**BIOCARD Data Analysis Proposal**

**‘Relationship of physical activity to longitudinal rates of change in   
AD-specific and AD non-specific blood biomarkers’**

**Overall Goal of Analysis**

The goal of these analyses is to examine whether measures of physical activity relate to rates of change in blood-based biomarkers of Alzheimer’s disease (AD) pathology, neuronal injury, astrocytic activation, and neuroinflammation over time among individuals who were cognitively unimpaired at their baseline (i.e., first available) physical activity measure. As a secondary goal, the analyses will examine whether APOE-e4 genetic status (i.e., the largest genetic risk factor for late-onset AD) or sex impact the relationship between physical activity and longitudinal blood biomarker changes. Physical activity engagement, our primary predictor of interest, is measured by two separate methods: (1) self-reported frequency of engagement in physical activities, as reported through the CHAMPS activity questionnaire; and (2) total volume of physical activity, as measured objectively using actigraphy. Longitudinal blood biomarker values will be usedas the outcome measures. All data come from the BIOCARD Study.

The two measures of AD pathology are:

* AB42AB40: a measure of amyloid protein accumulation in the brain (this variable reflects the ratio of amyloid beta 42 over amyloid beta 40); this measure decreases in blood in individuals with AD.
* PTAU181: a measure of phosphorylated tau, which increases in AD.

The measure of neurodegeneration is:

* NFL: neurofilament light chain; levels increase in AD and other neurodegenerative diseases.

The two measures of neuroinflammation are:

* YKL40: also known as chitinase-3-like protein 1; higher levels tend to indicate more inflammation and levels tend to increase in AD.
* sTREM2: soluble triggering receptor expressed on myeloid cells 2; higher levels correspond to more inflammation and levels tend to increase in AD.

The measure of astrocytic activation is:

* GFAP: glial fibrillary acidic protein; levels increase in AD and higher levels indicate a greater cellular response to damage in the nervous system.

**Relevant Data Files**

Demographics – *BIOCARD\_Demographics\_2024.08.07\_Deidentified.xlsx*

* Variables:
  + - ‘Sex’ (recode as ‘Sex\_F’ where Female = 1, Male = 0)
    - ‘EDUC’
    - ‘Race’ (recode as ‘Race\_White’ where White (coded as 1 in the datafile) = 1, otherwise 0 (note: if 99 or missing, set to missing)

Diagnosis – *BIOCARD\_DiagnosisData\_2024.09.08\_Deidentified.xlsx*

* Variables:
  + - ‘AgeAtVisit’ (specifies participant age at each visit)
    - ‘Diagnosis’
    - ‘DECAGE’ (age at which participant developed clinical symptoms of Mild Cognitive Impairment, for those who developed cognitive impairment over the course of study follow-up)
    - Create dichotomous ‘fup\_Dx’ indicator: 1 if last available (i.e., *most recent*) ‘DIAGNOSIS’ = MCI or dementia, otherwise 0

Blood biomarkers – *BIOCARD\_NTK Blood biomarkers\_08.09.24\_JHU only.xlsx*

* Variables:
  + - AD-specific measures (*n* = 2): ‘PTAU181\_zscore’, ‘AB42AB40\_zscore’
    - AD non-specific measures (*n* = 4): ‘NFL\_zscore’, ‘YKL40\_zscore’, ‘sTREM2\_zscore’, ‘GFAP\_zscore’
    - Outlier indicators for each blood biomarker measure: ‘PTAU181\_outlier’, ‘AB42AB40\_outlier’, ‘NFL\_outlier’, ‘YKL40\_outlier’, ‘sTREM2\_outlier’, ‘GFAP\_outlier’

CHAMPS activity questionnaire (self-reported measure of physical activity) – *BIOCARD\_Leisure\_Activity\_Champs\_2023.09.10\_other coded\_Deidentified.xlsx*

