD** | **Cognitive Frailty Mean** | **SD** | **|t|-test** | **Corrected p-value** |
| **TNF-related apoptosis-inducing ligand (pg/mL)-(TRAIL)** | **0.030346550** | **0.028129602** | **0.033898305** | **0.03034655** | **77.35** | **44.29** | **65.53** | **19.93** | **< .0001** | **<0.0001** |
| **24-hour urinary creatinine (mg/24 hours)** | **0.025460108** | **0.014329518** | **0.008474576** | **0.025460108** | **979.14** | **333.91** | **767.17** | **306.40** | **< .0001** | **<0.0001** |
| **Fibrinogen (mg/dL)** | **0.015823506** | **0.009692466** | **0.021186441** | **0.015823506** | **351.80** | **72.83** | **388.15** | **80.03** | **< .0001** | **<0.0001** |
| **Lipids: HDL cholesterol (mg/dL)** | **0.014715469** | **0.011976521** | **0.016949153** | **0.014715469** | **56.27** | **14.72** | **53.80** | **16.43** | **0.0466** | **0.0466** |
| **Cystatin C (mg/L)** | **0.012966575** | **0.012313314** | **0.012711864** | **0.012966575** | **0.99** | **0.26** | **1.26** | **0.51** | **< .0001** | **<0.0001** |
| **Blood urea nitrogen (mg/dL)** | **0.012798018** | **0.015478663** | **0.016949153** | **0.012798018** | **37.14** | **9.44** | **41.67** | **19.73** | **< .0001** | **<0.0001** |
| **Soluble TNF-a receptor I (pg/mL)** | **0.011677247** | **0.005056691** | **0.016949153** | **0.011677247** | **1430.03** | **579.89** | **2091.58** | **82.89** | **<0.0001** | **<0.0001** |
| **Erythrocyte sedimentation rate (ESR) (mm/hour)** | **0.011479619** | **0.027685275** | **0.016949153** | **0.011479619** | **19.30** | **16.32** | **30.90** | **24.75** | **<0.0001** | **<0.0001** |
| **Creatine phosphokinase (U/L)** | **0.011252254** | **0.009828549** | **0.008474576** | **0.011252254** | **99.49** | **59.53** | **79.37** | **54.47** | **<0.0001** | **<0.0001** |
| **Omega-6 fatty acids as % of total fatty acid area** | **0.009927552** | **0.003755798** | **0.004237288** | **0.009927552** | **29.98** | **4.23** | **28.41** | **4.77** | **<0.0001** | **<0.0001** |
| **Dehydroepiandrosterone sulfate (µg/dL)** | **0.009699038** | **0.004094407** | **0.008474576** | **0.009699038** | **87.58** | **67.99** | **66.59** | **58.90** | **<0.0001** | **<0.0001** |
| **25(OH)-D (25-hydroxyvitamin D) (nmol/L)** | **0.009690317** | **0.011910741** | **0.008474576** | **0.009690317** | **52.59** | **36.24** | **35.70** | **29.34** | **<0.0001** | **<0.0001** |
| **Vitamin E alpha tocopherol, (µmol/L)** | **0.008586559** | **0.00937805** | **0.016949153** | **0.008586559** | **34.18** | **7.33** | **31.05** | **7.93** | **<0.0001** | **<0.0001** |
| **Parathyroid hormone, (pg/mL)** | **0.008545621** | **0.011291322** | **0.016949153** | **0.008545621** | **25.32** | **18.84** | **35.26** | **28.42** | **<0.0001** | **<0.0001** |
| **Soluble CD14 (ng/mL)** | **0.007818680** | **0.006603146** | **0.008474576** | **0.00781868** | **1741.70** | **334.78** | **1870.97** | **406.93** | **<0.0001** | **<0.0001** |
| **Uric acid (mg/dL)** | **0.007707399** | **0.004887846** | **0.012711864** | **0.007707399** | **5.13** | **1.37** | **5.47** | **1.76** | **0.009** | **0.0107** |
| **C-terminal telopeptide of type-1 collagen (ng/mL)** | **0.007200276** | **0.009389035** | **0.004237288** | **0.007200276** | **0.49** | **0.25** | **0.68** | **0.41** | **<0.0001** | **<0.0001** |
