

# Longitudinal Plasma Proteomic Remodelling Precedes MRI-Defined Small Vessel Disease in The Multi-Ethnic Study of Atherosclerosis (MESA)

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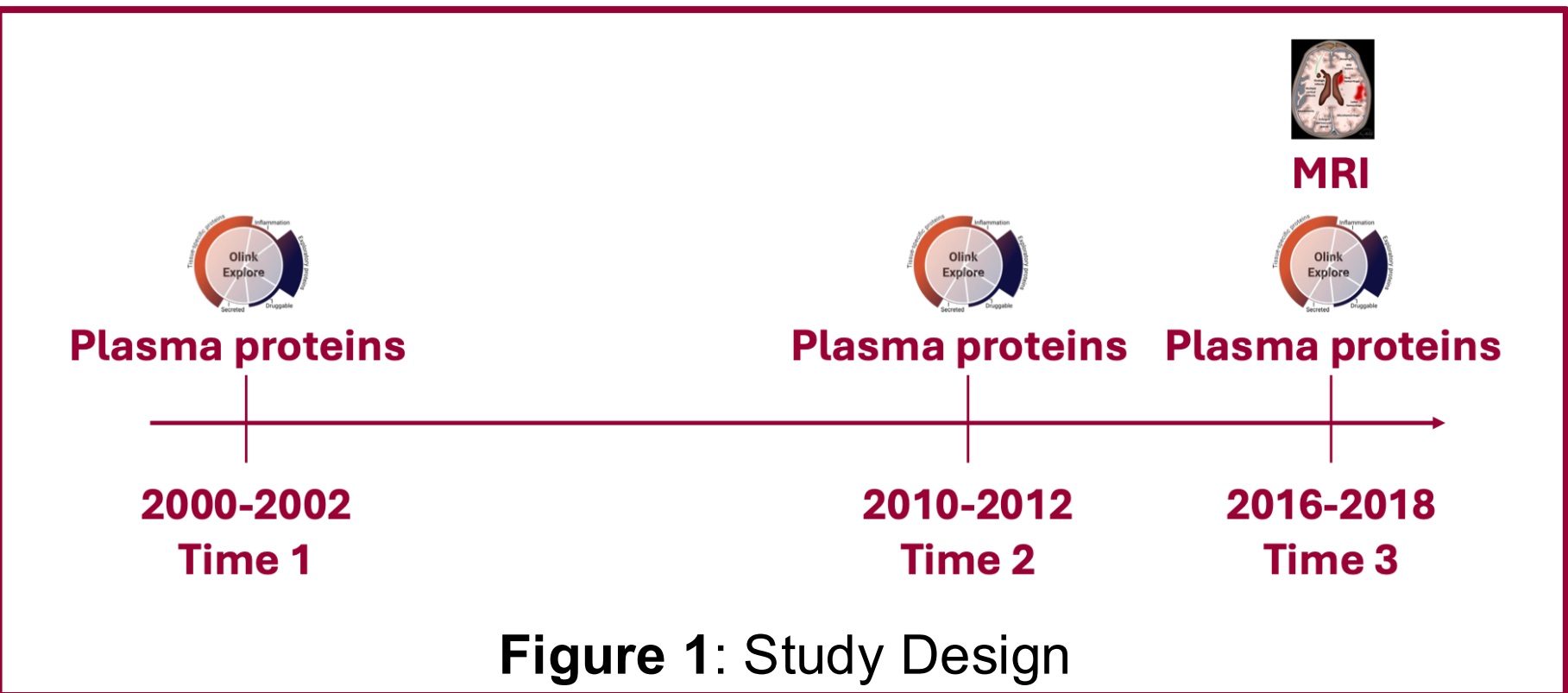
## BACKGROUND

- Small vessel disease (SVD) is a major contributor to vascular cognitive impairment and dementia (VCID).
- Multiple studies have identified plasma proteins that associate with brain magnetic resonance imaging (MRI) derived markers of SVD.
- However for many biological processes, disease risk is better reflected by within-person change over time rather than static levels, and few studies have interrogated changes in the plasma proteome over time for associations with SVD.

