Received: 10 October 2023 | Revised: 7 March 2024 | Accepted: 27 March 2024

DOI: 10.1002/alz.13848

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

SHORT REPORT

Post-GWAS multiomic functional investigation of the *TNIP1* locus in Alzheimer's disease highlights a potential role for GPX3

Daniel J. Panyard[1,2] | Lianne M. Reus[3,4,5] | Muhammad Ali[6,7,8] | Jihua Liu[9,10] | Yuetiva K. Deming[2,11,12] | Qiongshi Lu[9,10] | Gwendlyn Kollmorgen[13] | Margherita Carboni[14] | Norbert Wild[13] | Pieter J. Visser[3,4,15,16] | Lars Bertram[17,18] | Henrik Zetterberg[19,20,21,22,23] | Kaj Blennow[19,20] | Johan Gobom[19,20] | Dan Western[6,7,8] | Yun Ju Sung[6,7,8] | Cynthia M. Carlsson[11,12,24,25] | Sterling C. Johnson[11,12,24,25] | Sanjay Asthana[11,12,25] | Carlos Cruchaga[6,7,8] | Betty M. Tijms[3,4] | Corinne D. Engelman[2] | Michael P. Snyder[1]

Correspondence
Daniel J. Panyard, Department of Genetics, Stanford University School of Medicine, Stanford University, 3165 Porter Dr., Palo Alto, Stanford, CA 94304, USA.
Email: dpanyard@stanford.edu

Michael P. Snyder, Department of Genetics, Stanford University School of Medicine, Stanford University, Stanford, CA 94305, USA.
Email: mpsnyder@stanford.edu

Abstract

INTRODUCTION: Recent genome-wide association studies (GWAS) have reported a genetic association with Alzheimer's disease (AD) at the *TNIP1/GPX3* locus, but the mechanism is unclear.

METHODS: We used cerebrospinal fluid (CSF) proteomics data to test (n = 137) and replicate (n = 446) the association of glutathione peroxidase 3 (GPX3) with CSF biomarkers (including amyloid and tau) and the GWAS-implicated variants (rs34294852 and rs871269).

RESULTS: CSF GPX3 levels decreased with amyloid and tau positivity (analysis of variance $P = 1.5 \times 10^{-5}$) and higher CSF phosphorylated tau (p-tau) levels ($P = 9.28 \times 10^{-7}$). The rs34294852 minor allele was associated with decreased GPX3 (P = 0.041). The replication cohort found associations of GPX3 with amyloid and tau positivity ($P = 2.56 \times 10^{-6}$) and CSF p-tau levels ($P = 4.38 \times 10^{-9}$).

Funding information: NIA, Grant/Award Numbers: R01AG044546, R01AG064877, RF1AG053303, U01AG058922, R01AG064614, 1RF1AG074007, P30AG066444, P01AG003991, R01AG27161, R21AG067092, R01AG037639, R01AG021155, P30AG017266, T32AG000213; National Institutes of Health, Grant/Award Numbers: R01AG054047, RF1AG058501, P41GM108538; Wisconsin Alzheimer's Disease Research Center, Grant/Award Numbers: P50AG033514, P30AG062715; Clinical and Translational Science Award (CTSA) program through the NIH National Center for Advancing Translational Sciences (NCATS), Grant/Award Number: UL1TR000427; University of Wisconsin-Madison, Grant/Award Number: P2CHD047873; European Commission, Grant/Award Numbers: 115952, 806999, 101034344; Zon-MW, Redefining Alzheimer's disease, Grant/Award Number: #733050824736; Zon-MW, NCDC, Grant/Award Number: #73305095005; Swedish Research Council, Grant/Award Numbers: #2018-02532, #2017-00915; European Research Council, Grant/Award Number: #101053962; European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska'Curie, Grant/Award Number: #JPND2021-00694; Swedish State Support for Clinical Research, Grant/Award Number: #ALFGBG-720931; Alzheimer Drug Discovery Foundation, Grant/Award Number: #201809-2016862; Alzheimer's Association Research Fellowship, Grant/Award Number: 2019-AARF-643973; AD Strategic Fund and the Alzheimer's Association, Grant/Award Numbers: #ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C; Olav Thon Foundation, Grant/Award Number: #OT2016-101; Erling-Persson Family Foundation, Grant/Award Number: #EP2019-02; Stiftelsen för Gamla Tjänarinnor, Grant/Award Number: #SGT2021-09-Zetterberg; UK Dementia Research Institute, Grant/Award Number: #UKDRI-1003; Swedish Alzheimer Foundation, Grant/Award Numbers: #AF-930351, #AF-939721, #AF-968270; Hjärnfonden, Grant/Award Numbers: #FO2019-0228, #FO2017-0243, #ALZ2022-0006; Swedish Government and the County Councils; ALF-agreement, Grant/Award Numbers: #ALFGBG-715986, #ALFGBG-965240; Alzheimer's Association 2021 Zenith Award, Grant/Award Number: ZEN-21-848495; Alzheimerfonden, Grant/Award Number: #AF-930934; Foundation of Gamla Tjänarinnor; Chan Zuckerberg Initiative (CZI); ZonMW Memorabel, Grant/Award Numbers: #733050824, 10510022110012; Innovative Medicines Inititiative Joint Undertaking under the EMIF, Grant/Award Number: #115372

DISCUSSION: These results suggest variants in the *TNIP1* locus may affect the oxidative stress response in AD via altered GPX3 levels.

KEYWORDS
Alzheimer's disease, genomics, genome-wide association studies, glutathione peroxidase 3, proteomics

Highlights

- Cerebrospinal fluid (CSF) glutathione peroxidase 3 (GPX3) levels decreased with amyloid and tau positivity and higher CSF phosphorylated tau.
- The minor allele of rs34294852 was associated with lower CSF GPX3. levels when also controlling for amyloid and tau category.
- GPX3 transcript levels in the prefrontal cortex were lower in Alzheimer's disease than controls.
- rs34294852 is an expression quantitative trait locus for *GPX3* in blood, neutrophils, and microglia.

