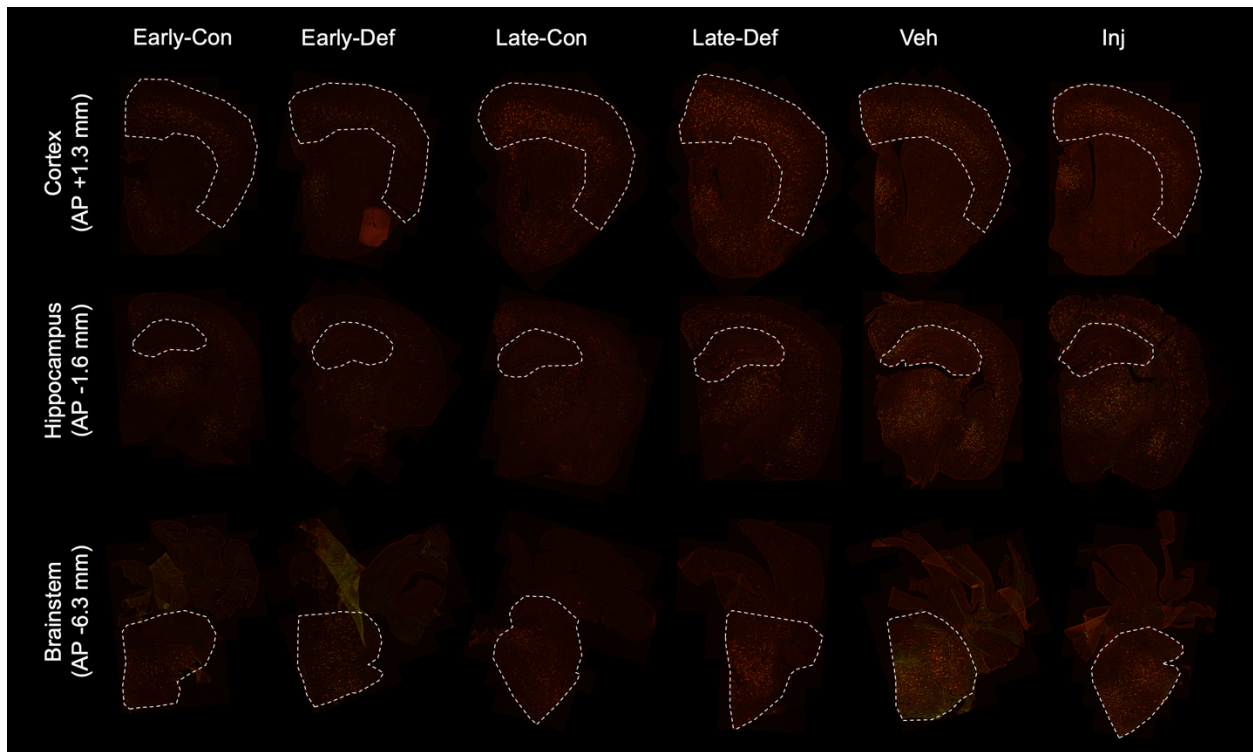# Supplementary Material

Vitamin D Reduces GABA-Positive Astrocytes in the 5xFAD Mouse Model of Alzheimer's Disease


