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PHGDH expression increases with progression of Alzheimer's disease pathology and symptoms

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Phosphoglycerate dehydrogenase (PHGDH) is required for the synthesis of serine, a modulator of synaptic plasticity (Guercio and Panizzutti, 2018). Human mutations in PHGDH can cause abnormal brain development due to serine deficiency while conditional knockout of PHGDH in the mouse hippocampus impairs synaptic plasticity and spatial memory (Le Douce et al., 2020; Neame et al., 2019; Yang et al., 2010). Given the importance of synaptic pathophysiology to Alzheimer's disease (AD), these results raise the possibility that abnormalities in PHGDH expression could contribute to AD pathogenesis. A missing key to this question is the direction of change of either serine level or PHGDH expression levels in AD. Despite extensive efforts, changes in L- or D-serine level have not been unequivocally correlated with AD, making it particularly important to assess changes in PHGDH expression that may occur in AD.

We read with great interest the recent publication in *Cell Metabolism* reporting that "expression of PHGDH is reduced in the AD brain" (Le Douce et al., 2020). Leveraging this result to bridge the gap between serine/PHGDH deficiency and AD pathogenesis, the authors suggested oral L-serine as "a ready-to-use therapy for AD" (Le Douce et al., 2020). In contrast to this report, our previous meta-analysis of the National Institute on Aging's

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SUPPLEMENTAL INFORMATION

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DECLARATION OF INTERESTS

S.Z. is a founder and board member of Genemo, Inc.

Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) consortium's RNA sequencing (RNA-seq) datasets revealed a consistent increase of PHGDH mRNA expression in AD in five brain regions from Mayo, ROSMAP, and Mount Sinai cohorts (Yan et al., 2020). Consistent with our previous findings, and in contrast to the work of Le Douce et al., here we report that PHGDH mRNA and protein levels are increased in the brains of two mouse models of AD and/or tauopathy, and are also progressively increased in human brains with no, early, and late AD pathology, as well as in people with no, asymptomatic, and symptomatic AD.

We report an increase in PHGDH expression in 3xTg-AD mice, an AD model that develops both amyloid-β and tau pathology. First, we reanalyzed an RNA expression dataset (GEO: GSE1 44459) of 3xTg-AD mice. Hippocampal PHGDH mRNA level in 3xTg-AD mice is not different (Bonferroni-corrected $p = 0.682$, t test) from non-transgenic (non-Tg) controls at age 3 months, when AD pathology has not developed, but is higher (Bonferroni-corrected $p = 0.015$, t test) than non-Tg controls at 12 months.

Next, we repeated Le Douce et al.'s immunofluorescent confocal analysis. To ensure reproducibility, we obtained hippocampal sections from five pairs of 6-month-old female 3xTg-AD and non-Tg controls. Hippocampal PHGDH immunostaining was increased in 3xTg-AD compared to non-Tg ($p = 0.0039$, n = 5/group, permutation test) (Figures S1A and S1B). Our five pairs of mice were raised by four different labs, strongly arguing against lab-to-lab variation. Additionally, we observed an increase in GFAP expression in 3xTg-AD compared to non-Tg (Figure S1A). Thus, hippocampal PHGDH expression is increased in 3xTg-AD mice compared to non-Tg controls.

We also detected an increase in PHGDH expression in human P301S tau transgenic mice (PS19). Recognizing that no single mouse model can fully recapitulate human AD, we asked if our observation in 3xTg-AD mice could be reproduced in PS19 mice, a model for tauopathy that is independent of amyloid-β. We assessed PHGDH protein level changes via immunohistochemistry and western blots. In PS19 mice, hippocampal PHGDH exhibited significant colocalization with GFAP, confirming astrocyte expression of PHGDH (Figure S1C) (Le Douce et al., 2020; Yang et al., 2010). Compared to non-Tg littermate controls, PHGDH protein levels were increased in the hippocampus of 10-month-old PS19 mice (p value for western blots < 0.0001, unpaired t test, n = 8 for PS19 and n = 7 for non-Tg) (Figures S1C–S1E), suggesting that expression of a human mutant tau transgene is sufficient to induce hippocampal PHGDH expression.

