TITLE

The Association Between SLIT2 in Human Vitreous Humor and Plasma and Neurocognitive Test Scores

RUNNING TITLE

SLIT2 Association with Neurocognitive Testing

AUTHORS

Sara I. Shoushtari[1,#], Easton Liaw[2,4,#], Sreevardhan Alluri[3], Zahra Sheikh[3], Sudhir Kumar[1,4], Courtney Huynh[1,4], Insa M. Schmidt[1,4], Steven Ness[3], Xuejing Chen[3], Nicole H. Siegel[3], Sushrut S. Waikar[1,4], Thor D. Stein[5,6,7], Weining Lu[1,4,*], Manju L. Subramanian[3,*]

AFFILIATIONS

1. Boston University Chobanian & Avedisian School of Medicine, Boston, MA 02118
2. Boston University Sargent College of Health and Rehabilitation Sciences, Boston, MA 02215
3. Boston Medical Center, Department of Ophthalmology, Boston, MA 02118
4. Boston University Chobanian & Avedisian School of Medicine, Nephrology Section, Department of Medicine, Boston, MA 02118
5. Boston University Alzheimer's Disease and CTE Center, Boston University Chobanian & Avedisian School of Medicine, Boston, MA 02118
6. Department of Pathology and Laboratory Medicine, Boston Medical Center, Boston, MA 02118
7. Department of Veterans Affairs Medical Center, VA Boston Healthcare System, Boston, MA 02130

[#]These authors contributed equally to this work

**Corresponding Authors*

Weining Lu, MD. Email: wlu@bu.edu

Manju L. Subramanian, MD. Email: manju.subramanian@bmc.org

CONFLICT OF INTEREST STATEMENT

The authors have declared that no conflict of interest exists.

ABSTRACT

Background: Slit Guidance Ligand 2 (SLIT2) binds Roundabout (ROBO) guidance receptors to direct axon pathfinding and neuron migration during nervous system development. SLIT2 expression has previously been linked to dementia risk.

Objective: To study the association between SLIT2 expression in human vitreous humor and plasma samples and neurocognitive test scores in a cross-sectional cohort study utilizing a novel, highly-sensitive Meso Scale Discovery (MSD) assay for SLIT2 detection.

Methods: Seventy-nine individuals with a mean age of 55.79 ± 12.03 years underwent eye surgery with collection of vitreous humor, blood (plasma) collection, and neurocognitive assessment. Vitreous humor and plasma samples were analyzed by SLIT2 MSD electrochemiluminescence immunoassay. Associations between SLIT2 levels in vitreous humor and plasma were analyzed using GraphPad Prism.

Results: We found up to a 7-fold higher level of SLIT2 in human vitreous humor compared to plasma. Lower vitreous SLIT2 levels were associated with a lower Montreal Cognitive Assessment (MoCA) score and Immediate Recall Verbatim (IRV) z-score, and higher plasma SLIT2 was associated with a lower MoCA score. In

multivariate analysis using single and multiple predictor models, the same significant associations were found when adjusted for age, sex, race, diabetic status, diabetic retinopathy status, glaucoma status, and Apolipoprotein E (APOE) genotype.

Conclusions: SLIT2 protein levels are significantly associated with MoCA score and IRV z-score in middle-aged individuals. The relationship remained significant when adjusted for demographics, co-morbidity, and APOE genotype, suggesting SLIT2 may be a sensitive biomarker for early detection of dementia and Alzheimer's disease, and warrants further studies.

KEYWORDS

ALZHEIMER'S DISEASE, DEMENTIA, NEUROCOGNITIVE TESTS, NEURODEGENERATION, NEUROPSYCHOLOGICAL TESTS, PLASMA, ROUNDABOUT PROTEINS, VITREOUS HUMOR

BACKGROUND

Neurodegenerative disorders can be a cause of neurocognitive impairment and are classified by pathological changes with potential destruction of neural tissue.[1–3] One change known in neurodegenerative disorders is an accumulation of proteins causing pathological damage.[4,5] Protein abnormalities that lead to inadequate clearance and subsequent aggregation can cause this pathological degeneration.[2,6] These changes can occur decades before the onset of cognitive symptoms.

Mild neurocognitive impairment has an approximate prevalence of 6% in individuals aged 60-64 and increases to 25% in individuals aged 80-84.[7] Approximately 5-10% of individuals with mild neurocognitive impairment progress to major neurocognitive disorder or dementia each year,[7,8] the most common of which is

70 Alzheimer's dementia,[9] with an estimated prevalence of 6.5% in middle-aged and senior
71 populations, or 5.7 million in the United States.[10] The prevalence of Alzheimer's disease
72 is expected to double in the next four decades.[10]

73 Neural-derived proteins have been demonstrated previously to have potential
74 relationships with neurocognitive dysfunction.[11] For example, increased neurofilament
75 light (NfL) protein levels have been associated with cognitive impairment.[12,13] Studies
76 have been conducted to assess a variety of proteins involved in the pathological
77 changes of neurodegeneration, such as beta-amyloid (Aβ), t-tau, p-T181-tau, and
78 GFAP.[14,15] Our group has studied protein biomarkers in ocular fluid and pathological
79 proteins associated with AD dementia and mild cognitive impairment.[16–20] Additionally,
80 we have demonstrated that relatively higher concentrations of neurodegenerative
81 biomarkers exist in ocular fluid, such as vitreous fluid, as compared with plasma.[16]

82 Slit Guidance Ligand 2 (SLIT2) protein is a ligand that binds to Roundabout
83 (ROBO) guidance receptors expressed during vascular and neuronal development.[21–23]
84 It is commonly found in cerebral tissue[24,25] and functions as a chemorepulsive guidance
85 to direct axon pathfinding and neuron migration,[23,26,27] such as the development of the
86 optic chiasm[28] and neurodegenerative disease.[29–31] Proteomic studies have found that
87 SLIT2 levels are increased in Aβ overexpression mouse models[32] and are correlated
88 with Aβ levels in human tissue.[33] SLIT2 has also been shown to co-localize with Aβ.[32]
89 We therefore hypothesize that SLIT2 may also be an additional associated
90 neurodegenerative biomarker.

91 Prior reports[31,33–35] have suggested a link between SLIT2 protein levels and
92 dementia and Alzheimer's Disease through studies on cognitive stimulation in the

93 workplace, proteomic profiling, and forward genetic screening. In a population-based
94 proteomic study, increased levels of plasma SLIT2 protein were shown to be associated
95 with an increased hazard ratio for dementia, suggesting that SLIT2 may be a potential
96 biomarker candidate for early detection of cognitive functional decline and possible
97 progression to dementia.[34] However, this proteomic finding has not been validated by a
98 current commercially available SLIT2 immunoassay due to their low sensitivity and
99 dynamic range.

100 SLIT2 protein is highly expressed in ocular neurons.[36] However, SLIT2 in the
101 vitreous humor and its association with cognitive status have not been previously
102 investigated. In this study, we hypothesize that the SLIT2 protein measured in human
103 vitreous humor and plasma is associated with cognitive function based on
104 neuropsychological assessment.

105 **METHODS**

106 *Study Design*

107 This is a cross-sectional cohort study conducted at Boston University Medical
108 Campus and Boston Medical Center (BUMC/BMC) to assess the association of
109 biomarkers in eye fluid and plasma with neurocognitive test scores. This study utilized
110 previously stored eye fluid and plasma samples from prior research that investigated the
111 association of biomarkers to eye disease and neurocognitive function. The BUMC/BMC
112 institutional review board provided approval and oversight (study reference number H-
113 37370, principal investigator MLS). The study was completed in accordance with
114 institutional ethical standards and the Declaration of Helsinki.

115 Study participant inclusion criteria included age 18 years or older, primary
116 language English or Spanish, and those scheduled for pars plana vitrectomy (PPV) in at
117 least one eye for a clinical eye condition. Surgical indications for vitrectomy included
118 eye diseases such as rhegmatogenous retinal detachment, macular hole, epiretinal
119 membrane, vitreous hemorrhage, and tractional retinal detachment. Study activities for
120 Spanish-speaking patients were completed with a bilingual research assistant. Written
121 informed consent was obtained from all patients who participated in the study, and
122 participants were not excluded for existing ocular or medical comorbidities. Upon
123 completing the consent process, the study activities included neuropsychological
124 testing, vitreous humor samples collected during PPV, and blood samples collected
125 during a research visit.

126 *Historical Data Collection*

127 Demographic information and clinical history of enrolled participants were
128 collected from participant-reported questionnaires and electronic medical records.
129 Demographic information included age, race, and sex. Clinical history included past
130 medical history and ophthalmic history. A baseline ophthalmic exam, including
131 assessment of common eye conditions such as glaucoma, age-related macular
132 degeneration, and diabetic retinopathy, was completed prior to eye surgery.

