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 detection of mild cognitive impairment and early dementia, 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.[3,6] These changes can occur decades before the onset of cognitive symptoms.

Mild neurocognitive impairment (MCI) 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 MCI progress to major neurocognitive disorder or dementia each year,[7,8] the most common of which is Alzheimer's dementia,[9] with an estimated

70 prevalence of 6.5% in middle-aged and senior populations, or 5.7 million in the United
71 States.[10] The prevalence of Alzheimer's disease is expected to double in the next four
72 decades.[10] Early-onset dementia is a subset making up approximately 4-6% of
73 Alzheimer's Disease cases, with an onset before age 65, and is notable for its
74 significant genetic predisposition and rapid progression.[11,12]

75 Neural-derived proteins have been demonstrated previously to have potential
76 relationships with neurocognitive dysfunction.[13] For example, increased neurofilament
77 light (NfL) protein levels have been associated with cognitive impairment.[14,15] Studies
78 have been conducted to assess a variety of proteins involved in the pathological
79 changes of neurodegeneration, such as beta-amyloid (Aβ), t-tau, p-T181-tau, and
80 GFAP.[16,17] In early-onset dementia, studies have found decreased Aβ and increased
81 total tau in CSF samples.[11] Our group has studied protein biomarkers in ocular fluid and
82 pathological proteins associated with late-onset Alzheimer's Disease and MCI.[18–22]
83 Additionally, we have demonstrated that relatively higher concentrations of
84 neurodegenerative biomarkers exist in ocular fluid, such as vitreous fluid, as compared
85 with plasma.[18]

86 Slit Guidance Ligand 2 (SLIT2) protein is a ligand that binds to Roundabout
87 (ROBO) guidance receptors expressed during vascular and neuronal development.[23–25]
88 It is commonly found in cerebral tissue[26,27] and functions as a chemorepulsive guidance
89 to direct axon pathfinding and neuron migration,[25,28,29] such as the development of the
90 optic chiasm[30] and neurodegenerative disease.[31–33] Proteomic studies have found that
91 SLIT2 levels are increased in Aβ overexpression mouse models[34] and are correlated
92 with Aβ levels in human tissue.[35] SLIT2 has also been shown to co-localize with Aβ.[34]

93 We therefore hypothesize that SLIT2 may also be an additional neurodegenerative
94 biomarker associated with cognitive impairment.

95 Prior reports[33,35–37] have suggested a link between SLIT2 protein levels and late-
96 onset dementia and Alzheimer's Disease through studies on cognitive stimulation in the
97 workplace, proteomic profiling, and forward genetic screening. In a population-based
98 proteomic study, increased levels of plasma SLIT2 protein were shown to be associated
99 with an increased hazard ratio for late-onset dementia, suggesting that SLIT2 may be a
100 potential biomarker candidate for cognitive functional decline and progression to
101 dementia.[36] However, this proteomic finding has not been validated by a current
102 commercially available SLIT2 immunoassay due to their low sensitivity and dynamic
103 range. Additionally, there is no currently published data on SLIT2 protein levels in the
104 early-onset dementia population.

105 SLIT2 protein is highly expressed in ocular neurons.[38] However, SLIT2 in the
106 vitreous humor and its association with cognitive status have not been previously
107 investigated. In this study, we hypothesize that the SLIT2 protein measured in human
108 vitreous humor and plasma is associated with cognitive function based on
109 neuropsychological assessment.

110 **METHODS**

111 *Study Design*

112 This is a cross-sectional cohort study conducted at Boston University Medical
113 Campus and Boston Medical Center (BUMC/BMC) to assess the association of
114 biomarkers in eye fluid and plasma with neurocognitive test scores. This study utilized
115 previously stored eye fluid and plasma samples from prior research that investigated the

116 association of biomarkers to eye disease and neurocognitive function. The BUMC/BMC
117 institutional review board provided approval and oversight (study reference number H-
118 37370, principal investigator MLS). The study was completed in accordance with
119 institutional ethical standards and the Declaration of Helsinki.

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

131 *Historical Data Collection*

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

138 *Neuropsychological testing*

139 Neuropsychological testing was conducted with all enrolled participants within a
140 3-month window before or after their surgery date. Our group collaborated with the
141 Boston University Alzheimer's Disease and Research Center, which recommended
142 neuropsychological testing within 180 days of fluid specimen collection and participated
143 in training research assistants to conduct the assessments. Validated tools for testing
144 included the Montreal Cognitive Assessment (MoCA)[39] and the Immediate Recall
145 Verbatim (IRV).[40] Test scores (MoCA raw score and IRV z-score) are standardized and
146 allow for comparison between individuals.[41,42]

147 *Biospecimen Collection*

148 Vitreous and blood samples were collected between September 2019 and
149 August 2021. Vitreous samples were collected at the start of each vitrectomy procedure
150 by the surgical team. 0.5–1.0 mL of undiluted vitreous fluid was aspirated via the
151 vitrectomy probe into an attached sterile 3-mL syringe. Infusion of saline into the
152 vitreous cavity was immediately undertaken in order to re-pressurize the posterior
153 chamber of the eye, and the vitrectomy subsequently proceeded according to the
154 standard of care for the respective ocular condition. The syringe containing the vitreous
155 specimen was capped using sterile technique and directly handed to a research
156 assistant, who labeled it with a predetermined non-identifiable study number and placed
157 the sample on ice for transport. Blood samples were collected from all study
158 participants at a research visit prior to surgery, and eighteen milliliters of whole blood
159 were drawn into Ethylenediaminetetraacetic acid (EDTA)-treated purple top tubes and
160 also placed on ice for transport. Once collected, vitreous and blood biospecimens were
161 immediately centrifuged, aliquoted into 200 microliter Eppendorf tubes at the Molecular

162 Genetics Core Laboratory (MGCL) at BU Medical Campus and stored in -80˚C freezers.
163 De-identified blood samples were processed into their component plasma and buffy
164 coat. Samples were thawed once prior to immunoassay analysis between August and
165 November 2023, with no additional freeze-thaw cycles. Plasma and vitreous samples
166 were utilized for SLIT2 protein analysis, and the buffy coat was used for Apolipoprotein
167 E (APOE) genotyping.