1 | BACKGROUND

Genomic studies in Alzheimer's disease (AD) have identified dozens of genetic associations,[1–4] but connecting variants with mechanistic pathways is challenging. Direct experimental validation to determine the consequences of mutations in implicated genes may be costly and time consuming. In recent years, AD research studies have collected a variety of multiomics data on a large scale, including proteomics, transcriptomics, and metabolomics. These molecular data sets can provide key intermediate information linking genes to AD risk.[5]

Several recent large-scale genome-wide association studies (GWAS) have reported and discussed the rs871269 and rs34294852 variants as protective variants for AD[6,7] (see Supplementary Note in supporting information). Here, we use proteomics data from four different AD cohorts along with existing transcriptomic and genomic annotation data to understand the biology underlying this GWAS locus, highlighting a potential connection to AD through the *GPX3* gene.

2 | METHODS

2.1 | Glutathione peroxidase 3 proteomics

Full details on each cohort, the generation of data, and their analysis are provided in the Supplementary Note. The discovery proteomics, genotyping, and cerebrospinal fluid (CSF) biomarker data set came from the University of Wisconsin Alzheimer's Disease Research Center (WI ADRC)[8] and Wisconsin Registry for Alzheimer's Prevention (WRAP)[9] cohorts (n = 137; 915 CSF proteins quantified), which have been described in detail previously[10] (Figure 1). The primary replication proteomics data set came from Alzheimer Center Amsterdam related studies,[11] including the Amsterdam Dementia Cohort (ADC),[12] the 90+ Study,[13] and the Twin Study[14,15] (total n = 446).

Two additional replication proteomics cohorts were also used. One came from the European Medical Information Framework for Alzheimer's Disease Multimodal Biomarker Discovery (EMIF-AD MBD) study (n = 242). The other came from the Memory and Aging Project (MAP) at the Knight Alzheimer's Disease Research Center (Knight ADRC; n = 948), Alzheimer's Disease Neuroimaging Initiative (ADNI; n = 758), the Dominantly Inherited Alzheimer Network (DIAN; n = 495), Pau (n = 232), and Ruiz (n = 632) studies.

All studies were approved by the relevant review board or committee, and informed consent was provided by participants (see Consent Statement).

Briefly, CSF glutathione peroxidase 3 (GPX3) levels were tested for association with amyloid and tau (AT) positivity categories using analysis of variance (ANOVA) and visualized with box plots. Linear regressions were used to test the association of CSF GPX3 levels with continuous measures of CSF amyloid and tau, and, for the discovery cohort, with a panel containing seven other biomarkers of neurodegeneration or neuroinflammation. In the discovery cohort, a simple Bonferroni correction was used to control for multiple testing of the nine total biomarkers in separate regressions. Additionally, in the discovery and primary replication cohorts, the ANOVA and linear regression models were repeated with the addition of the genotype of one of the AD GWAS variants (rs871269 or rs34294842), coded as the count of the number of minor alleles. In all analyses, age and sex were included as covariates along with additional study-specific covariates as appropriate (Supplementary Note).

2.2 | GPX3 expression quantitative trait loci analysis

Microglia expression quantitative trait loci (eQTL) summary statistics were accessed through the European Genome–Phenome Archive (EGAD00001005736) and the Wellcome Sanger Institute Data Access Committee. All sequence data sets were aligned to human genome assembly GRCh38. Simple linear regression was used to map eQTLs with 25 principal components (PCs).[16] The eQTL associations for *GPX3* were extracted for the analysis in this paper. Study designs and method details of the microglia eQTL mapping have been described elsewhere.[16] The presence of eQTLs for *GPX3* in other cell types was assessed in the public databases Open Targets and FIVEx.[17–19]

3 | RESULTS

3.1 | GPX3 proteomics

Using recently generated mass spectrometry (MS) CSF proteomics data from the University of Wisconsin (UW; mean age 66.1; 59.9% female; all of European ancestry; detailed description previously reported[10]), we searched for evidence supporting the role of genes near the GWAS-identified single nucleotide polymorphisms (SNPs) of rs871269 and rs34294852. Among the genes closest to these SNPs (*TNIP1* and *GPX3*), only GPX3 was identified and quantified in this discovery CSF data set; presumably, levels of TNIP1 were below the limit of detection.[10] We then conducted ANOVA analyses that examined the relationship of CSF GPX3 with CSF amyloid- and CSF tau-defined categories of AD (total n = 137; 56 A–T–, 39 A+T–, and 42 A+T+; see Section 2). We used these amyloid (A) and tau (T) categories because they are the major biomarkers central to AD, where amyloid tends to change first followed by tau (note that A–T+ is generally excluded as presumably non-AD pathology).[20,21] After correction for age and sex (see Section 2), CSF GPX3 levels were statistically significantly different across the AD continuum from A–T– to A+T– to A+T+ (ANOVA $P = 1.5 \times 10^{-5}$) with a significant decrease between the A–T– and A+T+ categories (t test $P = 1.3 \times 10^{-5}$; Figure 2A). GPX3 was also significantly associated with eight of the nine tested individual markers of neurodegeneration and neuroinflammation from the NeuroToolKit panel: phosphorylated tau (p-tau), the p-tau/amyloid beta (Aβ)42 ratio, alpha-synuclein, neurofilament light chain (NfL), neurogranin, chitinase-3-like protein (YKL-40), and soluble TREM2 (sTREM2) were all negatively correlated with GPX3, while the Aβ42/Aβ40 ratio was positively correlated (Figure 2B, Table 1, Supplementary Note). The remaining biomarker, interleukin 6, was not associated with GPX3 levels.

We then examined genotype effects on the GPX3 trajectory. According to ANOVA and pairwise t tests, there were no differences in GPX3 levels by just the minor allele count of either SNP alone (ANOVA P values were 0.95 and 0.10, respectively, for the genotypes for rs871269 and rs34294852; Figure 2C), indicating that no differences in GPX3 level were present by genotype across the population as a whole. However, when GPX3 levels were analyzed as the outcome in a multiple linear regression with both AT category and SNP minor allele count (numeric coding) as predictors, a significant decrease in GPX3 levels per copy of the minor allele was observed (P = 0.041 for rs34294852; Figure 2D), suggesting a potential dose–response relationship.

These associations with GPX3 levels were then assessed for replication in an independent set of MS-based CSF proteomics measurements: the Alzheimer Center Amsterdam related studies (n = 446; all of European ancestry). As in the UW cohort, GPX3 was not associated with clinical diagnosis categories (controls, mild cognitive impairment, and AD-type dementia; analysis of covariance [ANCOVA] P = 0.37, controlling for age at sample and sex), but GPX3 was associated with AT-based categories (ANCOVA $P = 2.56 \times 10^{-6}$), with GPX3 levels lower on average in the A+T+ group compared to the A+T– group (Figure S1 in supporting information), replicating what was seen in the UW data. With continuous values of the amyloid and tau (i.e., p-tau) biomarkers, the negative association of GPX3 with CSF p-tau levels was also replicated ($P = 4.38 \times 10^{-9}$, again controlling for age and sex), though the association with CSF amyloid levels was not replicated (P = 0.66). When the minor allele count of rs871269 or rs34294852 was added to the regression model (still with AT group, age, and sex as predictors), neither SNP was associated with GPX3 levels (P = 0.21 and P = 0.84 for rs871269 and rs34294852, respectively).

