Presymptomatic Increase of an Extracellular RNA in Blood Plasma Associates with the Development of Alzheimer’s Disease

Highlights

- Blood plasma exRNAs from a 15-year clinical follow-up are sequenced by SILVER-seq
- Brain expression levels of brain-specific genes correlate with presence of exRNA
- PHGDH exhibits AD-associated mRNA and protein increase in brain and exRNA increase
- Presymptomatic increase of plasma PHGDH exRNA predicts the clinical diagnosis of AD

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In Brief
Yan et al. report a concordant increase of plasma extracellular RNA with brain mRNA and protein levels of the PHGDH gene in Alzheimer’s subjects. A presymptomatic increase of PHGDH extracellular RNA in human blood plasma is predictive of the development of Alzheimer’s disease.

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Presymptomatic Increase of an Extracellular RNA in Blood Plasma Associates with the Development of Alzheimer’s Disease

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SUMMARY

The extracellular RNAs (exRNAs) from human biofluid have recently been systematically characterized. However, the correlations of biofluid exRNA levels and human diseases remain largely untested. Here, considering the unmet need for presymptomatic biomarkers of sporadic Alzheimer’s disease (AD), we leveraged the recently developed SILVER-seq (small-input liquid volume extracellular RNA sequencing) technology to generate exRNA profiles from a longitudinal collection of human plasma samples. These 164 plasma samples were collected from research subjects 70 years or older with up to 15 years of clinical follow-up prior to death and whose clinical diagnoses were confirmed by pathological analysis of their post mortem brains. The exRNAs of AD-activated genes and transposons in the brain exhibited a concordant trend of increase in AD plasma in comparison with age-matched control plasma. However, when we required statistical significance with multiple testing adjustments, phosphoglycerate dehydrogenase (PHGDH) was the only gene that exhibited consistent upregulation in AD brain transcriptomes from 3 independent cohorts and an increase in AD plasma as compared to controls. We validated PHGDH’s serum exRNA and brain protein expression increases in AD by using 5 additional published cohorts. Finally, we compared the time-course exRNA trajectories between “converters” and controls. Plasma PHGDH exRNA exhibited presymptomatic increases in each of the 11 converters during their transitions from normal to cognitive impairment but remained stable over the entire follow-up period in 8 out of the 9 control elderly subjects. These data suggest the potential utilities of plasma exRNA levels for screening and longitudinal exRNA changes as a presymptomatic indication of sporadic AD.

INTRODUCTION

Systematic Characterization of Human Blood exRNAs

Although extracellular RNAs (exRNAs) in human blood were discovered in early 2000 [1–3], the diversity of blood exRNAs has only recently been revealed [4–7]. The Extracellular RNA Communication Consortium and the greater research community have embarked on systematic assessments of experimental methods and characterization of human biofluid exRNAs [4, 7–11]. Despite these expositive progresses, it remains largely unknown to what extent blood exRNAs reflect human physiologic and disease statuses [7, 12–15].

Longitudinal Data: The Missing Link

Most published human exRNA sequencing data are based on cross-sectional studies [8]. The large inter-individual variation of exRNA levels reported from these studies make it challenging to identify biomarkers, based on power calculation and the currently feasible sample sizes [7, 10]. In theory, longitudinal studies that acquire time-course data from the same person can account for baseline inter-individual variations [16–19] and therefore increase statistical power and help to delineate disease-associated exRNAs. Unfortunately, the available longitudinal exRNA sequencing datasets to date were based on healthy individuals [14, 18]. Thus, longitudinal exRNA sequencing data from both healthy and disease donors were a missing link in evaluating whether exRNAs in human blood have the potential to be developed into disease biomarkers.

The Challenge of Developing a Presymptomatic Test of Sporadic AD

Blood tests represent an attractive means of noninvasively monitoring neurological changes [20], including the development of Alzheimer’s disease (AD) [21, 22]. To this end, measurements of protein/peptide concentrations in circulation that originated from brain tissue have exhibited the greatest promise. Recent breakthroughs revealed that serum neurofilament protein levels correlated with neurodegenerative disorders, including, but not specific to, AD [21, 23–27]. Plasma amyloid-β (Aβ) and tau correlated with Aβ and tau in cerebrospinal fluid (CSF) [28] and brain in sporadic AD [22, 29, 30]. Despite these exciting advances, Aβ,
tau, and neurofilament-based biomarkers have not exhibited a strong power for presymptomatic predication to sporadic AD, which accounts for more than 95% of AD patients [31–33]. Given the prodromal phase of approximately 15 years before onset of clinical symptoms, novel biomarkers of early AD development will greatly assist in providing diagnostic information in presymptomatic individuals [34, 35]. The low penetrance of AD-associated genetic variants in sporadic AD patients makes it challenging to identify appropriate research subjects before disease diagnosis [36, 37]. Thus, it remains challenging to obtain the necessary longitudinal data for developing presymptomatic biomarkers.

**Study Hypothesis and Design**

It remains unclear to what extent the blood exRNA profiles reflect a person’s physiological and pathological states. The premise of our study is that certain RNAs are consistently reported to be up-regulated in brain regions coinciding with neuropathologic changes in AD patients versus controls [38–42]. Our question is whether the plasma exRNAs of these established AD-associated genes are likewise more abundant in AD patients compared to controls.

To test this hypothesis, we have utilized four sets of genes. The first set is the ERV1 clade of transposons that, as a group, have been reported to exhibit higher expression in AD brains compared to controls [43]. Generally, it has been shown that transposon activity is elevated during aging and neuronal decline [44, 45] and, in AD, due to activation by pathogenic tau proteins [43, 46]. The second set, as a control, is the SINE clade of transposons that, as a group, did not exhibit an AD-versus-control difference in brain [43]. The third set is the AD-associated lipid metabolic processing genes [47–49]. The fourth set is derived from our meta-analysis of 6 RNA sequencing (RNA-seq) data-sets generated by the National Institute on Aging’s Accelerating Medicines Partnership-Alzheimer’s Disease (AMP-AD) consortium, covering 6 AD pathology-related (AD-related) brain regions from 3 cohorts (Table S1) [50, 51]. Our analyses of these gene sets revealed that AD-associated exRNA profiles are not completely independent of AD-associated brain expression profiles and thus opened the possibility of using blood exRNA profiles to monitor AD progression.

**Sequencing exRNA from a Droplet of Human Plasma**

The recently developed SILVER-seq (small-input liquid volume extracellular RNA sequencing) technology is able to efficiently sequence exRNAs from a small droplet (5 μL) of serum or plasma [11]. In contrast to other exRNA sequencing methods, which require several milliliters of input liquid biopsy (“alternative exRNA-seq”), SILVER-seq’s input volume is smaller than a typical drop of blood (30 μL). SILVER-seq has been validated to perform comparably in terms of accuracy and reproducibility to alternative exRNA-seq methods [11]. Tested on aliquots of the same liquid sample, the correlation of the measured exRNA levels between a SILVER-seq assay and an alternative exRNA-seq assay is comparable to that of two alternative exRNA-seq assays, and the correlation between two SILVER-seq assays was comparable to the correlation between a SILVER-seq assay and an alternative exRNA-seq assay [11]. SILVER-seq’s small input volume and validated accuracy and reproducibility make it possible to explore the potential of exRNAs from blood to be developed into disease biomarkers and eventually be measured by a micro-sampling assay, such as a convenient finger prick test that can be used for population screening [16].