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

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

182 *APOE Genotyping*

183 APOE is a major susceptibility gene, and relative allele copies can confer a
184 higher risk of Alzheimer's;[43–45] therefore, APOE genotyping is sometimes used as a
185 clinical risk evaluation tool.
186 To determine the APOE genotype, DNA was extracted from the buffy coat, and
187 single nucleotide polymorphisms from the APOE gene (National Center for
188 Biotechnology Information SNPs rs429358 and rs7412) were examined using TaqMan
189 assays (Applied Biosystems, Foster City, CA).
190 *Statistical Analysis*
191 Statistical analyses were performed using GraphPad Prism (Version 10.2.2 341)
192 for MacOS, GraphPad Software, Boston, MA, USA, www.graphpad.com). A p-value of
193 less than 0.05 was considered statistically significant.
194 SLIT2 protein levels and neurocognitive assessment score association testing,
195 using neurocognitive scores as a continuous variable, was performed using univariate
196 and multivariate linear regression. Univariate analysis included both SLIT2 protein
197 plasma and vitreous levels as predictors of MoCA raw score and IRV z-score.
198 Additionally, analysis was performed with participants grouped based on MoCA raw
199 score for normal cognition (MoCA ≥26) and potential cognitive impairment (MoCA <26).
200 Multivariate linear regression was completed with SLIT2 plasma and vitreous
201 protein level again utilized as a predictor of neurocognitive test score and adjusted for
202 the single predictors of age, sex, race, diabetic status, diabetic retinopathy status,
203 glaucoma, APOE genotype, and APOE risk category. APOE risk categories
204 (Supplemental Table 1) were constructed based on population genotype prevalence
205 patterns and associated likelihood of developing Alzheimer's disease, as previously

206 published.[43] Multiple predictor analysis was also completed and adjusted for
207 demographics, which included age, sex, and race, and for demographics with additional
208 medical and ophthalmic history variables. A complete model, which included
209 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category,
210 was also analyzed as applicable. Diabetes mellitus and diabetic retinopathy status could
211 not be simultaneously included in the complete model due to variable collinearity. For all
212 complete models, effect size was calculated to demonstrate the contribution of SLIT2 to
213 the variation in neurocognitive test scores. Additionally, power, with an assumed effect
214 size of 0.2 (α=0.05), was calculated to demonstrate the complete models' ability to
215 detect low-medium sized effects.

216 **RESULTS**

217 *Demographics*

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

226 *SLIT2 Protein Detection*

227 Our data showed that the newly developed highly sensitive SLIT2 MSD assay
228 can detect SLIT2 protein expression levels as low as 5 pg/ml, which is 200-fold more

229 sensitive than regular ELISA. In addition, we discovered a 7-fold higher SLIT2 level in
230 the vitreous humor compared to blood plasma samples in humans. In a Spearman
231 correlation, SLIT2 plasma and vitreous levels were not correlated with each other (ρ=-
232 0.05424, p=0.6833).

233 *Univariate Analysis*

234 In univariate linear regression, lower vitreous SLIT2 levels were associated with
235 lower MoCA raw scores (m=0.003897, r^2=0.07585, p=0.0332, Figure 2a) and lower IRV
236 z-scores (m=0.001196, r^2=0.09811, p=0.0224, Figure 2b). Alternatively, plasma levels
237 showed the opposite relationship, with higher plasma SLIT2 associated with lower
238 MoCA scores (m=-0.03559, r^2=0.09699, p=0.0206, Figure 3a). Plasma SLIT2 levels
239 were not associated with IRV z-scores (m=-0.003430, r^2=0.01830, p=0.3593, Figure
240 3b).

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

248 *Multivariate Analysis*

249 In single predictor adjustment models (Table 2), lower vitreous SLIT2 was
250 associated with lower MoCA score after adjusting separately for age, sex, race, diabetic
251 status, diabetic retinopathy status, and glaucoma. Multiple predictor models were

252 significant when adjusted for demographics (age, sex, and race; p=0.0225),
253 demographics with diabetic status (p=0.0162), demographics with diabetic retinopathy
254 status (p=0.0269), and demographics with glaucoma status (p=0.0111). A complete
255 model adjusting for demographics, diabetic retinopathy status, and glaucoma status
256 was significant (p=0.0141) with a large effect size (f^2=0.391) and sufficient power
257 (92.5%, f^2=0.2, α=0.05). APOE genotype and risk category were not included in the
258 complete model for vitreous SLIT2 because the single predictor analysis for both was
259 not significant.

260 Higher plasma SLIT2 was associated with lower MoCA score in single predictor
261 adjustment models adjusted separately for age, sex, race, diabetic status, diabetic
262 retinopathy status, glaucoma, APOE genotype, and APOE risk category (Table 3). In
263 multiple predictor analysis, similarly significant associations were found when adjusted
264 for demographics (age, sex, and race; p=0.0498), demographics with diabetic
265 retinopathy status (p=0.0198), demographics with APOE genotype (p=0.0418), and
266 demographics with APOE risk category (p=0.0413). A complete model adjusting for
267 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category
268 was not statistically significant (p=0.0522) but had a large effect size (f^2=0.5026),
269 signifying SLIT2 has a substantial contribution to the variation in MoCA score. In
270 addition, we found sufficient statistical power (87.7%, f^2=0.2, α=0.05) in the complete
271 model.

272 Vitreous SLIT2 and IRV z-score were also significant in single predictor models
273 adjusted separately for age, sex, race, diabetic status, diabetic retinopathy status,
274 glaucoma status, and APOE risk category (Table 4), and in multiple predictor models

275 adjusted for demographics (age, sex, and race; p=0.0142), demographics with diabetic
276 status (p=0.0149), demographics with diabetic retinopathy status (p=0.0175), and
277 demographics with glaucoma (p=0.0336). A complete model adjusting for
278 demographics, diabetic retinopathy status, glaucoma status, and APOE risk category
279 was not significant (p=0.1328) but had a large effect size (f^2=0.568), signifying SLIT2
280 has a substantial contribution to variation in IRV z-score. This complete model was also
281 found to be sufficiently powered (84.0%, f^2=0.2, α=0.05).

282 **DISCUSSION**

283 In this study, we found that vitreous and plasma SLIT2 protein were significantly
284 associated with Montreal Cognitive Assessment (MoCA) and Immediate Recall
285 Verbatim (IRV) scores. The relationship remained significant when adjusted for
286 covariates of age, sex, race, diabetes, and diabetic retinopathy in single and multiple
287 predictor models. We found up to a 7-fold increase in SLIT2 concentration in the
288 vitreous humor compared to plasma. As far as we are aware, this is the first study to
289 report relative concentrations of SLIT2 in vitreous and plasma and establish an
290 association between SLIT2 levels from both sources with cognitive function obtained by
291 MoCA and IRV z-score.

292 Our research partially supports previous studies that demonstrated associations
293 between SLIT2 and cognitive function. But while a prior report found that higher plasma
294 SLIT2 levels were associated with an increased risk of late-onset dementia,[36] our study
295 was conducted in a younger cohort of patients without known dementia diagnosis,
296 indicating that our findings may be more relevant for MCI and early-onset dementia.
297 There are no previous studies describing SLIT2 protein levels in early-onset dementia.

298 Additionally, our study further validates previous work done by our group that
299 demonstrated an association between vitreous protein levels and neurocognitive test
300 scores[19] and the relatively higher neurodegenerative protein concentrations in the
301 vitreous compared with plasma.[18] We have also found in a prior post-mortem study that
302 an elevated vitreous concentration may reflect an elevated cerebral concentration.[20] In
303 this study, our findings further demonstrate the potential for ocular fluids as a
304 biospecimen sampling source for early detection and diagnosis of neurocognitive
305 disorders, as well as the unexplored role of SLIT-ROBO signaling in the pathological
306 development of neurodegenerative disease.