| **Urine proteins (mg/dL)** | **0.007174622** | **0.011835413** | **0.008474576** | **0.007174622** | **0.98** | **7.78** | **2.80** | **10.35** | **0.0333** | **0.0352** |
| **Total testosterone (ng/mL)** | **0.006692034** | **0.004245555** | **0.008474576** | **0.006692034** | **2.37** | **2.06** | **1.74** | **1.75** | **<0.0001** | **<0.0001** |
| **Resistin (ng/mL)** | **0.006665635** | **0.003066128** | **0.012711864** | **0.006665635** | **3.81** | **1.86** | **4.94** | **2.82** | **<0.0001** | **<0.0001** |
| **Hemoglobin (g/dL)** | **0.006538294** | **0.001687461** | **0.004237288** | **0.006538294** | **13.90** | **1.29** | **12.95** | **1.60** | **<0.0001** | **<0.0001** |
| **Free thyroxine, fT4 (ng/dL)** | **0.006171355** | **0.00694256** | **0.008474576** | **0.006171355** | **1.45** | **0.31** | **1.56** | **0.50** | **0.002** | **0.003** |
| **Fatty acid C20:0 weight (mg/L)** | **0.006114046** | **0.003017798** | **0.008474576** | **0.006114046** | **2.87** | **2.84** | **2.52** | **1.94** | **0.0412** | **0.0427** |
| **Red cell distribution width (RDW) (%)** | **0.006079822** | **0.00257699** | **0.008474576** | **0.006079822** | **13.66** | **0.94** | **14.15** | **1.31** | **<0.0001** | **<0.0001** |
| **MCH concentration (MCHC) (g/dL)** | **0.005308074** | **0.00983547** | **0.004237288** | **0.005308074** | **33.84** | **1.00** | **33.30** | **1.11** | **<0.0001** | **<0.0001** |
| **Urine nitrites** | **0.004714047** | **0.002983739** | **0.004237288** | **0.004714047** | **0.10** | **0.42** | **0.32** | **0.71** | **0.0002** | **0.0003** |
| **Fatty acid C20:5 n-3 as % of total fatty acid weight** | **0.004676963** | **0.00545449** | **0.008474576** | **0.004676963** | **0.63** | **0.23** | **0.55** | **0.17** | **<0.0001** | **<0.0001** |
| **Homocysteine (µmol/L)** | **0.004184592** | **0.001820163** | **0.004237288** | **0.004184592** | **15.46** | **6.66** | **18.84** | **8.18** | **<0.0001** | **<0.0001** |
| **Beta-carotene (µmol/L)** | **0.004176619** | **0.001453662** | **0.008474576** | **0.004176619** | **0.43** | **0.27** | **0.37** | **0.24** | **0.0039** | **0.0052** |
| **Plasma insulin (mIU/L)** | **0.004084085** | **0.005935122** | **0.012711864** | **0.004084085** | **11.47** | **6.05** | **10.50** | **6.27** | **0.0429** | **0.0437** |
| **Alpha-1 globulin (%)** | **0.003832509** | **0.014051952** | **0.004237288** | **0.003832509** | **2.59** | **0.39** | **2.86** | **0.51** | **<0.0001** | **<0.0001** |
| **Total insulin-like growth factor-1, (ng/mL)** | **0.003743268** | **0.00657122** | **0.008474576** | **0.003743268** | **119.35** | **54.96** | **101.45** | **50.44** | **<0.0001** | **<0.0001** |
| **Alpha-2 globulin (%)** | **0.003735570** | **0.004502258** | **0.004237288** | **0.00373557** | **11.21** | **1.25** | **11.71** | **1.55** | **<0.0001** | **<0.0001** |
| **Advanced glycation endproduct (AGE): Carboxymethyl-lysine (ng/mL)** | **0.003691119** | **0.002535915** | **0.008474576** | **0.003691119** | **361.53** | **105.78** | **390.73** | **152.77** | **0.0107** | **0.0122** |