133 *Neuropsychological testing*

134 Neuropsychological testing was conducted with all enrolled participants within a
135 3-month window before or after their surgery date. Our group collaborated with the
136 Boston University Alzheimer's Disease and Research Center, which recommended
137 neuropsychological testing within 180 days of fluid specimen collection and participated

138 in training research assistants to conduct the assessments. Validated tools for testing
139 included the Montreal Cognitive Assessment (MoCA)[37] and the Immediate Recall
140 Verbatim (IRV).[38] Test scores (MoCA raw score and IRV z-score) are standardized and
141 allow for comparison between individuals.[39,40]

142 *Biospecimen Collection*

143 Vitreous and blood samples were collected between September 2019 and
144 August 2021. Vitreous samples were collected at the start of each vitrectomy procedure
145 by the surgical team. 0.5–1.0 mL of undiluted vitreous fluid was aspirated via the
146 vitrectomy probe into an attached sterile 3-mL syringe. Infusion of saline into the
147 vitreous cavity was immediately undertaken in order to re-pressurize the posterior
148 chamber of the eye, and the vitrectomy subsequently proceeded according to the
149 standard of care for the respective ocular condition. The syringe containing the vitreous
150 specimen was capped using sterile technique and directly handed to a research
151 assistant, who labeled it with a predetermined non-identifiable study number and placed
152 the sample on ice for transport. Blood samples were collected from all study
153 participants at a research visit prior to surgery, and eighteen milliliters of whole blood
154 were drawn into Ethylenediaminetetraacetic acid (EDTA)-treated purple top tubes and
155 also placed on ice for transport. Once collected, vitreous and blood biospecimens were
156 immediately centrifuged, aliquoted into 200 microliter Eppendorf tubes at the Molecular
157 Genetics Core Laboratory (MGCL) at BU Medical Campus and stored in -80˚C freezers.
158 De-identified blood samples were processed into their component plasma and buffy
159 coat. Samples were thawed once prior to immunoassay analysis between August and
160 November 2023, with no additional freeze-thaw cycles. Plasma and vitreous samples

161 were utilized for SLIT2 protein analysis, and the buffy coat was used for Apolipoprotein
162 E (APOE) genotyping.

163 *SLIT2 MSD Assay to measure protein expression in the vitreous and plasma*

164 To measure the low SLIT2 protein concentration in the human vitreous humor
165 and plasma samples, we developed a highly sensitive Meso Scale Discovery (MSD)
166 SLIT2 immunoassay using a SLIT2 antibody pair (Catalog # ab253658, Abcam,
167 Waltham, MA, USA) and GOLD 96-Well Small Spot Streptavidin SECTOR Plates
168 (Catalog # L45SA, Meso Scale Diagnostics, Rockville, MD, USA) with an
169 electrochemiluminescence detection technique, which has a greater dynamic range and
170 more precise and accurate readings for low concentrations of SLIT2 at picogram levels.
171 Details on this internally developed SLIT2 MSD assay are currently in preparation and
172 will be reported separately. In a process similar to a sandwich enzyme-linked
173 immunoabsorbent assay (ELISA), we detected and quantified standard SLIT2
174 recombinant protein and unknown SLIT2 concentration in human vitreous humor and
175 plasma samples using the MESO QuickPlex SQ120 instrument (Catalog # AI1AA-0,
176 Meso Scale Diagnostics, Rockville, MD, USA).

177 *APOE Genotyping*

178 APOE is a major susceptibility gene, and relative allele copies can confer a
179 higher risk of Alzheimer's;[41–43] therefore, APOE genotyping is sometimes used as a
180 clinical risk evaluation tool.

181 To determine the APOE genotype, DNA was extracted from the buffy coat, and
182 single nucleotide polymorphisms from the APOE gene (National Center for

183 Biotechnology Information SNPs rs429358 and rs7412) were examined using TaqMan
184 assays (Applied Biosystems, Foster City, CA).
185 *Statistical Analysis*
186 Statistical analyses were performed using GraphPad Prism (Version 10.2.2 341)
187 for MacOS, GraphPad Software, Boston, MA, USA, www.graphpad.com). A p-value of
188 less than 0.05 was considered statistically significant.
189 SLIT2 protein levels and neurocognitive assessment score association testing,
190 using neurocognitive scores as a continuous variable, was performed using univariate
191 and multivariate linear regression. Univariate analysis included both SLIT2 protein
192 plasma and vitreous levels as predictors of MoCA raw score and IRV z-score.
193 Additionally, analysis was performed with participants grouped based on MoCA raw
194 score for normal cognition (MoCA ≥26) and potential cognitive impairment (MoCA <26).
195 Multivariate linear regression was completed with SLIT2 plasma and vitreous
196 protein level again utilized as a predictor of neurocognitive test score and adjusted for
197 the single predictors of age, sex, race, diabetic status, diabetic retinopathy status,
198 glaucoma, APOE genotype, and APOE risk category. APOE risk categories
199 (Supplemental Table 1) were constructed based on population genotype prevalence
200 patterns and associated likelihood of developing Alzheimer's disease, as previously
201 published.[41] Multiple predictor analysis was also completed and adjusted for
202 demographics, which included age, sex, and race, and for demographics with additional
203 medical and ophthalmic history variables. A complete model, which included
204 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category,

205 was also analyzed as applicable. Diabetes mellitus and diabetic retinopathy status could
206 not be simultaneously included in the complete model due to variable collinearity.

207 **RESULTS**

208 *Demographics*

209 Seventy-nine participants were enrolled in the study; 79 vitreous samples and 67
210 blood samples were collected. Missing samples were due to challenges with specimen
211 collection, such as poor intravenous access, participant refusal of blood draws,
212 hemolyzed blood, and inadequate sample volume. Adequate material for the SLIT2
213 assay was obtained in 69 vitreous and 60 plasma samples. MoCA testing was
214 completed in 63 participants, and IRV testing was completed in 56. Figure 1
215 summarizes the biospecimen samples collected and the neurocognitive testing
216 completed. Table 1 describes the study demographic characteristics.

217 *SLIT2 Protein Detection*

218 Our data showed that the newly developed highly sensitive SLIT2 MSD assay
219 can detect SLIT2 protein expression levels as low as 5 pg/ml, which is 200 folds more
220 sensitive than regular ELISA. In addition, we discovered 7-fold higher SLIT2 levels in
221 the vitreous humor compared to blood plasma samples in humans. In a Spearman
222 correlation, SLIT2 plasma and vitreous levels were not correlated with each other (ρ=-
223 0.05424, p=0.6833).

224 *Univariate Analysis*

225 In univariate linear regression, lower vitreous SLIT2 levels were associated with
226 lower MoCA raw scores (m=0.003897, r^2=0.07585, p=0.0332, Figure 2a) and lower IRV
227 z-scores (m=0.001196, r^2=0.09811, p=0.0224, Figure 2b). Alternatively, plasma levels

228 showed the opposite relationship, with higher plasma SLIT2 associated with lower
229 MoCA scores (m=-0.03559, r^2=0.09699, p=0.0206, Figure 3a). Plasma SLIT2 levels
230 were not associated with IRV z-scores (m=-0.003430, r^2=0.01830, p=0.3593, Figure
231 3b).

232 Further analysis among only individuals with a MoCA score indicating possible
233 cognitive impairment (MoCA score below 26) was associated with lower vitreous SLIT2
234 (m=0.003660, r^2=0.09902, p=0.0353, Figure 4a) and higher plasma SLIT2 (m=-0.03311,
235 r^2=0.0073, p=0.0073, Figure 4b). In individuals with a MoCA score in the normal
236 cognition range (≥26), there was no significant association of MoCA scores with SLIT2
237 in plasma (m=-0.008733, r^2=0.03803, p=0.5231, Supplemental Figure 2a) or SLIT2 in
238 vitreous (m=0.003660, r^2=0.09902, p=0.2789, Supplemental Figure 2b).

239 *Multivariate Analysis*

240 In single predictor adjustment models (Table 2), lower vitreous SLIT2 was
241 associated with lower MoCA score after adjusting separately for age, sex, race, diabetic
242 status, diabetic retinopathy status, and glaucoma. Multiple predictor models were
243 significant when adjusted for demographics (age, sex, and race; p=0.0225),
244 demographics with diabetic status (p=0.0162), demographics with diabetic retinopathy
245 status (p=0.0269), and demographics with glaucoma status (p=0.0111). A complete
246 model adjusting for demographics, diabetic retinopathy status, and glaucoma status
247 was significant (p=0.0141) with a large effect size (f^2=0.399). APOE genotype and risk
248 category were not included in the complete model for vitreous SLIT2 because the single
249 predictor analysis for both was not significant.

250 Higher plasma SLIT2 (Table 3) was associated with lower MoCA score in single
251 predictor adjustment models adjusted separately for age, sex, race, diabetic status,
252 diabetic retinopathy status, glaucoma, APOE genotype, and APOE risk category. In
253 multiple predictor analysis, similarly significant associations were found when adjusted
254 for demographics (age, sex, and race; p=0.0498), demographics with diabetic
255 retinopathy status (p=0.0198), demographics with APOE genotype (p=0.0418), and
256 demographics with APOE risk category (p=0.0413). A complete model adjusting for
257 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category
258 was not statistically significant (p=0.0522) but had a large effect size (f^2=0.5712).
259 Vitreous SLIT2 and IRV z-score (Table 4) were also significant in single predictor
260 models adjusted separately for age, sex, race, diabetic status, diabetic retinopathy
261 status, glaucoma status, and APOE risk category, and in multiple predictor models
262 adjusted for demographics (age, sex, and race; p=0.0142), demographics with diabetic
263 status (p=0.0149), demographics with diabetic retinopathy status (p=0.0175), and
264 demographics with glaucoma (p=0.0336). A complete model adjusting for
265 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category
266 was not significant (p=0.1328) but had a large effect size (f^2=0.5514).

