

MESA-MIND-Longitudinal-Proteomics-cSVD

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Important Notes - read me first

- Always check that you have the most recent version of this document, which - unless I am sending you unfinalized work - is available [here](#).
 - An easy check for version control is to make sure this date: 2025-12-18. is the same as on the GitHub file [here](#).
- The code for this analysis available in the same repository ([targets master file here](#) and [individual functions here](#))

Summary

Step 1: Cleaning and Formatting Proteins

Input file names

- A table of protein abundances: SMP_IntensityNormalized_20251005.csv
- Sample information to link TOPMed IDs to unique MESA SHARe ID and exam combinations: Mapping_SMP_Plate_20251005.csv
- Keys to link Olink IDs to names compounds: MESAOLink3k_proteinKeys_03292023.csv
- A file to bridge SHARe ids (sidno) with MESA IDs (idno) MESA-SHARE_IDList_Labelled.csv

Raw file info

- The raw protein abundance file contained information on N=3040 protein assays, including those used for QC.
- When removing assays for QC, the raw protein abundance file contained information on N=2941 proteins.
- The protein abundance file contained information on N=14051 sample IDs (i.e., unique participant/exam combinations), including bridging samples.
- After removing QC samples (including bridging, controls, and one duplicate) the protein abundance file contained information on N=12739 sample IDs (i.e., unique participant/exam combinations).

Table 1: Final N by exam

Exam	N_Pps
1	5949
5	3917
6	2873

Formatting

- Bridging (and other QC) samples were removed.
- Protein assays used for QC were removed.
- Proteins that should be excluded due to QC warnings (variable “QC_warning” set to “EXCLUDED”) were removed, even though these do not have NPX values.
- Data were put into wide format, with “SampleID” as the unique ID, “OlinkID” forming the variable names (protein identifiers), and values taken from the “NPX” column.
 - In wide format, the file contained information on N=12739 unique sample IDs.
 - In wide format, the file contained information on N=0 duplicated sample IDs.¹
- SHARe IDs, and subsequently MESA IDs, were merged into the file with exam information.
- At this point, the range of unique SHARe ID by exam combinations was N=0 - 1. This indicates no sample ID were duplicated in the assays.
- The formatted protein file was used to calculate the coefficient of variation (CV) using the formula: $CV = \sqrt{2^2(\sigma^2)-1}$.
- A variable called “Retain” was created to indicate whether each protein was (1) unique (i.e., included on only one panel); (2) duplicated, and across all panels had the lowest CV; or (3) duplicated, and across all panels did not have the lowest CV.
- A final table of protein abundances, with additional variables for SHARe ID, MESA ID, Exam, TOPMed ID and Batch, was created after the steps above, with proteins duplicated across more than one panel cleaned such that only the one with the lowest CV is retained. This file was used in the analysis
- The number of participants, stratified by exam, in the final file is available in Table 1:

Step 2: Format Phenotypes

Input files

- Covariates from E1: MESAe1FinalLabel02092016.dta
- Covariates from E5: MESAe5_FinalLabel_20140613.dta
- Covariates from E6: MESAe6_FinalLabel_20220513.dta
- Afib info: SHARe_MesaEventsThruYear2020_AF_DS.txt
- ApoE info: MESA_ApoE_03102014.sas7bdat
- Incident CVD: MESAEvThru2020AllCohort_20241120.dta
- Microbleeds: MESAe6as253as301_BMRICMB_08052025.csv
- Perivascular spaces: MESAe6as253as301_BMRIPVS_20250310.csv
- White matter hyperintensities: MESAe6anyFIRST_BMRIWMHVol_20240422.csv
- Intracranial volumes: SHARe_AncilMesaAF_BMRIROIVol_DS.txt
- Fractional anisotropy: mesae6anyfirst_bmriTotalFAMUSE_20250828.csv
- White matter hyperintensities: MESAe6anyFIRST_BMRIWMHVol_20240422.csv

Formatting

Outcomes:

- Microbleeds were coded as 0/1, where 0= no microbleeds (value: 0) and 1 = presence of microbleeds (all non-zero values except missing). Then, those images with a low image quality (value = 4; N=0) were recoded to missing.