We also sought to replicate the CSF proteomics signal in two other population cohorts. Using MS-based proteomics data from the EMIF-AD MBD study (n = 242 participants; all of European ancestry), we performed an ANOVA to see if MS-derived CSF GPX3 levels were

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the Alzheimer's disease (AD) genome-wide association studies (GWAS) literature to examine reported results related to the *TNIP1/GPX3* locus. While two studies report AD associations at this locus, no clear mechanism of action nor causal genes were known.
2. **Interpretation**: Our cerebrospinal fluid (CSF) proteomics analysis of glutathione peroxidase 3 (GPX3) levels and follow-up transcriptomic, functional annotation, and expression quantitative trait locus investigation led us to hypothesize a potential mechanism of action for this observed GWAS finding: variants at the *TNIP1/GPX3* locus have a mediated effect on AD through altered GPX3 levels, perhaps reflecting an insufficient or deteriorating response to oxidative stress in AD.
3. **Future directions**: This article highlights a potential therapeutic target in AD. Specific experimental validation of this hypothesis with genetics or proteomics techniques in cell or animal models of AD is needed.

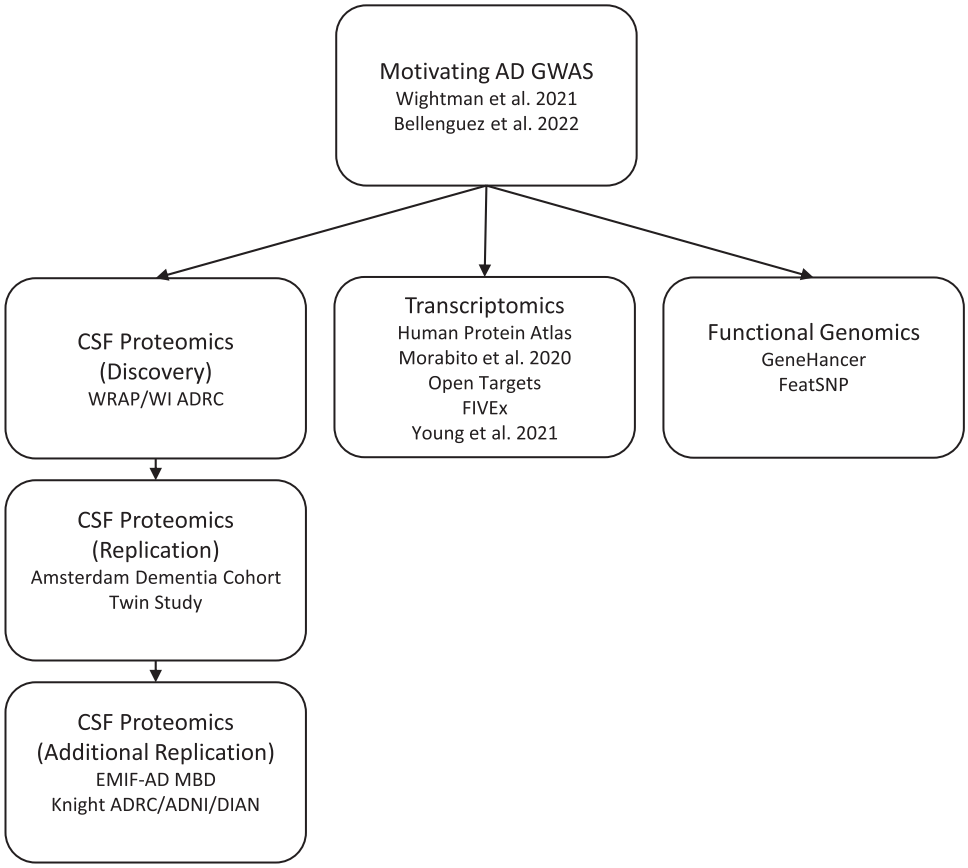

FIGURE 1 Study overview. An overview is provided of the motivating GWAS results, the novel CSF proteomics discovery and replication analyses, and the supporting transcriptomic and functional genomic data sets and resources that were used in this study. AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; DIAN, Dominantly Inherited Alzheimer Network; EMIF-AD MBD, European Medical Information Framework for Alzheimer's Disease Multimodal Biomarker Discovery; GWAS, genome-wide association studies; WRAP/WI ADRC, Wisconsin Registry for Alzheimer's Prevention/Wisconsin Alzheimer's Disease Research Center.

different by AT category. No such difference was observed ($P = 0.96$) in this cohort, even when stratified by rs34294852 genotypes (Figure S2 in supporting information) or controlling for age, sex, or study site. In the Knight ADRC's discovery and replication CSF proteomics data sets at Washington University in St. Louis ($n = 1168$ and 597 participants, respectively; >90% of participants in each data set were of European ancestry), CSF GPX3 levels were measured using an aptamer-based proteomics platform instead of MS. The abundance of GPX3 levels was not significantly different between A+T+ and A−T− individuals ($P = 0.90$ and $P = 0.51$, respectively; Figure S3 in supporting information).

