Association of 10 VEGF Family Genes with Alzheimer's Disease Endophenotypes at Single Cell Resolution

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Abstract

The cell-type specific role of the vascular endothelial growth factors (VEGFs) in the pathogenesis of Alzheimer's disease (AD) is not well characterized. In this study, we utilized a single-nucleus RNA sequencing dataset from Dorsolateral Prefrontal Cortex (DLFPC) of 424 donors from the Religious Orders Study and Memory and Aging Project (ROS/MAP) to investigate the effect of 10 VEGF genes (*VEGFA*, *VEGFB*, *VEGFC*, *VEGFD*, *PGF*, *FLT1*, *FLT4*, *KDR*, *NRP1*, and *NRP2*) on AD endophenotypes. Mean age of death was 89 years, among which 68% were females, and 52% has AD dementia. Negative binomial mixed models were used for differential expression analysis and for association analysis with β-amyloid load, PHF tau tangle density, and both cross-sectional and longitudinal global cognitive function. Intercellular VEGF-associated signaling was profiled using CellChat. We discovered prefrontal cortical *FLT1* expression was upregulated in AD brains in both endothelial and microglial cells. Higher *FLT1* expression was also associated with worse cross-sectional global cognitive function, longitudinal cognitive trajectories, and β-amyloid load. Similarly, higher endothelial *FLT4* expression was associated with more β-amyloid load. In contrast to the receptors, *VEGFB* showed opposing effects on β-amyloid load whereby higher levels in oligodendrocytes was associated with high amyloid burden, while higher levels in inhibitory neurons was associated with lower amyloid burden. Finally, AD cells showed significant reduction in overall VEGF signaling comparing to those from cognitive normal participants. Our results highlight key changes in VEGF receptor expression in endothelial and microglial cells during AD, and the potential protective role of VEGFB in neurons.

44 **Introduction**

45 Neurovascular unit dysfunction is a hallmark of progression of AD. The vascular
46 endothelial growth factor (VEGF) family of signaling proteins are responsible for the growth and
47 maintenance of cells involved in vascularization and neuralization [1]. The members of the
48 VEGF family include five ligand genes (*VEGFA*, *VEGFB*, *VEGFC*, *VEGFD*, and *PGF*), three receptor
49 genes (*FLT1*, *KDR*, and *FLT4*), and two co-receptor genes (*NRP1* and *NRP2*; **Figure 1a**). These
50 genes play a role in brain injury [2], stroke [3, 4], and the progression of neurodegenerative
51 disease [5], particularly Alzheimer's disease (AD). Within AD, disruptions in vascularization and
52 circulation of the brain have been shown to contribute to neurodegeneration and the
53 development of neuropathology [6–13]. As a result, VEGFs have become exciting targets for
54 research to better understand vascular contributions to AD.

55 The role of VEGFs in AD appears to vary depending on the gene family member. Some
56 family members have protective effects [9, 14, 15], while others contribute to the progression
57 of neurodegeneration [16–18]. Earlier work from our group observed that cerebrospinal fluid
58 levels of VEGFA displayed a protective effect [15]. When we expanded our analyses to brain
59 tissues we observed that *VEGFB*, *FLT1*, *PGF*, and *FLT4* all appear to contribute to the
60 progression of AD pathology and cognitive decline [16]. Within the prefrontal cortex, higher
61 expression of these four genes (*VEGFB*, *FLT1*, *PGF*, and *FLT4*) was associated with more rapid
62 cognitive decline and higher pathology burden [16]. Similar associations are observed when
63 measuring the protein abundance of these VEGF family members in brain tissue and extend to
64 other brain regions including the posterior cingulate gyrus [18].