Var1	Freq
0	1730
1	886

- Perivascular spaces (variable: epvs_wholebrain_vol) were recoded to missing where the variable ‘pvs_exclude’ was coded as 1 (N=0).
- White matter hyperintensities (variable: wm_wmh) were divided by 1000 to convert to ml (following Rizwan’s code), and those where the variable wmh_exclude had a code of 1 were set to missing.
- Fractional anisotropy (variable: wmma) was coded to missing where the variable fa_exclude had a value of 1.

Covariates

True time invariant covariates

- Race/ethnicity, gender, and highest education level were all taken from exam 1 data. ApoE information was taken from its own dataset (above).
 - ApoE was coded 0/1/2 where 0= no e4 isoform (codes 22, 23, 33), 1 = e4 isoform (24, 34, 44), and 2 = no isoform data. The ApoE variable was formatted as a factor.
 - Gender was coded such that female = 0 and male = 1.
 - Education was recoded 0/1, such that 0 = less than high school (codes: 0: NO SCHOOLING / 1: GRADES 1-8 / 2: GRADES: 9-11) and 1= high school or more (all other codes, excluding missing).
 - Race/ethnicity was recoded retaining the original MESA coded whereby 1=White American; 2= Chinese American, 3=Black, African-American, and 4 = Hispanic. Race/ethnicity was coded as a factor variable.

Pseudo-time invariant covariates

- Although some variables are technically time invariant, where they were included due to their effects on MRI data, since MRI data are only measured at one exam for this analysis (exam 6), these covariates were always taken from exam 6.
- These ‘pseudo time invariant covariates’ were: atrial fibrillation, myocardial infarction, congestive heart failure, LDL, systolic blood pressure, hypertension medication, and site (since site seems to affect MRI more than proteins??).
 - Afib, MI, and CHF were coded 0/1, such that 0= no diagnosis and 1= afib diagnosis. Missing data was left as missing (this is different to Rizwan who coded missing data as no diagnosis).
 - Site was arbitrarily coded as 0=Wake forest, 1= Columbia, 2=Johns Hopkins, 3=University of Minnesota, 4=Northwestern, 5=UCLA

Time varying covariates

- The following covariates were taken from the exam when the proteins were used, as these were seen to affect proteins more in the short term than they affect MRI (?): kidney function (egfr), BMI , cigarette smoking (never/former/current; coded as ordinal), diabetes status.
 - Smoking was harmonized and coded such that 0 = never smoker, 1= past smoker, 2=current smoker.

- Diabetes has harmonized and coded such that 0= no diabetes (including impaired fasting glucose), and 1 = diabetes (treated and untreated).
- The following covariates were taken from exam 6: age (age6c), kidney function (egfr; cegfr6c), BMI (bmi6c), systolic blood pressure (sbp6c), LDL (ldl6), site(site6c), the use of hypertension medication (htnmed6c; coded 0= no, and 1= yes), cigarette smoking (cig6c), diabetes status (dm036t)

Step 3: Sample descriptives

- There were N=1429 MESA participants with at least one MRI outcome after the exclusions above.
- Of those with MRI data, N=1307 participants had protein data, equating to N=1272 at exam 1, N=1256 at exam 5, and N=1270 at exam 6.
- Of those with protein data and at least one MRI outcome, N=941 participants had no missing covariate data and so were included in this analysis, equating to N=938 at exam 1, N=926 at exam 5, and N=940 at exam 6.
- See Table 2