3.2 | GPX3 transcriptomics

Next, we examined transcriptomics data sets to understand the relationship of *GPX3* expression in general and in an AD cohort. Across the cell types in the Human Protein Atlas, *GPX3* is most highly expressed in the proximal tubular cells of the kidney (20125.9 nTPM) and the Müller glia cells of the eye (5019.8 nTPM). Among brain tissues, the overall expression was lower, but *GPX3* was expressed in several types of neurons, microglia (3.7 nTPM), and astrocytes (1.0 nTPM; Figure S4 in supporting information).[22] Within an AD cohort, in which *GPX3* might be more relevant given the heterogeneity of the microglia transcriptome,[23] RNA-seq data from the prefrontal cortex from the Religious Orders Study Memory and Aging Project (ROSMAP) showed that *GPX3* transcript levels decreased in the prefrontal cortex from controls to AD diagnosis ($P = 7 \times 10^{-6}$; Figure S5 in supporting information), but not in other brain regions.[24]

In terms eQTL data, variant rs34294852 is an eQTL for *TNIP1* and *GPX3* in blood, and it is also an eQTL for *TNIP1* in monocytes and neutrophils and for *GPX3* for neutrophils.[17–19] We also investigated recent eQTL data for microglia and found that rs34294852 was an eQTL for GPX3 ($P = 0.038$).[16]

3.3 | GPX3 functional genomics

We also found evidence from functional genomics supporting a relationship between rs34294852 and *GPX3* transcription. In terms of genome functional annotation, rs34294852 is located within the sixth intron of *TNIP1* and downstream of the enhancer region

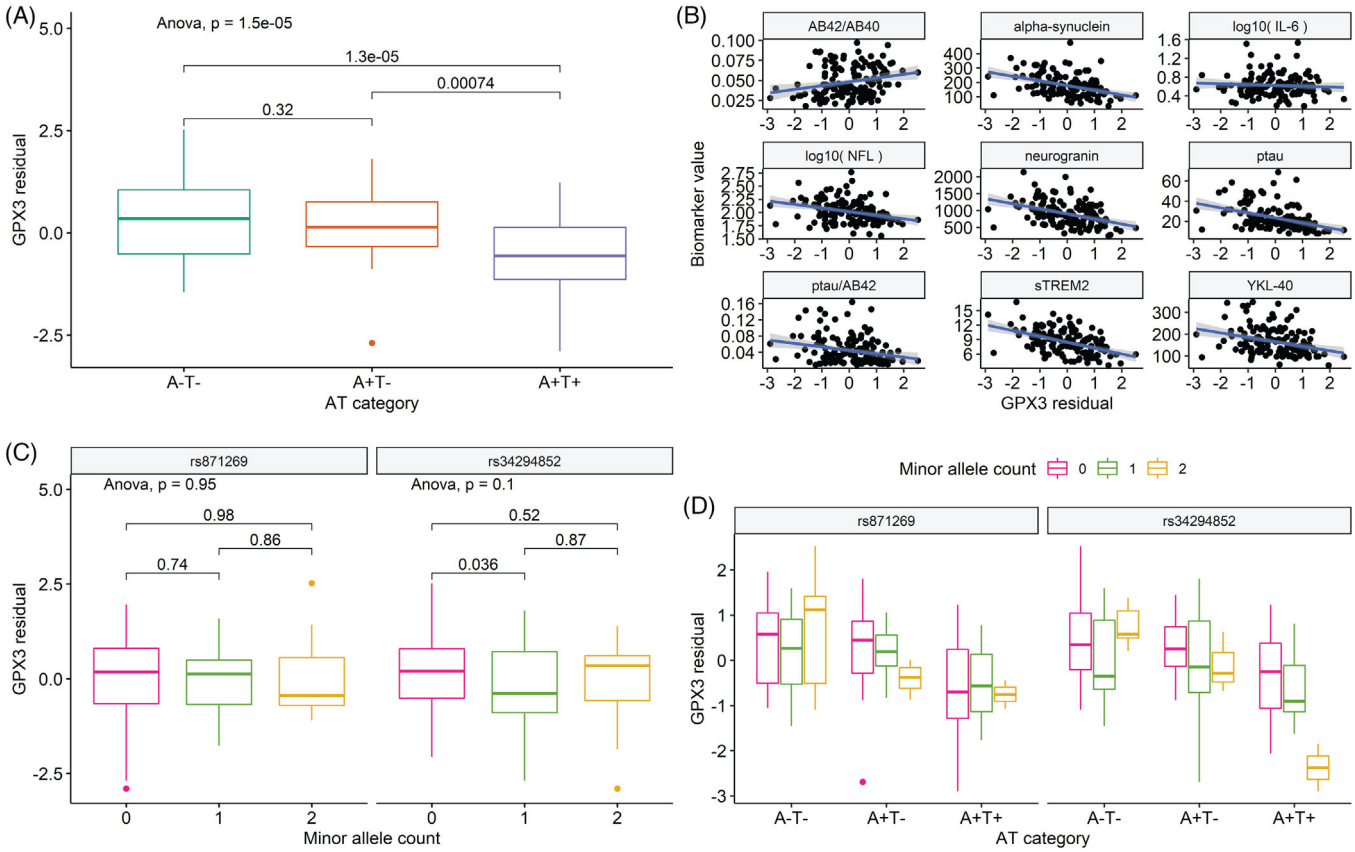

FIGURE 2 Associations of CSF GPX3 with AD-related measures in the WI ADRC and WRAP cohorts. A, CSF GPX3 levels (after regressing out the effects of age and sex) significantly decreased across amyloid and tau (AT) positivity categories ($n = 137$). B, CSF GPX3 levels were significantly associated with all CSF biomarkers of neurodegeneration and neuroinflammation except for IL-6 ($n = 137$). In each case, GPX3 levels decreased as biomarker values indicated a worse clinical profile. C, Across the whole sample ($n = 137$), no difference in GPX3 levels were observed by genotype of either AD-related variant alone. D, CSF GPX3 levels by both AT and genotype are shown for both relevant SNPs at the *TNIP1/GPX3* locus. Among participants who were A+T+ ($n = 42$), CSF GPX3 levels were significantly decreased for homozygous recessive carriers of the rs34294852 allele (10 total participants were homozygous recessive for rs34294852; $n = 5$ were A−T−; $n = 3$ were A+T−; $n = 2$ were A+T+). AD, Alzheimer's disease; CSF, cerebrospinal fluid; GPX3, glutathione peroxidase 3; GWAS, genome-wide association studies; IL-6, interleukin 6; SNP, single nucleotide polymorphism; WI ADRC, Wisconsin Alzheimer's Disease Research Center; WRAP, Wisconsin Registry for Alzheimer's Prevention.

TABLE 1 Association of GPX3 with CSF biomarkers of neurodegeneration and neuroinflammation.

Biomarker	Estimate	SE	*P*	R^2
Aβ42/Aβ40	0.0047	0.0016	0.0050	0.057
P-tau	−5.00	0.97	9.28E-07	0.164
P-tau/Aβ42	−0.0085	0.0029	0.0045	0.058
log10(NfL)	−0.068	0.018	0.0002	0.096
Alpha-synuclein	−32.3	6.3	1.01E-06	0.163
Neurogranin	−150.4	29.0	7.81E-07	0.166
sTREM2	−1.21	0.20	1.08E-08	0.216
log10(IL-6)	−0.017	0.021	0.4112	0.005
YKL-40	−20.6	5.3	0.0001	0.102

Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; GPX3, glutathione peroxidase 3; IL-6, interleukin 6; NfL, neurofilament light chain; p-tau, phosphorylated tau; SE, standard error; sTREM2, soluble TREM2; YKL-40, chitinase-3-like protein.