307 We found opposite directions of associations in the vitreous humor and plasma
308 between SLIT2 levels and MoCA score, the reasons for which remain unclear. SLIT2 is
309 a paracrine ligand associated with the extracellular membrane receptor ROBO, resulting
310 in high concentrations in local tissues surrounding an area of increased expression. As
311 SLIT2 is required for active axon pathfinding and new neuronal synapse formation in the
312 central nervous system (CNS), one possibility is that SLIT2 is more highly expressed in
313 the brain and ocular tissues compared to blood, and therefore lower SLIT2 in vitreous
314 humor may suggest decreased axon pathfinding and fewer new neuronal synapse
315 formations in the brain, observed as lower MoCA and IRV scores. Relatively higher
316 plasma SLIT2 may indicate the result of a systemic body reaction attempting to increase
317 SLIT2 levels and stimulate axon pathfinding and new neuronal synapse formations.
318 Similar inverse relationships have been noted in Aβ, with higher concentrations of
319 amyloid plaque deposition in the brain being associated with lower Aβ concentrations in
320 the CSF and plasma.[46–49] As previously mentioned, our group has found significantly

321 lower Aβ concentrations in the vitreous in individuals with lower cognitive scores (based
322 on mini-mental status examination), consistent with what is typically noted in the
323 cerebral spinal fluid (CSF) in those with MCI, early-onset dementia, and late-onset
324 Alzheimer's Disease.[11,19] Additionally, some insight might be gathered from the inverted
325 relationship expanding as neurocognitive function declines. It has been previously
326 demonstrated that neurodegeneration is associated with impaired blood-brain barrier
327 (BBB) and CNS vasculature functionality.[50] Increased BBB permeability[51] and
328 decreased function in pathological inflammatory states may contribute to the increased
329 concentration of SLIT2 in the plasma, as SLIT2 also plays a role in inflammation.[52,53]
330 The involvement of SLIT2 remains unclear, as SLIT2 overexpression in mouse models
331 have been shown to have increased or decreased BBB permeability.[54,55] Interestingly,
332 overexpression of SLIT1, which is a homologue of SLIT2 and highly expressed in the
333 brain, can facilitate functional neuronal regeneration in post-ischemic stroke mice by
334 enhancing new neuron migration through the brain glial meshwork to stroke lesions.[56]
335 Further investigation into the mechanism by which SLIT2 exits the CNS and enters the
336 systemic circulatory system is needed to better understand the relative concentration
337 differences between vitreous and plasma samples.

338 SLIT2 was significantly associated with diabetes, which has been previously
339 shown in rat models of type 1 diabetes[38] and human epidemiological studies.[57–59]
340 Diabetes has been shown to be associated with higher rates of late-onset Alzheimer's
341 disease [60]; elevated blood glucose, obesity, and metabolic disease have been shown to
342 be potential risk factors for early-onset dementia.[12,61,62] It is hypothesized that the

343 pathophysiological mechanisms of hyperglycemia induce and increase the
344 neuropathological changes.[60]
345 Limitations of this study include limited sample size and a lack of comprehensive
346 neurological testing. The sample size may limit the precision of regression estimates,
347 especially multivariate models with multiple predictors, increasing the potential for
348 variability in sampling. However, we were able to achieve adequately powered complete
349 models able to detect low-medium effects. While our sample was normally distributed
350 with regard to MoCA score, IRV z-score, and SLIT2 protein level (Supplemental Figure
351 1), the limited numbers made it difficult to make more specific comparisons.
352 Additionally, further confirmation of MCI and dementia diagnosis, including potentially
353 early-onset disease, in individuals with lower scores would require further testing and
354 functional assessment of participants. The limited sample also prevented analysis
355 adjusted for additional ocular co-morbidities, such as macular degeneration, which was
356 not present in any of our participants, likely due to our younger and more racially and
357 ethnically diverse study cohort. High prevalence of comorbidities known to be
358 associated with dementia risk, including early-onset dementia risk[12,61,62], such as
359 diabetes, likely contributed to the low average neurocognitive score in our young study
360 cohort. The large percentage of APOE E4 also biases our sample and reduces the
361 generalizability of our findings. This may have contributed to the low average
362 neurocognitive score, since it has been shown that diabetic patients are more
363 genetically predisposed to late-onset Alzheimer's Disease.[63] Further prospective studies
364 with larger recruitment goals may address these limitations. Additionally, participants for
365 this study were patients from the eye clinic at Boston Medical Center and, therefore,

366 were recruited based on ocular history, not neurological evaluation. While we adjusted
367 for any significant covariates, such as diabetes mellitus, diabetic retinopathy, and
368 glaucoma, the impact of eye and systemic diseases on SLIT2 levels remains unclear.
369 Because of this study's setting, only screening-level neuropsychological testing was
370 performed. Further studies validating our findings on the basis of neurodegenerative
371 diagnosis and staging could provide more specific information regarding the extent of
372 neurodegeneration associated with SLIT2 protein levels.

373 In conclusion, we have demonstrated that SLIT2 protein in the vitreous and
374 plasma is significantly associated with Montreal Cognitive Assessment score and
375 Immediate Recall Verbatim z-score, and the relationship remained significant when
376 adjusted for demographics, medical and ocular co-morbidities, and APOE genotype
377 factors. The utilization of a custom-made highly sensitive MSD immunoassay allowed
378 for quantification of SLIT2 at the pg/mL level. The inverse concentrations between
379 vitreous and plasma samples highlight the need to further understand the mechanisms
380 underlying neurodegeneration and its relationship with systemic circulation.

381 **DECLARATIONS**

382 **Ethics Approval and Consent to Participate**

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

385 **Data Availability**

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

388 **Competing Interest**

The authors have declared that no conflict of interest exists.

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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

412 Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Original
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416 **Consent For Publication**