* Create three continuous variables reflecting each participant’s frequency of engagement in low intensity physical activities, high intensity physical activities, and all physical activities (regardless of level of intensity):
  + - ‘LOW\_INT\_FREQ’ = ‘B11110a\_freq’ + ‘B11120a\_freq’ + ‘B11122a\_freq’ + ‘B11127a\_freq’ + ‘B11128a\_freq’ + ‘B11134a\_freq’ + ‘B11135a\_freq’ + ‘B11139a\_freq’ + (‘B11141a\_freq’ IF ‘B11141s\_CAT’ = ‘Physical-low’)
    - ‘HIGH\_INT\_FREQ’ = ‘B11107a\_freq’ + ‘B11109a\_freq’ + ‘B11114a\_freq’ + ‘B11115a\_freq’ + ‘B11116a\_freq’ + ‘B11119a\_freq’ + ‘B11121a\_freq’ + ‘B11123a\_freq’ + ‘B11124a\_freq’ + ‘B11125a\_freq’ + ‘B11126a\_freq’ + ‘B11129a\_freq’ + ‘B11130a\_freq’ + ‘B11131a\_freq’ + ‘B11132a\_freq’ + ‘B11133a\_freq’ + ‘B11136a\_freq’ + ‘B11137a\_freq’ + ‘B11138a\_freq’ + ‘B11140a\_freq’ + (‘B11141a\_freq’ IF ‘B11141s\_CAT’ = ‘Physical-modhi’)
    - ‘ALL\_INT\_FREQ’ = Sum of **all measures** included in the ‘LOW\_INT\_FREQ’ and ‘HIGH\_INT\_FREQ’ composite variables
* Note: if of interest, the method used to score the CHAMPS activity questionnaire data is described in this document: *BIOCARD\_CHAMPS\_Data Coding\_01.27.2025.docx*

Actigraphy (objective measure of physical activity) – *actigraphy\_data\_BIOCARD\_8\_7\_24\_from Daniel\_Deidentified.xlsx*

* Variable – ‘LTAC10’
  + - This represents total average activity counts over the 10 most active hours of the day, averaged across 3 days with valid data (the number of valid days is indicated by the ‘ndays’ variable).

Genetics (APOE genetic status) – *BIOCARD\_Genetics\_Data\_2023.03.28\_Deidentified.xlsx* **and** *New participants\_BIOCARD ApoE Genotypes 2023-2024\_Deidentified.xlsx*

* Variable – ‘APOECODE’
  + - Create a dichotomous ‘APOE4’ indicator: 0 if APOECODE = 2.2, 2.3, or 3.3;

1 if APOECODE = 2.4, 3.4, or 4.4 (note: if blank, set to missing)

* + - Individuals with one or two copies of the e4 allele of the *APOE* gene are at increased risk of AD compared to those with no e4 copies; having 2 copies is associated with greater risk than having 1.

Vital Signs/Sensory – *BIOCARD\_Vital\_Signs\_Sensory\_2023.04.17\_Deidentified.xlsx*

* Variable – ‘BMI’
  + - BMI will be included as a covariate because it can influence blood biomarker values, and may also correlate with physical activity levels.

**Participants in Analyses:**

* The primary **analytic sample** will be based on participants who have at least one CHAMPS assessment AND have blood biomarkers collected within 1.5 years of their baseline (i.e., first available) CHAMPS assessment (excluding values identified as extreme outliers, as noted below).
* Participants in actigraphy analyses will include the *subset of participants in the CHAMPS analyses* who had at least one actigraphy assessment.
* **Exclude the following:** participants with a diagnosis of MCI or dementia at their first available CHAMPS measure.

**Definition of Baseline**

* For the CHAMPS analyses (Part I): **‘baseline’** refers to *first available* CHAMPS assessment
  + - **Create ‘Age\_CHAMPS’ variable**, reflecting a participant’s age at their baseline CHAMPS assessment
* For actigraphy analyses (Part II): **‘baseline’** *first available* actigraphy assessment
  + - **Create ‘Age\_Act’ variable**, reflecting a participant’s age at their baseline actigraphy assessment