| **Interleukin-1 receptor antagonist (pg/mL)** | **0.003628467** | **0.007143239** | **0.004237288** | **0.003628467** | **151.95** | **111.77** | **194.04** | **178.49** | **0.0011** | **0.0016** |
| **C-reactive protein - high sensitivity (µg/mL)** | **0.003595036** | **0.004072452** | **0.004237288** | **0.003595036** | **4.81** | **8.05** | **7.91** | **13.73** | **0.0018** | **0.0025** |
| **Soluble TNF-a receptor II (pg/mL)** | **0.003553408** | **0.003953066** | **0.008474576** | **0.003553408** | **2709.69** | **709.84** | **3362.15** | **1054.91** | **<0.0001** | **<0.0001** |
| **Serum creatinine (mg/dL)** | **0.002671886** | **0.001135792** | **0.004237288** | **0.002671886** | **0.92** | **0.19** | **0.98** | **0.38** | **0.0217** | **0.0234** |
| **Soluble transferrin receptor (nmol/L)** | **0.002553661** | **0.003976923** | **0.004237288** | **0.002553661** | **16.66** | **5.65** | **18.30** | **8.56** | **0.0097** | **0.0113** |
| **Fatty acid C16:0 as % of total fatty acid weight** | **0.002533683** | **0.003256162** | **0.004237288** | **0.002533683** | **22.44** | **2.36** | **22.98** | **2.62** | **0.008** | **0.0097** |
| **Adiponectin via RIA (µg/mL)** | **0.002423759** | **0.003373917** | **0.004237288** | **0.002423759** | **13.24** | **9.50** | **17.84** | **12.39** | **<0.0001** | **<0.0001** |
| **Ca++ (mg/dL)** | **0.002412787** | **0.009697184** | **0.004237288** | **0.002412787** | **9.46** | **0.45** | **9.32** | **0.50** | **0.0004** | **0.0006** |
| **Alpha-2-macroglobulin (mg/dL)** | **0.002206422** | **0.003378556** | **0.004237288** | **0.002206422** | **210.52** | **68.30** | **223.74** | **68.06** | **0.0122** | **0.0134** |
| **Urinary Ca (mmol/L)** | **0.002203996** | **0.001244438** | **0.004237288** | **0.002203996** | **2.28** | **1.64** | **1.83** | **1.47** | **0.0004** | **0.0006** |
| **Beta globulins (%)** | **0.001988610** | **0.002508954** | **0.004237288** | **0.00198861** | **11.94** | **1.18** | **12.25** | **1.55** | **0.0065** | **0.0085** |
| **Albumin (%)** | **0.001497865** | **0.00144821** | **0.004237288** | **0.001497865** | **58.98** | **0.38** | **56.96** | **4.01** | **<0.0001** | **<0.0001** |
| **Lymphocytes (%)** | **0.001375674** | **0.000826441** | **0.008474576** | **0.001375674** | **30.92** | **8.02** | **28.56** | **8.17** | **0.0002** | **0.0003** |
| **White blood cells (WBC) (n, K/µL)** | **0.001029476** | **0.000265338** | **0.004237288** | **0.001029476** | **6.08** | **1.55** | **6.44** | **1.76** | **0.007** | **0.0089** |
| **Lipids: LDL cholesterol (mg/dL)** | **0.000972468** | **0.004258281** | **0.004237288** | **0.000972468** | **138.00** | **33.95** | **127.04** | **34.78** | **<0.0001** | **<0.0001** |
| **IGF binding protein-3, (ng/mL)** | **0.000969549** | **0.002657776** | **0.008474576** | **0.000969549** | **4279.38** | **1121.16** | **4009.81** | **1077.64** | **0.0018** | **0.0025** |
| **Omega-3 plasma fatty acid weight (mg/L)** | **0.000945649** | **0.000533488** | **0.004237288** | **0.000945649** | **109.63** | **42.53** | **96.43** | **34.25** | **<0.0001** | **<0.0001** |
| **Mean platelet volume (MPV) (fL)** | **0.000596140** | **0.000315899** | **0.004237288** | **0.00059614** | **11.14** | **0.97** | **10.94** | **1.00** | **0.0079** | **0.0097** |