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REFERENCES

1. Kovacs GG. Molecular pathology of neurodegenerative diseases: Principles and practice. *J Clin Pathol* 2019; 72: 725–735.
2. Rekatsina M, Paladini A, Piroli A, et al. Pathophysiology and Therapeutic Perspectives of Oxidative Stress and Neurodegenerative Diseases: A Narrative Review. Adv Ther 2020; 37: 113-139.
3. Dugger BN, Dickson DW. Pathology of Neurodegenerative Diseases. *Cold Spring Harb Perspect Biol* 2017; 9: a028035.
4. Webber CJ, Lei S (Eric), Wolozin B. The pathophysiology of neurodegenerative disease: Disturbing the balance between phase separation and irreversible aggregation. *Prog Mol Biol Transl Sci* 2020; 174: 187–223.
5. Zaib S, Javed H, Khan I, et al. Neurodegenerative Diseases: Their onset, epidemiology, causes and treatment. *ChemistrySelect*; 8: e202300225..
6. Relja M. Pathophysiology and Classification of Neurodegenerative Diseases. *EJIFCC* 2004; 15: 97-99.
7. Pérez Palmer N, Trejo Ortega B, Joshi P. Cognitive Impairment in Older Adults: Epidemiology, Diagnosis, and Treatment. *Psychiatric Clinics of North America* 2022; 45: 639–661.
8. Akushevich I, Yashkin AP, Kravchenko J, et al. Time Trends in the Prevalence of Neurocognitive Disorders and Cognitive Impairment in the United States: The Effects of Disease Severity and Improved Ascertainment. *Journal of Alzheimer's Disease* 2018; 64: 137–148.
9. Erkkinen MG, Kim MO, Geschwind MD. Clinical Neurology and Epidemiology of the Major Neurodegenerative Diseases. *Cold Spring Harb Perspect Biol* 2018; 10: a033118.
10. Thorpe KE, Levey AI, Thomas J. *May 2021 Neurodegenerative Disease Burden on US - FINAL* . 2021.
11. Mendez MF. Early-Onset Alzheimer Disease. *Neurol Clin* 2017; 35: 263–281.
12. Loi SM, Cations M, Velakoulis D. Young-onset dementia diagnosis, management and care: a narrative review. *Medical Journal of Australia* 2023; 218: 182–189.
13. Logroscino G, Urso D, Savica R. Descriptive Epidemiology of Neurodegenerative Diseases: What Are the Critical Questions? *Neuroepidemiology* 2022; 56: 309–318.
14. Olsson B, Portelius E, Cullen NC, et al. Association of Cerebrospinal Fluid Neurofilament Light Protein Levels with Cognition in Patients with Dementia, Motor Neuron Disease, and Movement Disorders. *JAMA Neurol* 2019; 76: 318–325.
15. Lin YS, Lee WJ, Wang SJ, et al. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. *Sci Rep* 2018; 8: 17368.
16. Kim KY, Shin KY, Chang KA. Brain-Derived Exosomal Proteins as Effective Biomarkers for Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Biomolecules* 2021; 11: 980.
17. Askenazi M, Kavanagh T, Pires G, et al. Compilation of reported protein changes in the brain in Alzheimer's disease. *Nat Commun* 2023; 14: 4466.

18. Sampani K, Ness S, Tuz-Zahra F, et al. Neurodegenerative biomarkers in different chambers of the eye relative to plasma: an agreement validation study. *Alzheimers Res Ther* 2024; 16: 192.
19. Wright LM, Stein TD, Jun G, et al. Association of cognitive function with amyloid-β and tau proteins in the vitreous humor. *Journal of Alzheimer's Disease* 2019; 68: 1429–1438.
20. Vig V, Garg I, Tuz-Zahra F, et al. Vitreous Humor Biomarkers Reflect Pathological Changes in the Brain for Alzheimer's Disease and Chronic Traumatic Encephalopathy. *J Alzheimers Dis* 2023; 93: 1181–1193.
21. Subramanian ML, Vig V, Chung J, et al. Neurofilament light chain in the vitreous humor of the eye. *Alzheimers Res Ther* 2020; 12: 111.
22. Fereshetian S, Agranat JS, Siegel N, et al. Protein and Imaging Biomarkers in the Eye for Early Detection of Alzheimer's Disease. *Journal of Alzheimer's Disease Reports* 2021; 5: 375–387.
23. Rama N, Dubrac A, Mathivet T, et al. Slit2 signaling through Robo1 and Robo2 is required for retinal neovascularization. *Nat Med* 2015; 21: 483–491.
24. Tong M, Jun T, Nie Y, et al. The role of the SLIT/Robo signaling pathway. *J Cancer* 2019; 10: 2694–2705.
25. Blockus H, Chédotal A. Slit-robo signaling. *Development (Cambridge)* 2016; 143: 3037–3044.
26. Sasaki T, Komatsu Y, Yamamori T. Expression patterns of SLIT/ROBO mRNAs reveal a characteristic feature in the entorhinal-hippocampal area of macaque monkeys. *BMC Res Notes* 2020; 13: 262.
27. Xu C, Fan CM. Expression of Robo/Slit and Semaphorin/Plexin/Neuropilin family members in the developing hypothalamic paraventricular and supraoptic nuclei. *Gene Expression Patterns* 2008; 8: 502–507.
28. Plachez C, Andrews W, Liapi A, et al. Robos are required for the correct targeting of retinal ganglion cell axons in the visual pathway of the brain. *Molecular and Cellular Neuroscience* 2008; 37: 719–730.
29. Thompson H, Andrews W, Parnavelas JG, et al. Robo2 is required for Slit-mediated intraretinal axon guidance. *Dev Biol* 2009; 335: 418–426.
30. Davison C, Zolessi FR. Slit2 is necessary for optic axon organization in the zebrafish ventral midline. Cells Dev 2021; 166: 203677.
31. Dickson BJ. Molecular Mechanisms of Axon Guidance. *Science (1979)* 2002; 298: 1959–1964.
32. Sherchan P, Travis ZD, Tang J, et al. The potential of Slit2 as a therapeutic target for central nervous system disorders. *Expert Opin Ther Targets* 2020; 24: 805–818.
33. Zhang L, Qi Z, Li J, et al. Roles and Mechanisms of Axon-Guidance Molecules in Alzheimer's Disease. *Mol Neurobiol* 2021; 58: 3290–3307.
34. Bai B, Wang X, Li Y, et al. Deep Multilayer Brain Proteomics Identifies Molecular Networks in Alzheimer's Disease Progression. *Neuron* 2020; 105: 975-991.e7.
35. Wang H, Dey KK, Chen PC, et al. Integrated analysis of ultra-deep proteomes in cortex, cerebrospinal fluid and serum reveals a mitochondrial signature in Alzheimer's disease. *Mol Neurodegener* 2020; 15: 43.