**Data Merging**

* For CHAMPS analyses (Part I): Where possible, merge datafiles using participant identifier (‘SUBJECT\_ID’) and visit number (‘VISITNO’). If a participant does not have blood biomarker measures collected at the same visit as their baseline CHAMPS assessment or their baseline actigraphy assessment, use blood biomarker values collected up to 1.5 years *before* the baseline CHAMPS measure. If a participant does not have blood biomarker measures collected at the same visit or up to 1.5 years before their baseline CHAMPS, use blood biomarker values collected up to 1.5 years *after* the baseline CHAMPS.
* For actigraphy analyses (Part II): Find the **subset of CHAMPS/Part I participants** that also have an actigraphy measure. Each participant’s first available actigraphy measure will be their ‘baseline’. As above, if a participant does not have blood biomarker measures collected at the same visit as their baseline actigraphy assessment, use blood biomarker values collected up to 1.5 years *before* the baseline actigraphy measure. If a participant does not have blood biomarker measures collected at the same visit or up to 1.5 years before their baseline actigraphy, use blood biomarker values collected up to 1.5 years *after* the baseline actigraphy.
  + - Note: we anticipate that the majority of participants will have blood biomarker values at the *same* visit as their baseline CHAMPS and baseline actigraphy assessment. When this is not the case, we are using the above-described matching in order to maximize the amount of longitudinal blood biomarker data included in the analyses.

**Exclusion of Blood Biomarker Outliers**

* Separately for each blood biomarker variable, exclude values identified as extreme outliers as per the corresponding variable names in the datafile (e.g., ‘{biomarker name}\_outlier’). For example, for PTAU181, outliers are coded 1 in the ‘PTAU181\_outlier’ column; for YKL40, outliers are coded 1 in the ‘YKL40\_outlier’ column, etc.

**Outcome Variables of Interest**

* Blood biomarkers collected over time (i.e., from ‘baseline’ physical activity measure to present)
* Standardization of each outcome variables: log-transform then z-score based on all available blood biomarker measures over time

**Primary Analytic Method:** Linear mixed effect regression models for examining baseline levels of, and rate of change in blood biomarker measures over time. Include random intercepts and slopes.

* For these analyses, baseline age refers to either Age\_CHAMPS or Age\_Act, depending on whether CHAMPS or actigraphy variables are being used as model predictors
* For model predictors: standardize all continuous variables before model fitting, **except for** **time**, which will be modeled in the unit of years since baseline (for easier interpretation)

**PART I: CHAMPS and Rate of Change in Blood Biomarker Measures Over Time**

**Model 1) CHAMPS and Blood Biomarker Trajectories Over Time**

Model description: Using all available blood biomarker measures over time as the dependent variable, run separate mixed effect regression models for the 6 blood biomarker measures listed above.

* Model predictors: Age\_CHAMPS, Sex\_F, APOE4, BMI, {CHAMPS measure}, time, and the interaction of each covariate with time
* Run separate models for each of the 3 {CHAMPS measure}s: ‘LOW\_INT\_FREQ’, ‘HIGH\_INT\_FREQ’, ‘ALL\_INT\_FREQ’
* Note: the time x time interaction does not need to be included
* In models for which there is a significant main effect of a {CHAMPS measure}, or a significant {CHAMPS measure}\*time interaction: create a figure depicting the covariate-adjusted associations by plotting the blood biomarker values over time separately for individuals with high vs. low values on the {CHAMPS measure}, by median split.

**Model 2) Interactions between CHAMPS and APOE4 in Relation to Blood Biomarker Trajectories**

Model description: Same as **Model 1** above, this time including the following interaction terms as *additional* model predictors: {CHAMPS measure}\*APOE4 and {CHAMPS measure}\*APOE4\*time. Please provide all model results (even if the interaction term is not significant). If the 3-way interaction is significant (*p* < 0.05), please run stratified analyses by APOE4 genetic status (i.e., carriers vs. non-carriers).

**Model 3) Interactions between CHAMPS and Sex in Relation to Blood Biomarker Trajectories**

Model description: Same as **Model 1** above, this time including the following interaction terms as *additional* model predictors: {CHAMPS measure}\*Sex\_F and {CHAMPS measure}\*Sex\_F\*time. Please provide all model results (even if the interaction term is not significant). If the 3-way interaction is significant (*p* < 0.05), please run stratified analyses by sex.