65 To better understand VEGF associations in the human brain within their cellular context,
66 we previously investigated single nucleus expression from 48 post-mortem DLPFC samples,
67 evaluating their expression in astrocytes, microglia, oligodendrocytes, oligodendrocyte
68 progenitor cells (OPCs), pericytes, endothelial cells, excitatory and inhibitory neurons [18].
69 Fascinatingly, we found that the robust *FLT1* and *VEGFB* signals that we had observed in bulk
70 tissue were driven by expression in microglia for both genes, along with endothelial cells,
71 oligodendrocytes, and OPCs for *VEGFB.* While results were encouraging, the small sample size
72 and few endothelial and microglial cells in this initial dataset made interpretation challenging.
73 The goal of this study is to further disentangle the cell-type specific VEGF expression
74 changes in relation to AD endophenotypes by analyzing a much larger AD single-nucleus RNA
75 sequencing (snRNA) cohort (N=424) [19, 20]. Specifically, we investigated 10 VEGF (*VEGFA*,
76 *VEGFB*, *VEGFC*, *VEGFD*, *PGF*, *FLT1*, *FLT4*, *KDR*, *NRP1*, and *NRP2*) family members' expression
77 within eight brain cell types to test for differential expression between cognitively normal
78 participants and those with AD, along with expression associations with global cognition (cross-
79 sectional and longitudinal trajectories) and AD pathology (β-amyloid and tau neurofibrillary
80 tangle density; **Figure 1b**). In addition, we evaluated cell-type specific changes in the VEGF-
81 associated signaling pathway during AD by conducting an intercellular communication analysis
82 for these 10 VEGF family members.

Methods

Participants

The participants included in this study were from two longitudinal clinical-pathological cohort studies including the Religious Orders Study and the Rush Memory and Aging Project (ROS/MAP). Each study was approved by an Institutional Review Board (IRB) of Rush University Medical Center. At enrollment, all participants were free of known dementia and agreed to annual clinical evaluation and the donation of their brain at the time of their death [21–23]. All participants signed informed and repository consents and an Anatomic Gift Act. Secondary analyses of this extant data were approved by the Vanderbilt University Medical Center IRB. ROS/MAP data can be accessed online at the Rush Alzheimer's Disease Center Resource Sharing Hub (https://www.radc.rush.edu/), as well as on the Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) Knowledge Portal (syn3219045).

Single nucleus RNA sequencing

Specimens were collected on availability of frozen pathologic materials from the dorsal lateral prefrontal cortex (DLPFC). The tissue specimens were collected by the Rush Alzheimer's Disease Center and processed at Columbia University Medical Center. Participants with a post-mortem interval < 41 hours and whole genome sequence data were selected to go through a series of rigorous quality control steps [24–26], which resulting in 424 participants. The participants had a median of 3,824 sequenced nuclei. In the final data, there were 8 major cell types, including astrocytes, endothelial cells, inhibitory neurons, microglia, oligodendrocytes, oligodendrocyte precursor cells (OPCs), and CUX2- and CUX2+ excitatory neurons. snRNAseq data can be downloaded from AD Knowledge Portal (Accession Number: syn31512863).

105 Additional filters applied before analyses included removing genes with expression in less than
106 10% of all cells, removing cells with less than 200 or more than 20,000 total RNA UMIs or had
107 more than 5% mitochondrial mapped reads.

108 **Measurements of Cognitive Function**

109 The measurement of global cognition was derived from 17 different neuropsychological
110 tests across five domains of cognition (semantic, episodic, and working memory, perceptual
111 speed, and perceptual orientation). The z-scores of all the available tests were averaged to
112 create a global cognition composite. Further details on the development of this composite have
113 been previously described [27]. For cross-sectional cognition function related analysis, global
114 cognitive score at last visit before death was used from 369 participants. Longitudinal cognitive
115 trajectory was derived from a linear mixed effects model with global cognition as the outcome
116 and the intercept and interval (years from last visit) entered as fixed and random effects. The
117 derived "cognition slope" was calculated as the sum of the random and fixed effects for each
118 participant. 423 participants are included in this analysis.

119 **Measurements of AD pathology**

120 Measures of pathology were previously characterized in ROS/MAP [21, 22]. Measured
121 by immunohistochemistry at autopsy, β-amyloid load and tau tangle density were quantified as
122 the average percent area occupied by β-amyloid (using antibodies specific to $A\beta_{42}$) or tau (using
123 antibodies specific to AT8 epitope of abnormally phosphorylated tau) across 8 brain regions at
124 autopsy: hippocampus, angular gyrus, and entorhinal, midfrontal, inferior temporal, calcarine,
125 anterior cingulate, and superior frontal cortices. Then, values were transformed to approximate
126 a normal distribution. 421 participants are included in β-amyloid load and tau density analysis.

127 **Statistical Analysis**

128 Statistical analyses were completed using RStudio (R version 4.3.1) and code is available
129 upon request from the corresponding author. Significance was set to a priori to α=0.05.
130 Additionally, p-values were corrected for all *VEGF* predictors across AD-associated outcomes
131 (290 models) using the false discovery rate (FDR) procedure.