Table 2: Sample Descriptives

Characteristic	Exam		
	1 N = 938 ¹	5 N = 926 ¹	6 N = 940 ¹
Age (y)	56.77 (8.22)	66.19 (8.09)	72.47 (8.04)
Gender			
Female	492 / 938 (52%)	486 / 926 (52%)	493 / 940 (52%)
Male	446 / 938 (48%)	440 / 926 (48%)	447 / 940 (48%)
Field Center			
Wake Forest	168 / 837 (20%)	166 / 827 (20%)	169 / 839 (20%)
Columbia	110 / 837 (13%)	107 / 827 (13%)	110 / 839 (13%)
Johns Hopkins	185 / 837 (22%)	183 / 827 (22%)	186 / 839 (22%)
Minnesota	170 / 837 (20%)	170 / 827 (21%)	170 / 839 (20%)
Northwestern	0 / 837 (0%)	0 / 827 (0%)	0 / 839 (0%)
UCLA	204 / 837 (24%)	201 / 827 (24%)	204 / 839 (24%)
Highest education level			
Up to and including high school	105 / 938 (11%)	103 / 926 (11%)	105 / 940 (11%)
More than high school	833 / 938 (89%)	823 / 926 (89%)	835 / 940 (89%)
Race or ethnicity			
Non-Hispanic White	380 / 938 (41%)	377 / 926 (41%)	380 / 940 (40%)
Chinese American	137 / 938 (15%)	138 / 926 (15%)	138 / 940 (15%)
Black/African-American	231 / 938 (25%)	224 / 926 (24%)	231 / 940 (25%)
Hispanic	190 / 938 (20%)	187 / 926 (20%)	191 / 940 (20%)
BMI (kg/m^2)	27.62 (4.97)	28.08 (5.19)	28.02 (5.33)
Smoking status			
Never	0 / 840 (0%)	0 / 866 (0%)	0 / 884 (0%)
Former	496 / 840 (59%)	441 / 866 (51%)	448 / 884 (51%)
Current	344 / 840 (41%)	425 / 866 (49%)	436 / 884 (49%)
LDL levels	107.55 (35.85)	107.40 (35.35)	107.50 (35.83)
systolic blood pressure	126.69 (20.49)	126.76 (20.49)	126.76 (20.53)
Diabetes status			

Normoglycemia/IFG	875 / 938 (93%)	780 / 926 (84%)	736 / 940 (78%)
Diabetes (treated or untreated)	63 / 938 (6.7%)	146 / 926 (16%)	204 / 940 (22%)
Takes hypertension medicine			
No	391 / 938 (42%)	386 / 926 (42%)	392 / 940 (42%)
Yes	547 / 938 (58%)	540 / 926 (58%)	548 / 940 (58%)
Atrial fibrillation			
No	795 / 938 (85%)	783 / 926 (85%)	796 / 940 (85%)
Yes	143 / 938 (15%)	143 / 926 (15%)	144 / 940 (15%)
Myocardial Infarction			
No	911 / 938 (97%)	899 / 926 (97%)	913 / 940 (97%)
Yes	27 / 938 (2.9%)	27 / 926 (2.9%)	27 / 940 (2.9%)
Coronary Heart Failure			
No	919 / 938 (98%)	907 / 926 (98%)	921 / 940 (98%)
Yes	19 / 938 (2.0%)	19 / 926 (2.1%)	19 / 940 (2.0%)
ApoE information			
No E4 isoform	669 / 938 (71%)	663 / 926 (72%)	671 / 940 (71%)
E4 isoform	259 / 938 (28%)	253 / 926 (27%)	259 / 940 (28%)
No ApoE data	10 / 938 (1.1%)	10 / 926 (1.1%)	10 / 940 (1.1%)
Kidney function (egfr)	82.44 (15.28)	82.29 (19.57)	76.72 (19.60)
Intracranial volume	1,360,159.33 (145,347.03)	1,360,711.30 (144,918.81)	1,360,215.07 (145,244.17)
Fractional anisotropy	0.39 (0.03)	0.39 (0.03)	0.39 (0.03)
White matter hyperintensities	6.79 (10.39)	6.70 (10.24)	6.80 (10.39)
Enlarged perivascular spaces	3,574.55 (2,228.27)	3,564.42 (2,230.86)	3,573.19 (2,226.09)
Presence of microbleeds?			
No	579 / 875 (66%)	572 / 864 (66%)	579 / 877 (66%)
Yes	296 / 875 (34%)	292 / 864 (34%)	298 / 877 (34%)

¹Mean (SD); n / N (%)

Step 5: Cross sectional Protein-Wide Association Studies (PWAS)

- This section includes PWAS where proteins were only used at one time point (exam 1 or exam 6), even if this was not the same time point as when the MRI data were used.
- All estimates are standardized.

