

# Plasma GFAP in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study

## INTRODUCTION

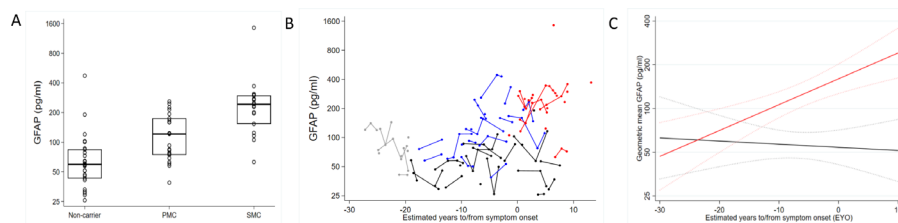
Glial fibrillar acidic protein (GFAP), a marker of astroglia activation, has been proposed as a biomarker of Alzheimer's disease (AD).<sup>1</sup> GFAP expression correlates with A $\beta$  plaque density and cerebrospinal fluid (CSF) concentration is elevated in symptomatic disease.<sup>1,2</sup> Ultrasensitive assays that reliably measure plasma GFAP show increases in AD that are relatively greater than in CSF.<sup>1</sup> Autosomal dominantly inherited familial AD (FAD) is a valuable model for characterising presymptomatic AD as mutations are highly penetrant and it has a young, reasonably predictable, age of onset.<sup>3</sup> We examined whether plasma GFAP concentration is altered in mutation carriers compared with non-carriers, and the timing of presymptomatic change.

## METHODS

We studied 69 participants within University College London's longitudinal study of FAD between 2010 and 2019; described previously.<sup>3</sup> Eligibility was either (1) a clinical diagnosis of FAD or (2) an FAD-affected parent, which means a 50% risk of inheriting a mutation and thereby of developing symptoms at a similar age to their affected parent.

FAD mutation status was determined using Sanger sequencing; participants and study clinicians were blinded to results. At each study visit, EDTA blood sampling and a participant and informant interview were conducted. Plasma samples were shipped frozen to Sahlgrenska University Hospital for blinded analysis using the GFAP single molecule array discovery kit (#102336) on an HD-X platform (Quanterix). Estimated years to/from symptom onset (EYO) was calculated by subtracting the age at which the participant's affected parent first developed progressive cognitive symptoms from the participant's age at blood sampling.

Baseline statistics and box plots of GFAP concentrations were produced for each participant group (symptomatic mutation carriers; presymptomatic carriers; non-carriers). Other analyses



**Figure 1** (A) Box plot for observed baseline plasma GFAP concentration. The measured plasma GFAP concentrations at baseline (first visit) are shown. Mutation carriers have been divided into those who are symptomatic (SMC) and those who are presymptomatic (PMC). The estimated geometric mean GFAP concentration in symptomatic carriers was 238% higher (95% CI 141% to 374%,  $p < 0.001$ ) than in non-carriers, and in presymptomatic carriers was 95% higher (95% CI 46% to 159%,  $p < 0.001$ ) than in non-carriers, after adjusting for age and sex. Age- and sex-adjusted geometric mean GFAP concentration was also significantly elevated in symptomatic compared with presymptomatic carriers (73% higher, 95% CI 26% to 138%,  $p = 0.001$ ). Boxes show the median and first and third quartiles. Dots represent individual observations. (B) Observed plasma GFAP concentration against estimated years to/from symptom onset. Symptomatic mutation carriers are shown in red, presymptomatic mutation carriers are shown in blue, non-carriers are shown in black. To preserve blinding to genetic status, all observed values for timepoints more than 19.3 years before expected symptom onset are shown in grey and some timepoints have been removed for at risk individuals. (C) Trajectory of plasma GFAP against estimated years to/from onset. Modelled geometric mean plasma against estimated years to/from symptom onset. Mutation carriers represented in red; non-carriers in black. Estimates are shown for a hypothetical male aged 41 years (the mean age at baseline). Dotted lines indicate 95% CIs. The y-axis scale is logarithmic in all panes. GFAP, glial fibrillar acidic protein.

used data from all visits. GFAP was log-transformed, with estimated coefficients back-transformed and expressed as multiplicative effects and geometric means. In non-carriers we assessed the association between GFAP and sex. Age-adjusted and sex-adjusted (1) differences in GFAP between patient groups and (2) relationship between GFAP and EYO were both modelled using mixed effects models. We estimated the age-adjusted and sex-adjusted difference in geometric mean GFAP between carriers and non-carriers for integer values of EYO between -30 and 20. The point when this estimate was statistically significantly different from zero ( $p \leq 0.05$ ) was interpreted cautiously as an indication of when the estimated trajectory of GFAP for carriers diverged from non-carriers. A sensitivity analysis refitted all models omitting participants (one symptomatic mutation carrier, one non-carrier) with high outlier GFAP values.