Note: Significant model results ranked by importance defined as gain with the univariate results, gain is the features importance in predicting cognitive frailty.

**Supplementary Data Table IV. Laboratory Biomarkers found to be statistically significantly different between healthy controls and individuals with cognitive frailty based on TMT-A and B ranked by importance (gain) from Model II**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cognitive Frailty Features** | **Gain** | **Cover** | **Frequency** | **Importance** | **TrailA** | **Control Mean** | **SD** | **Cognitive Frailty Mean** | **SD** | **|t|-test** | **Corrected p-value** | **TrailB** | **Control Mean** | **SD** | **Cognitive Frailty Mean** | **SD** | **|t|-test** | **Corrected p-value** |
| **Interleukin-6 via ELISA ultrasensitive (pg/mL)** | **0.041790268** | **0.019616729** | **0.014035088** | **0.041790268** |  | **1.57** | **1.81** | **3.14** | **7.22** | **0.0003** | **0.0004** |  | **1.46** | **1.77** | **2.42** | **2.57** | **< .0001** | **< .0001** |
| **Creatinine clearance, 24-hr urine (mL/minute)** | **0.030831483** | **0.018261243** | **0.014035088** | **0.030831483** |  | **88.69** | **29.71** | **68.68** | **25.64** | **<0.0001** | **<0.0001** |  | **9.48** | **30.34** | **74.67** | **25.66** | **< .0001** | **< .0001** |
| **Erythrocyte sedimentation rate (ESR) (mm/hour)** | **0.02106198** | **0.017790494** | **0.014035088** | **0.02106198** |  | **17.59** | **15.96** | **25.67** | **21.42** | **<0.0001** | **<0.0001** |  | **16.67** | **14.84** | **23.32** | **20.66** | **< .0001** | **< .0001** |
| **Creatine phosphokinase (U/L)** | **0.019570487** | **0.025241618** | **0.021052632** | **0.019570487** |  | **110.89** | **92.60** | **88.84** | **53.96** | **<0.0001** | **<0.0001** |  | **115.10** | **99.11** | **91.46** | **54.64** | **< .0001** | **< .0001** |
| **C-terminal telopeptide of type-1 collagen (ng/mL)** | **0.019104806** | **0.01358605** | **0.014035088** | **0.019104806** |  | **0.44** | **0.21** | **0.59** | **0.35** | **<0.0001** | **<0.0001** |  | **0.43** | **0.20** | **0.53** | **0.31** | **< .0001** | **< .0001** |
| **Cystatin C (mg/L)** | **0.016738529** | **0.018435368** | **0.028070175** | **0.016738529** |  | **0.91** | **0.21** | **1.13** | **0.42** | **<0.0001** | **<0.0001** |  | **0.88** | **0.19** | **1.07** | **0.35** | **< .0001** | **< .0001** |
| **"MCH concentration" (MCHC) (g/dL)** | **0.016397158** | **0.008585323** | **0.010526316** | **0.016397158** |  | **34.01** | **1.00** | **33.54** | **0.96** | **<0.0001** | **<0.0001** |  | **34.07** | **1.00** | **33.67** | **0.96** | **< .0001** | **< .0001** |
| **Vitamin B6 (ng/mL)** | **0.016303861** | **0.020917254** | **0.01754386** | **0.016303861** |  | **8.12** | **6.21** | **5.75** | **5.47** | **<0.0001** | **<0.0001** |  | **8.48** | **6.28** | **6.08** | **5.79** | **< .0001** | **< .0001** |
| **C-reactive protein - high sensitivity (µg/mL)** | **0.014035509** | **0.015325407** | **0.01754386** | **0.014035509** |  | **4.01** | **6.14** | **7.11** | **14.37** | **0.0004** | **0.0005** |  | **3.77** | **6.02** | **5.83** | **9.44** | **0.0004** | **.0006** |
| **Total testosterone (ng/mL)** | **0.013534893** | **0.018254719** | **0.010526316** | **0.013534893** |  | **2.57** | **2.17** | **1.85** | **1.87** | **<0.0001** | **<0.0001** |  | **2.68** | **2.20** | **1.87** | **1.84** | **< .0001** | **< .0001** |