**RESULTS**

**A 15-Year Follow-Up Study of Sporadic AD**

In order to generate longitudinal data on sporadic AD, we selected archived plasma samples from research subjects being followed at the University of California, San Diego (UCSD) Shiley-Marcos Alzheimer’s Disease Research Center during a 15-year period from 2000 to 2014. The criteria were subjects older than 70 years of age who were examined post mortem to confirm the clinical diagnosis of AD (pathology-confirmed participants); had multiple longitudinal blood samples spanning at least 5 years; and, in cases who transitioned from normal cognitive status to mild cognitive impairment (MCI) or dementia during the course of the study, provided samples prior to the change in cognitive status. A total of 35 pathology-confirmed participants from this 15-year follow-up satisfied our criteria (Table S2). These included 9 cognitively normal subjects (controls) who were not cognitively impaired during the entire follow-up period and whose post mortem neuropathological analyses confirmed that they lacked AD-associated changes (blue lines, Figure 1). Consistent with their advanced ages, post mortem examinations demonstrated Braak stage 1 or 2 for these individuals (Table S2). Two out the 9 controls carried one ε4 allele of the APOE gene, and the other 7 controls did not carry the ε4 allele. There were 15 subjects who were clinically diagnosed as probable AD when they first enrolled, and their post mortem examinations were consistent with a pathological diagnosis of AD with Braak stages 4–6 (red lines, Figure 1; Table S2). Five of the 15 AD subjects were homozygous for ε4, 2 AD subjects each carried a single ε4 allele, and the other 8 AD subjects did not carry the ε4 allele. Finally, there was a third group of 11 converters who were cognitively normal at enrollment, but, during their longitudinal follow-up period, their clinical diagnoses were changed to MCI. Post mortem examination of these individuals showed AD changes with Braak stages between 3 and 6 (green lines, Figure 1; Table S2). Five of 11 converters carried a single ε4 allele, and the other 6 converters did not carry the ε4 allele. A total of 164 plasma samples were collected from these 35 participants (dots, Figure 1).

**Correlation of the Expression of Brain-Specific Genes and the Chances of Detecting These Genes in Plasma**

We sequenced these 164 plasma samples using the SILVER-seq technique (Table S3). Genome-wide distributions of the transcripts per million (TPM) of known genes exhibited little difference between the earlier and later years of sample collection (Figure S1). We asked whether the expression level of a brain-specific gene in the brain correlates with the chance of this gene being detected in plasma by SILVER-seq. To this end, we retrieved 1,514 brain-specific genes from GTEx consortium’s summary of tissue-specific genes, which were based on GTEx consortium’s definition of tissue-specific score (TS_Score) and recommended threshold (TS_Score > 3) [52]. We categorized these brain-specific genes by their average TPM in...
GTEx-analyzed brain regions from low to high into four groups, which are TPM = (0, 1], (1, 10], (10, 100], and (100, infinity) (Figure 2A). The odds ratio of the brain-specific genes in each group and those genes detected in plasma increased as the average brain expression levels increased from group 1 to group 4 (Figure 2B; Table S4). This positive correlation was not abolished by changing the threshold for determining what genes are detected in plasma (SILVER-seq’s TPM > 3; Figure S2A).

To test whether sex and age affect the aforementioned correlation, we identified brain-specific genes in male, female, young, and old subjects based on the latest GTEx data (GTEx V8) and GTEx consortium’s recommended threshold for defining tissue specificity [TS_Score > 3; Figure S2B] [52]. The odds ratio of the male-brain-specific genes in each expression group and those genes detected in male plasma samples increased as the average male brain expression levels increased from group 1 to group 4 (Figure S2C). This positive correlation was repeated in the brain-specific genes identified from female subjects (Figure S2D) and those shared by male and female, young and old GTEx research subjects (Figure S2E).

Furthermore, we used stronger criteria for calling brain-specific expression from GTEx V8 data, which led to 106 genes. We termed these 106 genes “brain-exclusive genes” and divided them into four expression quartiles based on each gene’s average TPM in the GTEx-assayed brain regions. The odds ratio of the brain-exclusive genes in each expression quartile and those genes detected in plasma increased from the lowest to the highest quartile (Figure S2F). In comparison, when we re-ordered the brain-exclusive genes by each gene’s average TPM of the 41 peripheral tissues, the odds ratio became invariant from quartile to quartile, suggesting the chances of detecting these genes in plasma were not driven by their expression levels in peripheral tissues (Figure S2G). Taken together, the higher the level of brain expression of a brain-specific gene, the greater the chance of this gene being detected by SILVER-seq in plasma.

Lack of Genome-wide Correlations of AD-Associated Changes between Brain Gene Expression and Plasma exRNA Levels

We asked whether there are genome-wide correlations of AD-associated gene expression changes in brain and exRNA changes in plasma. To this end, we re-analyzed 6 RNA-seq datasets from 6 AD-related brain regions. These datasets were generated from 3 donor cohorts by the AMP-AD consortium [Table S1] [50, 51]. We used the t-statistic to represent the difference between AD and normal samples for each (ex)RNA and compared the t-statistics between plasma and each brain region (Figure S3A). The AD-associated plasma exRNA changes did not exhibit a genome-wide correlation to AD-associated changes in any analyzed brain region (all Pearson correlations < 0.04). The lack of genome-wide correlations was expected because plasma exRNAs come from many tissues other than the brain. Even if AD can influence plasma exRNA levels, AD is only one of many physiological and pathological conditions that may have such influences. We cannot control for all these physiological and pathological conditions in the research subjects in AMP-AD and our studies. In addition, there are significant technical differences in the experimental procedures for sequencing intracellular and extracellular RNAs. The lack of genome-wide correlations serves as an important baseline to the rest of our analyses.

Brain Transposon Activation in AD Is Detectable in Plasma exRNA

Considering the lack of global correlations, we focused on the RNA transcripts that are considered reliably upregulated in AD. We asked whether the genes reported to be reliably overexpressed in AD brains as compared to control brains also exhibit higher exRNA levels in AD plasma as compared to control plasma. Among all transposon clades and families, the ERV1 clade of transposons exhibited the largest AD-versus-control expression difference in dorsolateral prefrontal cortex (DPC), an AD-affected brain region [43]. We replotted the t-statistics from that study [43], comparing AD and control DPCs for every ERV1 transposon (dots, Figure 3A). The distribution of these t-statistics shifted to above 0 (boxplot, Figure 3A; p < 2.2 × 10^{-16}; t test), consistent with the previous report of higher expression of ERV1 transposons overall in AD brains [43]. Next, we calculated the t-statistic for every ERV1 transposon to compare AD and control plasma in our data. The distribution of these plasma-derived t-statistics shifted to above 0 (Figure 3B), suggesting that ERV1-derived exRNAs were more abundant in AD plasma than control plasma (p = 1.02 × 10^{-5};
AMP-AD genes. The t-statistic was used to represent the exRNA difference between AD plasma and control plasma for every gene. The average t-statistic of the AMP-AD genes was greater than that of all the genes (AMP-AD and all lanes, Figure 4A). However, this difference is not statistically significant (p = 0.141; permutation test), presumably due to the small number (28) of AMP-AD genes.

Next, we retrieved a total of 1,375 genes associated with “lipid metabolic process” (GO:0006629) [48, 49]. The average t-statistic of the lipid metabolic process genes was greater than that of all the genes (p < 0.0001; permutation test), suggesting an overall exRNA increase of lipid metabolic process genes in AD plasma. Twenty of these lipid metabolic process genes are genetically associated with AD (lipid-AD lane, Figure 4A), in which ACHE, APOE, ESR1, and APP ranked as the 4 exRNAs with the largest increase in AD plasma compared to control plasma (Table S5). However, none of them exhibited a statistically significant difference (smallest FDR = 0.28; ANOVA controlling for sex and APOE genetic status; Table S5). Taken together, the analyses of 4 gene groups, including ERV1 and SINE transposons and lipid metabolic process and AMP-AD genes, suggest that the brain-upregulated, AD-related transcripts exhibited weak but consistent trends of exRNA increases in AD plasma, indicating both promises and challenges of finding a single-gene exRNA biomarker of AD in plasma.