267 **DISCUSSION**

268 In this study, we found that vitreous and plasma SLIT2 protein were significantly
269 associated with Montreal Cognitive Assessment (MoCA) and Immediate Recall
270 Verbatim (IRV) scores. The relationship remained significant when adjusted for
271 covariates of age, sex, race, diabetes, and diabetic retinopathy in single and multiple
272 predictor models. We found up to a 7-fold increase in SLIT2 concentration in the

273 vitreous humor compared to plasma. As far as we are aware, this is the first study to
274 report relative concentrations of SLIT2 in vitreous and plasma and establish an
275 association of SLIT2 levels from both sources with cognitive function.

276 Our research supports previous studies that demonstrated associations between
277 SLIT2 and cognitive function. Prior reports by Kivimaki et al. found that higher plasma
278 SLIT2 levels were associated with an increased risk of dementia.[34] Our study further
279 validates previous work done by our group that demonstrated an association between
280 vitreous protein levels and neurocognitive test scores[17] and the relatively higher
281 neurodegenerative protein concentrations in the vitreous compared with plasma.[16] We
282 have also found in a prior post-mortem study that an elevated vitreous concentration
283 may reflect an elevated cerebral concentration.[18] In this study, our findings further
284 demonstrate the potential for ocular fluids as a biospecimen sampling source for early
285 detection and diagnosis of neurocognitive disorders, as well as the unexplored role of
286 SLIT-ROBO signaling in the pathological development of neurodegenerative disease.

287 We found opposite directions of associations in the vitreous humor and plasma
288 between SLIT2 levels and MoCA score, the reasons for which remain unclear. SLIT2 is
289 a paracrine ligand associated with the extracellular membrane receptor ROBO, resulting
290 in high concentrations in local tissues surrounding an area of increased expression. As
291 SLIT2 is required for active axon pathfinding and new neuronal synapse formation in the
292 CNS, one possibility is that it is more highly expressed in the brain and ocular tissues
293 compared to blood, and therefore lower SLIT2 in vitreous humor may suggest
294 decreased axon pathfinding and fewer new neuronal synapse formations in the brain,
295 observed as lower MoCA and IRV scores. Relatively higher plasma SLIT2 may indicate

296 the result of a systemic body reaction attempting to increase SLIT2 levels and stimulate
297 axon pathfinding and new neuronal synapse formations. Similar inverse relationships
298 have been noted in Aβ, with higher concentrations of amyloid plaque deposition in the
299 brain being associated with lower Aβ concentrations in the CSF and plasma.[44–47] As
300 previously mentioned, our group has found significantly higher Aβ concentrations in the
301 vitreous in individuals with lower cognitive scores (based on mini-mental status
302 examination), opposite of what is typically noted in the CSF in those with mild cognitive
303 impairment (MCI) or Alzheimer's Disease.[17] Additionally, some insight might be
304 gathered from the inverted relationship expanding as neurocognitive function declines. It
305 has been previously demonstrated that neurodegeneration is associated with impaired
306 blood-brain barrier (BBB) and CNS vasculature functionality.[48] Increased BBB
307 permeability[49] and decreased function in pathological inflammatory states may
308 contribute to the increased concentration of SLIT2 in the plasma, as SLIT2 also plays a
309 role in inflammation.[50,51] The involvement of SLIT2 remains unclear, as SLIT2
310 overexpression in mouse models has been shown to have increased or decreased BBB
311 permeability.[52,53] Interestingly, overexpression of SLIT1, which is a homologue of SLIT2
312 and highly expressed in the brain, can facilitate functional neuronal regeneration in post-
313 ischemic stroke mice by enhancing new neuron migration through the brain glial
314 meshwork to stroke lesions.[54] Further investigation into the mechanism by which SLIT2
315 exits the CNS and enters the systemic circulatory system is needed to better
316 understand the relative concentration differences between vitreous and plasma
317 samples.

318 SLIT2 was significantly associated with diabetes, which has been previously
319 shown in rat models of type 1 diabetes[36] and human epidemiological studies.[55–57]
320 Diabetes has been shown to be associated with higher rates of Alzheimer's disease; it
321 is hypothesized that the pathophysiological mechanisms of hyperglycemia induce and
322 increase the neuropathological changes found in Alzheimer's.[58]
323 Limitations of this study include limited sample size and a lack of comprehensive
324 neurological testing. While our sample was normally distributed with regard to MoCA
325 score, IRV z-score, and SLIT2 protein level (Supplemental Figure 1), the limited
326 numbers made it difficult to make more specific comparisons. Additionally, further
327 stratification in those with lower MoCA scores into MCI and dementia would require
328 functional assessment of participants. The limited sample also prevented analysis
329 adjusted for additional ocular co-morbidities, such as macular degeneration, which was
330 not present in any of our participants, likely due to our study cohort being younger and
331 more racially and ethnically diverse. High prevalence of comorbidities associated with
332 dementia risk, such as diabetes, likely contributed to the low average neurocognitive
333 score in our young study cohort, as well as the high percentage of patients with APOE
334 E4, since it has been shown that diabetic patients are more genetically predisposed to
335 Alzheimer's Disease.[59] We aim to address these limitations with further prospective
336 studies with larger recruitment goals. Additionally, participants for this study were
337 patients from the eye clinic at Boston Medical Center and, therefore, were recruited
338 based on ocular history, not neurological evaluation. While we adjusted for any
339 significant covariates, such as diabetes mellitus, diabetic retinopathy, and glaucoma,
340 the impact of eye and systemic diseases on SLIT2 levels remains unclear. Because of

341 this study's setting, only screening-level neuropsychological testing was performed.
342 Further studies validating our findings on the basis of neurodegenerative diagnosis and
343 staging could provide more specific information regarding the extent of
344 neurodegeneration associated with SLIT2 protein levels.

345 In conclusion, we have demonstrated that SLIT2 protein in the vitreous and
346 plasma is significantly associated with Montreal Cognitive Assessment score and
347 Immediate Recall Verbatim z-score, and the relationship remained significant when
348 adjusted for demographics, medical and ocular co-morbidities, and APOE genotype
349 factors. The utilization of a custom-made highly sensitive MSD immunoassay allowed
350 for quantification of SLIT2 at the pg/mL level. The inverse concentrations between
351 vitreous and plasma samples highlight the need for further understanding of the
352 mechanisms underlying neurodegeneration and its relationship with systemic
353 circulation.

354 **DECLARATIONS**

355 **Ethics Approval and Consent to Participate**

356 BUMC/BMC institutional review board provided approval and oversight (study reference
357 number H-37370, principal investigator MLS).

358 **Data Availability**

359 The data supporting the findings of this study are available on request from the
360 corresponding author.

361 **Competing Interest**

362 The authors have declared that no conflict of interest exists.

363 **Funding**

This work was supported in part by the National Institutes of Health grant R01-DK133940 (WL), R03AG063255 (MLS), the DOD grant E01HT9425-23-1-1058 (WL), a 2023 Boston University Ignition Award (WL), a 2024 B4D-ARC Award from the Evans Center of Boston University (MLS, WL) and funding support from Boston University Undergraduate Research Opportunities Program (EL). This publication is also supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health, through Boston University Clinical & Translational Science Institute (CTSI) Grant Number 1UL1TR001430. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Author contributions

SIS (Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft); EL (Conceptualization, Investigation, Methodology, Writing – Original Draft); SA (Data Curation, Investigation, Project Administration, Writing – Review & Editing); ZS (Data Curation, Investigation, Project Administration, Writing – Review & Editing); SK (Data Curation, Investigation, Writing – Review & Editing); CH (Data Curation, Investigation, Writing – Review & Editing); IMS (Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing); SN (Investigation, Writing – Review & Editing); XC (Investigation, Writing – Review & Editing); NHS (Investigation, Writing – Review & Editing); SSW (Conceptualization, Resources, Writing – Review & Editing); TS (Investigation, Methodology, Writing – Review & Editing); WL (Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Original Draft, Writing – Review & Editing); MLS (Conceptualization, Data Curation, Formal

Analysis, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Original Draft, Writing – Review & Editing)

Acknowledgments

We thank Dr. Weiming Xia and Dr. Michael Alosco of the Eye Biomarkers Study (EBS) and the BU Alzheimer's disease center for their support. We thank Dr. Janice Weinberg of the BU CTSI's Biostatistics, Epidemiology & Research Design (BERD) program for their statistical consultation.