| **Vitamin E alpha tocopherol, (µmol/L)** | **0.013064167** | **0.012399389** | **0.010526316** | **0.013064167** |  | **33.91** | **7.54** | **32.86** | **7.43** | **0.0367** | **0.0367** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| **Albumin (%)** | **0.012957738** | **0.006378732** | **0.010526316** | **0.012957738** |  | **59.71** | **3.34** | **58.09** | **3.51** | **<0.0001** | **<0.0001** |  | **59.99** | **3.27** | **58.52** | **3.45** | **< .0001** | **< .0001** |
| **Dehydroepiandrosterone sulfate (µg/dL)** | **0.012658594** | **0.011407339** | **0.007017544** | **0.012658594** |  | **116.98** | **95.79** | **77.99** | **67.71** | **<0.0001** | **<0.0001** |  | **125.8** | **100.15** | **80.97** | **67.13** | **< .0001** | **< .0001** |
| **Blood glucose (mg/dL)** | **0.012532317** | **0.010288275** | **0.014035088** | **0.012532317** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |  | **92.18** | **24.40** | **97.77** | **29.05** | **0.0028** | **0.0034** |
| **Alpha-2-macroglobulin (mg/dL)** | **0.009632353** | **0.015012622** | **0.010526316** | **0.009632353** |  | **201.68** | **64.3** | **221.07** | **73.22** | **<0.0001** | **<0.0001** |  | **197.37** | **61.88** | **223.54** | **74.06** | **< .0001** | **< .0001** |
| **Soluble TNF-a receptor II (pg/mL)** | **0.009249448** | **0.006819346** | **0.010526316** | **0.009249448** |  | **2473.75** | **654.70** | **3059.1** | **924.41** | **<0.0001** | **<0.0001** |  | **2399.93** | **623.03** | **2903.60** | **862.17** | **< .0001** | **< .0001** |
| **Soluble TNF-a receptor I (pg/mL)** | **0.008910896** | **0.011338502** | **0.007017544** | **0.008910896** |  | **1248.81** | **471.88** | **1763.25** | **914.92** | **<0.0001** | **<0.0001** |  | **1191.01** | **432.04** | **1592.49** | **741.76** | **< .0001** | **< .0001** |
| **Folate via RIA (ng/mL)** | **0.008433119** | **0.009100673** | **0.01754386** | **0.008433119** |  | **3.41** | **2.14** | **3.02** | **1.70** | **0.0013** | **0.0017** |  | **3.43** | **2.14** | **3.07** | **1.91** | **0.0078** | **.0087** |
| **24-hour urinary creatinine (mg/24 hours)** | **0.008193473** | **0.007541586** | **0.014035088** | **0.008193473** |  | **1082.84** | **374.84** | **833.83** | **294.48** | **<0.0001** | **<0.0001** |  | **1119.54** | **381.66** | **902.09** | **314.87** | **< .0001** | **< .0001** |
| **25(OH)-D (25-hydroxyvitamin D) (nmol/L)** | **0.007746372** | **0.010792335** | **0.014035088** | **0.007746372** |  | **57.82** | **36.01** | **40.97** | **34.34** | **<0.0001** | **<0.0001** |  | **58.33** | **33.73** | **47.92** | **40.02** | **< .0001** | **< .0001** |
| **Omega-6 fatty acids as % of total fatty acid weight** | **0.007517237** | **0.005039685** | **0.007017544** | **0.007517237** |  | **33.57** | **4.45** | **31.76** | **4.64** | **<0.0001** | **<0.0001** |  | **33.93** | **4.42** | **32.32** | **4.52** | **< .0001** | **< .0001** |
| **TNF-related apoptosis-inducing ligand (pg/mL)-(TRAIL)** | **0.00719579** | **0.004106597** | **0.014035088** | **0.00719579** |  | **76.51** | **42.76** | **71.47** | **19.74** | **0.0064** | **0.0074** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| **Interleukin-18 (pg/mL)** | **0.007054061** | **0.014543542** | **0.010526316** | **0.007054061** |  | **386.20** | **149.66** | **411.61** | **156.28** | **0.0146** | **0.0154** |  | **382.19** | **150.56** | **402.02** | **147.03** | **0.0478** | **.0478** |