White matter hyperintensity (WMH)

Model Specification

- The associations between WMH and proteins were analyzed via linear regression
- For WMH the numeric covariates included icv, age, egfr, BMI, sbp, ldl. ²
- For WMH the factor covariates included gender, race, site, edu, htnmeds, smoking, E4, AFprevalent, diabetes, MIprevalent, CHFprevalent. ²

WMH: Exam 6 proteins

- The E6 proteins -> E6 MRI for WMH included data from N=933 participants.
- A total of N=1 exam 6 proteins were significantly associated with WMH at an FDR corrected P<.05 (see Table 3 and Figure 1).

WMH: Exam 1 proteins

- The E1 proteins -> E6 MRI for WMH included data from N=913 participants.
- A total of N=0 exam 1 proteins were significantly associated with WMH at an FDR corrected P<.05

Enlarged Perivascular Spaces (EPVS)

Model Specification

- The associations between EPVS and proteins were analyzed via linear regression
- For EPVS the numeric covariates included icv, age, egfr, BMI, sbp, ldl. ²
- For EPVS the factor covariates included gender, race, site, edu, htnmeds, smoking, E4, AFprevalent, diabetes, MIprevalent, CHFprevalent. ²

EPVS: Exam 6 proteins

- The E6 proteins -> E6 MRI for EPVS included data from N=926 participants.
- A total of N=0 exam 1 proteins were significantly associated with EPVS at an FDR corrected P<.05

EPVS: Exam 1 proteins

- The E1 proteins -> E6 MRI for EPVS included data from N=906 participants.
- A total of N=0 exam 1 proteins were significantly associated with EPVS at an FDR corrected P<.05

Fractional Angiotensy (FA)

Model Specification

- The associations between FA and proteins were analyzed via linear regression
- For FA the numeric covariates included age, egfr, BMI, sbp, ldl.²
- For FA the factor covariates included gender, race, site, edu, htnmeds, smoking, E4, AFprevalent, diabetes, MIprevalent, CHFprevalent.²

FA: Exam 6 proteins

- The E6 proteins -> E6 MRI for FA included data from N=917 participants.
- A total of N=9 exam 6 proteins were significantly associated with FA at an FDR corrected P<.05 (see Table 4 and Figure 2).

FA: Exam 1 proteins

- The E1 proteins -> E6 MRI for FA included data from N=898 participants.
- A total of N=1 exam 1 proteins were significantly associated with FA at an FDR corrected P<.05 (see Table 5 and Figure 3).

Microbleeds (MB)

Model Specification

- The associations between MB and proteins were analyzed via logistic regression (MB coded as ‘present’ vs. ‘absent’ as above)
- For MB the numeric covariates included age, egfr, BMI, sbp, ldl. ²
- For MB the factor covariates included gender, race, site, edu, htnmeds, smoking, E4, AFprevalent, diabetes, MIprevalent, CHFprevalent. ²

MB: Exam 6 proteins

- The E6 proteins -> E6 MRI for MB included data from N= 873 participants.
- A total of N=0 exam 6 proteins were significantly associated with MB at an FDR corrected P<.05 (see XX and XX).

MB: Exam 1 proteins

- The E1 proteins -> E6 MRI for FA included data from N=854 participants.
- A total of N=0 exam 1 proteins were significantly associated with MB at an FDR correct P<.05 (see Table 5 and Figure 3).