394 **REFERENCES**

395 1. Kovacs GG. Molecular pathology of neurodegenerative diseases: Principles and
396 practice. *J Clin Pathol* 2019; 72: 725–735.
397 2. Dugger BN, Dickson DW. Pathology of neurodegenerative diseases. *Cold Spring*
398 *Harb Perspect Biol*; 9. Epub ahead of print 2017. DOI:
399 10.1101/cshperspect.a028035.
400 3. Rekatsina Antonella Paladini Alba Piroli Panagiotis Zis Joseph Pergolizzi Giustino
401 Varrassi M V. Pathophysiology and Therapeutic Perspectives of Oxidative Stress
402 and Neurodegenerative Diseases: A Narrative Review. *Adv Ther*; 37. DOI:
403 10.6084/m9.figshare.10247852.
404 4. Zaib S, Javed H, Khan I, et al. Neurodegenerative Diseases: Their Onset,
405 Epidemiology, Causes and Treatment. *ChemistrySelect*; 8. Epub ahead of print
406 25 May 2023. DOI: 10.1002/slct.202300225.
407 5. Webber CJ, Lei S (Eric), Wolozin B. The pathophysiology of neurodegenerative
408 disease: Disturbing the balance between phase separation and irreversible
409 aggregation. *Prog Mol Biol Transl Sci* 2020; 174: 187–223.
410 6. Relja M. PATHOPHYSIOLOGY AND CLASSIFICATION OF
411 NEURODEGENERATIVE DISEASES. *Journal of International Federation of*
412 *Clinical Chemistry and Laboratory Medicine*; 97–99.
413 7. Pérez Palmer N, Trejo Ortega B, Joshi P. Cognitive Impairment in Older Adults:
414 Epidemiology, Diagnosis, and Treatment. *Psychiatric Clinics of North America*
415 2022; 45: 639–661.
416 8. Akushevich I, Yashkin AP, Kravchenko J, et al. Time Trends in the Prevalence of
417 Neurocognitive Disorders and Cognitive Impairment in the United States: The
418 Effects of Disease Severity and Improved Ascertainment. *Journal of Alzheimer's*
419 *Disease* 2018; 64: 137–148.
420 9. Erkkinen MG, Kim MO, Geschwind MD. Clinical neurology and epidemiology of
421 the major neurodegenerative diseases. *Cold Spring Harb Perspect Biol*; 10. Epub
422 ahead of print 1 April 2018. DOI: 10.1101/cshperspect.a033118.
423 10. Thorpe KE, Levey AI, Thomas J. *May 2021 Neurodegenerative Disease Burden*
424 *on US - FINAL* . 2021.
425 11. Logroscino G, Urso D, Savica R. Descriptive Epidemiology of Neurodegenerative
426 Diseases: What Are the Critical Questions? *Neuroepidemiology* 2022; 56: 309–
427 318.
428 12. Olsson B, Portelius E, Cullen NC, et al. Association of Cerebrospinal Fluid
429 Neurofilament Light Protein Levels with Cognition in Patients with Dementia,
430 Motor Neuron Disease, and Movement Disorders. *JAMA Neurol* 2019; 76: 318–
431 325.
432 13. Lin YS, Lee WJ, Wang SJ, et al. Levels of plasma neurofilament light chain and
433 cognitive function in patients with Alzheimer or Parkinson disease. *Sci Rep*; 8.
434 Epub ahead of print 1 December 2018. DOI: 10.1038/s41598-018-35766-w.
435 14. Kim KY, Shin KY, Chang KA. Brain-derived exosomal proteins as effective
436 biomarkers for Alzheimer's Disease: A systematic review and meta-analysis.
437 *Biomolecules*; 11. Epub ahead of print 1 July 2021. DOI: 10.3390/biom11070980.

438 15. Askenazi M, Kavanagh T, Pires G, et al. Compilation of reported protein changes
439 in the brain in Alzheimer's disease. *Nat Commun*; 14. Epub ahead of print 1
440 December 2023. DOI: 10.1038/s41467-023-40208-x.
441 16. Sampani K, Ness S, Tuz-Zahra F, et al. Neurodegenerative biomarkers in
442 different chambers of the eye relative to plasma: an agreement validation study.
443 *Alzheimers Res Ther*; 16. Epub ahead of print 1 December 2024. DOI:
444 10.1186/s13195-024-01556-y.
445 17. Wright LM, Stein TD, Jun G, et al. Association of cognitive function with amyloid-β
446 and tau proteins in the vitreous humor. *Journal of Alzheimer's Disease* 2019; 68:
447 1429–1438.
448 18. Vig V, Garg I, Tuz-Zahra F, et al. Vitreous Humor Biomarkers Reflect Pathological
449 Changes in the Brain for Alzheimer's Disease and Chronic Traumatic
450 Encephalopathy. *J Alzheimers Dis* 2023; 93: 1181–1193.
451 19. Subramanian ML, Vig V, Chung J, et al. Neurofilament light chain in the vitreous
452 humor of the eye. *Alzheimers Res Ther*; 12. Epub ahead of print 17 September
453 2020. DOI: 10.1186/s13195-020-00677-4.
454 20. Fereshetian S, Agranat JS, Siegel N, et al. Protein and Imaging Biomarkers in the
455 Eye for Early Detection of Alzheimer's Disease. *Journal of Alzheimer's Disease*
456 *Reports* 2021; 5: 375–387.
457 21. Rama N, Dubrac A, Mathivet T, et al. Slit2 signaling through Robo1 and Robo2 is
458 required for retinal neovascularization. *Nat Med* 2015; 21: 483–491.
459 22. Tong M, Jun T, Nie Y, et al. The role of the SLIT/Robo signaling pathway. *J*
460 *Cancer* 2019; 10: 2694–2705.
461 23. Blockus H, Chédotal A. Slit-robo signaling. *Development (Cambridge)* 2016; 143:
462 3037–3044.
463 24. Sasaki T, Komatsu Y, Yamamori T. Expression patterns of SLIT/ROBO mRNAs
464 reveal a characteristic feature in the entorhinal-hippocampal area of macaque
465 monkeys. *BMC Res Notes*; 13. Epub ahead of print 27 May 2020. DOI:
466 10.1186/s13104-020-05100-7.
467 25. Xu C, Fan CM. Expression of Robo/Slit and Semaphorin/Plexin/Neuropilin family
468 members in the developing hypothalamic paraventricular and supraoptic nuclei.
469 *Gene Expression Patterns* 2008; 8: 502–507.
470 26. Plachez C, Andrews W, Liapi A, et al. Robos are required for the correct targeting
471 of retinal ganglion cell axons in the visual pathway of the brain. *Molecular and*
472 *Cellular Neuroscience* 2008; 37: 719–730.
473 27. Thompson H, Andrews W, Parnavelas JG, et al. Robo2 is required for Slit-
474 mediated intraretinal axon guidance. *Dev Biol* 2009; 335: 418–426.
475 28. Davison C, Zolessi FR. Slit2 is necessary for optic axon organization in the
476 zebrafish ventral midline. *Cells and Development*; 166. Epub ahead of print 1
477 June 2021. DOI: 10.1016/j.cdev.2021.203677.
478 29. Dickson BJ. Molecular Mechanisms of Axon Guidance. *Science (1979)* 2002;
479 298: 1959–1964.
480 30. Sherchan P, Travis ZD, Tang J, et al. The potential of Slit2 as a therapeutic target
481 for central nervous system disorders. *Expert Opin Ther Targets* 2020; 24: 805–
482 818.

483 31. Zhang L, Qi Z, Li J, et al. Roles and Mechanisms of Axon-Guidance Molecules in
484 Alzheimer's Disease. *Mol Neurobiol* 2021; 58: 3290–3307.
485 32. Bai B, Wang X, Li Y, et al. Deep Multilayer Brain Proteomics Identifies Molecular
486 Networks in Alzheimer's Disease Progression. *Neuron* 2020; 105: 975-991.e7.
487 33. Wang H, Dey KK, Chen PC, et al. Integrated analysis of ultra-deep proteomes in
488 cortex, cerebrospinal fluid and serum reveals a mitochondrial signature in
489 Alzheimer's disease. *Mol Neurodegener*; 15. Epub ahead of print 25 July 2020.
490 DOI: 10.1186/s13024-020-00384-6.
491 34. Kivimäki M, Walker KA, Pentti J, et al. Cognitive stimulation in the workplace,
492 plasma proteins, and risk of dementia: Three analyses of population cohort
493 studies. *The BMJ*; 374. Epub ahead of print 18 August 2021. DOI:
494 10.1136/bmj.n1804.
495 35. Bai G, Chivatakarn O, Bonanomi D, et al. Presenilin-dependent receptor
496 processing is required for axon guidance. *Cell* 2011; 144: 106–118.
497 36. Zhou W, Wang H, Yu W, et al. The expression of the Slit-Robo signal in the retina
498 of diabetic rats and the vitreous or fibrovascular retinal membranes of patients
499 with proliferative diabetic retinopathy. *PLoS One*; 12. Epub ahead of print 1
500 October 2017. DOI: 10.1371/journal.pone.0185795.
501 37. Daniel B, Agenagnew L, Workicho A, et al. Psychometric Properties of the
502 Montreal Cognitive Assessment (MoCA) to Detect Major Neurocognitive Disorder
503 Among Older People in Ethiopia: A Validation Study. *Neuropsychiatr Dis Treat*
504 2022; 18: 1789–1798.
505 38. Schroeder RW, Martin PK, Walling A. Neuropsychological Evaluations in Adults.
506 *American Family Physicians* 2019; 99: 101–108.
507 39. Weintraub S, Besser L, Dodge HH, et al. Version 3 of the Alzheimer Disease
508 Centers' Neuropsychological Test Battery in the Uniform Data Set (UDS).
509 *Alzheimer Dis Assoc Disord* 2018; 32: 10–17.
510 40. *Neuropsychological Battery Tests reported on Form C2, Uniform Data Set (UDS)*
511 *v3.0*, www.alz.washington.edu (2006).
512 41. Reiman EM, Arboleda-Velasquez JF, Quiroz YT, et al. Exceptionally low
513 likelihood of Alzheimer's dementia in APOE2 homozygotes from a 5,000-person
514 neuropathological study. *Nat Commun*; 11. Epub ahead of print 1 December
515 2020. DOI: 10.1038/s41467-019-14279-8.
516 42. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E
517 type 4 allele and the risk of Alzheimer's disease in late onset families. *American*
518 *Association for the Advancement of Science*,
519 http://www.sciencemag.org.ezproxy.bu.edu/ (1993).
520 43. Corder EH, Saunders AM, Risch N], et al. Protective effect of apolipoprotein E
521 type 2 allele for late onset Alzheimer disease. *Nat Genet* 1994; 7: 180–184.
522 44. Bateman RJ, Xiong C, Benzinger TLS, et al. Clinical and Biomarker Changes in
523 Dominantly Inherited Alzheimer's Disease. *New England Journal of Medicine*
524 2012; 367: 795–804.
525 45. Mehta PD, Pirttilä T, Mehta SP, et al. *Plasma and Cerebrospinal Fluid Levels of*
526 *Amyloid Proteins 1-40 and 1-42 in Alzheimer Disease*.
527 46. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and
528 prospects for the future. *Journal of Internal Medicine* 2018; 284: 643–663.

47. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016; 15: 673–684.
48. Cipollini V, Troili F, Giubilei F. Emerging biomarkers in vascular cognitive impairment and dementia: From pathophysiological pathways to clinical application. *Int J Mol Sci*; 20. Epub ahead of print 1 June 2019. DOI: 10.3390/ijms20112812.
49. Fakhoury M. Role of immunity and inflammation in the pathophysiology of neurodegenerative diseases. *Neurodegener Dis* 2015; 15: 63–69.
50. Fernando R, Grisolia ABD, Lu Y, et al. Slit2 Modulates the Inflammatory Phenotype of Orbit-Infiltrating Fibrocytes in Graves' Disease. *The Journal of Immunology* 2018; 200: 3942–3949.
51. Li Q, Huang L, Ding Y, et al. Recombinant Slit2 suppresses neuroinflammation and Cdc42-mediated brain infiltration of peripheral immune cells via Robo1–srGAP1 pathway in a rat model of germinal matrix hemorrhage. *J Neuroinflammation*; 20. Epub ahead of print 1 December 2023. DOI: 10.1186/s12974-023-02935-2.
52. Li JC, Han L, Wen YX, et al. Increased permeability of the blood-brain barrier and Alzheimer's disease-like alterations in slit-2 transgenic Mice. *Journal of Alzheimer's Disease* 2015; 43: 535–548.
53. Li G, He X, Li H, et al. Overexpression of Slit2 improves function of the paravascular pathway in the aging mouse brain. *Int J Mol Med* 2018; 42: 1935–1944.
54. Kaneko N, Herranz-Pérez V, Otsuka T, et al. New neurons use Slit-Robo signaling to migrate through the glial meshwork and approach a lesion for functional regeneration. *Sci Adv*; 4.
55. Zhang J, Chen C, Hua S, et al. An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease. *Diabetes Res Clin Pract* 2017; 124: 41–47.
56. Kang YE, Choung S, Lee JH, et al. The role of circulating slit2, the one of the newly batokines, in human diabetes mellitus. *Endocrinology and Metabolism* 2017; 32: 383–388.
57. Wang Y, Zhao S, Peng W, et al. The Role of Slit-2 in Gestational Diabetes Mellitus and Its Effect on Pregnancy Outcome. *Front Endocrinol (Lausanne)*; 13. Epub ahead of print 23 June 2022. DOI: 10.3389/fendo.2022.889505.
58. Jash K, Gondaliya P, Kirave P, et al. Cognitive dysfunction: A growing link between diabetes and Alzheimer's disease. *Drug Dev Res* 2020; 81: 144–164.
59. Ouyang F, Yuan P, Ju Y, et al. Alzheimer's disease as a causal risk factor for diabetic retinopathy: a Mendelian randomization study. *Front Endocrinol (Lausanne)*; 15. Epub ahead of print 2024. DOI: 10.3389/fendo.2024.1340608.

571 **TABLES**

572 **Table 1: Participant Demographics and Medical History**

Characteristic	Sample Breakdown (n=70)
Sex	
Male	41 (59%)
Female	29 (41%)
Age (years, mean ± SD)	55.79 ± 12.03
Race	
White	13 (19%)
Asian	3 (4%)
Black or African American	23 (33%)
Hispanic	30 (43%)
American Indian or Alaskan Native	1 (1%)
Language	
English	44 (63%)
Spanish	23 (36%)
Medical History	
Non-Diabetic	19 (27%)
Type 1 Diabetes Mellitus	8 (11%)
Type 2 Diabetes Mellitus	43 (61%)
No Diabetic Retinopathy	28 (40%)
Diabetic Retinopathy	42 (60%)
Stage 1	1 (1%)
Stage 2	1 (1%)
Stage 3	1 (1%)
Stage 4	31 (44%)
Unknown Stage	8 (11%)
Glaucoma	19 (27%)
Neurocognitive Tests	

MoCA (n=61, mean ± SD)	21.21 ± 5.15
MoCA score <26	46 (75%)
MoCA score ≥26	15 (25%)
Immediate Recall Verbatim (Z-Score, n=53, mean ± SD)	-0.76 ± 1.19
Biomarkers (pg/mL)	
SLIT Plasma (n=60, mean ± SD)	123.5 ± 48.38
SLIT Vitreous (n=69, mean ± SD)	577.6 ± 350.2

573

Table 2: Predictor Effects on the Association of SLIT2 Vitreous and MoCA Score. The single predictor model applied each variable separately to determine its individual impact on the association of SLIT2 Vitreous and MoCA Score. In the multiple predictor model, all demographic variables (age, sex, and race) were additionally combined with medical variables. APOE genotype and risk category were not included in the multiple predictor or complete models because the single predictor model did not find a significant association. The complete model did meet statistical significance with a large effect size (f^2=0.399).

Effect	Estimate	Standard Error	Confidence Interval (95%)	P
Single Predictor				
Age	0.003743	0.00179	0.000147 to 0.007338	0.0416
Sex	0.004392	0.00180	0.0007784 to 0.008006	0.0181
Race	0.004022	0.00189	0.0002259 to 0.007818	0.0382
Diabetic Status	0.004088	0.00184	0.0004033 to 0.007773	0.0303
Diabetic Retinopathy Status	0.003779	0.00181	0.0001360 to 0.007422	0.0423
Glaucoma	0.004361	0.00186	0.0006289 to 0.008094	0.0228
APOE Genotype	0.003007	0.00184	-0.0007016 to 0.006715	0.1096
APOE Risk Category	0.003172	0.00181	-0.0004808 to 0.006825	0.0872
Multiple Predictor				
Demographics	0.004345	0.00184	0.0006379 to 0.008051	0.0225
Demographics + Diabetic Status	0.004735	0.00190	0.0009117 to 0.008559	0.0162
Demographics + Diabetic Retinopathy Status	0.004299	0.00188	0.0005113 to 0.008087	0.0269
Demographics + Glaucoma	0.005080	0.00193	0.001211 to 0.008948	0.0111
Complete Model	0.004991	0.00196	0.0001052 to 0.008930	0.0141

Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative Diabetic Retinopathy
Glaucoma: No glaucoma (reference group), Glaucoma
APOE Genotype: 3/3 (reference group), 2/2, 2/4, 3/4, 4/4
APOE Risk Category (see Supplementary Table 1): 2/3 and 3/3 (reference group), 2/2, 2/4 and 3/4, 4/4

593 **Table 3: Predictor Effects on the Association of SLIT2 Plasma and MoCA Score**.
594 The single predictor model applied each variable separately to determine its individual
595 impact on the association of SLIT2 plasma and MoCA Score. In the multiple predictor
596 model, all demographic variables (age, sex, and race) were additionally combined with
597 medical variables. For this analysis, APOE risk category was included in the complete
598 model because the single predictor model was significantly associated with plasma
599 SLIT2. The complete model did not meet statistical significance but had a large effect
600 size (f^2=0.5712).