132 Negative binomial mixed models implemented by the Nebula R package [28](v1.4.2)
133 were used to analyze the association between each *VEGF* gene within each of the eight cell
134 types and AD diagnosis, global cognition longitudinally and at last visit before death, and β-
135 amyloid load and PHF tau tangle density at death. For analysis of diagnosis, 299 individuals
136 were included: 142 normal cognition controls (cogdx = 1) and 157 AD dementia patients (cogdx
137 = 4 or 5). All models covaried for age at death, sex, post-mortem interval (PMI), and the interval
138 between last cognitive visit and death in years (for cognition). One participant didn't have PMI
139 data, hence, was removed from association analysis. The gene count matrix input of the models
140 was the UMI count data from the RNA assay normalized and scaled by the "sctransform" R
141 package (https://github.com/satijalab/sctransform).

142 **VEGF-mediated Intercellular Communication Profiling**

143 Intercellular communication pattern involving these 10 VEGF genes among cell types
144 was analyzed using the 'CellChat' R package [29](v1.0). Due to lack of some of the evident
145 ligand-receptor pairs involving these 10 VEGF genes from the default CellChat database
146 (CellChatDB), we manually updated CellChatDB by incorporating those curated additionally
147 from the CellTalk DB (v1.0, https://github.com/ZJUFanLab/CellTalkDB/tree/master/database).
148 All VEGF ligand-receptor pairs that were included in this study can be found in **Supplemental**

149 **Table 2.** We performed the rest of the analysis according to the default setting of CellChat,
150 skipping projecting gene expression data onto human protein-protein interaction network.
151
152 **Results**
153 **Participant Characteristics**
154 The snRNAseq data used for our analysis consisted of 424 non-Latinx White participants
155 (**Table 1**) with an average PMI of 7.7 hours (SD = 5.1 hours), 68% of whom are females, 52% are
156 clinical AD dementia patients (cogdx = 4 or 5), and 27% carried at least one APOE4 allele. The
157 cohort has a mean age at death at 89 years old with an average of 13 years of education.
158 ***VEGFs* Associations with Cognition**
159 Higher expression of *FLT1* in endothelial cells and microglia was associated with worse
160 cognitive performance at last visit before death (endothelial-*FLT1* (logFC= -0.089, FDR= 0.009)
161 and microglia-*FLT1* (logFC= -0.176, FDR= 0.009)) and faster cognitive decline (endothelial-*FLT1*
162 (logFC= -0.771, FDR= 0.041) and microglia-*FLT1* (logFC= -1.964, p= 0.009)). Additionally, higher
163 expression of *VEGFB* in oligodendrocytes (logFC= -0.561, FDR= 0.019) was also associated with
164 faster cognitive decline. **Table 2** listed all statistically significant associations between 10 VEGF
165 gene expression and various AD outcomes tested (the complete result can be found in
166 **Supplemental Table 1**).
167 ***VEGFs* Associations with AD Pathology**
168 Multiple cell types and *VEGF* genes were associated with β-amyloid load (**Table 2**).
169 Higher microglia-*FLT1* (logFC= 0.053, FDR= 0.001), oligodendrocyte-*VEGFB* (logFC= 0.014, FDR=
170 0.009), endothelial-*FLT4* (logFC= 0.027, FDR= 0.013), astrocyte-*VEGFD* (logFC= 0.016, FDR=