GH05J151051 from GeneHancer.[25,26] Variant rs34294852 is predicted to alter the binding of the transcription factor MZF1 according to FeatSNP,[27] which is a database that aggregates brain-specific epigenetic data to examine the effects of genetic variants.

4 | DISCUSSION

GPX3 is a secreted glutathione peroxidase that protects the body from oxidative damage by reducing hydroperoxides.[28] AD has long been linked to oxidative stress, as have aging processes in general.[29–31] Connections between GPX activity and AD have been noted in previous studies of AD, usually finding decreased GPX activity in AD compared to healthy controls.[32–36]

Based on our results here, we developed a hypothesis regarding the GWAS signal at the *TNIP1/GPX3* locus (Figure 3). First, as oxidative stress rises with the preclinical pathophysiological processes of AD, the body combats that stress in part with GPX3. As AD progresses

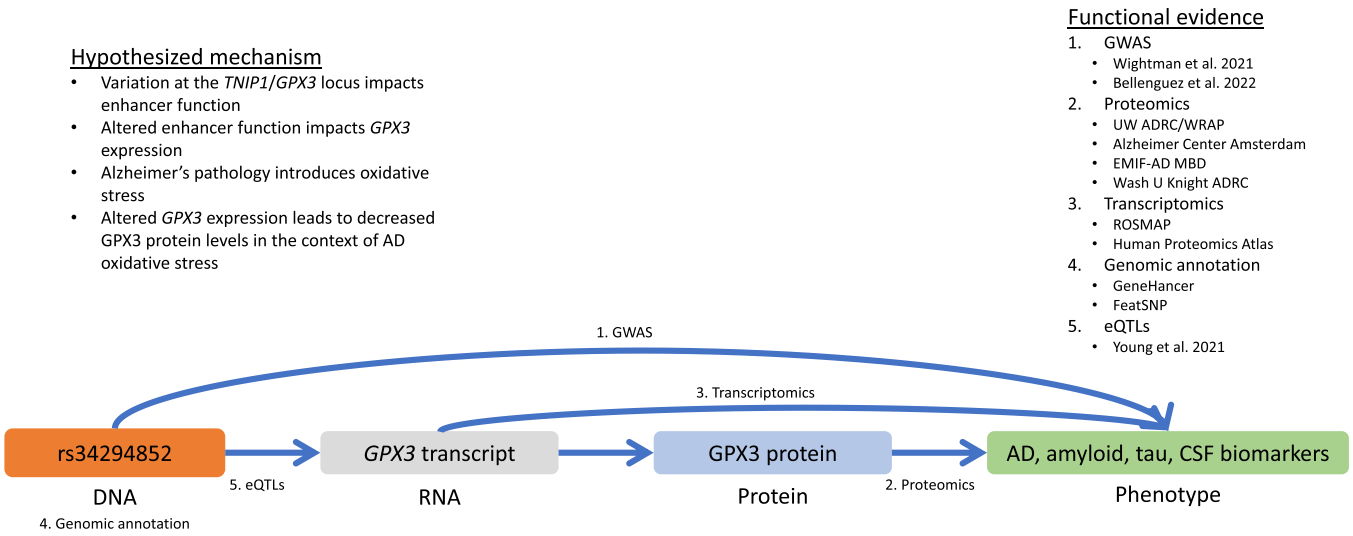

FIGURE 3 Proposed functional mechanism of the *TNIP1/GPX3* locus in AD. Our hypothesis for a functional mechanism connecting the variant rs34294852 to AD outcomes is overlaid onto a map of the major types of omics data analyzed here. Major lines of post-GWAS functional evidence supporting this hypothesis are summarized in the right-hand list. AD, Alzheimer's disease; CSF, cerebrospinal fluid; EMIF-AD MBD, European Medical Information Framework for Alzheimer's Disease Multimodal Biomarker Discovery; eQTL, expression quantitative trait loci; GWAS, genome-wide association studies; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; UW ADRC, University of Wisconsin Alzheimer's Disease Research Center; WRAP, Wisconsin Registry for Alzheimer's Prevention.

to the accumulation of tau tangles and beyond, GPX3 transcript and protein levels drop, perhaps reflecting growing disruption to normal oxidative stress protective measures, decompensation after some protective element is depleted (e.g., decreased selenium availability), or some other event.[36] The presence of certain genetic variants at the *TNIP1/GPX3* locus may affect GPX3 levels or the capacity to respond to oxidative stress through GPX3 expression by affecting enhancer activity at this genetic locus. Moreover, the data we present here might help explain some of the inconsistency in the GPX–AD associations observed in prior work if those associations are indeed affected by variants at the *TNIP1/GPX3* locus. Strengthening our findings here in AD is a recent study of the *TNIP1/GPX3* locus in amyotrophic lateral sclerosis (ALS) that also found GPX3 levels to drop in more progressed disease and with the putative risk allele,[37] providing further support for this proposed hypothesis and evidence that GPX3 may be a useful therapeutic target.

This hypothesized GPX3 trajectory also presents an explanation for the subtlety of the signal in the data sets examined here. Depending on the population studied, the availability of data points at different times in the trajectory of AD, and how groups are defined (by clinical diagnosis, AT group, or something else), the statistical signal of what is happening with GPX3 may be obscured. For instance, the lack of signal when looking at AD dementia cases versus controls might be due to comparing levels of GPX3 that have decreased due to pathology with cognitively healthy individuals who either do not have AD or have not yet experienced enough oxidative stress to merit a rise in GPX3 levels in the first place. Focusing on AT-defined categories may have helped uncover this signal by differentiating between different stages of preclinical AD.