Table 3: Associations Between Exam 6 Proteins and White Matter Hyperintensity Reaching an FDR-corrected $P < .05$ Equivalent

Protein	N	Beta	SE	P	P-FDR	Olink ID	Protein CV	UniProt ID
CKMT1A_CKMT1B	932	-0.14	0.03	3.33×10^{-6}	9.74×10^{-3}	OID20721	66.95	P12532

Table 4: Associations Between Exam 6 Proteins and Fractional Angiotensy Reaching an FDR-corrected $P < .05$ Equivalent

Protein	N	Beta	SE	P	P-FDR	Olink ID	Protein CV	UniProt ID
REG4	916	-0.13	0.03	1.04×10^{-4}	3.87×10^{-2}	OID20784	40.16	Q9BYZ8
FABP3	917	-0.15	0.04	1.05×10^{-4}	3.87×10^{-2}	OID30351	53.39	P05413
CLEC5A	917	-0.13	0.03	1.06×10^{-4}	3.87×10^{-2}	OID20165	47.48	Q9NY25
GDF15	917	-0.15	0.04	1.53×10^{-4}	4.96×10^{-2}	OID20251	39.14	Q99988
PLA2G2A	917	-0.13	0.03	2.35×10^{-5}	2.29×10^{-2}	OID20274	43.92	P14555
SSC5D	917	-0.13	0.03	1.23×10^{-5}	1.79×10^{-2}	OID20351	34.66	A1L4H1
ANG	917	-0.12	0.03	8.10×10^{-5}	3.87×10^{-2}	OID20403	33.03	P03950
OMG	917	0.14	0.03	2.88×10^{-6}	8.42×10^{-3}	OID21352	71.71	P23515
HAVCR1	917	-0.13	0.03	5.31×10^{-5}	3.87×10^{-2}	OID21422	95.89	Q96D42

Table 5: Associations Between Exam 1 Proteins and Fractional Angiotensy Reaching an FDR-corrected $P < .05$ Equivalent

Protein	N	Beta	SE	P	P-FDR	Olink ID	Protein CV	UniProt ID
LILRA5	898	-0.14	0.03	1.55×10^{-5}	4.54×10^{-2}	OID20209	34.07	A6NI73

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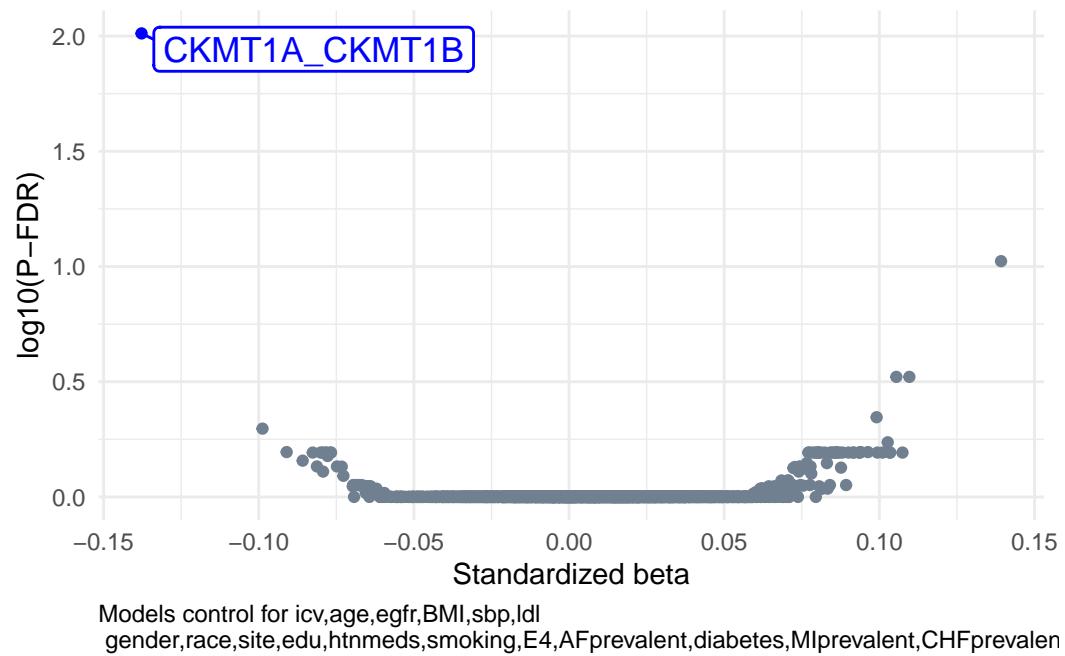