**Objective** To examine whether ~15-year trajectories of the plasma proteome associate with subsequent SVD burden, as assessed by MRI.

## STUDY POPULATION AND DESIGN

- N=1, 307 older US adults participating of the Multi-Ethnic Study of Atherosclerosis (Table 1).
- Plasma proteins were measured via the Olink Explore 3072/384 assay at three time points between 2000 and 2018 (Fig 1).
- Four measures of SVD were assessed via MRI at the final visit (Fig 1).
  - White matter hyperintensity volume (WMH), perivascular space of the basal ganglia (BG PVS), white matter fractional anisotropy (WMFA), and microbleeds across the whole brain (MBs).



## ANALYSES

- First, cross-sectional models assessed associations between protein abundances at each time point with SVD at the final follow-up.
- Next, multilevel linear models assessed associations between prior protein changes over time and subsequent SVD.
  - Within-person trajectories in the abundances for each of 2,941 plasma proteins (outcome) over time (predictor) were separately modelled.
  - An interaction term between time and SVD indicated whether prior protein trajectories over time differed by subsequent SVD.
- Where greater than 50 associations reached significance with an SVD measure, a parsimonious set of predictive proteins was determined by jointly modelling all proteins reaching  $P < .05$  after a false discovery rate (FDR) correction in an association model that included a penalty function (elastic net).
- All models were adjusted for baseline age, sex, race/ethnicity, education, smoking, presence/absence of APOE  $\epsilon 4$ , and kidney function.

## SAMPLE CHARACTERISTICS

**Table 1:** Means (+/- Standard Deviations) or Frequency (n, %) for Sample Characteristics at Each Time Point

|                           | TIME 1<br>(2000-2002) | TIME 2<br>(2010-2012) | TIME 3<br>(2016-2018) |
|---------------------------|-----------------------|-----------------------|-----------------------|
| Frequency, N              | 1,307                 | 1,281                 | 1,284                 |
| Age, y                    | 56.4 (8.0)            | 65.8 (7.8)            | 72.2 (7.8)            |
| Gender, n (%)             |                       |                       |                       |
| Female                    | 689 (53%)             | 676 (53%)             | 676 (53%)             |
| Race and Ethnicity, n (%) |                       |                       |                       |
| Black/African-American    | 350 (27%)             | 340 (27%)             | 345 (27%)             |
| Hispanic                  | 247 (19%)             | 240 (19%)             | 240 (19%)             |
| Chinese-American          | 157 (12%)             | 156 (12%)             | 157 (12%)             |
| Non-Hispanic White        | 553 (42%)             | 545 (43%)             | 542 (42%)             |
| BMI, kg/m <sup>2</sup>    | 28.0 (5.2)            | 28.4 (5.4)            | 28.4 (5.4)            |
| Diabetes status, n (%)    |                       |                       |                       |
| Prevalent diabetes        | 84 (6.4%)             | 204 (16%)             | 265 (21%)             |
| APOE isoform, n (%)       |                       |                       |                       |
| No $\epsilon 4$ isoform   | 916 (72%)             | 899 (72%)             | 916 (72%)             |
| $\epsilon 4$ isoform      | 348 (27%)             | 340 (27%)             | 348 (27%)             |
| Unknown                   | 10 (<1%)              | 10 (<1%)              | 10 (<1%)              |

## RESULTS

### Single Time Point Proteins

**Table 2:** Associations Between SVD and Single Time Point Proteins for up to N=1,307 MESA participants

|                         | TIME 1<br>PROTEINS<br>(2000-2002) | TIME 2<br>PROTEINS<br>(2010-2012) | TIME 3<br>PROTEINS<br>(2016-2018) |
|-------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| MRI measure (2016-2018) |                                   |                                   |                                   |
| WMH                     | 0                                 | 7                                 | 0                                 |
| BG PVS                  | 0                                 | 0                                 | 0                                 |
| WMFA                    | 628 / 26*                         | 8                                 | 33                                |
| MBs                     | 0                                 | 0                                 | 1                                 |