Effect	Estimate	Standard Error	Confidence Interval (95%)	P
Single Predictor				
Age	-0.03469	0.01488	-0.06454 to -0.004826	0.0237
Sex	-0.03584	0.01504	-0.06602 to -0.005657	0.0209
Race	-0.03208	0.01562	-0.06346 to -0.0007018	0.0453
Diabetic Status	-0.03431	0.01551	-0.06543 to -0.003179	0.0314
Diabetic Retinopathy Status	-0.04446	0.01556	-0.07569 to -0.01323	0.0061
Glaucoma	-0.03749	0.01560	-0.06882 to -0.006153	0.0200
APOE Genotype	-0.03605	0.01559	-0.06741 to -0.004692	0.0252
APOE Risk Category	-0.03512	0.01555	-0.06639 to -0.003847	0.0285
Multiple Predictor				
Demographics	-0.03096	0.01538	-0.06190 to -0.0000256	0.0498
Demographics + Diabetic Status	-0.03012	0.01612	-0.06259 to 0.002351	0.0682
Demographics + Diabetic Retinopathy Status	-0.03876	0.01605	-0.97198 to -0.006437	0.0198
Demographics + Glaucoma	-0.03266	0.01663	-0.06622 to 0.0009003	0.0562
Demographics + APOE Genotype	-0.03402	0.01619	-0.06672 to -0.001326	0.0418
Demographics + APOE Risk Category	-0.03375	0.01603	-0.06610 to -0.001401	0.0413
Complete Model	-0.03620	0.01803	-0.07276 to 0.0003654	0.0522

601
602 Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
603 Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative
604 Diabetic Retinopathy
605 Glaucoma: No glaucoma (reference group), Glaucoma

606 APOE Genotype: 3/3 (reference group), 2/2, 2/4, 3/4, 4/4
607 APOE Risk Category (see Supplementary Table 1): 2/3 and 3/3 (reference group), 2/2,
608 2/4 and 3/4, 4/4
609

610 **Table 4: Predictor Effect on the Association of SLIT2 Vitreous and IRV z-score.**

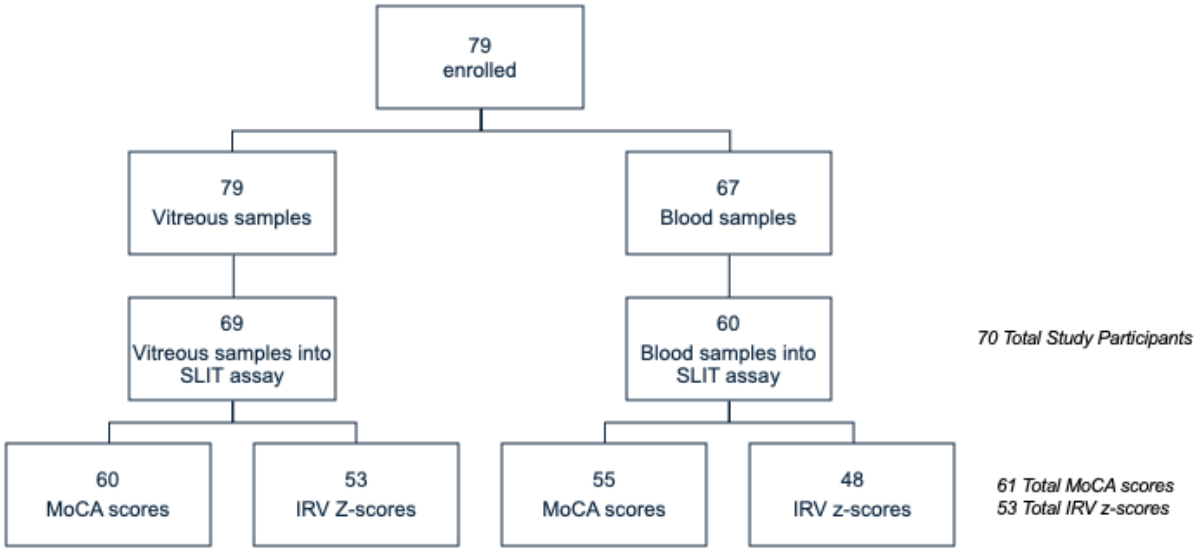
611 The single predictor model applied each variable separately to determine its individual
612 impact on the association of SLIT2 vitreous and IRV z-score. In the multiple predictor
613 model, all demographic variables (age, sex, and race) were additionally combined with
614 medical variables. The complete model did not meet statistical significance but had a
615 large effect size (f^2=0.5514).

Effect	Estimate	Standard Error	Confidence Interval (95%)	P
Single Predictor				
Age	0.00115	0.0004934	0.001588 to 0.002141	0.0239
Sex	0.001276	0.0005092	0.0002535 to 0.002299	0.0155
Race	0.001268	0.0005337	0.0001942 to 0.002342	0.0217
Diabetic Status	0.001249	0.0005158	0.002129 to 0.002286	0.0192
Diabetic Retinopathy Status	0.001155	0.0005102	0.0001306 to 0.002180	0.0279
Glaucoma	0.001107	0.0005086	0.00008515 to 0.002129	0.0343
APOE Genotype	0.001083	0.0005381	-0.000004023 to 0.002169	0.0508
APOE Risk Category	0.001078	0.0005268	0.00001528 to 0.002141	0.0469
Multiple Predictor				
Demographics	0.001332	0.0005220	0.0002807 to 0.002383	0.0142
Demographics + Diabetic Status	0.001345	0.0005353	0.0002755 to 0.002415	0.0149
Demographics + Diabetic Retinopathy Status	0.001300	0.0005266	0.0002387 to 0.002361	0.0175
Demographics + Glaucoma	0.001178	0.0005364	0.00009582 to 0.002259	0.0336
Demographics + APOE Genotype	0.001000	0.0006345	-0.0002880 to 0.002288	0.1240
Demographics + APOE Risk Category	0.001053	0.0005828	-0.0001287 to 0.002235	0.0791
Complete Model	0.0009285	0.0006025	-0.0002972 to 0.002154	0.1328

616 Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
617 Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative
618 Diabetic Retinopathy
619 Glaucoma: No glaucoma (reference group), Glaucoma
620 APOE Genotype: 3/3 (reference group), 2/2, 2/4, 3/4, 4/4
621 APOE Risk Category (see Supplementary Table 1): 2/3 and 3/3 (reference group), 2/2,
622 2/4 and 3/4, 4/4

623
624 **FIGURES**

625 **Figure 1**

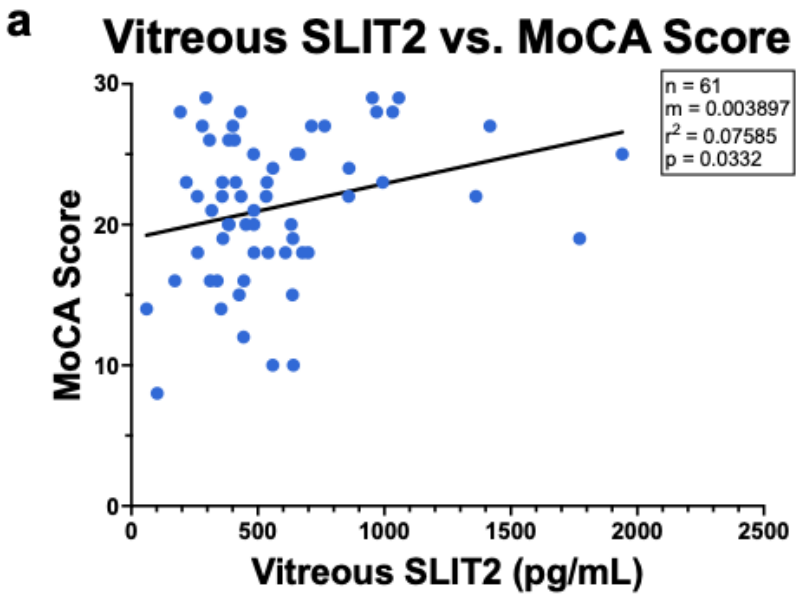


626

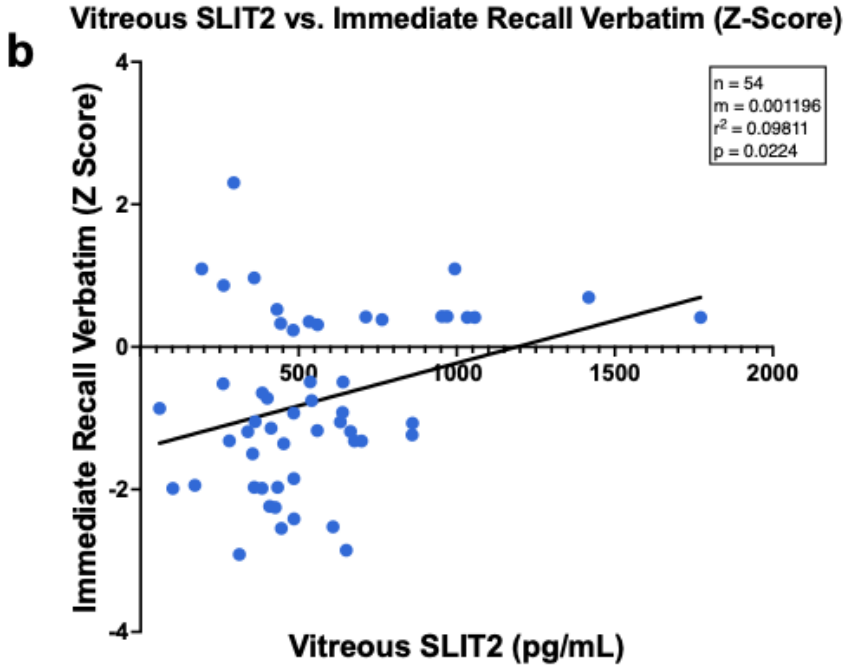
627 **Figure 1. Enrolled Participant Flow Diagram.** A total of 79 patients were enrolled in
628 the study. Missing samples were due to challenges with specimen collection, such as
629 poor intravenous access, participant refusal of blood draws, hemolyzed blood.
630 Additionally, there was inadequate sample volume for SLIT assay analysis for some
631 collected samples. Lastly, neurocognitive testing was not completed or unable to be
632 obtained for all participants.