171 0.029), and oligodendrocyte precursor cell-*NRP1* (logFC= 0.018, FDR= 0.046) were all associated
172 with higher β-amyloid load. On the contrary, within inhibitory neurons, higher expression of
173 *VEGFB* was associated with lower β-amyloid load (logFC= -0.012, FDR= 0.019). On the other
174 hand, we only found one gene that associated with tau density, which was higher astrocyte-
175 expressed NRP1 was associated with lower tau density (logFC= -0.014, FDR= 0.009, **Table 2**).
176 To examine whether contribution of micro-*FLT1* and oligo-*VEGFB* to more amyloid load
177 influences the association of their expression with worse cognition performance, we performed
178 a competitive hierarchical analysis ("gene expression ~ sex + age at death + PMI + amyloid +
179 cognition (either cross sectional score at last visit or longitudinal trajectory)" using the same
180 Nebula setting for other association analyses). We found that when covarying for amyloid, both
181 gene associations with cognitive performance were attenuated about 20% (by comparison of
182 logFC values), but remained statistically significant for micro-*FLT1* (p = 0.011 for both cross-
183 sectional and longitudinal cognitive performance models) and fell just below statistical
184 significance in the case of oligo-*VEGFB* (p = 0.065 for longitudinal cognitive performance
185 model). Together our analysis indicated that the associations of micro-*FLT1* and oligo-*VEGFB* to
186 cognitive performance are only partially explained by their association with amyloid load.
187 **Differential Expression of *VEGFs in AD***
188 We also tested for differences in *VEGF* expression between neuropathologically
189 confirmed AD dementia cases and cognitively unimpaired. We observed that AD patients had
190 higher expression levels of *FLT1* within microglia (logFC= 0.452, FDR= 0.009) and endothelial
191 cells (**Table 2**, logFC= 0.195, FDR= 0.014).
192

193 **VEGF-associated Intercellular Communication Profile**

194 Overall VEGF communication strength (calculated by summing communication
195 probabilities of all curated VEGF pathway ligand-receptor (L-R) pairs among all cell types
196 (**Supplemental Table 2**)) was significantly reduced (Wilcoxon test; $p<0.05$) in the AD group
197 compared to the cognitively normal group, and the reduction was observed for both incoming
198 and outgoing VEGF signaling strength (calculated by in-degree and out-degree network
199 centrality scores respectively) among cells (**Figure 2a**). VEGFA was the most abundantly
200 expressed VEGF ligand among investigated, primarily in astrocytes, hence, it was no surprise to
201 see many of the significant interactions (L-R pairs) for VEGF pathway were with VEGFA for both
202 AD and cognitively normal groups. As a result, overall VEGF information flow among these brain
203 cells was from astrocytes to other cell types including themselves (**Figure 2b**). But interestingly,
204 the top receiver (cell type) of VEGF communications for the cognitively normal group was
205 excitatory neurons, whereas for AD group, it was endothelial cells (**Figure 2c**). The underlying
206 cause of this difference lay in the significant change of the relative contribution of top L-R pairs
207 to the overall VEGF communications when comparing these two diagnoses groups. Specifically,
208 "VEGFA-GRIN2B" was 37.8% in AD vs 39.5% in cognitive normal group, "VEGFA-SIRPA" was
209 10.2% vs 10.7% (AD vs cognitive normal), and "VEGFA-EGFR" was 10.2% vs 9.5% (AD vs
210 cognitive normal). But probably the most important change is for "VEGFA-FLT1", which
211 contributed 13.8% of all VEGF signals in AD dementia patients comparing to 9.5% in cognitive
212 normal individuals. VEGFA-FLT1 signal is from astrocytes to endothelial cells exclusively, and it
213 showed a comparable communication probability between cells in AD and cognitive normal
214 participants (adjusted probability of 0.073 vs 0.076), which can be seen as an outsider

215 considering the overall reduction of VEGF signals in AD group (**Supplemental Table 3** presented
216 the complete result). The significant elevated *FLT1* expression in endothelial cells from AD
217 patients might have facilitated this change.
218
219 **Discussion**
220 In this study we provided the most comprehensive exploration into cell-specific effects
221 of the VEGF family in the context of AD. Our results build on previous evidence from bulk tissue
222 at the protein and transcriptomic level that *VEGFB, FLT1, FLT4,* and *NRP1* relate to the clinical
223 progression and neuropathology of AD [16, 18]. The single cell resolution here builds on our
224 early single nucleus data [18] to provide the clearest picture of the important and distinct roles
225 the VEGF family members play in various brain cell types. Our results particularly highlight the
226 diverse associations for VEGF family members across endothelial cells, microglial cells,
227 astrocytes, oligodendrocytes, and neurons. While *FLT1* showed strong upregulation in the AD
228 brain in endothelial cells as might be expected, we also observed strong upregulation of *FLT1* in
229 microglial cells. Yet, regardless of the upregulation of *FLT1*, our intercellular communication
230 analyses suggest that FLT1-associated canonical VEGF signaling pathways themselves are in fact
231 comparable in the AD brain to normal brain. In contrast to the consistent effects observed for
232 *FLT1*, we observed opposing direction of effect on β-amyloid load for *VEGFB* expression in
233 oligodendrocytes vs inhibitory neurons, whereby higher levels of oligodendrocyte *VEGFB*
234 related to higher pathology, while higher levels of neuronal *VEGFB* related to lower pathology.
235 Together, these results suggest that in addition to the classic vascular signaling pathway
236 underlying the associations between VEGF family members and AD, there may also be immune

237 (e.g., microglial *FLT1*) and neuronal (e.g., neuronal *VEGFB*) signaling cascades that contribute to
238 AD pathogenesis.