\*Selected by the penalized model

- With the exception of WMFA, select SVD measures were associated with a modest number of proteins measured at a single time point (Table 2).
- There was little overlap in proteins associated across SVD measures, and within a measure across protein time point (Fig 2)

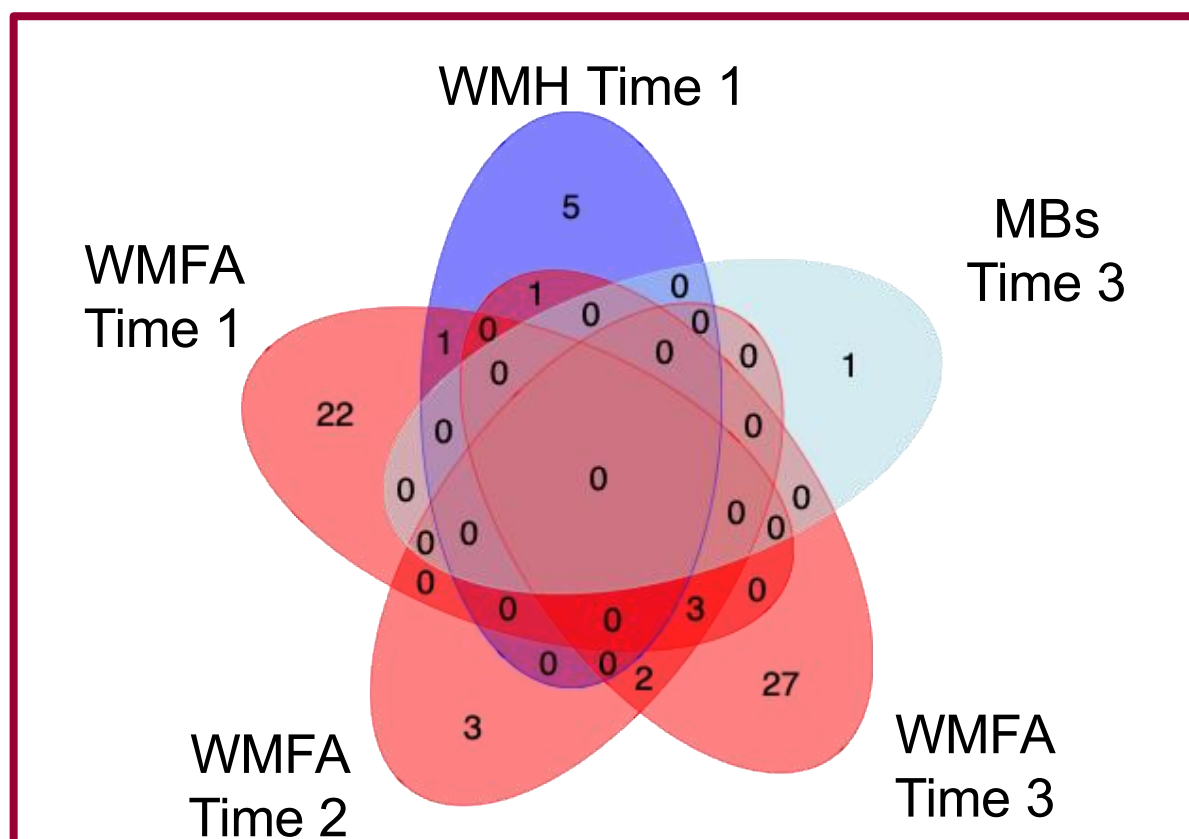


**Figure 2:** Overlap in Proteins Measured at a Single Time Point and SVD

## RESULTS

### Longitudinal Protein Trajectories

- All SVD measures were associated with a substantial number of prior protein trajectories (Table 3).
- There was notable overlap across MRI measures in the proteins whereby prior trajectories predicted SVD (Fig 3)
- However, for "optimal protein sets" selected by penalized models, the proteins whereby prior trajectories associated with subsequent SVD showed a more measure-specific pattern of associations (Fig 4)