Supplementary Figure 1. Representative histologic findings of the hemisphere images staining Aβ plaques (Thioflavin S; Green) and microglia (Iba1; Red). Dashed lines indicate the regions of interest used for the quantification of Aβ plaques and microglia based on Paxinos and Franklin's the Mouse Brain. Aβ, amyloid-β; Iba1, ionized calcium-binding adaptor molecule 1
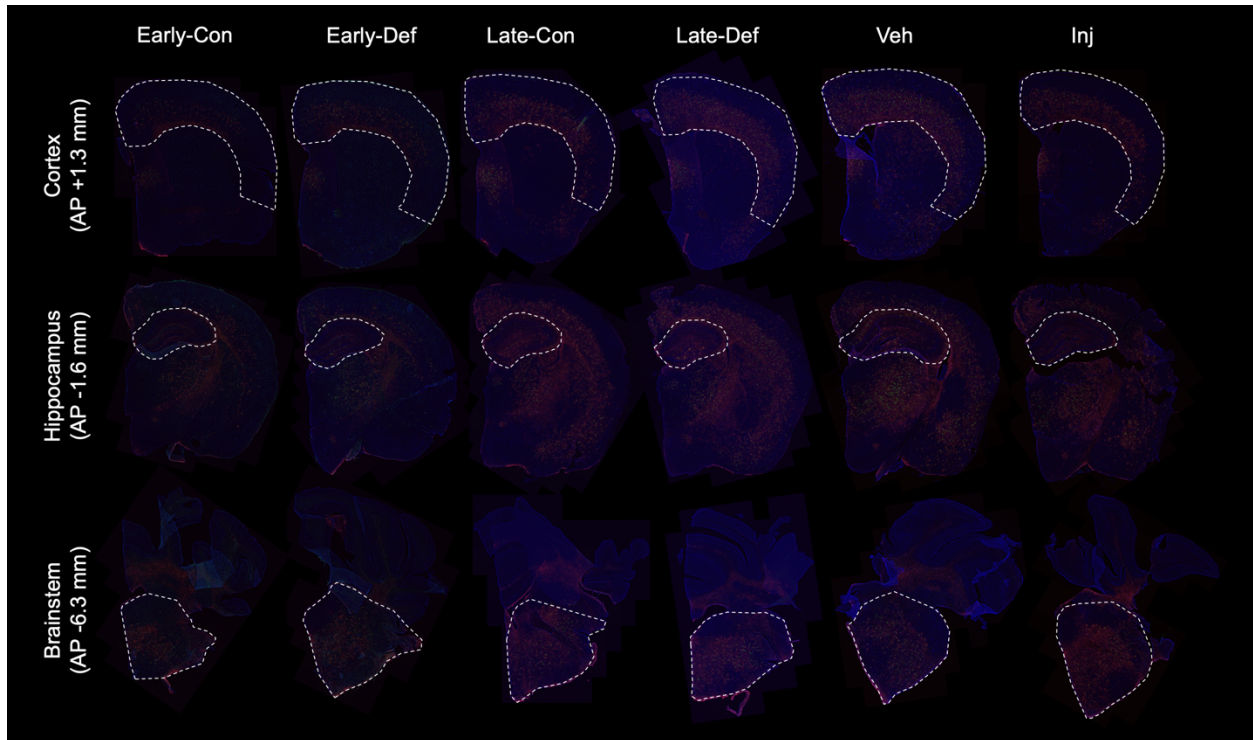
Supplementary Figure 2. Representative histologic findings of the hemisphere images staining Aβ plaques (Thioflavin S; Green), astrocytes (GFAP; Red), and GABA (Blue). Dashed lines indicate the regions of interest used for the quantification of Aβ plaques and astrocytes. Aβ, amyloid-β; GFAP, glial fibrillary acidic protein; GABA, gamma-aminobutyric acid