239 The central role of VEGF signaling is angiogenesis under both health and disease
240 conditions [30, 31]. Pathological angiogenesis is a hallmark of AD, which is believed to both
241 drive and respond to multiple AD pathologies driving hypoxia, oxidative stress, inflammation,
242 and Aβ accumulation [32]. Indeed, previous work has identified a marked upregulation of
243 angiogenic factors and mediators in the AD brain [33, 34]. In the present results, the
244 upregulation of *FLT1* in endothelial cells among AD patients could reflect a repair response. The
245 increase in vascularization and blood flow could be a response to AD neuropathology similar to
246 the response that has been indicated in other neurodegenerative and neuropsychiatric diseases
247 such as ALS [35] and schizophrenia [36]. On the other hand, the elevated endothelial *FLT1* could
248 drive AD neuropathology. For example, it has been shown that VEGF-induced pathological
249 angiogenesis can facilitate Aβ generation [37]. VEGF-induced pathological angiogenesis can also
250 worsen AD pathology by disrupting permeability of the blood-brain barrier (BBB) [38, 39]. BBB
251 is formed by physical interaction of multiple brain cell types, with a common structure
252 consisting of blood capillaries formed by endothelial cells, pericytes, and the astrocytic end feet
253 [40]. As a natural protective barrier of the central nervous system (CNS), BBB homeostasis is
254 strictly regulated, disruption of which can lead to many neurological conditions including AD
255 [41, 42]. Our intercellular communication profile showed that signals communicated among
256 these 10 VEGF genes were predominantly from astrocytes to endothelial cells, hinting at the
257 canonical angiogenesis pathway at the blood brain barrier as a primary driver here. In fact,
258 evidence is already emerging that demonstrates CNS angiogenesis can be regulated through

259 VEGF-dependent signaling at the BBB [43]. Characterizing the interplay between VEGF family
260 genes and BBB integrity in the context of AD may provide more insight into the mechanistic
261 pathways underlying the associations observed here.

262 Our results also suggest roles of some of the VEGF family genes in regulating the
263 immune response, including the upregulation of microglial *FLT1* in AD group. Increased
264 microglia function in AD has been observed by many [44–48] in the presence of β-amyloid
265 peptides and facilitates an inflammatory response [49]. FLT1 was believed to be one of the 20
266 or so proteins that has been found to be enriched in Aβ plaques [50], and has recently been
267 showed to interact with VCAM1 and plays a role in VCAM1-dependent chemotaxis of microglia,
268 which enhances microglia clearance of Aβ [51]. Studies have also shown the critical role that
269 FLT1 plays in microglial proliferation and monocyte-macrophage migration [52, 53]. With all
270 these under consideration, elevation of microglial *FLT1* in AD should not be a surprise. The
271 primary ligand for *FLT1* is *VEGFB*, so it is possible that the *VEGFB* association in
272 oligodendrocytes may reflect upregulation of this same pathway. It is well known that
273 oligodendrocytes and microglia work together during regulation of myelination, and this is
274 particularly noticeable during neurodegenerative conditions [54]. Future work confirming these
275 cell-specific transcriptomic effects at the protein level is necessary.