We report sequential increase of PHGDH mRNA expression with concomitant AD pathology in humans. We reanalyzed 80,660 single-nucleus transcriptomes from 24, 15, and 9 individuals with no, early, and late AD pathology (Mathys et al., 2019). Consistent with reported astrocyte expression of PHGDH (Yang et al., 2010), PHGDH was detected in approximately 10%–25% of astrocytes (Ast), oligodendrocytes (Oli), and oligodendrocyte precursor cells (OPCs), and less than 4% of excitatory (Ex) and inhibitory (In) neurons, endothelial cells (ECs), and microglia (Mic) (Figure S1F). PHGDH exhibits a sequential increase of expression from no to early and to late pathology in astrocytes ($p = 0.0016$, ANOVA controlling for sex; no multiple hypothesis testing is involved) (Figure S1F). The

fraction of PHGDH-expressing astrocytes does not increase from no to early pathology (green versus blue Ast columns, Figure S1G), suggesting that the early pathology-associated PHGDH expression increase (green versus blue Ast columns, Figure S1F) is due to an increase in the expression level per cell rather than an increase in the proportion of PHGDH-expressing cells.

We identified sequential increases in PHGDH protein expression with AD pathology and symptoms. We reanalyzed two human mass spectrometry datasets (Hondius et al., 2016; Seyfried et al., 2017). PHGDH protein levels increase with increasing Braak stage in an Amsterdam cohort of 40 individuals ($p = 0.013$, ANOVA controlling for sex, age, and postmortem delay [PMD]) (Figures S1H and S1I). PHGDH expression also increases from controls to asymptomatic AD (ASYMAD; these people exhibited no clinical symptom but have autopsy-identified pathology) and to AD (people with symptoms and autopsy-confirmed pathology) in dorsolateral prefrontal cortex from a Baltimore cohort of 42 individuals ($p = 0.040$, ANOVA controlling for sex, age, and PMD) (Figures S1J and S1K). The longest PMD in our reanalyzed mass spectrometry datasets is 30 h (Hondius et al., 2016; Seyfried et al., 2017), and the PMD distributions in AD and control are not statistically different (Figures S1I and S1K). These data suggest an increase in PHGDH protein expression during both pathological and symptomatic progression of AD.

To validate the mass spectrometry results, we carried out PHGDH immunostaining on 21 hippocampal samples from age-matched cases and controls. We compared 10 samples at Braak stages 0–3 (negative-early group [NE]) with 11 samples at Braak stages 5–6 (advanced AD group [AD]). PHGDH immunostaining is higher in AD hippocampi than in NE hippocampi ($p < 0.02$, ANOVA controlling for sex) (Figures S1L and S1M), confirming the mass spectrometry result from the Amsterdam cohort. Next, we separately analyzed CA1, CA3, and dentate gyrus (DG). PHGDH immunostaining is increased in all three regions in AD compared to NE. This AD-associated increase is pronounced in CA1 and CA3 ($p < 0.006$, ANOVA controlling for sex) (Figure S1N), but not significant in DG ($p = 0.72$, ANOVA controlling for sex). The longest PMD of the human hippocampal samples used in our immunostaining analysis is 18 h. These data corroborate the AD-pathology-associated increase of PHGDH expression.

To validate the correlation of PHGDH protein level with AD's symptomatic development, we utilized the Dementia Rating Scale-2 (DRS), a metric of a patient's overall level of cognitive functioning (larger DRS indicates better overall cognitive ability). Ten of our analyzed hippocampal samples have corresponding DRS. In these samples, PHGDH immunostaining decreases as the donor's DRS increases ($p < 0.0001$, ANOVA controlling for sex). Next, we separately analyzed CA1, CA3, and DG. PHGDH immunostaining decreases in each region as the DRS increases ($p < 0.0015$ in CA1 and CA3; Figure S1O; $p < 0.02$ in DG). These data suggest an increase of hippocampal PHGDH expression as a patient's overall cognitive function declines, corroborating the AD-symptom-associated increase of PHGDH in the Baltimore cohort.

The reproducible increase of PHGDH expression during both pathological and symptomatic developments of AD suggests different underlying mechanisms between PHGDH deficiency

and AD. Le Douce et al.'s human AD samples had longer PMD than their control samples because five of their six controls (83%) as compared to only five out of their 15 AD samples (33%) had less than 30 h of PMD (Le Douce et al.'s Table 1). Considering that human PHGDH protein is sensitive to protease cleavage at room temperature, PMD-related protein degradation may explain the lower PHGDH levels in Le Douce's AD samples. Thus, we feel "oral L-serine as a ready-to-use therapy to AD" (Le Douce et al., 2020) warrants precaution. Despite being a cognitive enhancer, some evidence suggests that long-term use of "D-serine contributes to neuronal death in AD through excitotoxicity" (Guercio and Panizzutti, 2018). Furthermore, D-serine, as a coagonist of NMDAR, would be expected to oppose NMDAR antagonists, which have proven clinical benefits in treating AD.