The Supplementary Figures 3-5 presented in this supplementary section have been reprocessed from our previous study (Kang et al., 2022) [1]. This reprocessing was conducted to provide additional insights and to complement the findings discussed in the main text on the reviewer's request. All reprocessing procedures adhere to ethical guidelines and maintain the integrity of the original data.
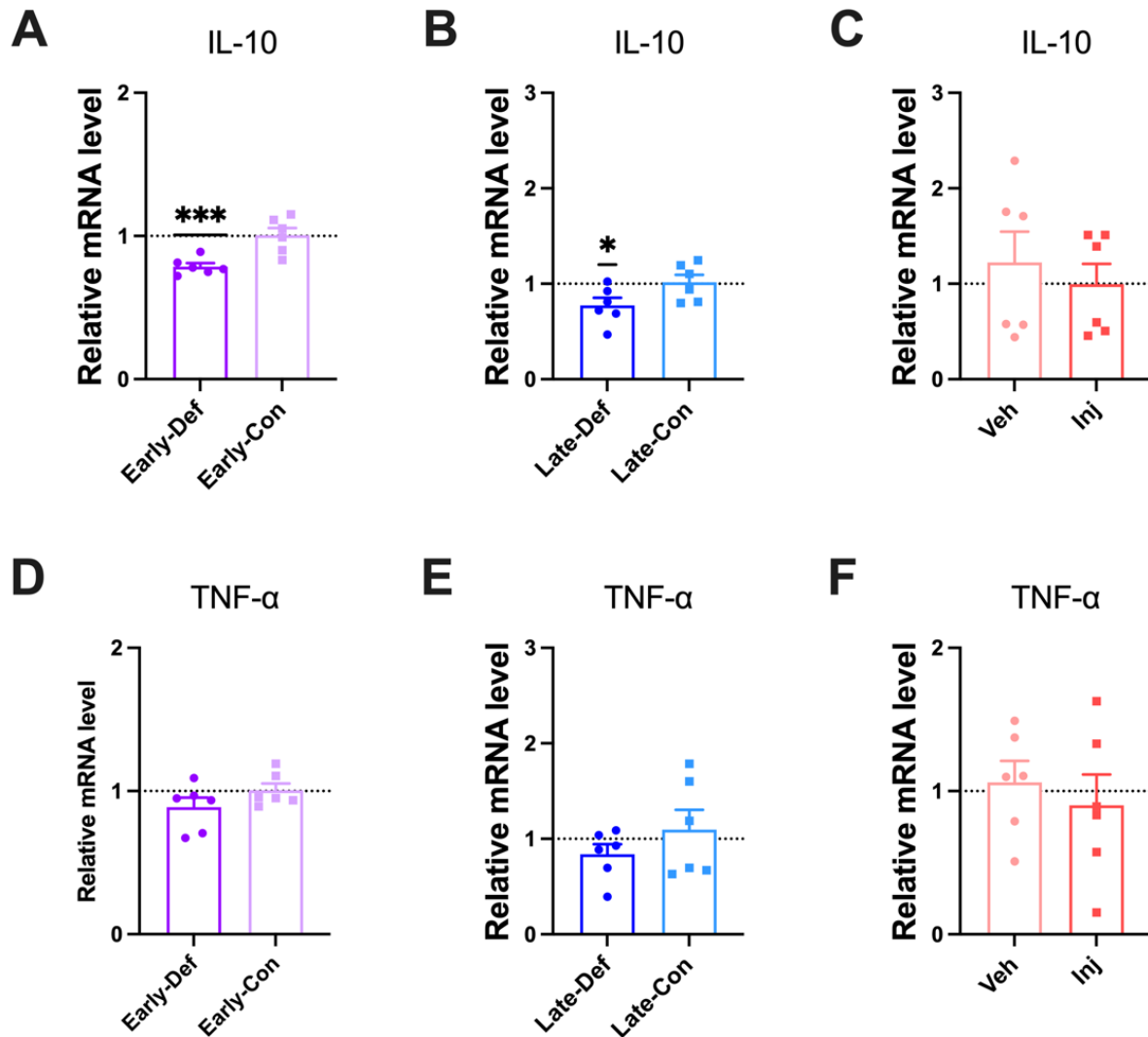
Supplementary Figure 3. Vitamin D deficiency decreased mRNA levels of IL-10 in the brain.
A) The results from the qRT-PCR analysis of the transcriptional levels of IL-10 between Early-Def (n=6) and Early-Con (n=6) groups are shown. B) The mRNA levels of IL-10 between Late-Def (n=6) and Late-Con (n=6) groups are illustrated. C) The mRNA levels of IL-10 between Veh (n=6) and Inj (n=6) groups are illustrated. D) The mRNA levels of TNF-α between Early-Def and Early-Con are shown. E) The mRNA levels of TNF-α between Late-Def and Late-Con are shown. (F) The mRNA levels of TNF-α between Veh and Inj are shown. Data are presented as mean ± SEM. $*p < 0.05$ by Student's t-test. Supplementary Figure 3 showcases a re-representation of the data originally published by Kang et al., 2022 [1]. This re-representation aims to enhance understanding and provide a different perspective in the context of the current study on the reviewer's request. IL-10, interleukin-10; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; TNF-α, tumor necrosis factor α