While multiple layers of omic data described here support a role of GPX3 in AD, the proteomic associations were not consistently observed across the cohorts analyzed. Beyond the issues raised above, this inconsistency at the protein level might arise from several other confounding factors. Given the rarity of the minor allele, this mechanism might be hard to detect in some cohorts (as was the case here) and perhaps then only under certain disease conditions or at specific times in the development of AD. This pathway might also be difficult to detect for other reasons: (1) the original GWAS effect was small, (2) the pathway might only be relevant to a subset of cell types, and (3) and the effect might be harder to observe for homozygous dominant or heterozygous rs34294852 genotypes. Moreover, the effect allele for rs34294852 (C) has a minor allele frequency ranging from 0.16 for East Asians on the low end to 0.25 for African/African Americans on the high end,[38] which would make homozygous recessive individuals relatively uncommon, as was the case in the data sets analyzed here, which could mean that direct observation of this genotype-mediated pathway at the protein level would be more difficult. Adding to the difficulty of replicating the proteomics signal are differences in populations, proteomics technologies (MS vs. aptamer-based),[39] and amyloid and tau phenotyping between the cohorts, which could also hinder replication. Finally, it is important to note that the data here supporting a role of GPX3 do not rule out an effect through TNIP1 as well; more work will be needed to examine other potential effects of these variants on AD.

Nevertheless, the observed multiomic evidence connecting variation at this locus to AD to GPX3 expression, combined with our understanding of oxidative stress in AD and GPX proteins' role in combating such stress, provide an intriguing hypothesis for the functional mechanism of these GWAS variants in AD. Several functional experiments would be a reasonable next step in exploring this hypothesis: (1) a gene-editing experiment to validate the impact of genetic variation at rs34294852 on *GPX3* expression, (2) proteomics analysis in brain or other relevant cell types to examine GPX3 levels in AD, and

(3) a cell or organismal model of AD to assess the expression of GPX proteins in connection with oxidative stress burden and its relationship to changes in tau level. Furthermore, longitudinal observational CSF proteomics data starting from the A–T– preclinical stage of AD would help test this hypothesized mechanism as well. Nevertheless, the post-GWAS analyses here demonstrate the utility of multiomic cohort data in the investigation of GWAS loci and their mechanisms of action, which can lead to new insights into AD with the ultimate goal of identifying new therapeutic targets.

AUTHOR CONTRIBUTIONS

Daniel J. Panyard conceived the idea for the study, conducted the analyses of the various Wisconsin data sets, prepared the figures and tables, and led the writing of the manuscript. Lianne M. Reus conducted the analyses in the Alzheimer Center Amsterdam and EMIF-AD MBD cohorts. Michael P. Snyder helped conceive the idea for the study and interpret the results. Muhammad Ali conducted the analyses in the Knight ADRC cohort. Jihua Liu and Qiongshi Lu provided the eQTL results in microglia. Yuetiva K. Deming contributed to the interpretation of the results. Henrik Zetterberg, Kaj Blennow, Gwendlyn Kollmorgen, Margherita Carboni, and Norbert Wild contributed to the generation of the University of Wisconsin NTK CSF biomarker data set. Corinne D. Engelman, Cynthia M. Carlsson, Sterling C. Johnson, Sanjay Asthana, Henrik Zetterberg, and Kaj Blennow contributed resources or funding. Cynthia M. Carlsson, Sterling C. Johnson, and Sanjay Asthana assisted with the Wisconsin Alzheimer's disease cohort studies and the data and analyses conducted there for this work. Dan Western, Yun Ju Sung, and Carlos Cruchaga assisted with the Knight ADRC cohorts and the data and analyses conducted there for this work. Pieter J. Visser, Lars Bertram, Henrik Zetterberg, Kaj Blennow, Johan Gobom, and Betty M. Tijms assisted with the EMIF-AD MBD cohorts and the data and analyses conducted there for this work. All authors contributed to and critically reviewed the manuscript.

AFFILIATIONS

[1]Department of Genetics, Stanford University School of Medicine, Stanford University, Stanford, California, USA

[2]Department of Population Health Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA

[3]Alzheimer Center Amsterdam, Neurology, Vrije Universiteit Amsterdam, Amsterdam UMC location VUmc, Amsterdam, The Netherlands

[4]Amsterdam Neuroscience, Neurodegeneration, Amsterdam, The Netherlands

[5]Center for Neurobehavioral Genetics, University of California, Los Angeles, California, USA

[6]Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri, USA

[7]NeuroGenomics and Informatics Center, Washington University School of Medicine, St. Louis, Missouri, USA

[8]Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, Missouri, USA

[9]Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, Wisconsin, USA

[10]Department of Statistics, University of Wisconsin-Madison, Madison, Wisconsin, USA

[11]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin-Madison, Madison, Wisconsin, USA

[12]Department of Medicine, University of Wisconsin-Madison, Madison, Wisconsin, USA

[13]Roche Diagnostics GmbH, Penzberg, Germany

[14]Roche Diagnostics International Ltd, Rotkreuz, Switzerland

[15]Department of Psychiatry, Maastricht University, Maastricht, The Netherlands

[16]Department of Neurobiology, Care Sciences and Society, Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

[17]Lübeck Interdisciplinary Platform for Genome Analytics, Institutes of Neurogenetics and Cardiogenetics, University of Lübeck, Lübeck, Germany

[18]Department of Psychology, University of Oslo, Oslo, Norway

[19]Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[20]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[21]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[22]UK Dementia Research Institute at UCL, London, UK

[23]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

[24]Wisconsin Alzheimer's Institute, University of Wisconsin-Madison, Madison, Wisconsin, USA

[25]William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA

ACKNOWLEDGMENTS

We would like to thank the participants and staff of the WRAP, WI ADRC, EMIF-AD MBD, and Knight ADRC. Without their efforts this research would not be possible. This research is supported by National Institute on Aging (NIA) grants R01AG27161 (Wisconsin Registry for Alzheimer Prevention: Biomarkers of Preclinical AD), National Institutes of Health, (NIH) grant R01AG054047 (Genomic and Metabolomic Data Integration in a Longitudinal Cohort at Risk for Alzheimer's Disease), P41GM108538 (National Center for Quantitative Biology of Complex Systems), R01AG037639 (White Matter Degeneration: Biomarkers in Preclinical Alzheimer's Disease), R01AG021155 (The Longitudinal Course of Imaging Biomarkers in People at Risk of AD), R21AG067092 (Identifying Metabolomic Risk Factors in Plasma and CSF for Alzheimer's Disease), and P50AG033514 and P30AG062715 (Wisconsin Alzheimer's Disease Research Center Grant), the Clinical and Translational Science Award (CTSA) program through the NIH National Center for Advancing Translational Sciences (NCATS) grant UL1TR000427, and the University of Wisconsin-Madison Office of the Vice Chancellor for Research and Graduate Education with funding from the Wisconsin Alumni Research Foundation. Computational resources were supported by a core grant to the Center for Demography and Ecology at the University of Wisconsin-Madison (P2CHD047873). We also acknowledge use of the facilities of the Center for Demography of Health and Aging at the University of Wisconsin-Madison, funded by NIA Center grant P30AG017266. L.M.R. was funded by the Memorabel fellowship (ZonMW project number: 10510022110012). Y.K.D. was supported by a training grant from the National Institute on Aging (T32AG000213). P.J.V. was supported by grants from the European Commission, IMI (AMYPAD: 115952; RADAR-AD: 806999; and EPND: 101034344),