| **24-hour urinary cortisol (µg/24 hours)** | **0.00650171** | **0.007939441** | **0.010526316** | **0.00650171** |  | **108.98** | **32.17** | **93.34** | **57.87** | **0.0001** | **0.0001** |  | **109.66** | **50.09** | **96.01** | **67.41** | **0.0018** | **0.0023** |
| **Serum cortisol (µg/dL)** | **0.00583663** | **0.007164907** | **0.014035088** | **0.00583663** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |  | **13.67** | **5.06** | **12.73** | **4.37** | **0.0026** | **0.0033** |
| **Soluble CD14 (ng/mL)** | **0.005597229** | **0.008704836** | **0.010526316** | **0.005597229** |  | **1670.14** | **331.90** | **1824.72** | **386.26** | **<0.0001** | **<0.0001** |  | **1653.78** | **323.41** | **1760.93** | **361.97** | **< .0001** | **< .0001** |
| **Urinary creatinine (mg/dL)** | **0.005467574** | **0.004446002** | **0.003508772** | **0.005467574** |  | **78.09** | **38.10** | **66.78** | **32.12** | **<0.0001** | **<0.0001** |  | **80.74** | **39.52** | **68.38** | **33.02** | **< .0001** | **< .0001** |
| **Homocysteine (µmol/L)** | **0.005058175** | **0.01368253** | **0.014035088** | **0.005058175** |  | **14.16** | **5.57** | **17.39** | **7.78** | **<0.0001** | **<0.0001** |  | **13.88** | **5.59** | **16.17** | **6.47** | **< .0001** | **< .0001** |
| **Urinary Na (mmol/L)** | **0.005040676** | **0.00568806** | **0.003508772** | **0.005040676** |  | **99.50** | **45.59** | **90.34** | **38.51** | **0.0014** | **0.0018** |  | **101.58** | **44.78** | **88.47** | **39.39** | **< .0001** | **< .0001** |
| **Alpha-1 globulin (%)** | **0.004905499** | **0.00071443** | **0.003508772** | **0.004905499** |  | **2.53** | **0.38** | **2.69** | **0.43** | **<0.0001** | **<0.0001** |  | **2.51** | **0.39** | **2.64** | **0.43** | **< .0001** | **< .0001** |
| **Fatty acid C20:5 n-3 as % of total fatty acid area** | **0.004842838** | **0.004365281** | **0.007017544** | **0.004842838** |  | **0.48** | **0.20** | **0.44** | **0.19** | **0.027** | **0.0275** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| **Lymphocytes (%)** | **0.00400406** | **0.005602415** | **0.003508772** | **0.00400406** |  | **31.96** | **8.07** | **29.53** | **8.13** | **<0.0001** | **<0.0001** |  | **32.45** | **8.16** | **29.87** | **8.16** | **< .0001** | **< .0001** |
| **Neutrophils (%)** | **0.00388351** | **0.007716045** | **0.010526316** | **0.00388351** |  | **59.15** | **8.64** | **61.46** | **8.47** | **<0.0001** | **<0.0001** |  | **58.64** | **8.83** | **61.19** | **8.42** | **< .0001** | **< .0001** |
| **Fibrinogen (mg/dL)** | **0.003655515** | **0.008378834** | **0.010526316** | **0.003655515** |  | **339.88** | **72.16** | **367.95** | **78.12** | **<0.0001** | **<0.0001** |  | **334.56** | **73.08** | **360.54** | **72.38** | **< .0001** | **< .0001** |
| **Omega-6 plasma fatty acid weight (mg/L)** | **0.00365299** | **0.002054096** | **0.003508772** | **0.00365299** |  | **1069.85** | **241.60** | **1022.35** | **216.50** | **0.0024** | **0.0029** |  | **1086.09** | **239.82** | **1034.56** | **223.86** | **0.0016** | **0.0021** |
| **Hematocrit (%)** | **0.003643905** | **0.003572639** | **0.003508772** | **0.003643905** |  | **41.02** | **3.28** | **40.06** | **3.64** | **<0.0001** | **<0.0001** |  | **41.07** | **3.23** | **40.51** | **3.48** | **0.0147** | **0.0153** |
| **Adiponectin (µg/mL)** | **0.003007961** | **0.003092498** | **0.003508772** | **0.003007961** |  | **12.66** | **9.11** | **15.85** | **11.71** | **<0.0001** | **<0.0001** |  | **12.41** | **9.13** | **14.66** | **11.45** | **0.0028** | **0.0034** |