Relative mRNA levels compared to control or vehicle group
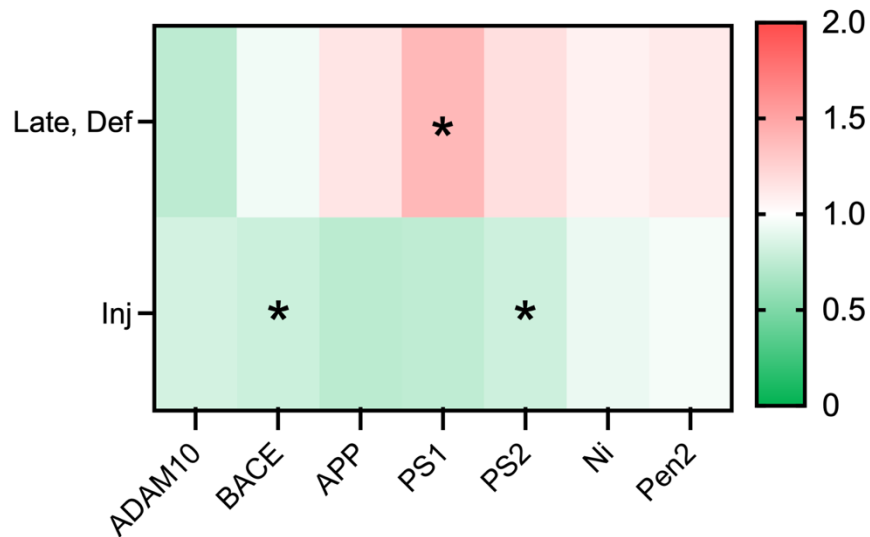
Supplementary Figure 4. Lower mRNA levels of genes related to Aβ production in the vitamin D supplementation group. A heatmap of the qRT-PCR results of Aβ pathology-related gene expression is shown. mRNA levels of Late-Def (n=6) and Inj (n=5) groups were compared to Late-Con (n=6) and Veh (n=6) groups, respectively. The data are presented by the mean ± SEM of each group. *p < 0.05 by the Student's t-test. Supplementary Figure 4 showcases a re-representation of the data originally published by Kang et al., 2022 [1]. This re-representation aims to enhance understanding and provide a different perspective in the context of the current study on the reviewer's request. ADAM-10, A Disintegrin and Metalloproteinase (ADAM) family; APP, amyloid precursor protein; BACE, β-secretase; Ni, nicastrin; Pen2, presenilin-enhancer 2; PS1, presenilin 1; PS2, presenilin 2
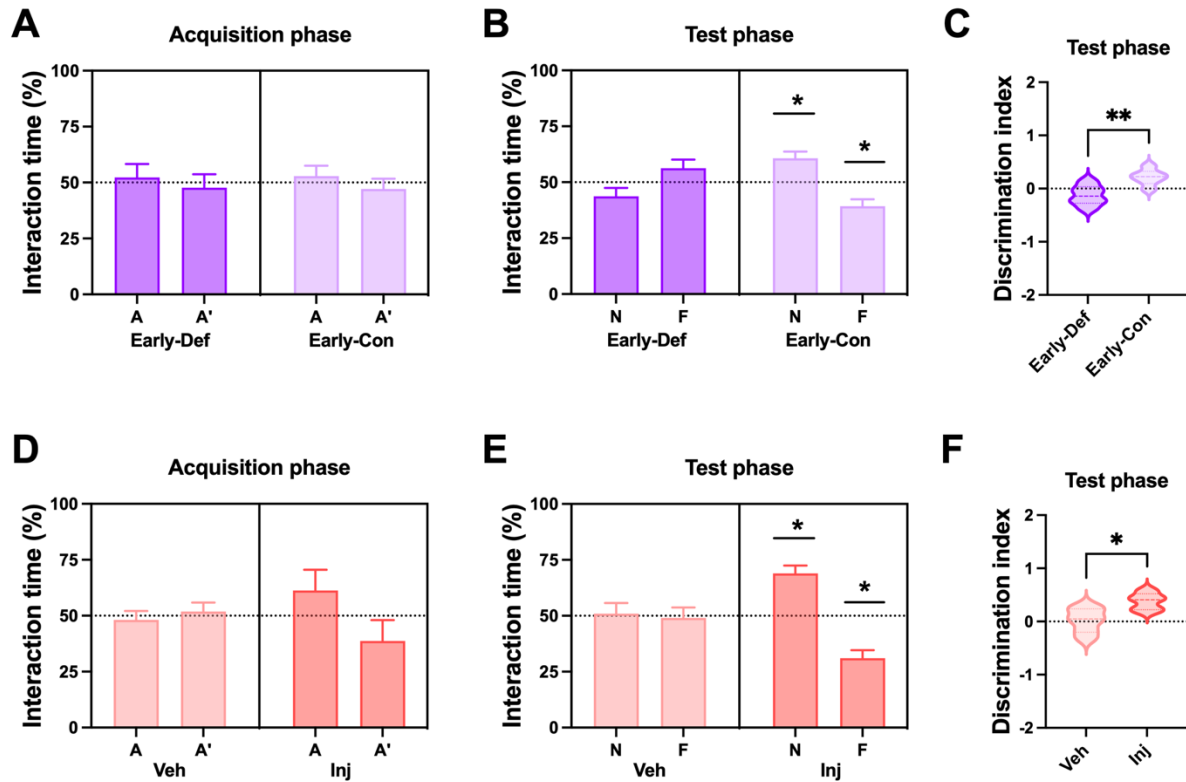
Supplementary Figure 5. The effects of vitamin D on memory impairment in AD mouse model. A, B) The interaction time spent in each location of the Early-Def (n = 6) and Early-Con (n = 6) groups in (A) the acquisition trial and (B) the test trial are shown. C) The discrimination indexes of the Early-Def and Early-Con groups are illustrated. D, E) The interaction time spent in each location of the Veh (n = 6) and Inj (n = 5) groups in (D) the acquisition trial and (E) the test trial are shown. F) The discrimination indexes of the Veh and Inj groups are illustrated. The data are presented by the mean ± SEM of each group. *p < 0.05 by the Student's t-test (one-sample t-test compared to theoretical mean). Supplementary Figure 5 showcases a re-representation of the data originally published by Kang et al., 2022 [1]. This re-representation aims to enhance understanding and provide a different perspective in the context of the current study on the reviewer's request.