633

634 **Figure 2**



635



636

637 **Figure 2. The relationship of SLIT2 in the vitreous with neurocognitive tests.**

638 Linear regression analysis demonstrated a significant relationship between SLIT2

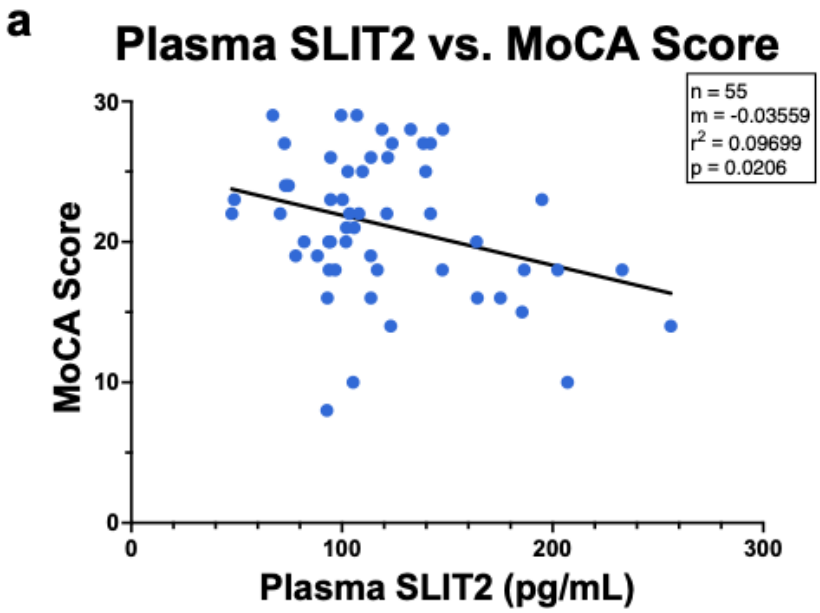
639 vitreous protein level and MoCA score (a, r^2=0.076, p=0.0332) and Immediate Recall

640 Verbatim Z-Score (b, r^2=0.098, p=0.0224).

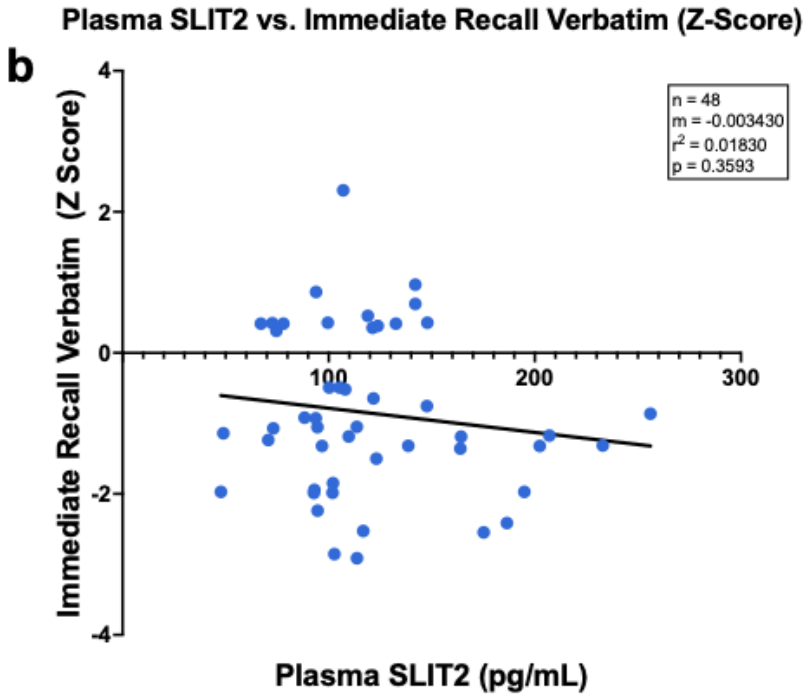
641

642

643 **Figure 3**



644



645

646 **Figure 3. The relationship of SLIT2 plasma levels with neurocognitive tests.** Linear

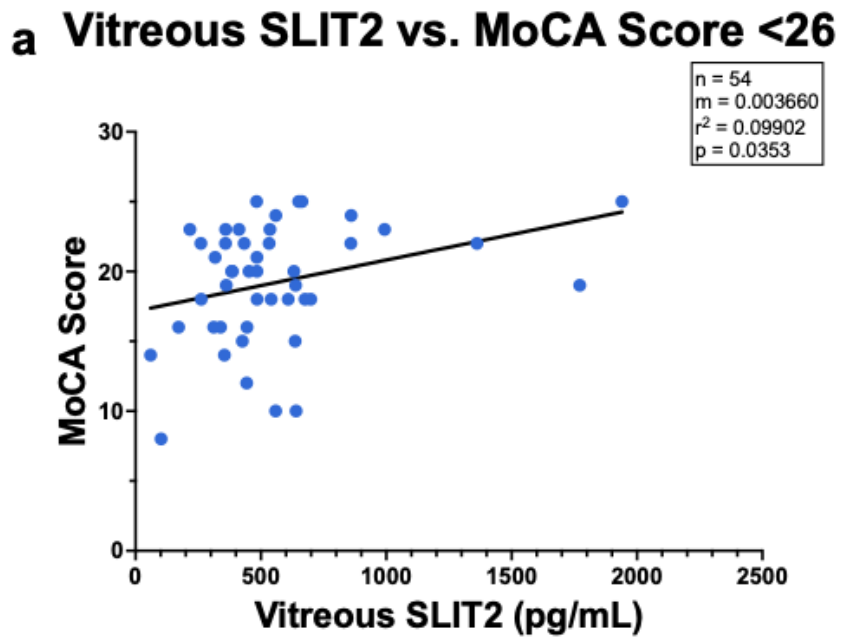
647 regression analysis demonstrated a significant relationship between SLIT2 plasma

648 protein levels and MoCA scores (a, r^2=0.097, p=0.0206). There was no relationship

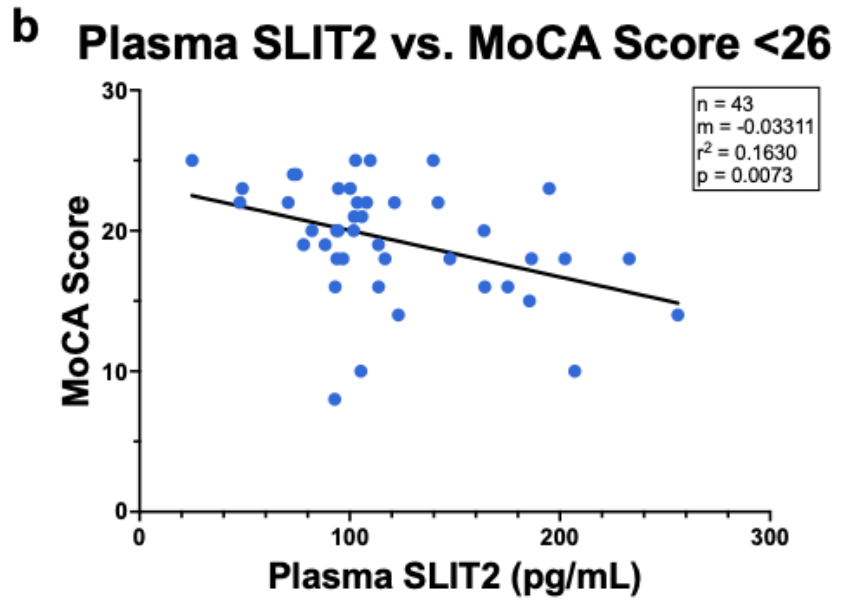
649 between SLIT2 plasma protein levels and Immediate Recall Verbatim Z-scores (b,

650 r^2=0.01830, p=0.3593).

651 **Figure 4**



652



653

654 **Figure 4. The relationship between SLIT2 and Montreal Cognitive Assessment**

655 **(MoCA) Scores <26, the cut off value for mild cognitive impairment (MCI).** Linear

656 regression analysis demonstrated the relationship between SLIT2 vitreous protein level

657 and MoCA scores <26 was also significant (a, r^2=0.099, p=0.0353). There was also a

658 significant relationship between SLIT2 plasma protein level and MoCA scores <26 (b,

659 r^2=0.163, p=0.007).