Phosphoglycerate Dehydrogenase (PHGDH) Exhibited the Largest AD-Associated Increase in Plasma and Consistent Upregulation in Brain
The AMP-AD gene with the largest AD-associated exRNA increase in plasma was PHGDH (last bar on the right, Figure 4B). PHGDH exhibited higher expression in AD patients (red bars) than control donors (blue bars) in 5 brain regions, including temporal cortex, dorsolateral prefrontal cortex, superior temporal gyrus, parahippocampal gyrus, and inferior frontal gyrus (* marked columns, Figure 4C), based on the AMP

Modest Consistency of AD-Associated Increases of mRNAs in the Brain and Plasma
We tested whether the coding genes with reported AD-associated expression changes in the brain exhibited corresponding changes in plasma. To identify a reliable set of AD upregulated mRNAs in brain, we re-analyzed 6 RNA-seq datasets from 6 AD-related brain regions. These datasets were generated from 3 donor cohorts by the AMP-AD consortium (Table S1) [50, 51]. A total of 28 coding genes were upregulated in at least 5 of these 6 brain regions in AD (false-discovery rate [FDR] < 0.05 in each brain region), which hereafter will be referred to as the
The lack of a global correlation does not rule out the possibility that AD affects the exRNA profiles of a subset of genes. We tested whether the genes that have been associated with AD by genetic association studies exhibited any correlated AD-versus-control changes between the two cohorts. To this end, we leveraged the DisGeNET database [47] that integrated geno-type-phenotype relationship datasets, including genome-wide association study (GWAS) data from multiple databases. DisGeNET documented a total of 1,926 AD-associated genes, among which 83 genes have been reviewed by experts and are termed “expert-curated” AD-associated genes. The 1,926 genes did not exhibit correlated changes between the two cohorts (Pearson correlation = 0.016; \(p = 0.43\); Figure 4F), although the Pearson correlation is approximately 2-fold larger than that of the all genes (Pearson correlation = 0.009). Moreover, the 83 expert-curated genes were not completely uncorrelated between the two cohorts (Pearson correlation = 0.287; \(p = 0.009\); Figure 4G). These increasing correlations from all genes to expert-curated genes suggest that, despite significant technical differences, AD-versus-control changes from two cohorts are not completely uncorrelated on the subset of genes that are relevant to AD.

For an external validation, we checked the PHGDH exRNA levels in AD and control samples from the Burgos dataset. PHGDH exRNA levels were higher in AD sera than in control sera in the Burgos cohort (fold change = 2.4; \(t\)-test; \(p = 0.095\)). There is no multiple hypotheses testing in this case. Compared to the 1,926 DisGeNET-documented AD-associated genes, PHGDH was among the most reproducibly upregulated exRNAs in both cohorts (\(p = 1.8 \times 10^{-5}\); permutation test; Figures 4F, S4A, and S4B). Compared to the 83 expert-curated genes, PHGDH was also among the most reproducibly upregulated exRNAs in both cohorts (\(p = 0.0001\); permutation test; Figures 4G, S4C, and S4D). Taken together, PHGDH exRNA was increased in AD in both cohorts. No other AD-associated gene in the DisGeNET database exhibited a statistically significant and reproducible increase in these two cohorts.
Figure 4. Changes of Plasma exRNA Levels of the AMP-AD Genes

(A) Distributions of t-statistics (AD plasma versus control plasma) for all genes, lipid metabolic process genes, and AMP-AD genes (columns).
(B) The log fold change for each AMP-AD gene (column).
(C) PHGDH expression levels in each brain region (x axis) in control (blue) and AD (red). The cohort name of each study is given in brackets. FDR is based on t test. *FDR < 0.05.
PHGDH Protein Changes in 5 AD Brain Regions from 4 Cohorts

Taking the above analyses together, PHGDH is the only gene that exhibited consistent AD-versus-control increases from multiple brain regions and plasma/serum in the total of 5 independent cohorts (3 AMP-AD cohorts, our cohort, and Burgos cohort). We proceeded to test whether brain PHGDH protein levels are changed in AD. To this end, we re-analyzed 3 published proteomics studies. Each study examined 1 or 2 brain regions, which are hippocampus [53], dorsolateral prefrontal cortex and precuneus [54], and anterior cingulate gyrus and frontal cortex [55] (Table S6). Hippocampal PHGDH protein levels increased with Braak stages (ANOVA p = 7.5 \times 10^{-3}; Figure 4H). Both dorsolateral prefrontal cortex and precuneus PHGDH protein levels exhibited sequential increases from controls to asymptomatic AD (intact cognition subjects who exhibited AD lesions at autopsy) and to symptomatic AD (p = 0.045; two-way ANOVA; Figures 4I and 4J). In addition, both anterior cingulate gyrus and frontal cortex PHGDH protein levels were significantly increased in AD subjects (p < 8 \times 10^{-5}) and in AD and Parkinson’s disease (AD&PD) co-morbid subjects as compared to controls (p < 0.001; t test; Figures 4K and 4L). Taken together, PHGDH protein exhibited significant increases in all 5 analyzed brain regions from 4 independent cohorts.

Difference of PHGDH Protein Changes in AD and PD

We asked whether brain PHGDH exhibited similar protein expression changes in PD as in AD. PD-versus-control PHGDH protein differences were much smaller than AD-versus-control differences or AD&PD-versus-control differences in anterior cingulate gyrus (Figure 4K). Frontal cortex PHGDH protein levels did not exhibit a significant difference between PD and controls (p = 0.87; t test) but significant AD-versus-control and AD&PD-versus-control differences (Figure 4L). These data suggest a disease-type specificity of brain PHGDH levels.

Longitudinal Changes of Plasma PHGDH exRNA

Differential Converters from Controls

We proceeded to evaluate plasma PHGDH as a presymptomatic biomarker for AD-related cognition impairment. To this end, we utilized the longitudinal data from the converter group of 11 subjects, from which the majority of the plasma samples were collected prior to each subject’s diagnosis of MCI (vertical dash lines, Figure 5A). Importantly, the converter group has not been used in any of the analyses presented above and thus presents a different (un-analyzed) set of research subjects. We quantified the longitudinal changes in plasma PHGDH levels in everyone using a simple linear regression of all the measured PHGDH levels of this individual (Figure 5A). Considering most samples (38 samples) from the converter group were collected on or before the clinical diagnosis of cognitive impairment (78% of the total 49 samples), we did not leave out any data points from the regression analysis. We chose a simple analysis method over sophisticated methods to minimize the chances of over-fitting the data. Remarkably, the estimated linear regression coefficient ($\beta$) was positive in each of these 11 subjects (Figure 5A), suggesting an increase of plasma PHGDH over time in every converter.

Next, we checked whether the longitudinal changes ($\beta$s) were different between the converter group and the control group. The $\beta$s of the converters were greater than those of the controls ($p < 0.0026$; t test; Figure 5B). The $\beta$s of the control group were not significantly different from 0 ($p = 0.38$; t test; blue boxplot, Figure 5B), suggesting that plasma PHGDH was relatively stable over time in cognitively normal control subjects. Consistent with these results, a mixed model that accounts for sex and age reported a significant interaction between time and group (converter or control; $p = 0.030$; ANOVA), whereas the time effect is insignificant in the presence of the interaction term ($p = 0.922$; ANOVA).