36. Kivimäki M, Walker KA, Pentti J, et al. Cognitive stimulation in the workplace, plasma proteins, and risk of dementia: three analyses of population cohort studies. *BMJ* 2021; 374: n1804.
37. Bai G, Chivatakarn O, Bonanomi D, et al. Presenilin-dependent receptor processing is required for axon guidance. *Cell* 2011; 144: 106–118.
38. Zhou W, Wang H, Yu W, et al. The expression of the Slit-Robo signal in the retina of diabetic rats and the vitreous or fibrovascular retinal membranes of patients with proliferative diabetic retinopathy. *PLoS One* 2017; 12: e0185795.
39. Daniel B, Agenagnew L, Workicho A, et al. Psychometric Properties of the Montreal Cognitive Assessment (MoCA) to Detect Major Neurocognitive Disorder Among Older People in Ethiopia: A Validation Study. *Neuropsychiatr Dis Treat* 2022; 18: 1789–1798.
40. Schroeder RW, Martin PK, Walling A. Neuropsychological Evaluations in Adults. *American Family Physicians* 2019; 99: 101–108.
41. Weintraub S, Besser L, Dodge HH, et al. Version 3 of the Alzheimer Disease Centers' Neuropsychological Test Battery in the Uniform Data Set (UDS). *Alzheimer Dis Assoc Disord* 2018; 32: 10–17.
42. *Neuropsychological Battery Tests reported on Form C2, Uniform Data Set (UDS) v3.0*, www.alz.washington.edu (2006).
43. Reiman EM, Arboleda-Velasquez JF, Quiroz YT, et al. Exceptionally low likelihood of Alzheimer's dementia in APOE2 homozygotes from a 5,000-person neuropathological study. *Nat Commun* 2020; 11: 667.
44. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; 261: 921-923.
45. Corder EH, Saunders AM, Risch N], et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* 1994; 7: 180–184.
46. Bateman RJ, Xiong C, Benzinger TLS, et al. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *New England Journal of Medicine* 2012; 367: 795–804.
47. Mehta PD, Pirttilä T, Mehta SP, et al. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol* 2000; 57: 100-105.
48. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. *Journal of Internal Medicine* 2018; 284: 643–663.
49. 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.
50. Cipollini V, Troili F, Giubilei F. Emerging Biomarkers in Vascular Cognitive Impairment and Dementia: From Pathophysiological Pathways to Clinical Application. *Int J Mol Sci* 2019; 20: 2812.
51. Fakhoury M. Role of immunity and inflammation in the pathophysiology of neurodegenerative diseases. *Neurodegener Dis* 2015; 15: 63–69.
52. 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.

53. 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* 2023; 20: 249.
54. 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.
55. 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.
56. 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* 2018; 4: eaav0618.
57. 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.
58. 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.
59. 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)* 2022; 13: 889505.
60. 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.
61. Li R, Li R, Xie J, et al. Associations of socioeconomic status and healthy lifestyle with incident early-onset and late-onset dementia: a prospective cohort study. *Lancet Healthy Longev* 2023; 4: e693–e702.
62. Bosi M, Malavolti M, Garuti C, et al. Environmental and lifestyle risk factors for early-onset dementia: a systematic review. *Acta Biomed* 2022; 93: e2022336.
63. 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)* 2024; 15: 1340608.

TABLES

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

596

597 **Table 2: Predictor Effects on the Association of SLIT2 Vitreous and MoCA Score.**
598 The single predictor model applied each variable separately to determine its individual
599 impact on the association of SLIT2 Vitreous and MoCA Score. In the multiple predictor
600 model, all demographic variables (age, sex, and race) were additionally combined with
601 medical variables. APOE genotype and risk category were not included in the multiple
602 predictor or complete models because the single predictor model did not find a
603 significant association. The complete model did meet statistical significance (p=0.0141)
604 with a large effect size (f^2=0.391) and sufficient power (92.5%, f^2=0.2, α=0.05).

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

605 Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
606 Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative
607 Diabetic Retinopathy
608 Glaucoma: No glaucoma (reference group), Glaucoma
609 APOE Genotype: 3/3 (reference group), 2/2, 2/4, 3/4, 4/4
610 APOE Risk Category (see Supplementary Table 1): 2/3 and 3/3 (reference group), 2/2,
611 2/4 and 3/4, 4/4
612
613

614
615

616 **Table 3: Predictor Effects on the Association of SLIT2 Plasma and MoCA Score**.
617 The single predictor model applied each variable separately to determine its individual
618 impact on the association of SLIT2 plasma and MoCA Score. In the multiple predictor
619 model, all demographic variables (age, sex, and race) were additionally combined with
620 medical variables. For this analysis, APOE risk category was included in the complete
621 model because the single predictor model was significantly associated with plasma
622 SLIT2. The complete model did not meet statistical significance (p=0.0522) but had a
623 large effect size (f^2=0.5026) and sufficient power (87.7%, f^2=0.2, α=0.05).

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

624
625 Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
626 Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative
627 Diabetic Retinopathy
628 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

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

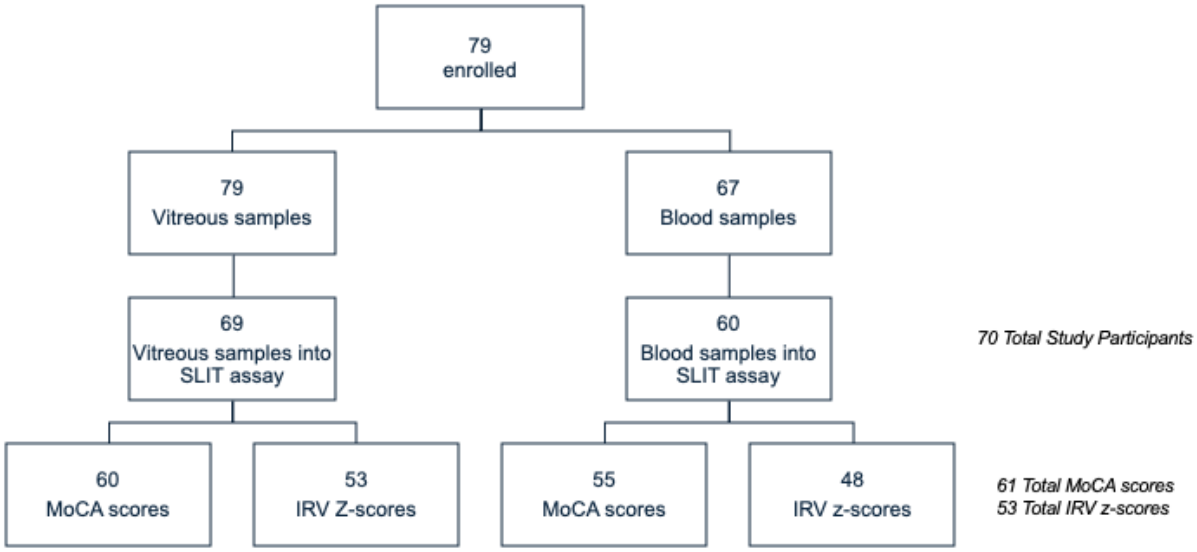
634 The single predictor model applied each variable separately to determine its individual
635 impact on the association of SLIT2 vitreous and IRV z-score. In the multiple predictor
636 model, all demographic variables (age, sex, and race) were additionally combined with
637 medical variables. The complete model did not meet statistical significance (p=1328) but
638 had a large effect size (f^2=0.568) and sufficient power (84.0%, f^2=0.2, α=0.05).