**Table 3:** Associations Between SVD and Prior Protein Trajectories for up to N=1,284 MESA participants

|                         | PROTEIN<br>TRAJECTORIES<br>(2000-2018) |
|-------------------------|----------------------------------------|
| MRI measure (2016-2018) |                                        |
| WMH                     | 293 / 15*                              |
| BG PVS                  | 63 / 7*                                |
| WMFA                    | 301 / 27*                              |
| MBs                     | 160 / 17*                              |

\*Selected by the penalized model

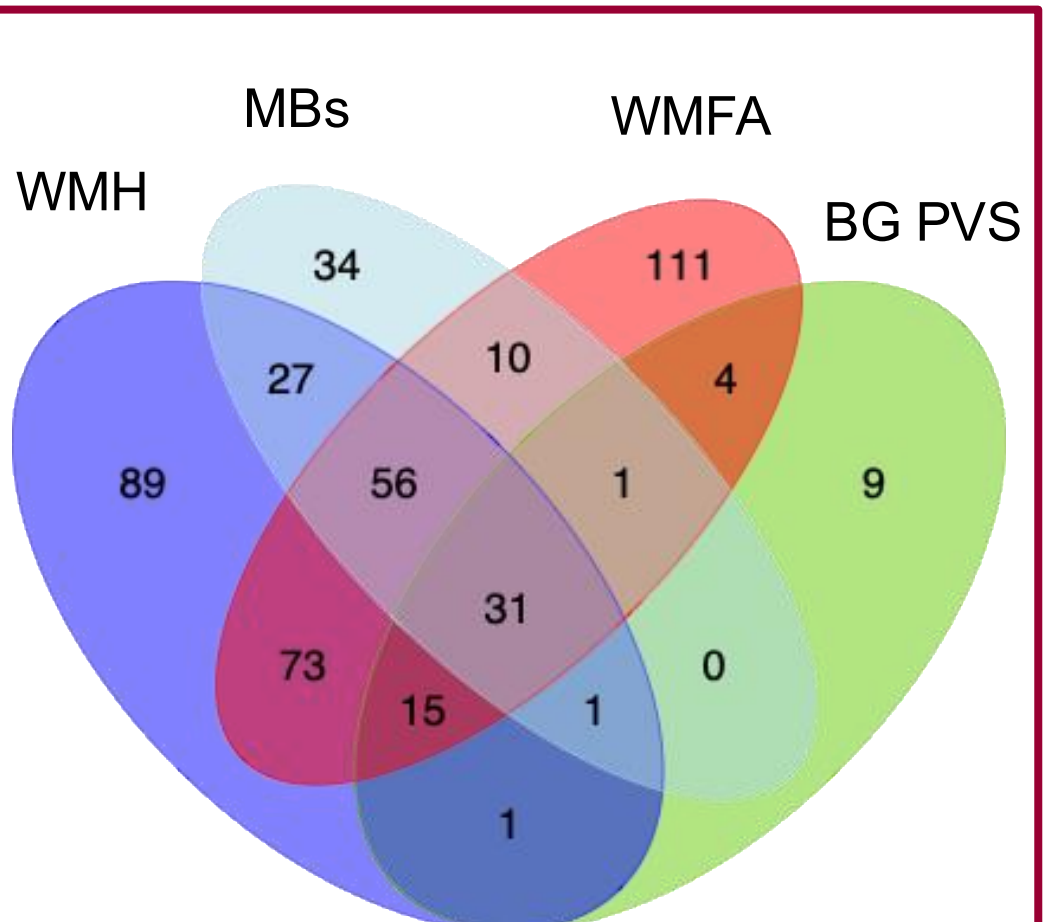


**Figure 3:** Overlap in Longitudinal Protein Trajectories Associated with SVD in Non-Penalized Models

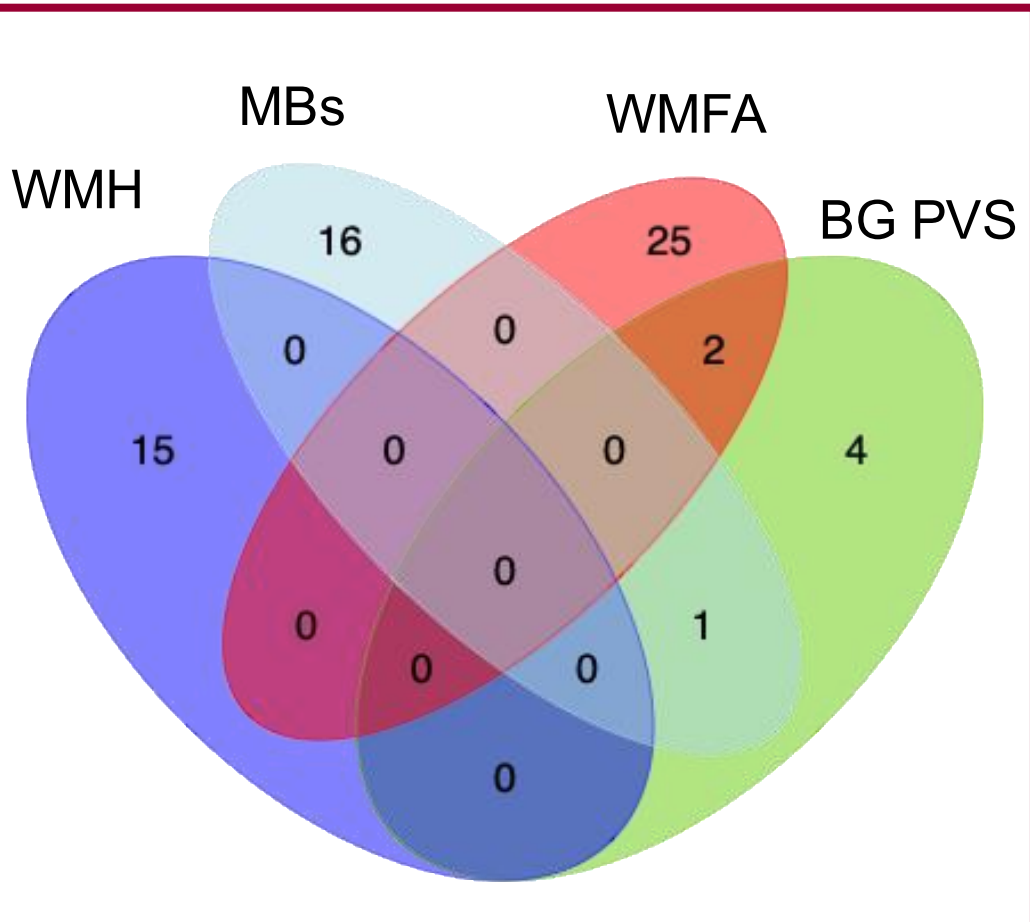


**Figure 4:** Overlap in Longitudinal Protein Trajectories Associated with SVD in Penalized Models

## CONCLUSIONS

- While cross-sectional differences in a modest number of proteins related to SVD, within-person remodelling of the proteome across mid- to late-life captured subsequent SVD at greater resolution.
- Non-penalized models captured shared proteins across measures, while the addition of a penalty term to reduce bias due to correlated predictors revealed heterogeneity in the proteins underlying different SVD phenotypes.

## SUMMARY

- SVD may be preceded by long-term biological shifts in the proteome, providing a potential framework for risk stratification and discovery of the mechanisms linking SVD to the development of VCID.

## ACKNOWLEDGEMENTS AND FUNDING

- We are grateful to the MESA participants for their time and efforts
- Supported by the following funding:
  - NIH: NIH R01 AG058969; NIH R01 HL127659
  - USDA/ARS: USDA CRIS 3092-5100