Supplementary Table 1. Results of a systematic rapid review of previous studies investigating the association between vitamin D and tau pathology in Alzheimer's disease (published, 2022-2023)

Author, year	Country	Samples	Subject	Results	References
Sadeghzadeh et al., 2023	Iran	32 male mice model with surgical procedure to induce brain ischemia	BALB/C mice with surgical procedure to induce brain ischemia for vascular dementia model	While vitamin D supplements decreased inflammation and prevented apoptosis, there are no significant effects on p-tau.	Sadeghzadeh J, Jafarzadeh J, Hadinezhad P, Nazari A, Sohrabi S, Musazadeh V, Barzegar A, Shahabi P. Profiling inflammatory mechanisms, hyperphosphorylated tau of hippocampal tissue and spatial memory following vitamin D3 treatment in the mice model of vascular dementia. Int Immunopharmacol. 2023 Jul;120:110314. doi: 10.1016/j.intimp.2023.110314. Epub 2023 May 21. PMID: 37220695.
Patel et al., 2022	India			Vitamin D significantly improve the cognitive function and lower hyperphosphorylated tau proteins in the scopolamine-induced rats.	Patel P, Shah J. Vitamin D3 supplementation ameliorates cognitive impairment and alters neurodegenerative and inflammatory markers in scopolamine induced rat model. Metab Brain Dis. 2022 Dec;37(8):2653-2667. doi: 10.1007/s11011-022-01086-2. Epub 2022 Sep 26. PMID: 36156759.
Lin et al., 2022	China	50 APP/PS1 transgenic mice and 10 WT mice	Male APP/PS1 transgenic mice for AD model	Vitamin D significantly reduced the levels of Aβ, cortical APP, tau, and p-tau in APP/PS1 mice.	Lin J, Niu Z, Xue Y, Gao J, Zhang M, Li M, Peng Y, Zhang S, Li W, Zhang Q, Li X. Chronic vitamin D3 supplementation alleviates cognition impairment via inhibition of oxidative stress regulated by PI3K/AKT/Nrf2 in APP/PS1 transgenic mice. Neurosci Lett. 2022 Jul 13;783:136725. doi: 10.1016/j.neulet.2022.136725. Epub 2022 Jun 10. PMID: 35697158.
Wu et al., 2022	China	APP/PS1 transgenic mice (n=10)	Female APP/PS1 transgenic mice for AD model	Activation of the vitamin D receptor reduced the phosphorylation of Tau via inhibiting Tyr216 in the APP/PS1 AD model mice.	Wu TY, Zhao LX, Zhang YH, Fan YG. Activation of vitamin D receptor inhibits Tau phosphorylation is associated with reduction of iron accumulation in APP/PS1 transgenic mice. Neurochem Int. 2022 Feb;153:105260. doi: 10.1016/j.neuint.2021.105260. Epub 2021 Dec 22. PMID: 34953963.

Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid-β protein precursor; WT, wild-type

Supplementary Table 2. Results of a systematic rapid review of previous studies investigating the association between vitamin D and Alzheimer's disease (published, 2022-2023)

Author, year	Country	Samples	Subject	Results	References	Associations
Richter et al., 2023	Netherlands	Serum samples from patients (n=25)	Patients with AD	Low vitamin D status was associated with CSF Aβ levels.	Richter AL, Diepeveen-de Bruin M, Balvers MGJ, De Groot LCPGM, De Deyn PP, Engelborghs S, Witkamp RF, Vermeiren Y. Association between low vitamin D status, serotonin and clinico-bio-behavioral parameters in Alzheimer's disease. Dement Geriatr Cogn Disord. 2023 Oct 6. doi: 10.1159/000534492. Epub ahead of print. PMID: 37806302.	▲
Mohanad et al., 2023	Egypt	Aluminum-chloride-D-galactose (AlCl3-D-gal)-induced AD rat model	AD rat model (non-genetic animal model)	Vitamin D may attenuate cognitive impairments by restoring normal mitochondrial function and reducing inflammatory and oxidative stress.	Mohanad M, Mohamed SK, Aboulhoda BE, Ahmed MAE. Neuroprotective effects of vitamin D in an Alzheimer's disease rat model: Improvement of mitochondrial dysfunction via calcium/calmodulin-dependent protein kinase kinase 2 activation of Sirtuin1 phosphorylation. Biofactors. 2023 Oct 6. doi: 10.1002/biof.2013. Epub ahead of print. PMID: 37801071.	▲
Evlice et al., 2023	Turkey	132 patients with AD and 38 controls	Patients with AD	Vitamin D deficiency can aggregate and trigger ischemia in AD.	Evlice A, Sanli ZS, Boz PB. The importance of Vitamin-D and Neutrophil-Lymphocyte Ratio for Alzheimer's Disease. Pak J Med Sci. 2023 May-Jun;39(3):799-803. doi: 10.12669/pjms.39.3.7024. PMID: 37250565; PMCID: PMC10214823.	▲
Melo van Lent et al., 2022	Germany	250 patients with all-cause dementia and 209 patients with AD	Patients with all-cause dementia and AD	While vitamin A and E were not associated to AD and dementia, vitamin D deficiency increased risk to for AD and dementia.	Melo van Lent D, Egert S, Wolfsgruber S, Kleineidam L, Weinhold L, Wagner-Thelen H, Stoffel-Wagner B, Bickel H, Wiese B, Weyerer S, Pentzek M, Jessen F, Schmid M, Maier W, Scherer M, Riedel-Heller SG, Ramirez A, Wagner M. Low Serum Vitamin D Status Is Associated with Incident Alzheimer's Dementia in the Oldest Old. Nutrients. 2022 Dec 23;15(1):61. doi: 10.3390/nu15010061. PMID: 36615719; PMCID: PMC9824107.	▲
Soares et al., 2022	Norway	100 outpatients aged above 65 years with cognitive impairment and 76 cognitively	Participants with cognitive impairment	Participants with higher CSF vitamin D levels showed lower CSF levels of tau protein and	Soares JZ, Valeur J, Šaltytė Benth J, Knapskog AB, Selbæk G, Bogdanovic N, Pettersen R. Associations Between Intrathecal Levels of Vitamin D, Cytokines, and Core Biomarkers of Alzheimer's Disease: A Cross-Sectional Study. J Alzheimers Dis. 2022;89(3):825-834. doi: 10.3233/JAD-220407. PMID: 35938253.	▲