**Descriptive Statistics for Participants Included in the CHAMPS Analyses – Model 1**

Provide descriptive statistics for all participants included in Model 1.

|  |  |
| --- | --- |
|  | Participants in analyses |
| *N* | 209 |
| *N* with a diagnosis of MCI or dementia at last visit (‘fup\_Dx’ = 1) | 91 |
| *N* participants with ‘Subject\_ID’ ≥ 400 \* |  |
| Mean (SD) Age\_CHAMPS |  |
| Range (min, max) Age\_CHAMPS |  |
| N (%) female sex (‘Sex\_F’ = 1) |  |
| Mean (SD) years of education (‘EDUC’) |  |
| N (%) White race (‘Race\_White’ = 1) |  |
| N (%) APOE4 carriers (‘APOE4’ = 1) |  |
| Mean (SD) years of follow-up (baseline CHAMPS to last blood biomarker measure) |  |
| Range (min, max) years of follow-up (baseline CHAMPS to last blood biomarker measure) |  |
| Mean (SD) number of blood biomarker measures over time |  |
| Range (min, max) number of blood biomarker measures over time |  |
| Mean (SD) time (in years) between baseline CHAMPS and associated baseline blood biomarker measure |  |
| Range (min, max) time (in years) between baseline CHAMPS and associated baseline blood biomarker measure |  |
| Mean (SD) LOW\_INT\_FREQ |  |
| Mean (SD) HI\_INT\_FREQ |  |
| Mean (SD) ALL\_INT\_FREQ |  |
| Mean (SD) BMI |  |
| Mean (SD) GFAP at baseline CHAMPS |  |
| Mean (SD) NFL at baseline CHAMPS |  |
| Mean (SD) PTAU181 at baseline CHAMPS |  |
| Mean (SD) AB42AB40 at baseline CHAMPS |  |
| Mean (SD) PTAU181\_AB42AB40 at baseline CHAMPS |  |
| Mean (SD) sTREM2 at baseline CHAMPS |  |
| Mean (SD) YKL40 at baseline CHAMPS |  |

\* Individuals with a SUBJECT\_ID > 400 are recent study enrollees. If any of these participants are include in the analyses, we will need to describe their recruitment/enrollment in the manuscript text. We’ve included this item as a reminder to describe this, if needed.

**PART II:** **Actigraphy and Rate of Change in Blood Biomarker Measures Over Time**

The models below are identical to the CHAMPS models described above, but they use the actigraphy based measure of physical activity rather than the CHAMPS self-report measures. Because an individual may have had their CHAMPS and actigraphy data collected in different years, the definition of ‘baseline’ for the blood biomarker measures may be different than the definition of ‘baseline’ used in Part I.

**Model 4) Actigraphy and Blood Biomarker Trajectories Over Time**

Model description: Using all available blood biomarker measures over time as the dependent variable, run separate mixed effect regression models for the 6 blood biomarker measures listed above.

* Model predictors: Age\_Act, Sex\_F, APOE4, BMI, LTAC10, time, and the interaction of each covariate with time
* Note: the time x time interaction does not need to be included
* In models for which there is a significant main effect of the {actigraphy measure}, or a significant {actigraphy measure}\*time interaction: create a figure depicting the covariate-adjusted associations by plotting the blood biomarker values over time separately for individuals with high vs. low values on the {CHAMPS measure}, by median split.

**Model 5) Interactions between Actigraphy and APOE4 in Relation to Blood Biomarker Trajectories**

Model description: Same as **Model 4** above, this time including the following interaction terms as *additional* model predictors: LTAC10\*APOE4 and LTAC10\*APOE4\*time. Please provide all model results (even if the interaction term is not significant). If the 3-way interaction is significant (*p* < 0.05), please run stratified analyses by APOE4 genetic status (i.e., carriers vs. non-carriers).

**Model 6) Interactions between Actigraphy and Sex in Relation to Blood Biomarker Trajectories**

Model description: Same as **Model 4** above, this time including the following interaction terms as *additional* model predictors: LTAC10\*Sex\_F and LTAC10\*Sex\_F\*time. Please provide all model results (even if the interaction term is not significant). If the 3-way interaction is significant (*p* < 0.05), please run stratified analyses by sex.