Further details on study procedures and analyses are provided in online supplemental material.

## RESULTS

Online supplemental table 1 shows baseline characteristics. Fifty participants were asymptomatic (23 mutation carriers 27 non-carrier controls). Baseline and longitudinal observed GFAP data are shown in figure 1A,B. Within non-carriers, estimated geometric mean

plasma GFAP was higher in females compared with males (54% higher, 95% CI 2% to 133%,  $p = 0.039$ ), with no meaningful difference after omitting the outlier. After adjusting for age at visit and sex, geometric mean GFAP concentrations were estimated to be higher in both symptomatic and presymptomatic carriers compared with non-carriers ( $p < 0.001$  for both comparisons). These results remained significant after removing the two outliers (online supplemental figure 1). The age- and sex-adjusted geometric mean GFAP concentration in carriers was first significantly higher ( $p = 0.04$ ) than in non-carriers at EYO of 16 years (figure 1C).

## DISCUSSION

This study found plasma GFAP was increased in both presymptomatic and symptomatic mutation carriers compared with non-carriers, and in symptomatic compared with presymptomatic carriers. Plasma GFAP levels diverged between carriers and non-carriers around 16 years before estimated symptom onset. This is consistent with recent findings of higher plasma GFAP in amyloid-positive versus amyloid-negative cognitively normal older adults and with GFAP increases being associated with subsequent decline in global cognition, amyloid accumulation and conversion to dementia.<sup>1,2,4</sup> Overall these results

support plasma GFAP being a biomarker of early AD pathology.

The timing of GFAP change is consistent with a response to amyloid, supporting previous findings that plasma GFAP is associated with amyloid burden and p-tau181 levels.<sup>1,2</sup> Additionally, plasma levels partially mediate the association between amyloid burden and tau positron emission tomography (PET) signal, and are not increased in amyloid negative, tau positive individuals or in dementia with Lewy bodies and frontotemporal dementia.<sup>1,2</sup> This suggests that plasma GFAP is a marker of amyloid-related astrogliosis. However, plasma GFAP may also be an indicator of blood-brain barrier and/or glymphatic dysfunction—astrocytes form part of the neurovascular unit and may directly release GFAP into blood, perhaps explaining the greater magnitude of increases in plasma versus CSF GFAP, and the lack of association between plasma GFAP and other CSF inflammatory markers.<sup>1,2,5</sup>

Symptomatic carriers, on average, had geometric mean plasma GFAP concentrations greater than three times that of non-carriers, with presymptomatic carriers having a mean concentration twice that of non-carriers, approaching midway between symptomatic and non-carrier groups. These are remarkable differences given this is a blood-based assay. Nonetheless, the overlap between groups, the outliers and the within-individual variability suggests that plasma GFAP may be most useful in combination with other AD blood biomarkers. This is consistent with recent studies in sporadic AD showing higher diagnostic yield for detecting underlying amyloid/AD pathology when GFAP was used in combination with other plasma markers.<sup>1</sup>

Plasma GFAP demonstrated considerable intraindividual and interindividual variability; fluctuations in plasma concentrations of NfL and p-tau181 have previously been shown in the same cohort.<sup>3</sup> It is unlikely that AD-related processes are solely responsible for these fluctuations as variability occurred in carrier and non-carrier groups. Variability in plasma levels may be partly attributable to responses to nonspecific CNS injury, with increases previously being reported in traumatic brain injury, stroke, epilepsy and COVID-19-related delirium.<sup>5</sup>

Our study has limitations. The sample size, due to the rarity of FAD, was relatively small. Additionally, we

used parental age at symptom onset to estimate timing of future symptomatic decline. This provides a reasonable estimate of future age at onset, but it is not without error due to variability in age at onset. Further, prospective studies are needed to assess diagnostic accuracy and investigate sources of variability.