| **Parathyroid hormone, (pg/mL)** | **0.002996903** | **0.008428031** | **0.007017544** | **0.002996903** |  | **22.68** | **16.32** | **31.12** | **23.23** | **<0.0001** | **<0.0001** |  | **22.36** | **17.44** | **28.13** | **17.47** | **< .0001** | **< .0001** |
| **Omega-3 fatty acids as % of total fatty acid area** | **0.002933676** | **0.009319042** | **0.007017544** | **0.002933676** |  | **2.04** | **0.62** | **1.88** | **0.56** | **0.0002** | **0.0003** |  | **2.07** | **0.63** | **1.96** | **0.60** | **0.0049** | **0.0056** |
| **Interleukin-1 receptor antagonist (pg/mL)** | **0.002864988** | **0.008290595** | **0.007017544** | **0.002864988** |  | **146.60** | **97.39** | **177.16** | **154.61** | **0.0015** | **0.0019** |  | **142.80** | **95.94** | **174.54** | **153.13** | **0.0007** | **0.001** |
| **Uric acid (mg/dL)** | **0.002571323** | **0.005440035** | **0.003508772** | **0.002571323** |  | **4.98** | **1.28** | **5.22** | **1.56** | **0.0148** | **0.0154** |  | **4.93** | **1.29** | **5.19** | **1.36** | **0.0036** | **0.0042** |
| **Resistin via EIA (ng/mL)** | **0.00233496** | **0.005168944** | **0.007017544** | **0.00233496** |  | **3.75** | **1.87** | **4.33** | **2.19** | **<0.0001** | **<0.0001** |  | **3.67** | **1.66** | **4.05** | **2.23** | **0.0094** | **0.01** |
| **IGF binding protein-3, (ng/mL)** | **0.00204864** | **0.002395909** | **0.003508772** | **0.00204864** |  | **4452.55** | **1077.41** | **4158.20** | **1124.84** | **0.0001** | **0.0001** |  | **4517.72** | **1060.85** | **4238.12** | **1166.19** | **0.0004** | **0.0006** |
| **Blood urea nitrogen (mg/dL)** | **0.001848406** | **0.00356917** | **0.007017544** | **0.001848406** |  | **32.61** | **7.77** | **37.42** | **14.90** | **<0.0001** | **<0.0001** |  | **32.12** | **7.20** | **0.27** | **13.06** | **< .0001** | **< .0001** |
| **Lycopene (µmol/L)** | **0.001844727** | **0.004465825** | **0.003508772** | **0.001844727** |  | **0.71** | **0.34** | **0.65** | **0.31** | **0.0042** | **0.005** |  | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| **Endogenous secretory receptor for AGEs (ng/mL)** | **0.001712395** | **0.000343816** | **0.003508772** | **0.001712395** |  | **0.43** | **0.2** | **0.48** | **0.25** | **0.0077** | **0.0083** |  | **0.43** | **0.18** | **0.48** | **0.27** | **0.0086** | **0.0094** |
| **Estradiol (pg/mL)** | **0.001620834** | **0.002680514** | **0.003508772** | **0.001620834** |  | **13.29** | **15.99** | **9.52** | **7.18** | **<0.0001** | **<0.0001** |  | **14.30** | **17.34** | **9.35** | **6.52** | **< .0001** | **< .0001** |
| **Urinary Ca (mmol/L)** | **0.001387468** | **0.004556416** | **0.003508772** | **0.001387468** |  | **2.50** | **1.74** | **1.90** | **1.18** | **<0.0001** | **<0.0001** |  | **2.52** | **1.63** | **2.15** | **1.64** | **0.0001** | **0.0001** |
| **Total insulin-like growth factor-1, (ng/mL)** | **0.001353188** | **0.004284569** | **0.007017544** | **0.001353188** |  | **139.07** | **69.69** | **106.54** | **49.24** | **<0.0001** | **<0.0001** |  | **145.42** | **71.16** | **113.21** | **55.61** | **< .0001** | **< .0001** |
| **Urinary cortisol (µg/mL)** | **0.001072581** | **0.004113434** | **0.003508772** | **0.001072581** |  | **1082.84** | **374.84** | **833.83** | **294.48** | **<0.0001** | **<0.0001** |  | **1119.54** | **381.66** | **902.09** | **314.87** | **< .0001** | **< .0001** |