and the ZonMW (Redefining Alzheimer's disease, #733050824736, and NCDC, #73305095005). H.Z. is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#101053962), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the Alzheimer's Association Research Fellowship (2019-AARF-643973), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation (#OT2016-101), the Erling-Persson Family Foundation (#EP2019-02), Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228, #SGT2021-09-Zetterberg), the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No #JPND2021-00694 (MIRIADE), and the UK Dementia Research Institute at UCL (#UKDRI-1003). Author KB is supported by the Swedish Research Council (#2017-00915), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, and #AF-968270), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. J.G. is supported by Alzheimerfonden (#AF-930934) and the Foundation of Gamla Tjänarinnor. C.C. receives support from the Identifying Rare Variants that Increase Risk for Alzheimer-s Disease (R01AG044546, R01AG064877, RF1AG053303, RF1AG058501, U01AG058922, R01AG064614, 1RF1AG074007), and the Chan Zuckerberg Initiative (CZI). The recruitment and clinical characterization of research participants at Washington University were supported by NIA P30AG066444, and P01AG003991. This work was supported by access to equipment made possible by the Hope Center for Neurological Disorders, the NeuroGenomics and Informatics Center (NGI: https://neurogenomics.wustl.edu/) and the Departments of Neurology and Psychiatry at Washington University School of Medicine. L.M.R., P.J.V., and B.M.T. are supported by the ZonMW Memorabel grant programma (#733050824), and P.J.V. is additionally supported by the Innovative Medicines Initiative Joint Undertaking under the EMIF grant agreement (#115372). ELECSYS, COBAS and COBAS E are trademarks of Roche. The Roche NeuroToolKit robust prototype assays are for investigational purposes only and are not approved for clinical use. We thank the University of Wisconsin Madison Biotechnology Center Gene Expression Center for providing Illumina Infinium genotyping services. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

CONFLICT OF INTEREST STATEMENT

C.C. receives research support from Biogen, EISAI, Alector, GSK and Parabon; these funders of the study had no role in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. C.C. is a member of the advisory board of Vivid Genomics, Halia Therapeutics, and ADx Healthcare. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. G.K. is a full-time employee of Roche Diagnostics GmbH. M.C. is a full-time employee and shareholder of Roche Diagnostics International Ltd. N.W. is a full-time employee of Roche Diagnostics GmbH. S.C.J. serves as a consultant to Roche Diagnostics and receives research funding from Cerveau Technologies. M.P.S. is a cofounder and scientific advisor of Personalis, SensOmics, Qbio, January AI, Fodsel, Filtricine, Protos, RTHM, Iollo, Marble Therapeutics, Crosshair Therapeutics, NextThought, and Mirvie. He is a scientific advisor of Jupiter, Neuvivo, Swaza, Mitrix, Yuvan, TranscribeGlass, and Applied Cognition. Other authors have no competing interests to declare. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

The data sets analyzed from the WI ADRC and WRAP studies may be requested at https://www.adrc.wisc.edu/apply-resources. Microglia eQTL summary statistics may be requested through the European Genome-Phenome Archive (EGAD00001005736) and the Wellcome Sanger Institute Data Access Committee. The EMIF-AD proteomics data may be requested from the ProteomeXchange Consortium via the PRIDE partner repository with the data set identifier https://doi.org/10.6019/PXD019910. The Knight ADRC proteomic data are available at NIAGADS: NG00102 collection and can be interactively explored at http://ngi.pub:3838/ONTIME_Proteomics/.

CONSENT STATEMENT

WI ADRC and WRAP cohorts: this study was performed as part of the GeneRations Of WRAP (GROW) study, which was approved by the University of Wisconsin Health Sciences Institutional Review Board; participants in the WI ADRC and WRAP studies provided written informed consent. Alzheimer Center Amsterdam cohort: All studies were approved by the Medical Ethics Committee. Informed consent, either from the patient or from the legal representative, was obtained from all participants. EMIF-AD MBD cohort: Written informed consent was obtained from all participants or surrogates, and the procedures for this study were approved by the institutional review boards of all participating institutions (see Bos et al. for full listing and Supplementary Note). Knight ADRC cohort: All participants provided informed consent to allow their data and biospecimens to be included; the

study was approved by an institutional review board at Washington University School of Medicine in St. Louis.