Finally, we defined a simple rule to call which research subject exhibited a longitudinal increase in PHGDH exRNA, which is “$\beta$ minus the standard deviation of $\beta$ is greater than 0” (error bars above 0, Figure 5C). Based on this rule, all 11 converters and 1 out of the 9 controls were called exhibiting longitudinal increases of PHGDH. Thus, the simple rule of ($\beta$ – standard deviation of $\beta$) > 0, which means having a clear upward change, classified converters from controls with 100% sensitivity and 89% specificity in this cohort of elderly people. These data suggest that the longitudinal increase in plasma PHGDH is predictive of the clinical diagnosis of cognition impairment.

Large Individual Variations in Post-diagnosis Longitudinal Changes

Although post-symptomatic changes are not directly relevant to our primary interest of identifying a presymptomatic biomarker, we examined the longitudinal changes in the AD group to further explore whether PHGDH levels continue to rise following AD diagnosis or whether they simply remain elevated. The average $\beta$ of the AD group was not significantly different from 0 ($p = 0.99$; t test; red boxplot, Figure 5B), suggesting the lack of a consistent direction of change. This was similar to the control group (blue boxplot, Figure 5B). However, the $\beta$s of the AD group were not clustered as tightly around 0 as those of the control group (red versus blue, Figure 5B), suggesting greater longitudinal variability of PHGDH exRNA after conversion of cognitive

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(D) Plasma PHGDH levels in control (blue) and AD (red). FDR is based on ANOVA test controlling for sex and APOE status. *FDR < 0.05.
(E–G) Comparison of exRNA changes between two cohorts. The AD-versus-control changes for each exRNA (dot) is represented by a t-statistic from our cohort (x axis) and from the Burgos cohort (y axis). The correlations increased from all genes (E), the 1,926 database recorded genes and PHGDH (red dot; F), and the 83 expert-curated genes and PHGDH (red dot; G).
(H–L) Changes of PHGDH protein levels in brain.
(I and J) Distributions dorsolateral prefrontal cortex (I) and precuneus (J) PHGDH protein levels (violin plots) in controls (blue), asymptomatic AD (orange), and AD (red).
(K and L) Distributions of anterior cingulate gyrus (K) and frontal cortex (L) PHGDH protein levels (violin plots) in controls (blue), Parkinson’s disease (PD) (light blue), AD and PD co-morbid patients (ADPD) (orange), and AD (red).
See also Figures S3 and S4 and Tables S1, S5, and S6.
status. The βs of the converter group were greater than those of the AD group (p = 0.022; t test; green versus red, Figure 5B), suggesting that a longitudinal increase in PHGDH exRNA was more consistently detected presymptomatically.

DISCUSSION

The Possibility of Assessing Brain Gene Expression by Blood exRNAs

Despite extracellular RNase activity, circulating exRNAs can be protected by exosomes and extracellular carriers, including lipoprotein complexes and ribonucleoproteins [6, 7]. These protections keep plasma exRNAs at an equilibrium concentration of approximately 10 pmol/L [14]. Clinical utility of plasma exRNA was demonstrated in a special case, in which placentally derived exRNAs were utilized to prenatally detect chromosomal aneuploidy [56]. It had remained an open question as to whether exRNAs may reflect the physiological status of the brain.

Conceptually, brain-derived exRNAs can enter the bloodstream by going through either the lymphatic system or the blood-brain barrier (BBB). The clearance of brain waste by the lymphatic system into the blood circulation has been proposed as a means of protection against neurological disorders [57, 58]. Exosomes with their RNA cargos can go across healthy BBB [59]. The BBB leakiness in early AD likely makes it more permissive to the passage of all types of extracellular molecules [60, 61]. Consistent with these concepts, the brain expression level of a brain-specific gene is positively correlated with the chances of detecting this gene in plasma (Figure 2), suggesting that circulating exRNAs may be a class of overlooked molecules for assessing the expression of genes within the central nervous system. In this study, we reported a tendency of concordant AD-associated expression changes in brain and plasma in elderlies, thus the possibility of expanding candidate blood biomarkers from Aβ and tau proteins and peptides to a wide range of genes that are activated during AD development. This expansion is much needed for research of early indications of AD because the initial molecular changes may well predate the accumulation of toxic Aβ and tau species [62].

Limitations of This Study

The APOE genetic statuses are not the same in AD, controls, and converters. Whereas 5 of 15 AD subjects were homozygous for ε4, none of the 9 controls and the 11 converters was homozygous for ε4. This difference is consistent with the increased AD risks of the ε4/ε4 genotype. We have controlled for APOE genetic status in all comparison among these research subjects.

This is not a double-blind randomized prospective study and thus cannot control for all possible confounders. The treatments received by the AD patients can be a confounder to the identified differences between AD and controls. However, this concern is ameliorated by the difference of the longitudinal changes in the prodromal phase of the converters and the longitudinal changes in controls, because the converters did not receive any treatments until the last year or two prior to their full-blown clinical conversion.

SILVER-seq has not been tested as a method for measuring the absolute concentrations of exRNAs. We have therefore devoted all data analyses to the relative changes of normalized SILVER-seq read counts between samples or sample groups. Furthermore, TPM may not be an ideal metric for quantifying exRNA levels of coding genes and long non-coding RNA genes, because the exRNAs derived from these genes are often short RNA fragments [11, 14]. Future developments of statistical methods are needed to account for the size distribution and the actual genomic origins of exRNAs as well as possible background noises in SILVER-seq data.
The number of converters whose diagnoses have changed from normal to cognitive impairment is limited. It will be useful for future work to follow additional research subjects over a long period (>10 years) of time and carry out randomized double-blinded analyses. We recognize that the challenges of such a future study include the difficulty to find and follow many converters before symptoms due to the lack of power in using genetic variants to predict sporadic AD. This unique collection of plasma samples from pathology-confirmed participants analyzed in our study is the result of a decades-long effort to longitudinally assess AD and control subjects through the generous support of the National Institute on Aging.

It is difficult to define what is “absolutely normal” in people of age 70 or older because nearly all of them experience some memory or cognitive decline. Indeed, all the control individuals in this study exhibited some pathological changes as expected (Braak stage 1 or 2), although at lower degrees than those in the AD and converter groups (Braak stages 3–6). Therefore, the “clean” data from people who had both clinical diagnosis and pathological confirmation generated in this study could be particularly useful for explorations of new biomarkers that may or may not be related to the accumulation of Aβ or toxic tau in brain [62]. Because the control group all show pathological signs of aging, especially those related to AD-associated changes, albeit to a much lower degree, they are not in this sense absolutely normal. Given this and heterogeneity between individuals, finding any specific molecular differences between the control and the disease groups may be an exceedingly difficult task. In this context, the observed differentiation between the converter groups by PHGDH dynamics as compared to control or AD group is even more noteworthy.

Considering these limitations, we compared our data with the data of other cross-sectional studies. These comparisons revealed a consistent trend of AD-associated upregulation of the ERV1 clade of transposons and AMP-AD genes in plasma and in brain. In particular, the AD-associated increase of PHGDH was consistent across 3 cohorts in which brain transcriptomes were analyzed (Mayo, ROSMAP, Mount Sinai) [50, 51], 2 cohorts that analyzed circulating exRNAs [8], and 4 additional cohorts that analyzed brain protein levels [53–55].