276 One fascinating discovery in the present results is that higher expression of *VEGFB*
277 within inhibitory neurons was associated with lower β-amyloid load, which was the only
278 protective effect of any VEGF we tested in this dataset. This result is consistent with the
279 previously reported evidence of the benefits of *VEGFB* expression on neuronal survival and
280 nerve regeneration [55–58]. Perhaps in the early stage of AD when β-amyloid load is low,

281 inhibitory neurons manage to inhibit apoptosis and promote neurogenesis through
282 upregulating VEGFB. Upregulation of VEGFB could be one of the molecular mechanisms that
283 enables inhibitory neurons to be more resistant to AD neuropathology comparing to excitatory
284 neurons [59]. Interestingly, a study of APP/PS1 amyloidopathy mice did show an increase of
285 inhibitory synapses in hippocampus before pathology develops and then decreased at 12
286 months of age when robust AD pathology was present [60]. Hence, the protective effect of
287 VEGFB from inhibitory neurons to the development of AD pathology could function in a time-
288 sensitive matter. One also cannot rule out the possibility that increased *VEGFB* expression
289 within inhibitory neurons could help with the clearance of β-amyloid.

290 We also observed higher expression of *FLT4* within endothelial cells related to higher β-
291 amyloid load. The primary ligand for *FLT4* is *VEGFD* and we also observed an association
292 between elevated *VEGFD* in oligodendrocytes and β-amyloid load. In contrast to the other VEGF
293 receptors, the FLT4/VEGFD signaling axis is primarily involved in lymphangiogenesis. Certainly
294 meningeal lymphatics has been implicated in β-amyloid clearance through a complex interplay
295 with the immune system [61], so it is quite possible that lymphatics underly the observed
296 *VEGFD* and *FLT4* associations with β-amyloid. In previous work, we identified an associated
297 between higher expression of *FLT4* in the dorsal lateral prefrontal cortex and worse
298 neuropathology [16], but also identified higher expression of *FLT4* in the caudate nucleus
299 associated with lower β-amyloid levels and tau levels [18]. Others have also observed these
300 flips in direction with associations depending on the brain region [62]. Combined, these results
301 suggest that tissue and cell-type are important considerations when looking at the role of *FLT4*
302 in the brain within the context of AD.

303 Finally, we observed a cell-type specific effect of NRP1. Higher expression of *NRP1* in
304 astrocytes was associated with lower levels of neurofibrillary tangles, but higher expression of
305 *NRP1* within oligodendrocyte precursor cells was associated with higher β-amyloid load. Other
306 work from our group has also observed differing roles of *NRP1* when pertaining to AD
307 neuropathology [18]. However, this is the first time we have observed statistically significant
308 associations within specific cell-types. All above results further highlight the complexity of the
309 *VEGF* family and their role within neurodegeneration. To fully understand the clinical potential
310 of *VEGF* as a biomarker and therapeutic target, in-depth understanding of each of the tissue
311 and cell-type is necessary.
312 This study has many strengths. ROS/MAP is a well-characterized cohort with longitudinal
313 cognitive data, comprehensive neuropathology data with multiple cell-type measures of *VEGF*
314 expression. Additionally, this study reports novel discoveries and replication of the effects of
315 *VEGF* family members on AD phenotypes including pathology and cognition at the
316 transcriptomic level within a large sample size. Because of the larges sample size and cell-type
317 specific expression measurements, we were able to provide more comprehensive data and
318 hypotheses about cellular context.
319 Despite these strengths, this study also has limitations. ROS/MAP is enriched for non-
320 Hispanic white, highly educated individuals which limits the generalizability to more
321 representative populations. Further, while being able to examine cell-type specific effects in a
322 large sample size, we are limited to the dorsolateral prefrontal cortex tissue.
323 To conclude, this study provides an extensive characterization within a large sample size
324 of the *VEGF* signaling family members in the context of cognitive aging and AD. Our results

provide evidence that further supports the role of *VEGFB*, *VEGFD*, *FLT1*, *FLT4* and *NRP1* play inside and outside of classic VEGF signaling axis in AD, with support for both an endothelial and microglial signaling cascade that could underly these well-established associations. For the first time, we provide evidence that *VEGFB* expression in inhibitory neurons may relate to a protective effect against AD neuropathology as has been hypothesized previously and observed in models of Parkinson's disease. These results provide compelling evidence that VEGFB and FLT1 may have potential as therapeutic targets in AD, but there remains a need to confirm these single nucleus transcriptomic results at the protein level and establish causality through experimental approaches.

Acknowledgements

This project is supported by R01-AG061518, R01-AG074012, and R01-AG059716. ROSMAP is supported by P30AG10161, P30AG72975, R01AG15819, R01AG17917, U01AG46152, and U01AG61356. ROSMAP resources can be requested at https://www.radc.rush.edu and www.synpase.org.