**Descriptive Statistics for Participants Included in the Actigraphy Analyses – Model 4**

Provide descriptive statistics for all participants included in Model 4.

|  |  |
| --- | --- |
|  | Participants in analyses |
| *N* |  |
| *N* with a diagnosis of MCI or dementia at last visit (‘fup\_Dx’ = 1) |  |
| *N* participants with ‘Subject\_ID’ ≥ 400 \* |  |
| Mean (SD) Age\_Act |  |
| Range (min, max) Age\_Act |  |
| N (%) female sex (‘Sex\_F’ = 1) |  |
| Mean (SD) years of education (‘EDUC’) |  |
| N (%) White race (‘Race\_White’ = 1) |  |
| N (%) APOE4 carriers (‘APOE4’ = 1) |  |
| Mean (SD) years between Age\_CHAMPS and Age\_Act |  |
| Range (min, max) years between Age\_CHAMPS and Age\_Act |  |
| Mean (SD) years of follow-up (baseline actigraphy to last blood biomarker measure) |  |
| Range (min, max) years of follow-up (baseline actigraphy to last blood biomarker measure) |  |
| Mean (SD) number of blood biomarker measures over time |  |
| Range (min, max) number of blood biomarker measures over time |  |
| Mean (SD) time (in years) between baseline actigraphy and associated baseline blood biomarker measure |  |
| Range (min, max) time (in years) between baseline actigraphy and associated baseline blood biomarker measure |  |
| Mean (SD) time (in years) between baseline CHAMPS and baseline actigraphy |  |
| Range (min, max) time (in years) between baseline CHAMPS and baseline actigraphy |  |
| Mean (SD) LTAC10 |  |
| Mean (SD) BMI |  |
| Mean (SD) GFAP at baseline actigraphy |  |
| Mean (SD) NFL at baseline actigraphy |  |
| Mean (SD) PTAU181 at baseline actigraphy |  |
| Mean (SD) AB42AB40 at baseline actigraphy |  |
| Mean (SD) PTAU181\_AB42AB40 at baseline actigraphy |  |
| Mean (SD) sTREM2 at baseline actigraphy |  |
| Mean (SD) YKL40 at baseline actigraphy |  |

\* Included as a reminder to describe the enrollment of new participants when writing-up the manuscript.

**PART III: Correlations Between Measures**

**Correlations between baseline blood biomarker measures**

Complete the below table, showing the correlations between baseline (i.e., coinciding with first available CHAMPS) blood biomarker measures. Please provide *r* and *p*-values.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | PTAU181 | AB42AB40 | PTAU181\_AB42AB40 | NFL | GFAP | sTREM2 | YKL40 |
| PTAU181 |  |  |  |  |  |  |  |
| AB42AB40 |  |  |  |  |  |  |  |
| PTAU181\_AB42AB40 |  |  |  |  |  |  |  |
| NFL |  |  |  |  |  |  |  |
| GFAP |  |  |  |  |  |  |  |
| sTREM2 |  |  |  |  |  |  |  |
| YKL40 |  |  |  |  |  |  |  |

**Correlations between baseline physical activity measures**

Complete the below table, showing the correlations between baseline (i.e., coinciding with first available) CHAMPS and actigraphy measures. Please provide *r* and *p*-values.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | LOW\_INT\_FREQ | HIGH\_INT\_FREQ | HIGH\_INT\_FREQ | LTAC10 |
| LOW\_INT\_FREQ |  |  |  |  |
| HIGH\_INT\_FREQ |  |  |  |  |
| ALL\_INT\_FREQ |  |  |  |  |
| LAC10 |  |  |  |  |

Note for future analyses: If results differ for the CHAMPS (Part I) and actigraphy (Part II) analyses, the CHAMPS models may need to be re-run in the subset of participants that have both CHAMPS *and* actigraphy data from the same visit.

Please send results to: Corinne Pettigrew, Anja Soldan, Jiangxia Wang, and Elizabeth Paitel