660 **Supplemental Materials**

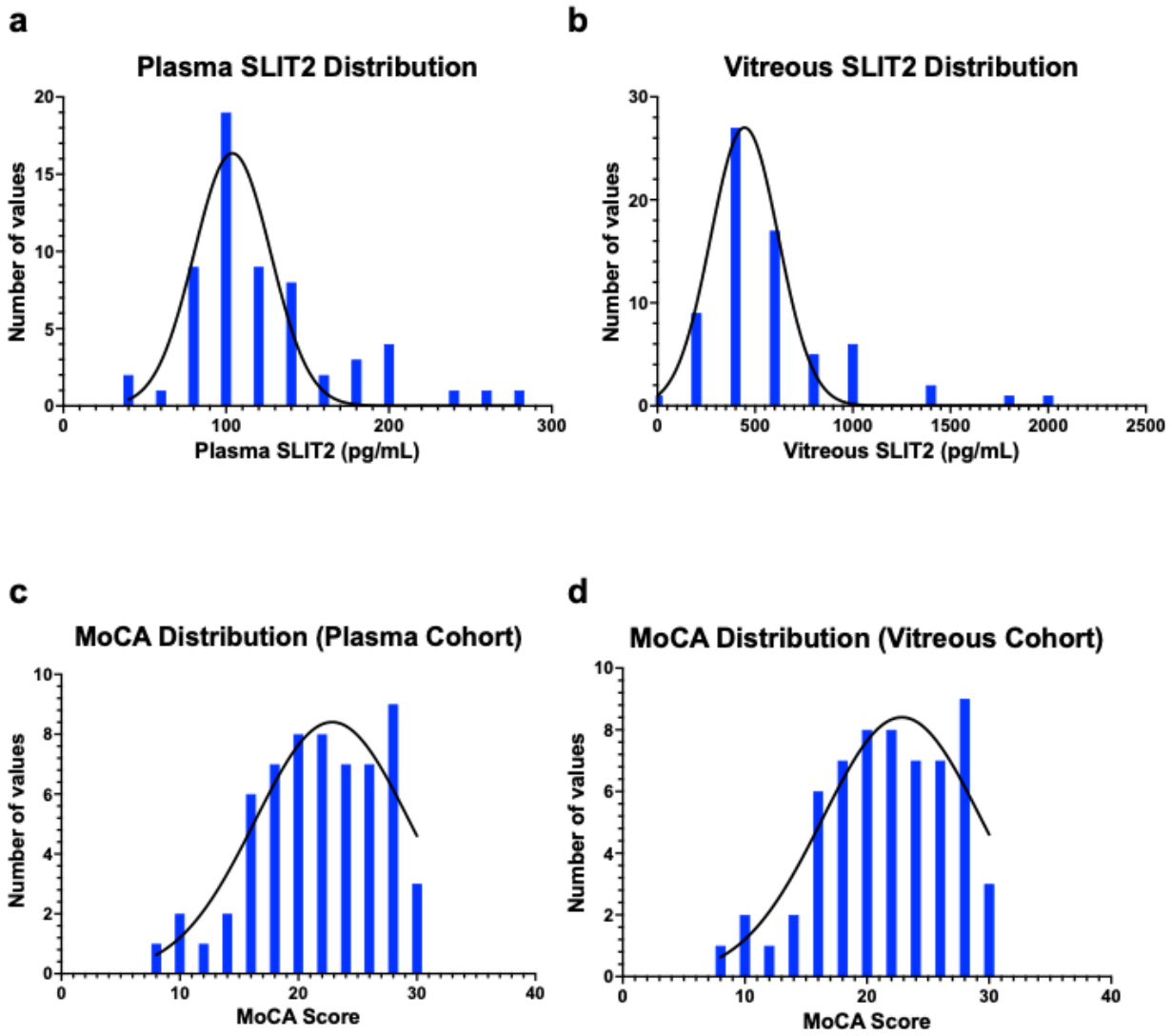
661 **Supplemental Table 1: APOE Allele Risk Categories**
662

		Plasma SLIT2			Vitreous SLIT2		
Risk Category	APOE Genotype	n	Mean	SD	n	Mean	SD
1	2/2	2	83.87	15.2	2	538.6	454.5
2	2/3 3/3	3	135.7	57.85	3	722.3	242.4
3	2/4 3/4	38	121.4	38.62	40	611.7	424.3
4	4/4	13	129.9	58.76	13	486.9	182.0

663

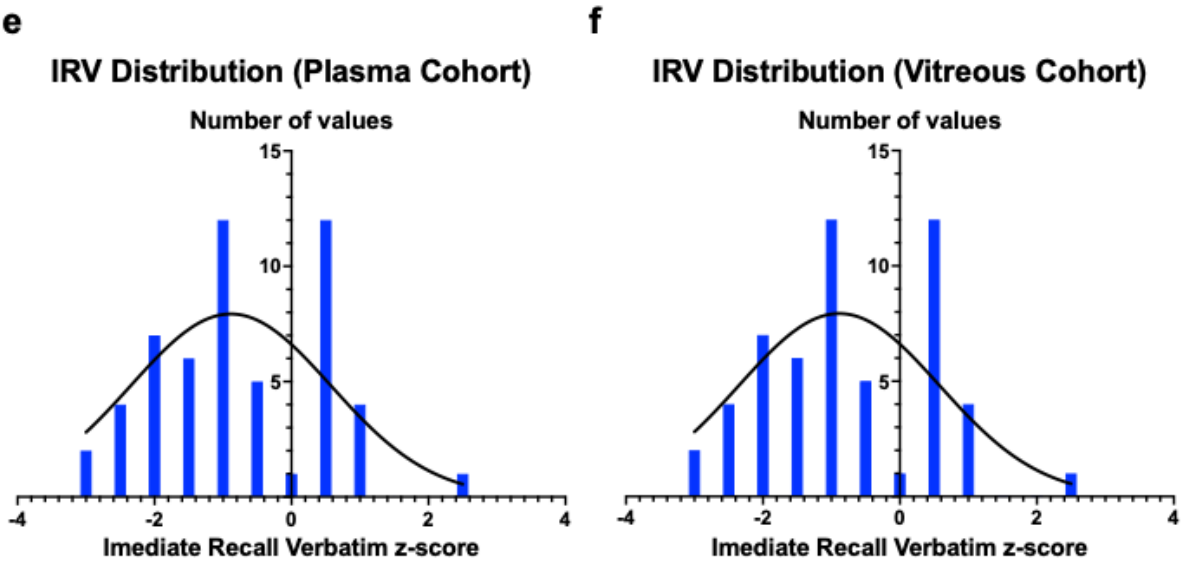
664

665 **Supplemental Figure 1**



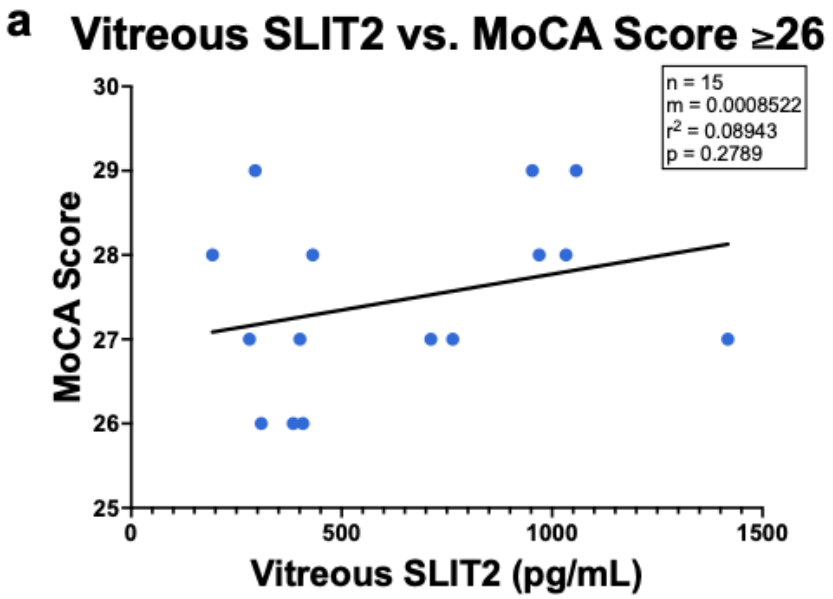
666

667

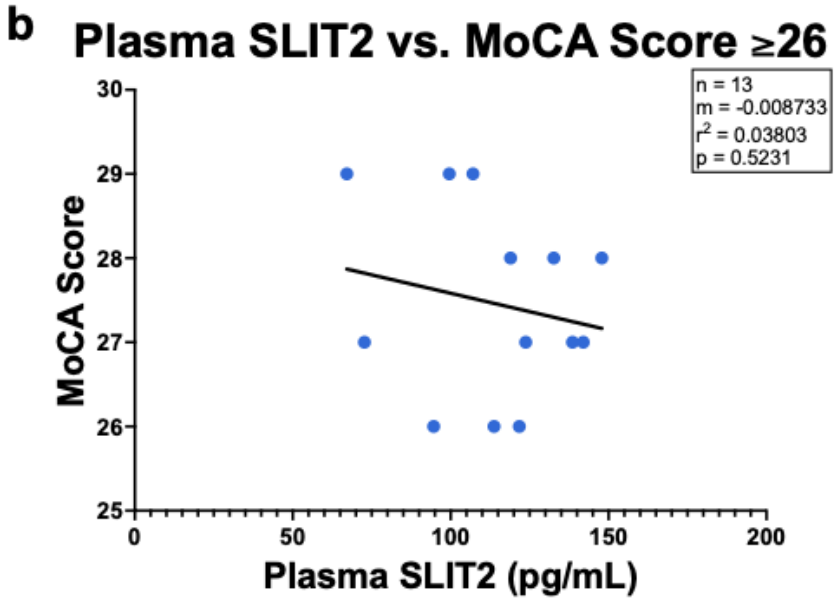


Supplemental Figure 1. Distribution of SLIT2 protein levels and neurocognitive testing. Distribution of study predictor, SLIT2 plasma (a) and vitreous (b) level, and outcomes, Montreal Cognitive Assessment score (MoCA, c-d) and Immediate Recall Verbatim z-score (IRV, e-f) with overlying normalized curve.

674 **Supplemental Figure 2**



675



676

677 **Supplemental Figure 2. The relationship between SLIT2 and Montreal Cognitive**

678 **Assessment (MoCA) Scores ≥26, the cut off value for mild cognitive impairment**

679 **(MCI).** Linear regression analysis demonstrated a moderately decreased relationship

680 between SLIT2 protein in the plasma and MoCA scores >26 (a, r^2=0.03803, p=0.5231)

681 and a moderately increased relationship between SLIT2 protein in the vitreous and

682 MoCA scores >26 (b, r^2=0.08943, p=0.2789).

683

684