A Hypothetical Model of PHGDH’s Role in AD Etiology

Our hypothetical model for the relevance of PHGDH to AD is as follows. PHGDH catalyzes serine biosynthesis in the brain, which is required for glycine production, as shown in conditional knockout animals [63]. Glycine and serine are both agonists at the glycine binding site on NMDA receptors, which must be bound along with glutamate to induce calcium influx via this receptor [63–65]. Overactivity of NMDA receptors can lead to excitotoxicity [66]. PHGDH is almost exclusively expressed in astrocytes [67] where serine is initially synthesized and stored, whereas the D-serine isomer is found in neurons and microglia, predominantly in regions of the brain that coincide with NMDA receptor expression [68]. Both endogenous glycine and D-serine have been found to potentiate excitotoxicity [69, 70], and the increased release of these co-modulators have been linked to synaptic damage in a range of neuropathologies, including AD [71–74]. Consistent with this idea, increased D-serine in the CSF has shown potential to distinguish probable AD patients from controls [72].

Recently, the discovery of a self-amplifying feedforward loop between Aβ-independent excessive activities in a fraction of neurons at early stages and Aβ-mediated hyperactivation at late stages of AD adds to the complexity of AD-associated brain changes [75]. Because sustained baseline activation is required for initiating this vicious circle of excessive activation [75], increased PHGDH expression may theoretically represent a necessary step in the early presymptomatic disease stages, where both pre-existing baseline activation and subsequent excitotoxicity take place.

A Potential Companion Diagnosis for NMDA-Receptor Inhibitors

In order to prevent excitotoxicity in AD, NMDA receptor inhibitors have been widely utilized as AD medications. Among them, memantine is an US Food and Drug Administration (FDA)-approved drug for AD treatment [76]. Unfortunately, the drug has limited efficacy [77]. One explanation for the limited efficacy of memantine may be due to the inability to identify the subpopulation with NMDA receptor overactivity that may most benefit from this treatment approach. Currently, memantine is approved for moderate-to-severe AD individuals [77]. Our data suggest that PHGDH exRNA levels in peripheral blood potentially identify patients whose NMDA receptor activities in brain may be elevated, and if so, they may be more likely to respond to memantine and other NMDA-receptor inhibitors. Consequently, it may be very informative to test plasma PHGDH exRNA levels as a biomarker to predict treatment efficacy of memantine in AD individuals.

These data also point to the exploration of exRNA biomarkers for other neurological diseases. Activation of several other classes of transposable elements has been reported in other neurodegenerative diseases, including ALS [45, 78, 79]. It would be useful to examine whether those classes of transposons were also upregulated in plasma exRNA. Additionally, NMDA receptor overactivation is also a molecular feature of schizophrenia [80], ALS [81], epilepsy [82], and drug addiction [83, 84]. It would be useful to test whether temporal changes of plasma PHGDH are associated with these disorders.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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### REFERENCES

1. Ng, E.K., Tsui, N.B., Lam, N.Y., Chiu, R.W., Yu, S.C., Wong, S.C., Lo, E.S., Rainer, T.H., Johnson, P.J., and Lo, Y.M. (2002). Presence of filterable and nonfilterable mRNA in the plasma of cancer patients and healthy individuals. Clin. Chem. 48, 1212-1217.

2. Ng, E.K., Tsui, N.B., Lau, T.K., Leung, T.N., Chiu, R.W., Yu, S.C., Wong, S.C., Lo, E.S., Rainer, T.H., Johnson, P.J., and Lo, Y.M. (2003). mRNA of placental origin is readily detectable in maternal plasma. Proc. Natl. Acad. Sci. USA 100, 4748–4753.

3. García, J.M., García, V., Peña, C., Domínguez, G., Silva, J., Díaz, R., Espinosa, P., Citores, M.J., Collado, M., and Bonilla, F. (2008). Extracellular plasma RNA from colon cancer patients is confined in a vesicle-like structure and is mRNA-enriched. RNA 14, 1424–1432.

4. Freedman, J.E., Gerstein, M., Mick, E., Rozowsky, J., Levy, D., Kitchen, R., Kohli, M., Boardman, L., Patel, T., and Wang, L. (2016). Plasma extracellular RNAs are widely detected in human plasma. Nat. Commun. 7, 11106.

5. Yer, A., Courtright, A., Reiman, R., Carlson, E., Beecroft, T., Janss, A., Siniard, A., Richholt, R., Bslak, C., Rozowsky, J., et al. (2017). Total extracellular small RNA profiles from plasma, saliva, and urine of healthy subjects. Sci. Rep. 7, 44061.

6. Yuan, T., Huang, X., Woodcock, M., Du, M., Dittmar, R., Wang, Y., Tsai, S., Kohli, M., Boardman, L., Patel, T., and Wang, L. (2016). Plasma extracellular RNA profiles in healthy and cancer patients. Sci. Rep. 6, 19413.

7. Murillo, O.D., Thistlethwaite, W., Rozowsky, J., Subramanian, S.L., Lucero, R., Shah, N., Jackson, A.R., Srivinasa, S., Chung, A., Laurent, C.D., et al. (2019). exRNA atlas analysis reveals distinct extracellular RNA cargo types and their carriers present across human biofluids. Cell 177, 463–477.e15.

8. Burgos, K., Malenica, I., Metpally, R., Courtright, A., Rakela, B., Beach, T., Shill, H., Adler, C., Sabbagh, M., Villa, S., et al. (2014). Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer’s and Parkinson’s diseases correlate with disease status and features of pathology. PLoS ONE 9, e94839.

9. Lee, M.Y., Baxter, D., Scherer, K., Kim, T.K., Wu, X., Abu-Amaru, D., Florio, J., Yehuda, R., Marmar, C., Jett, M., et al. (2019). Distinct profiles of cell-free microRNAs in plasma of veterans with post-traumatic stress disorder. J. Clin. Med. 8, E963.

10. Srinivasan, S., Yeri, A., Cheah, P.S., Chung, A., Danielson, K., De Hoff, P., Filant, J., Laurent, C.D., Laurent, L.D., Magee, R., et al. (2019). Small RNA sequencing across diverse biofluids identifies optimal methods for exRNA isolation. Cell 177, 446–462.e16.

11. Zhou, Z., Wu, Q., Yan, Z., Zheng, H., Chen, C.J., Liu, Y., Qi, Z., Calandrèlli, R., Chen, Z., Chien, S., et al. (2019). Extracellular RNA in a single droplet of human serum reflects physiologic and disease states. Proc. Natl. Acad. Sci. USA 116, 19200–19208.

12. Yurkovich, J.T., and Hood, L. (2019). Blood is a window into health and disease. Clin. Chem. 65, 1204–1206.

13. Momen-Heravi, F., Saha, B., Kody, S., Catalano, D., Satishchandran, A., and Szabo, G. (2015). Increased number of circulating exosomes and their microRNA cargos are potential novel biomarkers in alcoholic hepatitis. J. Transl. Med. 13, 261.

14. Max, K.E.A., Bertram, K., Akat, K.M., Bogardus, K.A., Li, J., Morozov, P., Ben-Dov, I.Z., Li, X., Weiss, Z.R., Aiziazian, A., et al. (2018). Human plasma and serum extracellular small RNA reference profiles and their clinical utility. Proc. Natl. Acad. Sci. USA 115, E5334–E5343.

15. Kunz, F., Kontopoulou, E., Reinhardt, K., Soldier, M., Strachan, S., Reinhardt, D., and Thakur, B.K. (2019). Detection of AML-specific mutations in pediatric patient plasma using extracellular vesicle-derived RNA. Ann. Hematol. 98, 595–603.

16. Farrington, D.P. (1991). Longitudinal research strategies: advantages, problems, and prospects. J. Am. Acad. Child Adolesc. Psychiatry 30, 369–374.

17. Chen, R., Xia, L., Tu, K., Duan, M., Kukurba, K., Li-Pook-Than, J., Xie, D., and Snyder, M. (2018). Longitudinal personal DNA methylome dynamics in a human with a chronic condition. Nat. Med. 24, 1930–1939.