Our results lead to a hypothetic model of positive feedback between increased astrocyte PHGDH expression and excitotoxicity. In this model, an increase in PHGDH expression in brain astrocytes leads to an increase in the basal level of NMDA receptor-dependent synaptic activities (Neame et al., 2019), with an increase in the probability of initiating an amyloid-β-dependent "vicious cycle of neuronal hyperactivation," eventually leading to excitotoxicity (Zott et al., 2019). Excitotoxicity and pathological protein deposition induce astrogliosis, which further increases astrocyte PHGDH level and thus creates an ongoing vicious cycle of increased astrocyte PHGDH, excitotoxicity, and astrogliosis. Consistent with this idea, reduction of D-serine by knocking out serine racemase, the L-serine to D-serine conversion enzyme, has a protective effect on amyloid-β toxicity in a mouse model (Inoue et al., 2008).

Our new results also support the prior observation of an AD-associated increase in PHGDH extracellular RNA (exRNA) in human plasma (Yan et al., 2020). Further, they help explain why the longitudinal increase of PHGDH exRNA can predict AD's clinical diagnosis (Yan et al., 2020). Together, these data nominate circulating PHGDH exRNA as a possible diagnostic biomarker of late-onset AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Guercio GD, and Panizzutti R (2018). Potential and challenges for the clinical use of d-Serine as a cognitive enhancer. Front. Psychiatry 9, 14. [PubMed: 29459833]

Hondius DC, van Nierop P, Li KW, Hoozemans JJ, van der Schors RC, van Haastert ES, van der Vies SM, Rozemuller AJ, and Smit AB (2016). Profiling the human hippocampal proteome at all pathologic stages of Alzheimer's disease. Alzheimers Dement. 12, 654–668. [PubMed: 26772638]

Inoue R, Hashimoto K, Harai T, and Mori H (2008). NMDA- and beta-amyloid1-42-induced neurotoxicity is attenuated in serine racemase knock-out mice. J. Neurosci 28, 14486–14491. [PubMed: 19118183]

Le Douce J, Maugard M, Veran J, Matos M, Jégo P, Vigneron PA, Faivre E, Toussay X, Vandenberghe M, Balbastre Y, et al. (2020). Impairment of glycolysis-derived l-serine production in astrocytes contributes to cognitive deficits in Alzheimer's disease. Cell Metab. 31, 503–517.e8. [PubMed: 32130882]

Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Menon M, He L, Abdurrob F, Jiang X, et al. (2019). Single-cell transcriptomic analysis of Alzheimer's disease. Nature 570, 332–337. [PubMed: 31042697]

Neame S, Safory H, Radzishevsky I, Touitou A, Marchesani F, Marchetti M, Kellner S, Berlin S, Foltyn VN, Engelender S, et al. (2019). The NMDA receptor activation by d-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle. Proc. Natl. Acad. Sci. U S A 116, 20736–20742. [PubMed: 31548413]

Seyfried NT, Dammer EB, Swarup V, Nandakumar D, Duong DM, Yin L, Deng Q, Nguyen T, Hales CM, Wingo T, et al. (2017). A multi-network approach identifies protein-specific co-expression in asymptomatic and symptomatic Alzheimer's disease. Cell Syst. 4, 60–72.e4. [PubMed: 27989508]

Yan Z, Zhou Z, Wu Q, Chen ZB, Koo EH, and Zhong S (2020). Presymptomatic increase of an extracellular RNA in blood plasma associates with the development of Alzheimer's disease. Curr. Biol 30, 1771–1782.e3. [PubMed: 32220323]

Yang JH, Wada A, Yoshida K, Miyoshi Y, Sayano T, Esaki K, Kinoshita MO, Tomonaga S, Azuma N,Watanabe M, et al. (2010). Brain-specific Phgdh deletion reveals a pivotal role for L-serine biosynthesis in controlling the level of D-serine, an N-methyl-D-aspartate receptor co-agonist, in adult brain. J. Biol. Chem 285, 41380–41390. [PubMed: 20966073]

Zott B, Simon MM, Hong W, Unger F, Chen-Engerer HJ, Frosch MP, Sakmann B, Walsh DM, and Konnerth A (2019). A vicious cycle of β amyloid-dependent neuronal hyperactivation. Science 365, 559–565. [PubMed: 31395777]