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

639 Diabetic Status: Non-Diabetic (reference group), Type 1 Diabetes, Type 2 Diabetes
640 Diabetic Retinopathy Status: No Diabetic Retinopathy (reference group), Proliferative
641 Diabetic Retinopathy
642 Glaucoma: No glaucoma (reference group), Glaucoma
643 APOE Genotype: 3/3 (reference group), 2/2, 2/4, 3/4, 4/4
644 APOE Risk Category (see Supplementary Table 1): 2/3 and 3/3 (reference group), 2/2,
645 2/4 and 3/4, 4/4

646
647 **FIGURES**

648 **Figure 1**

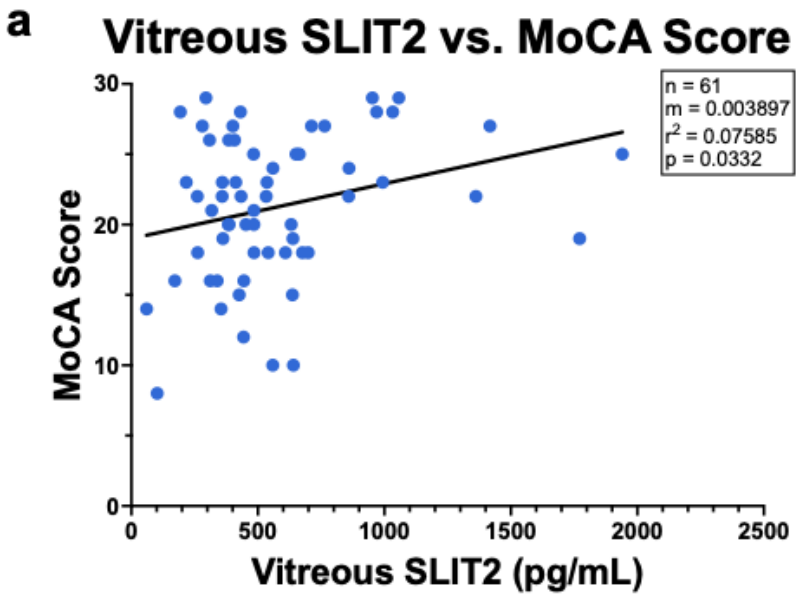


649

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

656

657 **Figure 2**



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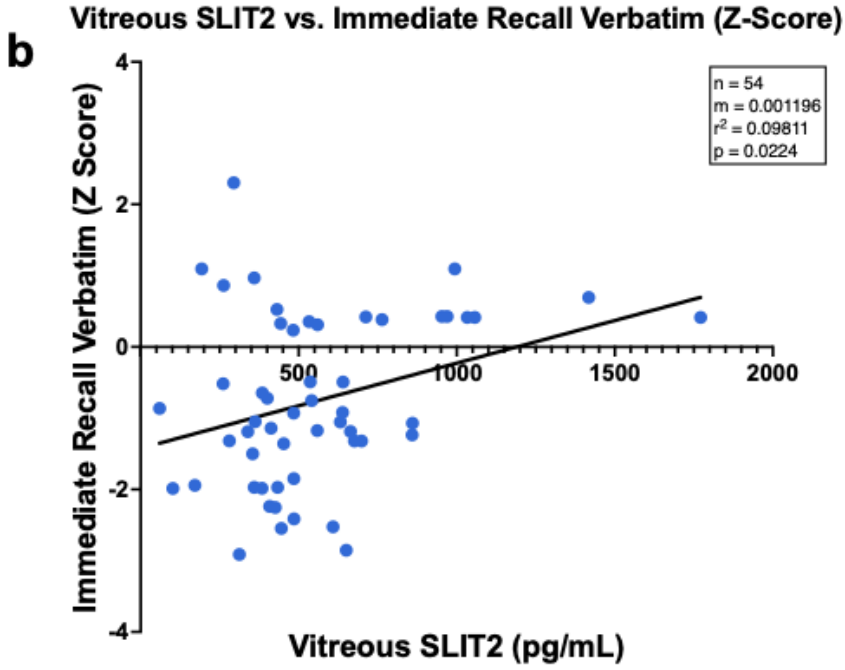


659

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

661 Linear regression analysis demonstrated a significant relationship between SLIT2

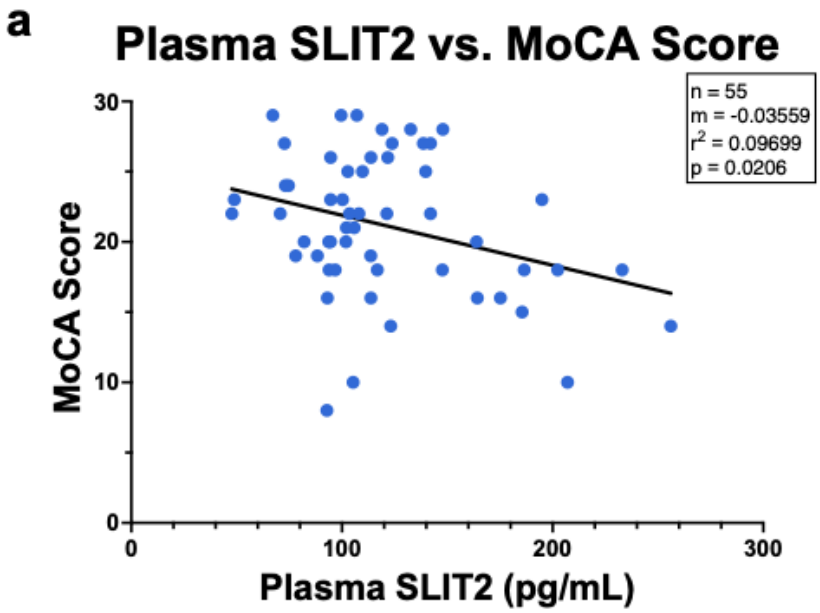
662 vitreous protein level and MoCA score (a, r^2=0.076, p=0.0332) and Immediate Recall

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

664

665

666 **Figure 3**



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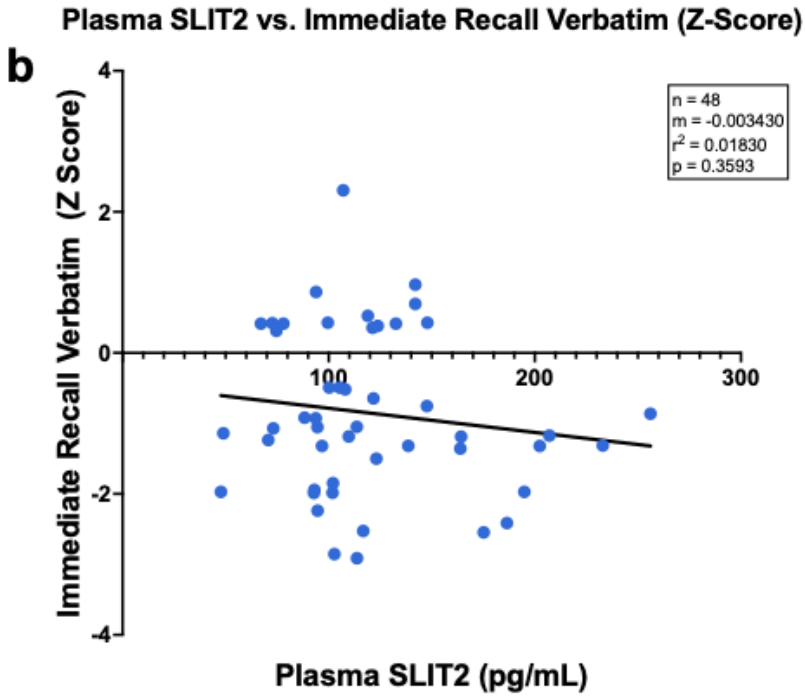