18. Jiang, C., Wang, X., Li, X., Iniora, J., Wang, T., Liu, Q., and Snyder, M. (2018). Dynamic human environmental exposome revealed by longitudinal personal monitoring. Cell 175, 277–291.e31.

19. Zhou, W., Sailani, M.R., Contrepeis, K., Zhou, Y., Ahadi, S., Leopold, S.R., Zhang, M.J., Rao, V., Avina, M., Mishra, T., et al. (2019). Longitudinal multiomics of host-microbe dynamics in prediabetes. Nature 569, 663–671.

20. Lopez, J.P., Fiori, L.M., Cruceanu, C., Lin, R., Labonte, B., Cates, H.M., Heller, E.A., Vialou, V., Ku, S.M., Gerald, C., et al. (2017). MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. Nat. Commun. 8, 15497.

21. Preische, O., Schultz, S.A., Apel, A., Kuhle, J., Kaeser, S.A., Barro, C., Gräber, S., Kuder-Buletta, E., LaFougere, C., Laske, C., et al.: Dominantly Inherited Alzheimer Network (2019). Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer’s disease. Nat. Med. 25, 277–283.

22. Nakamura, A., Kaneko, N., Villemagne, V.L., Kato, T., Doecke, J., Doré, V., Fowler, C., Li, Q.X., Martins, R., Rowe, C., et al. (2018). High performance plasma amyloid-β biomarkers for Alzheimer’s disease. Nature 554, 249–254.

23. Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., et al. (2016). Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 97, 494–496.

24. Weston, P.S.J., Poole, T., Ryan, N.S., Nair, A., Liang, Y., Macpherson, K., Druyeh, R., Malone, I.B., Ahsan, R.L., Pemberton, H., et al. (2017). Serum neurofilament light in familial Alzheimer disease: a marker of early neurodegeneration. Neurology 89, 2167–2175.

25. Lin, Y.S., Lee, W.J., Wang, S.J., and Fuh, J.L. (2018). Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci. Rep. 8, 17368.
26. Mattsson, N., Andreasson, U., Zetterberg, H., and Blennow, K. (2017). Alzheimer’s Disease Neuroimaging Initiative (2017). Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 74, 557–566.

27. Benedet, A.L., Ashton, N.J., Pascoal, T.A., Leuzy, A., Mathotaarachchi, S., Kang, M.S., Therriault, J., Savard, M., Chamoun, M., Schöll, M., et al. (2019). Plasma neurofilament light associates with Alzheimer’s disease metabolic decline in amyloid-positive individuals. Alzheimers Dement. (Amst.) 17, 679–689.

28. Jia, L., Qiu, Q., Zhang, H., Chu, L., Du, Y., Zhang, J., Zhou, C., Liang, F., Shi, S., Wang, S., et al. (2019). Concordance between the assessment of Aβ42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid. Alzheimers Dement. 15, 1071–1080.

29. Schindler, S.E., Bollinger, J.G., Ogud, V., Mauwenegga, K.G., Li, Y., Gordon, B.A., Holtzman, D.M., Morris, J.C., Benzinga, T.L.S., Xiong, C., et al. (2019). High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology 93, e1647–e1659.

30. Lim, C.Z., Zhang, Y., Chen, Y., Zhao, H., Stephenson, M.C., Ho, N.R.Y., Chen, Y., Chung, J., Reilhac, A., Loh, T.P., et al. (2019). Subtyping of circulating exosome-bound amyloid β reflects brain plaque deposition. Nat. Commun. 10, 1144.

31. Reiman, E.M., Langbaum, J.B., Fleisher, A.S., Caselli, R.J., Chen, K., Ayutyanont, N., Quiroz, Y.T., Kosik, K.S., Lopera, F., and Tariot, P.N. (2011). Alzheimer’s Prevention Initiative: a plan to accelerate the evaluation of presymptomatic treatments. J. Alzheimers Dis. 26 (Suppl 3), 321–329.

32. Frisoni, G.B., Boccardi, M., Barkhof, F., Blennow, K., Cappa, S., Chiotis, K., Démonet, J.F., Garibotto, V., Giannakopoulos, P., Gietl, A., et al. (2017). Strategic roadmap for an early diagnosis of Alzheimer’s disease based on biomarkers. Lancet Neurol. 16, 661–676.

33. Kapogiannis, D., Mustapic, M., Shardell, M.D., Berkowitz, S.T., Diehl, T.C., Spangler, R.D., Tran, J., Lazaropoulos, M.P., Chawla, S., Gulyani, S., et al. (2019). Association of extracellular vesicle biomarkers with Alzheimer disease in the Baltimore Longitudinal Study of Aging. JAMA Neurol. Published online July 15, 2019. https://doi.org/10.1001/jamaneurol.2019.2462.

34. Silverberg, N., Elliott, C., Ryan, L., Masliah, E., and Hodes, R. (2018). NIA commentary on the NIA-AA Research Framework: towards a biological definition of Alzheimer’s disease. Alzheimers Dement. 14, 576–578.

35. Allen, M., Wang, X., Burgess, J.D., Watzlawik, J., Serie, D.J., Younkin, S., and Younkin, G. (2016). Accelerating medicines partnership—Alzheimer’s disease (AMP-AD) knowledge portal aids Alzheimer’s drug discovery through open data sharing. Expert Opin. Ther. Targets 20, 389–391.

36. De Jager, C.L., Hu, Y., McCabe, C., Xu, J., Vardarajan, B.N., Folsky, D., Klein, H.U., White, C.C., Peters, M.A., Lodgson, B., et al. (2017). Alzheimer’s brains show inter-related changes in RNA and lipid metabolism. Neurobiol. Dis. 106, 1–13.

37. Sato, N., and Morishita, R. (2015). The roles of lipid and glucose metabolism in modulation of β-amyloid, tau, and neurodegeneration in the pathogenesis of Alzheimer disease. Front. Aging Neurosci. 7, 199.

38. Yang, R.Y., Quan, J., Sodaei, R., Aguet, F., Segrè, A.V., Allen, J.A., Lanza, T.A., Reinhart, V., Crawford, M., Hasson, S., et al. (2018). A systematic survey of human tissue-specific gene expression and splicing reveals new opportunities for therapeutic target identification and evaluation. bioRxiv. https://doi.org/10.1101/311563.

39. Hondius, D.C., van Nierop, P., Li, K.W., Hoozemans, J.J., van der Schors, R.C., van Haastert, E.S., van der Vies, S.M., Rozenmuller, A.J., and Smit, A.B. (2016). Profiling the human hippocampal proteome at all pathologic stages of Alzheimer’s disease. Alzheimers Dement. 12, 654–668.

40. Seyfried, N.T., Dammer, E.B., Swarup, V., Nandakumar, D., Duong, D.M., Yin, L., Deng, Q., Nguyen, T., Hales, C.M., Wingo, T., et al. (2017). A multi-network approach identifies protein-specific co-expression in asymptomatic and symptomatic Alzheimer’s disease. Cell Syst. 4, 80–72.e4.

41. Ying, L., Duong, D.M., Yin, L., Gearing, M., Slatkin, L., and Sutcliffe, J.S. (2018). Global quantitative analysis of the human brain proteome in Alzheimer’s and Parkinson’s disease. Sci. Data 5, 180036.

42. Lo, Y.M.D., Sun, F.M., Chen, K.C.A., Tsui, N.B.Y., Chong, K.C., Lai, T.K., Leung, T.Y., Zee, B.C.Y., Cantor, C.R., and Chiu, R.W.K. (2017). Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proc. Natl. Acad. Sci. USA 104, 13116–13121.