Note: Significant model results ranked by importance as defined by gain with the univariate results, gain is the features importance in predicting cognitive frailty; NS = not significant

References

1. Ferrucci L, Bandinelli S, Benvenuti E, et al. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc*. 2000;48(12):1618-1625.

2. Kelaiditi E, Cesari M, Canevelli M, et al. Cognitive frailty: rational and definition from an (I.A.N.A./I.A.G.G.) international consensus group. *J Nutr Health Aging*. 2013;17(9):726-734. doi:10.1007/s12603-013-0367-2

3. Sargent L, Nalls M, Starkweather A, et al. Shared biological pathways for frailty and cognitive impairment: A systematic review. *Ageing Res Rev*. 2018;47:149-158. doi:10.1016/J.ARR.2018.08.001

4. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: a review and practical application. *Aging health*. 2008;4(3):311-320. doi:10.2217/1745509X.4.3.311

5. Lewinsohn PM, Seeley JR, Roberts RE, Allen NB. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychol Aging*. 1997;12(2):277-287. doi:10.1037/0882-7974.12.2.277

6. Radloff LS. A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas*. 1977;1(3):385-401. doi:10.1177/014662167700100306

7. Delrieu J, Andrieu S, Cantet C, Cesari M, Ousset P.J., Voisin T, Fougere B, Gillette S, Carrie I and VB. Neuropsychological Profile of “Cognitive Frailty” Subjects in MAPT Study. 2016;116(8):1477-1490. doi:10.14283/jpad.2016.94

8. Reitan RM. *Validity of the Trail Making Test as an Indicator of Organic Brain Damage*. Vol 8. @ Southern Universities Press; 1958.

9. Ashendorf L, Jefferson AL, Connor MKO, et al. Trail Making Test Errors in Normal Aging, Mild Cognitive Impairment, and Dementia. 2008;23(2):129-137. doi:10.1016/j.acn.2007.11.005.Trail

10. Strauss E, Sherman EMS, Spreen O. *A Compendium of Neuropsychologial Tests: Adiminstration, Norms, and Commentary*. Oxford University Press; 2006.

11. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-56.

12. Ferrucci L, Bandinelli S, Benvenuti E, et al. Subsystems Contributing to the Decline in Ability to Walk: Bridging the Gap Between Epidemiology and Geriatric Practice in the InCHIANTI Study. *J Am Geriatr Soc*. 2000;48(12):1618-1625. doi:10.1111/j.1532-5415.2000.tb03873.x

13. Stenholm S, Ferrucci L, Vahtera J, et al. Natural Course of Frailty Components in People Who Develop Frailty Syndrome: Evidence From Two Cohort Studies. *Journals Gerontol Ser A*. August 2018. doi:10.1093/gerona/gly132

14. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189-198.

15. Chen, Tianqi, He, Tong, Benesty, Michael, Khotilovich, Vadim, Tang Y. ’xgboost’-Extreme Gradient Boosting. 2017. doi:10.1145/2939672.2939785>

16. Friedman J, Hastie T, Tibshirani R. Additive logistic regression: a statistical view of boosting (With discussion and a rejoinder by the authors). *Ann Stat*. 2000;28(2):337-407.

17. Purcell, S, Neale, B, Todd-Brown, K, Thomas, L, Ferreira, MAR, Bender, D, Maller, J, Sklar, P, de Bakker, PIW, Daly, MJ, Sham P. PLINK: a toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet*. 2007;81.

18. Marschner I. ’glm2’-Fitting GEneralized Linear Models.

19. Christensen R. ’Ordinal’- Regression Models for Ordinal Data.

20. Purcell S. PLINK V1.07.