Conflict of Interest

Dr. Timothy Hohman is a Deputy Editor for Alzheimer's & Dementia: Translational Research and Clinical Intervention. All other co-authors declare no conflict of interest.

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514 **Table 1. Characteristics of Study Participants**

	Cognitive Normal Controls (N=143)	**AD Patients** (N=157)	**Total Cohort** (N=424)	**P Value**
Age at death (years)	87.20 ± 7.26	91.15 ± 6.56	89.19 ± 6.82	<0.001
Education (years)	16.28 ± 3.45	16.55 ± 3.22	16.31 ± 3.47	0.48
Post-mortem interval (hours)	7.80 ± 4.99	7.25 ± 4.32	7.71 ± 5.11	0.31
Global cognition slope	-0.04 ± 0.04	-0.19 ± 0.09	-0.11 ± 0.09	<0.001
β-amyloid load	3.12 ± 3.77	5.93 ± 4.46	4.46 ± 4.50	<0.001
Tau density	2.97 ± 3.36	9.36 ± 9.15	5.98 ± 7.15	<0.001
Male, no. (%)	49 (34)	42 (27)	136 (32)	0.16
APOE4 carrier, no. (%)	27 (19)	60 (38)	109 (27)	<0.001

515 Values are presented as mean ± standard deviation unless otherwise indicated. P values of

516 comparison between cognitive normal controls and AD patients were derived from Welch Two

517 Sample t-test for continuous variables and Pearson's Chi-squared test for categorical variables.

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527 **Table 2. FDR Significant Cell-Type Specific VEGF Associations with Cognition and AD Pathology**

Gene	Outcome	Cell Type	logFC	SE	P Value	FDR
FLT1	Last cognition before death	Endothelial cells	-0.089	0.024	1.768E-04	0.009
FLT1	Last cognition before death	Microglia	-0.176	0.046	1.492E-04	0.009
FLT1	Cognition trajectory	Endothelial cells	-0.771	0.248	0.002	0.041
FLT1	Cognition trajectory	Microglia	-1.964	0.504	9.716E-05	0.009
VEGFB	Cognition trajectory	Oligodendrocytes	-0.561	0.166	7.232E-04	0.019
FLT1	Diagnosis	Endothelial cells	0.195*	0.055	4.232E-04	0.014
FLT1	Diagnosis	Microglia	0.452*	0.113	6.378E-05	0.009
FLT1	β-amyloid load	Microglia	0.053	0.011	2.700E-06	7.830E-04
VEGFB	β-amyloid load	Oligodendrocytes	0.014	0.004	1.428E-04	0.009
VEGFB	β-amyloid load	Inhibitory neurons	-0.012	0.004	6.529E-04	0.019
FLT4	β-amyloid load	Endothelial cells	0.027	0.008	3.579E-04	0.013
VEGFD	β-amyloid load	Astrocytes	0.016	0.005	0.001	0.029
NRP1	β-amyloid load	Oligodendrocyte precursor cells	0.018	0.006	0.002	0.046
NRP1	Tau density	Astrocytes	-0.014	0.004	2.229E-04	0.009

528 *logFC values of diagnosis is log2 fold change of gene expression comparing AD to cognitive

529 normal participants.