43. Absinta, M., Ha, S.K., Nair, G., Sati, P., Luciano, N.J., Palisoc, M., Louveau, A., Zaghloul, K.A., Pittaluga, S., and Dubnau, J. (2013). Activation of transposable elements during aging and neuronal decline in Drosophila. Nat. Neurosci. 16, 529–531.

44. Raper, D., et al. (2018). Functional aspects of meningial lymphatics in ageing and Alzheimer’s disease. Nature 560, 185–191.
59. Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M.J. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat. Biotechnol. 29, 341–345.
60. van de Haar, H.J., Burgmans, S., Jansen, J.F., van Osch, M.J., van Buchem, M.A., Muller, M., Hofman, P.A., Verhey, F.R., and Backes, W.H. (2016). Blood-brain barrier leakage in patients with early Alzheimer disease. Radiology 281, 527–535.
61. Sweeney, M.D., Sagare, A.P., and Zlokovic, B.V. (2018). Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat. Rev. Neurol. 14, 133–150.
62. Chen, X.Q., and Mobley, W.C. (2019). Alzheimer disease pathogenesis: insights from molecular and cellular biology studies of oligomeric Aβ and tau species. Front. Neurosci. 13, 659.
63. Yang, J.H., Wada, A., Yoshida, K., Miyoshi, Y., Sayano, T., Esaki, K., Kinoshita, M.O., 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.
64. Zhu, S., Stein, R.A., Yoshioka, C., Lee, C.H., Goehring, A., Mchaourab, H.S., and Gouaux, E. (2016). Mechanism of NMDA receptor inhibition and activation. Cell 165, 704–714.
65. Le Bail, M., Martineau, M., Sacchi, S., Yatsenko, N., Radziszewsky, I., Conrod, S., At Ouares, K., Wolosker, H., Pollegioni, L., Billard, J.M., and Mothe, J.P. (2015). Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proc. Natl. Acad. Sci. USA 112, E204–E213.
66. Hynd, M.R., Scott, H.L., and Dodd, P.R. (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer’s disease. Neurochem. Int. 45, 583–595.
67. Ehmsen, J.T., Ma, T.M., Sason, H., Rosenberg, D., Ogo, T., Furuya, S., Snyder, S.H., and Wolosker, H. (2013). D-serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase. J. Neurosci. 33, 12464–12469.
68. Hashimoto, A., Nishikawa, T., Oka, T., and Takahashi, K. (1993). Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. J. Neurochem. 60, 783–786.
69. Katsuki, H., Watanabe, Y., Fujimoto, S., Kume, T., and Akaike, A. (2007). Contribution of endogenous glycine and d-serine to excitotoxic and ischemic cell death in rat cerebrocortical slice cultures. Life Sci. 81, 740–749.
70. Sasabe, J., Chiba, T., Yamada, M., Okamoto, K., Nishimoto, I., Matsuoka, M., and Aiso, S. (2007). D-serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. EMBO J. 26, 4114–4119.
71. Perez, E.J., Tapanes, S.A., Loris, Z.B., Balu, D.T., Sick, T.J., Coyle, J.T., and Liebl, D.J. (2017). Enhanced astrocytic d-serine underlies synaptic damage after traumatic brain injury. J. Clin. Invest. 127, 3114–3125.
72. Madeira, C., Loureno, M.V., Vargas-Lopes, C., Suemoto, C.K., Brandão, C.O., Reis, T., Leite, R.E., Laks, J., Jacob-Filho, W., Pasqualucci, C.A., et al. (2015). d-serine levels in Alzheimer’s disease: implications for novel biomarker development. Transl. Psychiatry 5, e561.
73. Mustafa, A.K., Ahmad, A.S., Zeynalov, E., Gazi, S.K., Sikka, G., Ehmsen, J.T., Barrow, R.K., Coyle, J.T., Snyder, S.H., and Doré, S. (2010). Serine racemase deletion protects against cerebral ischemia and excitotoxicity. J. Neurosci. 30, 1413–1416.
74. Sasabe, J., Miyoshi, Y., Suzuki, M., Mita, M., Konno, R., Matsuoka, M., Hamase, K., and Aiso, S. (2012). D-amino acid oxidase controls motoneuron degeneration through D-serine. Proc. Natl. Acad. Sci. USA 109, 627–632.
75. Zott, B., Simon, M.M., Hong, W., Unger, F., Chen-Engerer, H.J., Frosch, M.P., Sakmann, B., Walsh, D.M., and Konnerth, A. (2019). A vicious cycle of β amyloid-dependent neuronal hyperactivation. Science 359, 559–565.
76. Witt, A., Macdonald, N., and Kirkpatrick, P. (2004). Memantine hydrochloride. Nat. Rev. Drug Discov. 3, 109–110.
77. McShane, R., Westby, M.J., Roberts, E., Minakaran, N., Schneider, L., Farrimond, L.E., Maayan, N., Ware, J., and Debarros, J. (2019). Memantine for dementia. Cochrane Database Syst. Rev. 3, CD003154.
78. Liu, L., Lee, M.H., Henderson, L., Tyagi, R., Bachani, M., Steiner, J., Campanac, E., Hoffman, D.A., von Geldern, G., Johnson, K., et al. (2015). Human endogenous retrovirus-K contributes to motor neuron disease. Sci. Transl. Med. 7, 307ra153.
79. Liu, E.Y., Russ, J., Cali, C.P., Phan, J.M., Amiel-Wolf, A., and Lee, E.B. (2019). Loss of nuclear TDP-43 is associated with decondensation of LINE retrotransposons. Cell Rep. 27, 1409–1421.e6.
80. Balu, D.T. (2016). The NMDA receptor and schizophrenia: from pathophysiology to treatment. Adv. Pharmacol. 76, 351–382.
81. Spalloni, A., Nutini, M., and Longone, P. (2013). Role of the N-methyl-d-aspartate receptors complex in amyotrophic lateral sclerosis. Biochim. Biophys. Acta 1832, 312–322.
82. Ghasemi, M., and Schachter, S.C. (2011). The NMDA receptor complex as a therapeutic target in epilepsy: a review. Epilepsy Behav. 22, 617–640.
83. Tomek, S.E., Lacrosse, A.L., Nemirovsky, N.E., and Olive, M.F. (2013). NMDA receptor modulators in the treatment of drug addiction. Pharmaceuticals (Basel) 6, 251–268.
84. Chen, J., Ma, Y., Fan, R., Yang, Z., and Li, M.D. (2018). Implication of genes for the N-methyl-D-aspartate (NMDA) receptor in substance addictions. Mol. Neurobiol. 55, 7567–7578.
85. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
86. Jeong, H.H., Yalamanchili, H.K., Guo, C., Shulman, J.M., and Liu, Z. (2018). An ultra-fast and scalable quantification pipeline for transposable elements from next generation sequencing data. Pac. Symp. Biocomput. 23, 168–179.
87. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30, 923–930.
88. R Development Core Team (2013). R: A language and environment for statistical computing (R Foundation for Statistical Computing).
89. Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
90. Rozowsky, J., Kitchen, R.R., Park, J.J., Galeev, T.R., Diao, J., Warell, J., Thistletwaite, W., Subramanian, S.L., Milosavljevic, A., and Gerstein, M. (2019). exceRpt: a comprehensive analytic platform for extracellular RNA profiling. Cell Syst. 8, 352–357.e3.
STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Biological Samples** |        |            |
| Human plasma        | UCSD Shiley-Marcos Alzheimer’s Disease Research Center | N/A |
| **Deposited Data**  |        |            |
| Raw and processed SILVER-seq data | This paper | GEO: GSE136243 |
| Human reference genome GRCh38/hg38 | UCSC Genome Browser | http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg38 |
| Ensembl gene annotation (GTF file) | Ensembl Genome Browser | ftp://ftp.ensembl.org/pub/release-84/gtf/homo_sapiens/ |
| Tissue specific genes derived from GTEx V6 data | GTEx Consortium paper [52] | https://doi.org/10.1101/311563 |
| GTEx V8 data | GTEx Portal | https://gtexportal.org/home/datasets |
| RNA-seq datasets of AD and control brains | AMP-AD Knowledge Portal | https://www.synapse.org/#!Synapse:syn9702085 |
| Serum exRNA sequencing data of the Burgos study | exRNA Atlas | EXR-KJENS1sPivS2-AN |
| Proteomics dataset from the Hondius study | Table S3 of their published paper [53] | https://doi.org/10.1016/j.jalz.2015.11.002 |
| Proteomics dataset from the Seyfried study | Synapse platform | syn3606086 |
| Proteomics dataset from the Ping study | Synapse platform | syn10239444 |
| **Software and Algorithms** |        |            |
| Trimmomatic (version 0.36) | [85] | http://www.usadellab.org/cms/?page=trimmomatic |
| STAR (version 2.5.4b) | [86] | https://github.com/alexdobin/STAR |
| featureCounts (version 1.6.1) | [87] | http://bioinf.wehi.edu.au/featureCounts/ |
| SalmonTE (version 0.4) | [86] | https://github.com/LiuzLab/SalmonTE |
| DisGeNET Database 6.0 | [47] | http://www.disgenet.org/ |
| R (version 3.6.0) | [88] | https://www.r-project.org/ |
| R lme4 package (version 1.1-21) | [89] | https://cran.r-project.org/web/packages/lme4/index.html |