668

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

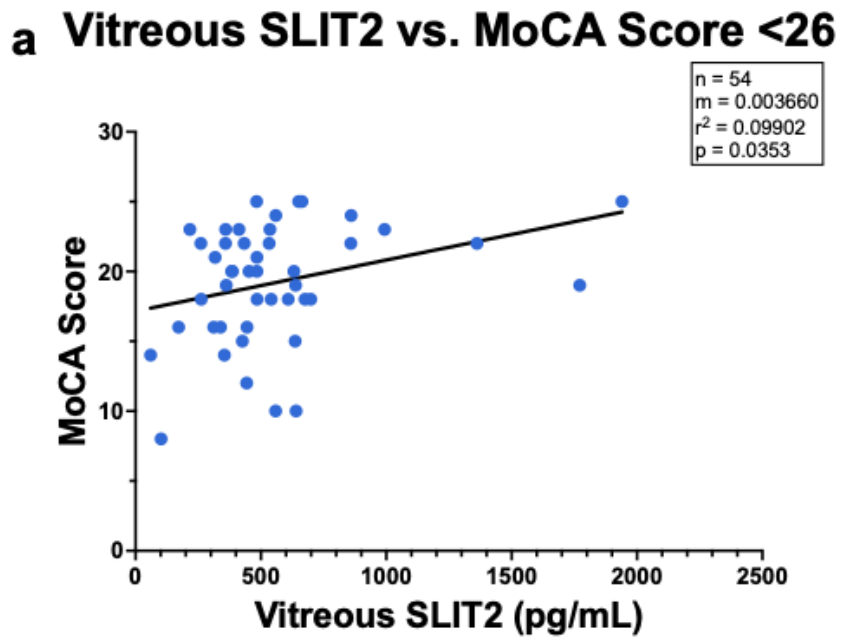
670 regression analysis demonstrated a significant relationship between SLIT2 plasma

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

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

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

674 **Figure 4**



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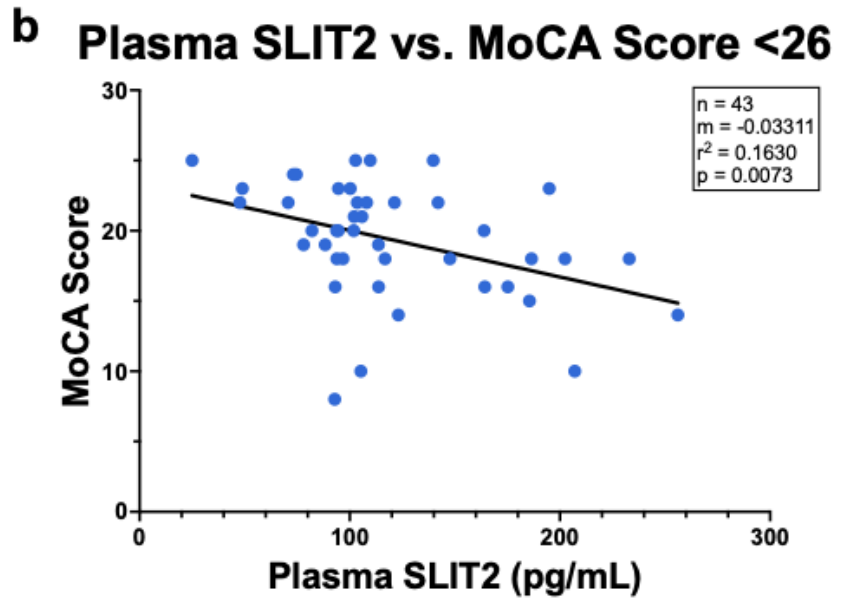


676

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

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

679 regression analysis demonstrated the relationship between SLIT2 vitreous protein level

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

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

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

683 **Supplemental Materials**

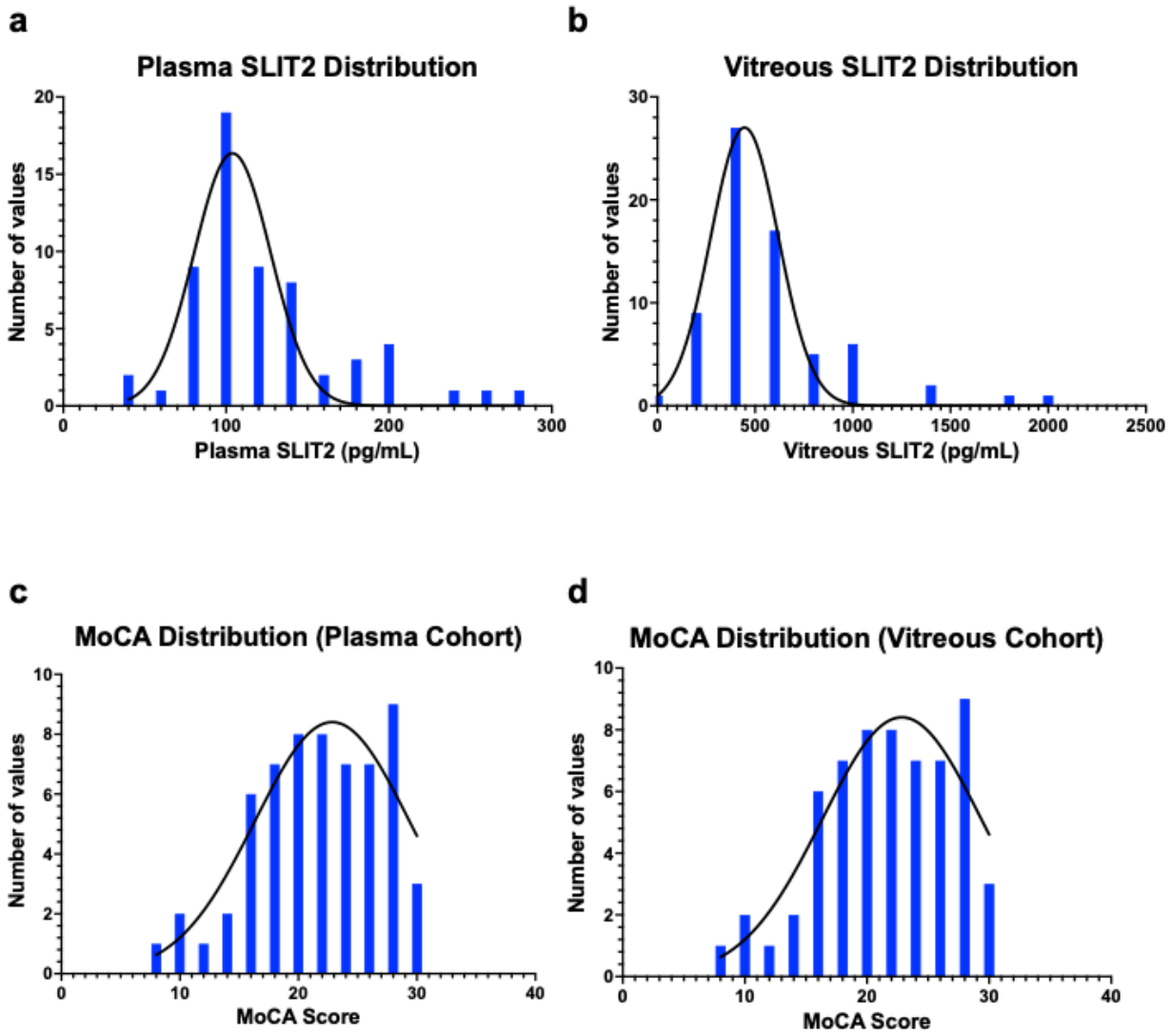
684 **Supplemental Table 1: APOE Allele Risk Categories**
685