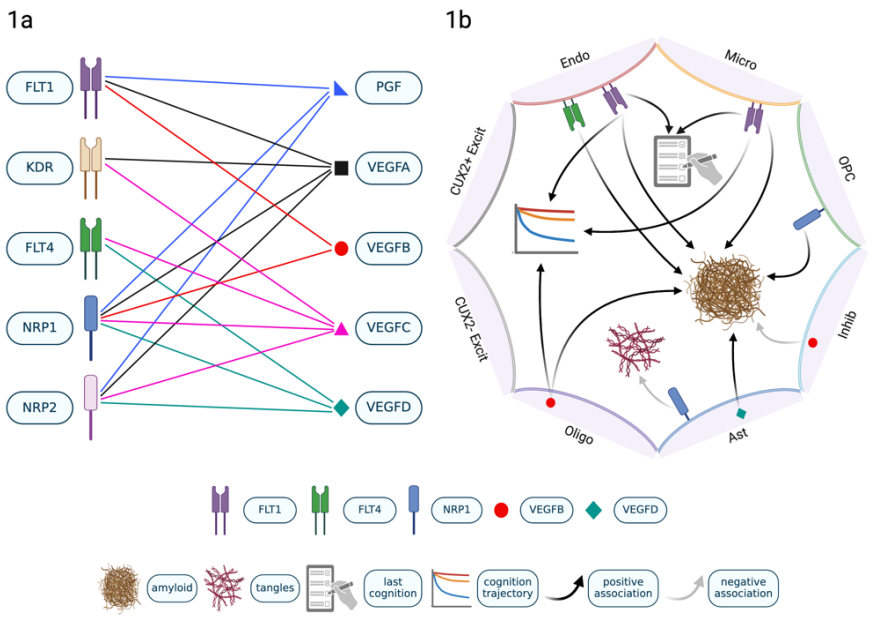

Figure 1. 10 VEGF System and Their Cell-Type Specific Effect on AD Endophenotypes
1a. 10 VEGF ligand-receptor system. **1b**. Association of VEGF and AD endophenotypes in DLPFC brain cells. Cell type abbreviations are as follows, Endo, Endothelial Cells; Micro, Microglia; OPC, Oligodendrocyte Precursor Cells; Inhib, Inhibitory Neurons; Ast, Astrocytes; Oligo, Oligodendrocytes; CUX2+ Excit, CUX2+ Excitatory Neurons; CUX2- Excit, all Excitatory Neurons that don't express CUX2-. This figure was created with BioRender.com.

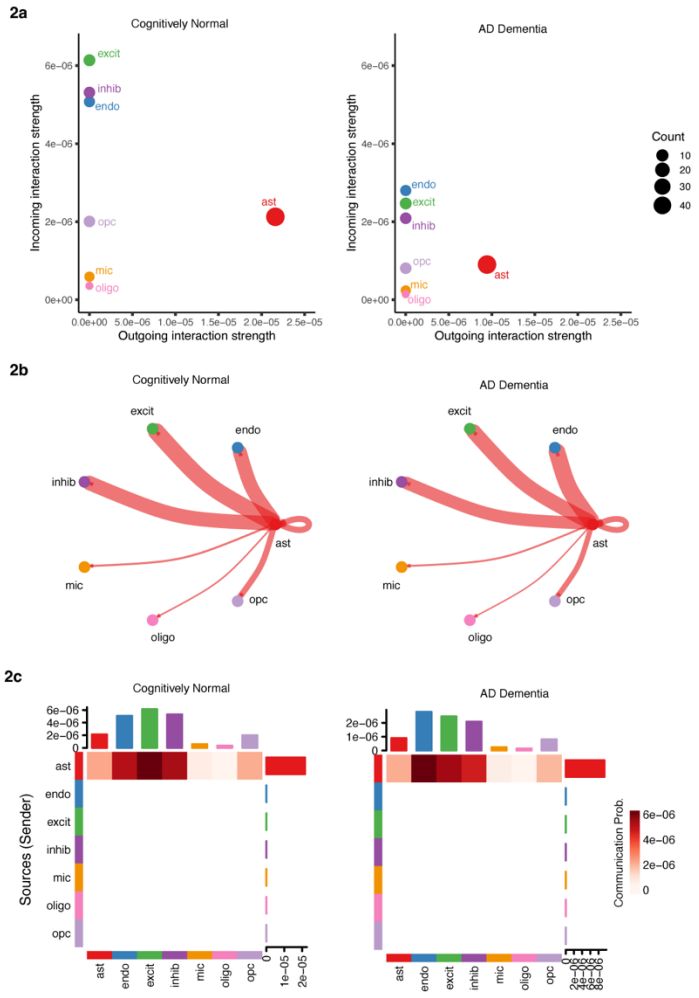

Figure 2. 10 VEGFs-Associated Signaling Pathway Profile in AD vs Cognitively Normal Groups

2a. Overall incoming and outgoing VEGF signaling strength comparison. **2b**. Circle plot of overall VEGF information flow. **2c**. heatmap of overall VEGF information flow. Cell type abbreviations are as follows, Endo, Endothelial Cells; Micro, Microglia; OPC, Oligodendrocyte Precursor Cells; Inhib, Inhibitory Neurons; Ast, Astrocytes; Oligo, Oligodendrocytes; CUX2+ Excit, CUX2+ Excitatory Neurons; CUX2- Excit, all Excitatory Neurons that don't express CUX2-.