Figure 1: Associations Between Exam 6 Proteins and White Matter Hyperintensity

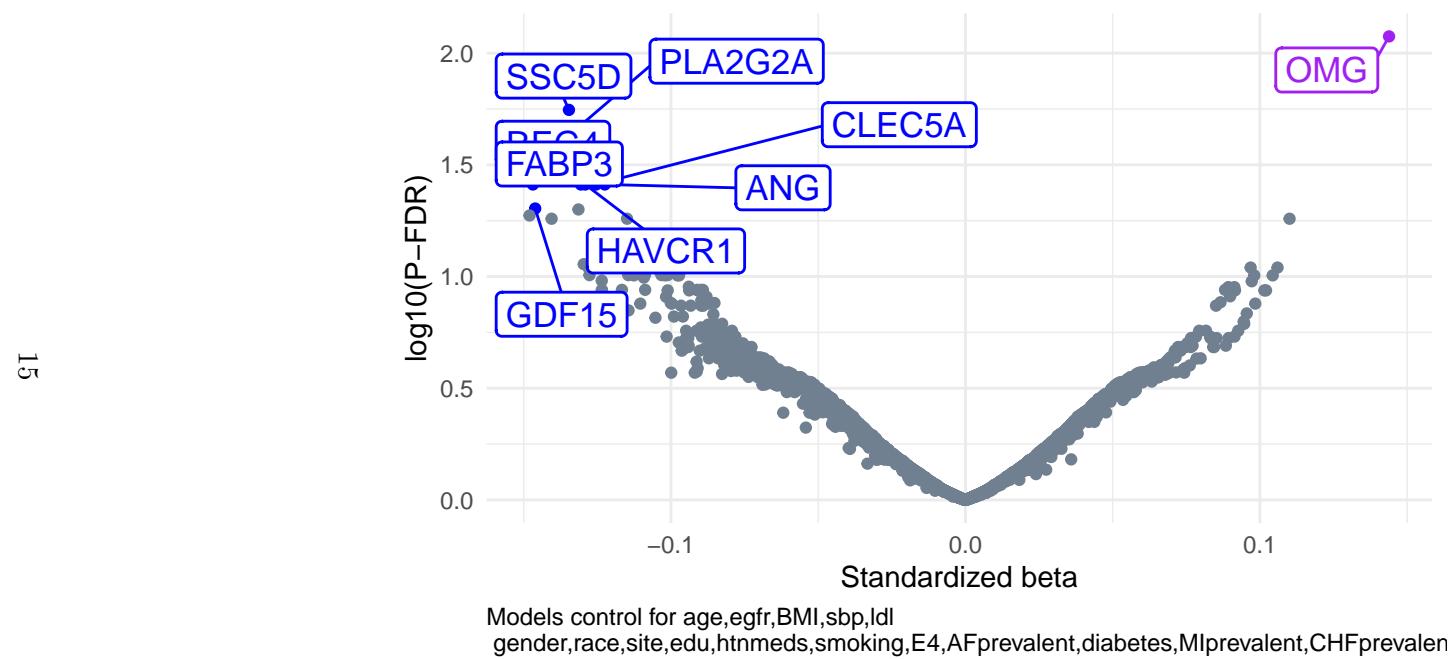


Figure 2: Associations Between Exam 6 Proteins and Fractional Angiotensy

91

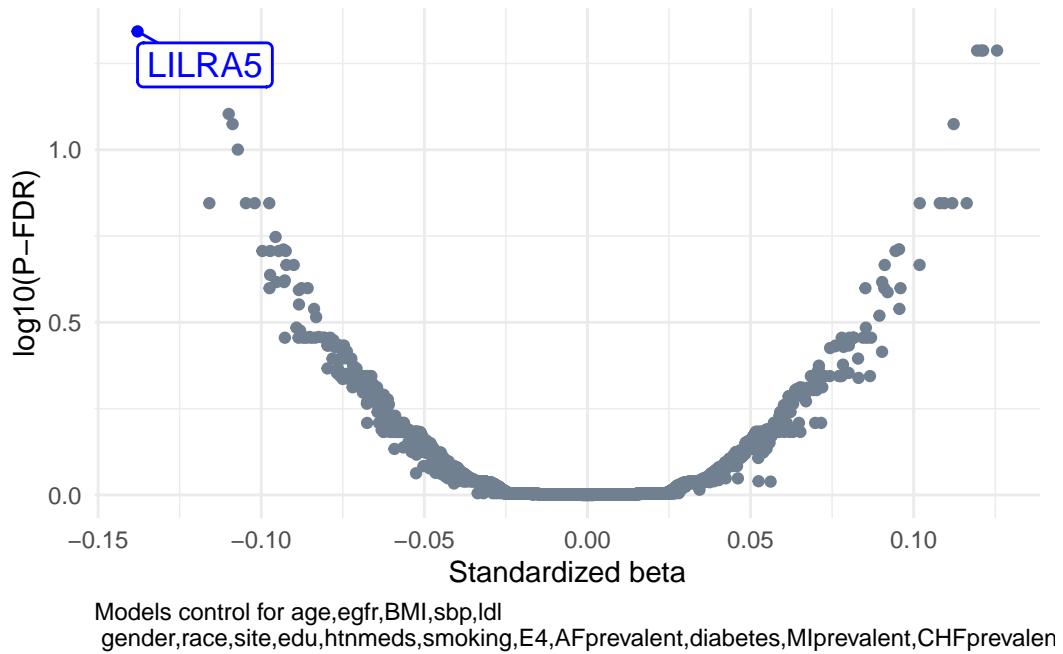


Figure 3: Associations Between Exam 1 Proteins and Fractional Angiotensy

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Notes

Footnotes

¹ This is a reproducible file for many runs, containing many data checks. Values of 0 or NULL are expected, and just indicate no problem with the data.

² These are intentionally written as variable names to avoid reproducibility errors.

Session Info

For reproducibility

```
- Session info -----
  setting  value
  version   R version 4.5.2 (2025-10-31)
  os        Linux Mint 21
  system    x86_64, linux-gnu
  ui        X11
  language  (EN)
  collate   en_US.UTF-8
  ctype     en_US.UTF-8
  tz        America/Chicago
  date      2025-12-18
  pandoc   3.2 @ /usr/lib/rstudio-server/bin/quarto/bin/tools/x86_64/ (via rmarkdown)
  quarto    1.8.26 @ /usr/local/bin/quarto

- Packages -----
  package      * version date (UTC) lib source
  backports     1.5.0   2024-05-23 [1] CRAN (R 4.5.0)
  base64url     1.4     2018-05-14 [1] CRAN (R 4.5.1)
  callr        3.7.6   2024-03-25 [1] CRAN (R 4.5.1)
  cards         0.7.1   2025-12-02 [1] CRAN (R 4.5.2)
  cli           3.6.5   2025-04-23 [1] CRAN (R 4.5.2)
  codetools     0.2-20  2024-03-31 [4] CRAN (R 4.5.0)
  colorspace    2.1-1   2024-07-26 [1] CRAN (R 4.5.0)
  commonmark    1.9.5   2025-03-17 [1] CRAN (R 4.5.0)
  data.table    1.17.8  2025-07-10 [1] CRAN (R 4.5.1)
  digest        0.6.37  2024-08-19 [1] CRAN (R 4.5.1)
```