		healthy controls		phosphorylated tau protein.		
Lai et al., 2022	Taiwan	APP/PS1 mice (n=4-7) and older adults (n=14,648)	AD mouse model (genetic animal model) and human cohort	Faster disease progression after vitamin D supplementation were shown.	Lai RH, Hsu CC, Yu BH, Lo YR, Hsu YY, Chen MH, Juang JL. Vitamin D supplementation worsens Alzheimer's progression: Animal model and human cohort studies. Aging Cell. 2022 Aug;21(8):e13670. doi: 10.1111/acel.13670. Epub 2022 Jul 12. PMID: 35822270; PMCID: PMC9381901.	▼
Dimitrakis et al., 2022	Greece	90 patients with AD and 103 healthy controls	Southeastern European Caucasian population	Vitamin D receptor gene TaqI TT allele was found to increase risk of AD.	Dimitrakis E, Katsarou MS, Lagiou M, Papastefanopoulou V, Stanitsa E, Spandidos DA, Tsatsakis A, Papageorgiou S, Moutsatsou P, Antoniou K, Kroupis C, Drakoulis N. Association of vitamin D receptor gene TaqI polymorphism with Alzheimer's disease in a Southeastern European Caucasian population. Exp Ther Med. 2022 May;23(5):341. doi: 10.3892/etm.2022.11271. Epub 2022 Mar 22. PMID: 35401802; PMCID: PMC8988159.	▲
Broberg et al., 2022	Canada	56 APP/PS1 mice	AD mouse model (genetic animal model)	Vitamin D deficiency group showed impaired gait performance in AD mice.	Broberg DN, Wong D, Bellyou M, Montero-Odasso M, Beauchet O, Annweiler C, Bartha R. Effects of Memantine and High Dose Vitamin D on Gait in Male APP/PS1 Alzheimer's Disease Mice Following Vitamin D Deprivation. J Alzheimers Dis. 2022;85(4):1755-1766. doi: 10.3233/JAD-215188. PMID: 34958027.	▲
Bao et al., 2020	China	40 APP/PS1 rats	AD rat model (genetic animal model)	Vitamin D improved memory function and morphological defects in hippocampal neurons.	Bao Z, Wang X, Li Y, Feng F. Vitamin D Alleviates Cognitive Dysfunction by Activating the VDR/ERK1/2 Signaling Pathway in an Alzheimer's Disease Mouse Model. Neuroimmunomodulation. 2020;27(4):178-185. doi: 10.1159/000510400. Epub 2021 Feb 18. PMID: 33601398.	▲
Mehrabadi et al., 2020	Iran	60 rats with control group, sham group, AD group with intra-hippocampal $A\beta_{1-40}$ injection (Total, n=60)	AD rat model (nongenetic animal model)	Vitamin D and E and their combination improved memory and learning impairment and decreased neuronal loss and oxidative stress.	Mehrabadi S, Sadr SS. Administration of Vitamin D3 and E supplements reduces neuronal loss and oxidative stress in a model of rats with Alzheimer's disease. Neurol Res. 2020 Oct;42(10):862-868. doi: 10.1080/01616412.2020.1787624. Epub 2020 Jul 4. PMID: 32627720.	▲

Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid-β protein precursor; CSF, cerebrospinal fluid; WT, wild-type
▲ The positive effects of vitamin D on AD were shown. (The negative effects of vitamin D deficiency on AD were shown.)
▼ The negative effects of vitamin D on AD were shown. (The positive effects of vitamin D deficiency on AD were shown.)

REFERENCES

[1]   Kang J, Park M, Lee E, Jung J, Kim T (2022) The role of vitamin D in Alzheimer's disease: a transcriptional regulator of amyloidopathy and gliopathy. *Biomedicines* **10**, 1824.