REFERENCES

1. Naj AC, Jun G, Beecham GW, et al. Common variants in MS4A4/MS4A6E, CD2uAP, CD33, and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet*. 2011;43:436-441. doi:10.1038/ng.801
2. Lambert J-C, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45:1452-1458. doi:10.1038/ng.2802
3. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51:404-413. doi:10.1038/s41588-018-0311-9
4. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. 2019;51:414. doi:10.1038/s41588-019-0358-2
5. Odenkirk MT, Zheng X, Kyle JE, et al, Deciphering ApoE genotype-driven proteomic and lipidomic alterations in Alzheimer's disease across distinct brain regions. *J Proteome Res*. 2024. doi:10.1021/acs.jproteome.3c00604
6. Bellenguez C, Küçükali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nat Genet*. 2022;54:412-436. doi:10.1038/s41588-022-01024-z
7. Wightman DP, Jansen IE, Savage JE, et al, A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nat Genet*. 2021;53:1276-1282. doi:10.1038/s41588-021-00921-z
8. Melah KE, Lu SY-F, Hoscheidt SM, et al. CSF markers of Alzheimer's pathology and microglial activation are associated with altered white matter microstructure in asymptomatic adults at risk for Alzheimer's disease. *J Alzheimers Dis*. 2016;50:873-886. doi:10.3233/JAD-150897
9. Johnson SC, Koscik RL, Jonaitis EM, et al. The Wisconsin Registry for Alzheimer's Prevention: A review of findings and current directions. *Alzheimers Dement*. 2017;10:130-142. doi:10.1016/j.dadm.2017.11.007
10. Panyard DJ, McKetney J, Deming YK, et al. Large-scale proteome and metabolome analysis of CSF implicates altered glucose and carbon metabolism and succinylcarnitine in Alzheimer's disease. *Alzheimers Dement*. 2023;19:5447-5470. doi:10.1002/alz.13130
11. Tijms BM, Vromen EM, Mjaavatten O, et al. Cerebrospinal fluid proteomics in patients with Alzheimer's disease reveals five molecular subtypes with distinct genetic risk profiles. *Nat Aging*. 2024:1-15. doi:10.1038/s43587-023-00550-7
12. van der Flier WM, Scheltens P. Amsterdam Dementia Cohort: performing research to optimize care. *J Alzheimer Dis*. 2018;62:1091-1111. doi:10.3233/JAD-170850
13. Legdeur N, Badissi M, Carter SF, et al. Resilience to cognitive impairment in the oldest-old: design of the EMIF-AD 90+ study. *BMC Geriatr*. 2018;18:289. doi:10.1186/s12877-018-0984-z
14. Boomsma DI, Geus EJC de, Vink JM, et al. Netherlands Twin Register: from twins to twin families. *Twin Res Hum Genet*. 2006;9:849-857. doi:10.1375/twin.9.6.849
15. Konijnenberg E, Carter SF, Ten Kate M, et al. The EMIF-AD PreclinAD study: study design and baseline cohort overview. *Alzheimers Res Ther*. 2018;10:75. doi:10.1186/s13195-018-0406-7
16. Young AMH, Kumasaka N, Calvert F, et al. A map of transcriptional heterogeneity and regulatory variation in human microglia. *Nat Genet*. 2021;53:861-868. doi:10.1038/s41588-021-00875-2
17. Kwong A, Boughton AP, Wang M, et al. FIVEx: an interactive eQTL browser across public datasets. *Bioinformatics*. 2021:btab614. doi:10.1093/bioinformatics/btab614
18. Chen L, Ge B, Casale FP, et al. Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell*. 2016;167:1398-1414.e24 doi:10.1016/j.cell.2016.10.026
19. Võsa U, Claringbould A, Westra H-J, et al. Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis. *bioRxiv*. 2018:447367. doi:10.1101/447367
20. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14:535-562. doi:10.1016/j.jalz.2018.02.018
21. Jack CR Jr, Knopman DS, Jagust WJ, et al. Update on hypothetical model of Alzheimer's disease biomarkers. *Lancet Neurol*. 2013;12:207-216. doi:10.1016/S1474-4422(12)70291-0
22. Karlsson M, Zhang C, Méar L, et al. A single–cell type transcriptomics map of human tissues. *Sci Advances*. 2021;7:eabh2169. doi:10.1126/sciadv.abh2169
23. Masuda T, Sankowski R, Staszewski O, Prinz M. Microglia heterogeneity in the single-cell era. *Cell Rep*. 2020;30:1271-1281. doi:10.1016/j.celrep.2020.01.010
24. Morabito S, Miyoshi E, Michael N, Swarup V. Integrative genomics approach identifies conserved transcriptomic networks in Alzheimer's disease. *Hum Mol Genet*. 2020;29:2899-2919. doi:10.1093/hmg/ddaa182
25. Fishilevich S, Nudel R, Rappaport N, et al. GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. *Database*. 2017;2017:bax028. doi:10.1093/database/bax028
26. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards Suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinformatics*. 2016;54:1.30.1-1.30.33. doi:10.1002/cpbi.5
27. Ma C, Madden P, Gontarz P, Wang T, Zhang B. FeatSNP: an interactive database for brain-specific epigenetic annotation of human SNPs. *Front Genet*. 2019;10:262.
28. O'Leary NA, Wright MW, Brister JR, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016;44:D733-D745. doi:10.1093/nar/gkv1189
29. Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog Neurobiol*. 1999;57:301-323. doi:10.1016/S0301-0082(98)00055-0
30. Butterfield DA, Mattson MP. Apolipoprotein E and oxidative stress in brain with relevance to Alzheimer's disease. *Neurobiol Dis*.. 2020;138:104795. doi:10.1016/j.nbd.2020.104795
31. Tönnies E, Trushina E. Oxidative stress, synaptic dysfunction, and Alzheimer's disease. *J Alzheimers Dis*. 2017;57:1105-1121. doi:10.3233/JAD-161088
32. García-Blanco A, Baquero M, Vento M, Gil E, Bataller L, Cháfer-Pericás C. Potential oxidative stress biomarkers of mild cognitive impairment due to Alzheimer disease. *J Neurol Sci*. 2017;373:295-302. doi:10.1016/j.jns.2017.01.020
33. Võsa U, Claringbould A, Westra HJ. et al. Large-scale *cis*- and *trans*-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet* 2021;53:1300-1310. doi:10.1038/s41588-021-00913-z
34. Puertas MC, Martínez-Martos JM, Cobo MP, Carrera MP, Mayas MD, Ramírez-Expósito MJ. Plasma oxidative stress parameters in men and women with early stage Alzheimer type dementia. *Exp Gerontol*. 2012;47:625-630. doi:10.1016/j.exger.2012.05.019
35. Torres LL, Quaglio NB, de Souza GT, et al. Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis*. 2011;26:59-68. doi:10.3233/JAD-2011-110284
36. Zimetti F, Adorni MP, Marsillach J, et al. Connection between the altered HDL antioxidant and anti-inflammatory properties and the risk to develop Alzheimer's disease: a narrative review. *Oxid Med Cell Longev*. 2021;2021:e6695796. doi:10.1155/2021/6695796
37. Restuadi R, Steyn FJ, Kabashi E, et al. Functional characterisation of the amyotrophic lateral sclerosis risk locus GPX3/TNIP1. *Genome Med*. 2022;14:7. doi:10.1186/s13073-021-01006-6

38. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434-443. doi:10.1038/s41586-020-2308-7
39. Raffield LM, Dang H, Pratte KA, et al. Comparison of proteomic assessment methods in multiple cohort studies. *Proteomics*. 2020;20:e1900278. doi:10.1002/pmic.201900278

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Panyard DJ, Reus LM, Ali M, et al. Post-GWAS multiomic functional investigation of the *TNIP1* locus in Alzheimer's disease highlights a potential role for GPX3. *Alzheimer's Dement*. 2024;20:5044–5053. https://doi.org/10.1002/alz.13848