dplyr	1.1.4	2023-11-17	[1]	CRAN	(R 4.5.0)
evaluate	1.0.5	2025-08-27	[1]	CRAN	(R 4.5.1)
farver	2.1.2	2024-05-13	[1]	CRAN	(R 4.5.0)
fastmap	1.2.0	2024-05-15	[1]	CRAN	(R 4.5.0)
fs	1.6.6	2025-04-12	[1]	CRAN	(R 4.5.0)
generics	0.1.4	2025-05-09	[1]	CRAN	(R 4.5.1)
ggplot2	3.5.2	2025-04-09	[1]	CRAN	(R 4.5.0)
ggrepel	0.9.6	2024-09-07	[1]	CRAN	(R 4.5.0)
glue	1.8.0	2024-09-30	[1]	CRAN	(R 4.5.0)
gt	1.1.0	2025-09-23	[1]	CRAN	(R 4.5.2)
gttable	0.3.6	2024-10-25	[1]	CRAN	(R 4.5.0)
gtsummary	2.5.0	2025-12-05	[1]	CRAN	(R 4.5.2)
htmltools	0.5.8.1	2024-04-04	[1]	CRAN	(R 4.5.0)
igraph	2.1.4	2025-01-23	[1]	CRAN	(R 4.5.0)
jsonlite	2.0.0	2025-03-27	[1]	CRAN	(R 4.5.0)
knitr	1.50	2025-03-16	[1]	CRAN	(R 4.5.2)
labeling	0.4.3	2023-08-29	[1]	CRAN	(R 4.5.0)
later	1.4.2	2025-04-08	[1]	CRAN	(R 4.5.0)
lifecycle	1.0.4	2023-11-07	[1]	CRAN	(R 4.5.0)
litedown	0.7	2025-04-08	[2]	CRAN	(R 4.5.1)
magrittr	2.0.4	2025-09-12	[1]	CRAN	(R 4.5.1)
markdown	2.0	2025-03-23	[2]	CRAN	(R 4.5.1)
munsell	0.5.1	2024-04-01	[1]	CRAN	(R 4.5.0)
pillar	1.11.1	2025-09-17	[1]	CRAN	(R 4.5.1)
pkgconfig	2.0.3	2019-09-22	[1]	CRAN	(R 4.5.0)
prettyunits	1.2.0	2023-09-24	[1]	CRAN	(R 4.5.0)
processx	3.8.6	2025-02-21	[2]	CRAN	(R 4.5.1)
ps	1.9.1	2025-04-12	[1]	CRAN	(R 4.5.0)
purrr	1.1.0	2025-07-10	[1]	CRAN	(R 4.5.1)
quarto	1.5.1	2025-09-04	[1]	CRAN	(R 4.5.2)
R6	2.6.1	2025-02-15	[1]	CRAN	(R 4.5.0)
Rcpp	1.0.14	2025-01-12	[1]	CRAN	(R 4.5.0)
rlang	1.1.6	2025-04-11	[1]	CRAN	(R 4.5.0)
rmarkdown	2.29	2024-11-04	[1]	CRAN	(R 4.5.0)
rstudioapi	0.17.1	2024-10-22	[1]	CRAN	(R 4.5.0)
scales	1.3.0	2023-11-28	[1]	CRAN	(R 4.5.0)
secretbase	1.0.5	2025-03-04	[1]	CRAN	(R 4.5.1)
sessioninfo	1.2.3	2025-02-05	[1]	CRAN	(R 4.5.1)
targets	1.11.4	2025-09-13	[1]	CRAN	(R 4.5.1)
tibble	3.3.0	2025-06-08	[1]	CRAN	(R 4.5.1)
tidyverse	1.3.1	2024-01-24	[1]	CRAN	(R 4.5.0)
tidyselect	1.2.1	2024-03-11	[1]	CRAN	(R 4.5.0)
vctrs	0.6.5	2023-12-01	[1]	CRAN	(R 4.5.0)

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withr      3.0.2   2024-10-28 [1] CRAN (R 4.5.0)
xfun       0.53    2025-08-19 [1] CRAN (R 4.5.1)
xml2       1.4.0   2025-08-20 [1] CRAN (R 4.5.1)
yaml       2.3.10  2024-07-26 [1] CRAN (R 4.5.0)

[1] /home/awood/R/x86_64-pc-linux-gnu-library/4.5
[2] /usr/local/lib/R/site-library
[3] /usr/lib/R/site-library
[4] /usr/lib/R/library
```