		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

686

687

688 **Supplemental Figure 1**



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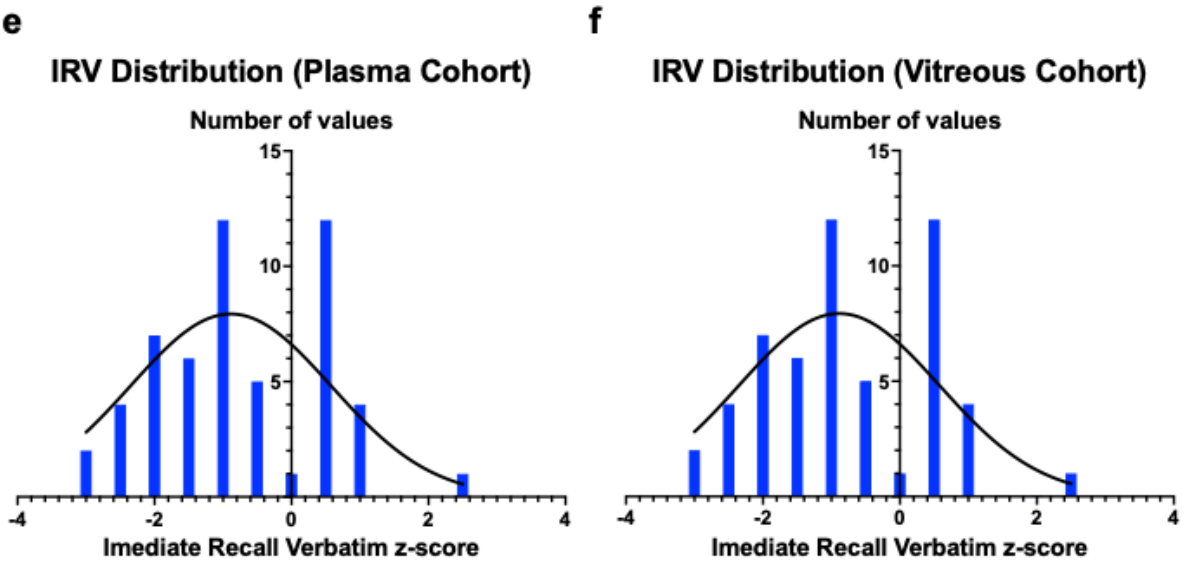
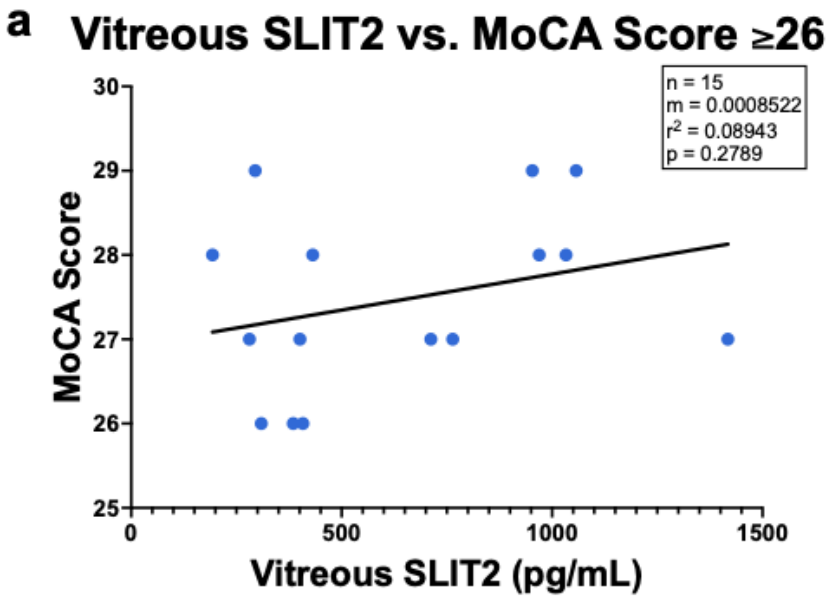


Supplemental Figure 1. Distribution of SLIT2 protein levels and neurocognitive testing. Distribution of study predictor, SLIT2 plasma (a) and vitreous (b) level, and outcomes, Montreal Cognitive Assessment score (MoCA, c-d) and Immediate Recall Verbatim z-score (IRV, e-f) with overlying normalized curve.

697 **Supplemental Figure 2**



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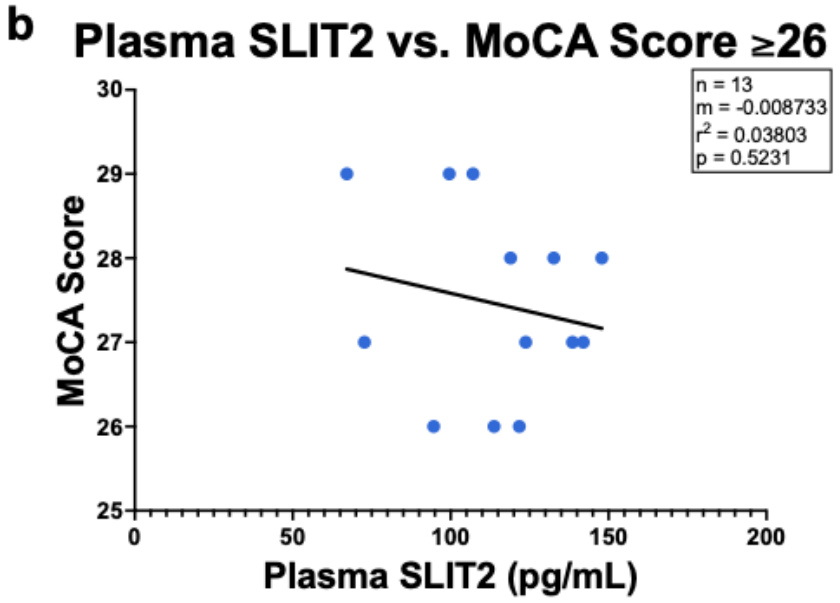

699

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

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

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

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

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

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

706

707