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Sheng Zhong (szhong@ucsd.edu). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human plasma samples

Plasma collection and analyses were approved by the University of California San Diego Human Research Protection Office. Every research subject or next of kin with guardianship of the subject, if necessary, who enters the UCSD Shiley-Marcos Alzheimer’s Disease Research Center agrees to a postmortem examination as part of the entry criteria. Written informed consent was obtained from each participant. Venous blood was drawn by an ADRC staff member trained in phlebotomy. This blood was drawn in the morning (08:00-10:00) to minimize circadian variability of plasma analytes but fasting was not required. EDTA plasma was prepared by letting 2 samples (16 mL) stand in a vacutainer tube for 30 minutes, followed by centrifugation at room temperature at 3,500 g x 15 minutes, and aliquoting the plasma into 0.5 mL aliquots in polypropylene cryotubes (0.5 mL, Sarstedt) to which with barcoded labels were applied. Aliquots were then flash frozen and stored at −80°C.

A total of 164 plasma samples from 35 research subjects were collected. Information on these research subjects are listed in Table S2.
METHOD DETAILS

SILVER-seq analysis of human plasma samples
Thawed plasma was aliquoted into 5 μl per sample and was subjected to SILVER-seq [11]. A total of 164 samples were sequenced to yield on average 19.2 million 75 bp single-end reads per sample (Table S3).

SILVER-seq data processing
Adapters and low-quality bases were trimmed by Trimomatic (version 0.36) [85]. The trimmed sequences were aligned to human reference genome (GRCh38/hg38) by STAR (version 2.5.4b) [86] and de-duplicated by UMI. Read counts per gene were calculated by featureCounts (version 1.6.1) [87] with the Ensembl gene annotation GTF file (release 84) and subsequently converted to Transcripts Per Million (TPM).

Retrieving the summary of tissue-specific expression from GTEx
We retrieved the GTEx consortium’s summary table of tissue specific expression [52]. This summary table was based on GTEx consortium’s definition of tissue-specific score (TS_Score), recommended threshold (TS_Score > 3), and GTEx V6p data release that included 8,527 samples from 13 brain regions and 36 other tissues [52]. Based on this summary table, we retrieved 1,514 brain-specific genes with TS_Score > 3 in at least one brain region and TS_Score ≤ 3 in all peripheral tissues. To account for the recent data release, we re-calculated TS_Scores [52] based on the latest GTEx dataset (V8), including 17,382 samples from 13 brain regions and 41 other tissues. We also separately calculated TS_Scores based on young (age < 60), old (age ≥ 60), female, and male samples. Our stronger criteria for defining brain specific expression are: TS_Score > 3 in at least one brain region, and TS_Score ≤ 3 in all peripheral tissues, and the average TPM of the 13 brain regions > 0.1, and the maximum TPM of the 41 peripheral tissues < 0.1.

Quantification of transposon exRNA expression levels
We re-used the annotations of transposons, transposon clades as described previously [86]. Expression levels of transposons were calculated by SalmonTE (version 0.4) with default parameters [86].

RNA-seq of AD and control brains
Pre-processed RNA-seq datasets of AD and control brains generated by the AMP-AD consortium were downloaded from AMP-AD Knowledge Portal (https://www.synapse.org/#!Synapse:syn9702085).

Serum exRNA sequencing data
The exRNA sequencing dataset of the Burgos study was downloaded from exRNA Atlas (https://exrna-atlas.org/) by accession ID EXR-KJENS1sPlvS2-AN [7]. The read counts on Gencode genes were produced by the ERCC consortium. As quality control, the samples with at least 1 mapped read (read count > 0) on 9,500 or more genes were retained for further analysis. The read counts per gene were converted to normalized CPM (counts per million) values for downstream analyses [90].

AD-associated genes from DisGeNET database
A total of 1,981 documented and 84 expert curated genes were downloaded from DisGeNet (http://www.disgenet.org/) by querying the gene-disease associations with “Alzheimer’s Disease” [47]. Among them, 1,926 of the 1,981 documented and 83 of the 84 expert curated genes had Ensembl (GRCh38 release 84) gene IDs and were used in our analysis.

Published proteomics datasets
The processed proteomics dataset from the Hondius study was retrieved in their Table S3 [53]. The processed datasets of the Seyfried study and the Ping study were downloaded from the Synapse platform (https://www.synapse.org/) by accession IDs syn3606086 and syn10239444, respectively [54, 55].

Analysis of longitudinal changes by a mixed model
The mixed model is specified as:

\[ Y_{ij} = \beta_0 + \beta_1 A_{ij} + R_{ij} \]

and,

\[ \beta_0 = \gamma_{00} + \gamma_{01} G_i + \gamma_{02} S_i + U_{0i} \]

\[ \beta_1 = \gamma_{10} + \gamma_{11} G_i + \gamma_{12} S_i \]

where,

\[ U_{0i} \sim \mathcal{N}(0, \tau_{00}) \]
In this model, the indices are $i$: research subject, and $j$: sample. The response variable is PHGDH’s exRNA level: $Y$. The observed data of the response variable is the log transformed TPM: $\log_2(\text{TPM}+1)$. The fixed effects are Time (A, age), Group (G, converter or control), and Sex (S, male or female). $\beta_0^i$ is the intercept that accounts for group and sex, where $U_0^i$ is the error term for each sample. $\beta_1^i$ includes the contribution of time to the intercept ($\gamma_{10} \times A^i$), the interaction of group and time ($\gamma_{11} G^i \times A^i$) and the interaction of sex and time ($\gamma_{12} S^i \times A^i$). We implemented this model using the lme4 package in R [89].

QUANTIFICATION AND STATISTICAL ANALYSIS

All the statistical analyses were performed with R (version 3.6.0) [88]. t test and ANOVA were carried out with the t.test() and aov() functions. FDR was calculated with the p.adjust() function. Pearson correlation was calculated with the cor() function. Linear regression was carried out with the lm() function. Linear mixed model analysis was implemented using the lme4 package in R [89].

DATA AND CODE AVAILABILITY

The accession number for the data reported in this paper is GEO: GSE136243.