## CONCLUSION

Plasma GFAP concentration in FAD increases presymptomatically, with changes being detected over a decade prior to estimated symptom onset, supporting its further investigation as an accessible biomarker of AD-related astrogliosis.

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**Acknowledgements** AO'C acknowledges support from an Alzheimer's Society clinical research training fellowship (AS-CTF-18-001), and from the Rosetrees Trust.

**Contributors** AO'C, NCF and EA did the literature search. AO'C, EA, ALB, NA, KB, HZ and NCF designed the study. AO'C, PSJW and NR contributed to recruitment and data collection. Blood samples were processed and analysed by AJH, EA, NA, ALB and HZ. TP carried out the statistical analysis. JMP contributed to the genetic analysis. TP created the figures. All authors were involved in the interpretation of results and writing the report.

**Funding** HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712 and #101053962), Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), 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, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärtfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721 and #AF-968270), Hjärtfonden, 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), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236) and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). NR is supported by a University of London Chadburn Academic Clinical Lectureship. NCF acknowledges support from Alzheimer's Research UK, the UK Dementia Research Institute and the NIHR UCLH Biomedical Research Centre. This work was supported by the NIHR UCLH/UCL Biomedical Research Centre, the Rosetrees Trust, the MRC Dementia Platform UK and the UK Dementia Research Institute at UCL which receives its funding from UK DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK, and the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986). PSJW is supported by a Wellcome Trust Clinical Research Career Development Fellowship.

**Competing interests** HZ 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 (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a cofounder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. NCF has served on advisory boards or as a consultant for Biogen, Ionis, Lilly, and Roche (all payments to UCL) and has served on a data safety monitoring board for Biogen.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee (subsequently, National Research Ethics Service Committee, London Queen Square, Rec Reference ID 11/LO/0753). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jnnp-2022-329663>).

AO, EA, ALB and TP contributed equally.  
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**To cite** O'Connor A, Abel E, Benedet AL, *et al.* *J Neurol Neurosurg Psychiatry* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jnnp-2022-329663

Received 28 May 2022  
Accepted 19 July 2022

*J Neurol Neurosurg Psychiatry* 2022;**0**:1–3.  
doi:10.1136/jnnp-2022-329663

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## **Methods**

### **Study Procedures**

All individuals identified an informant who was interviewed separately for a collateral history. At each study visit, a semi-structured health questionnaire (including exclusion of recent head injury), neurological examination, and the clinical dementia rating (CDR) scale were performed (1). Individuals were defined as symptomatic if cognitive decline was reported by the participant and/or their informant and the global CDR was  $>0$ .

### **GFAP quantification in plasma**

Samples were collected, processed, aliquoted, and frozen at  $-80^{\circ}\text{C}$  according to standardised procedures. Non-fasting plasma samples were collected in 10 ml ethylenediaminetetraacetic acid coated tubes. Prior to analysis plasma samples were vortexed (2000rpm) and centrifugated (4000g, 10minutes) at room temperature. An 8-fold dilution was used. The intra- and inter-assay variation of internal quality control samples were  $<10\%$ .

### **Statistical analysis**

Age- and sex-adjusted differences in GFAP between patient groups were estimated using a mixed effects model that included random intercepts for individual and family membership, with residual variances differing for the groups being compared. Two presymptomatic individuals became symptomatic during follow-up, with their group membership changed appropriately in analyses.

The mixed effects model that examine the age- and sex-adjusted relationship between GFAP and EYO included: a random intercept for family; random intercept and slope for EYO at individual level allowing these to differ by genetic mutation status; residual variances differing by mutation status. We pre-specified inclusion of mutation status and EYO, plus their interaction, as fixed effects. We investigated quadratic and cubic terms for EYO, plus their interactions with mutation status, but there was no evidence to include these terms. The estimated geometric mean longitudinal GFAP concentration trajectories for mutation

carriers and non-carriers (and 95% confidence intervals) were plotted against EYO, standardised to a male aged 41 years (mean baseline age of all participants).

Analyses used Stata v17.

**Supplementary Table 1**

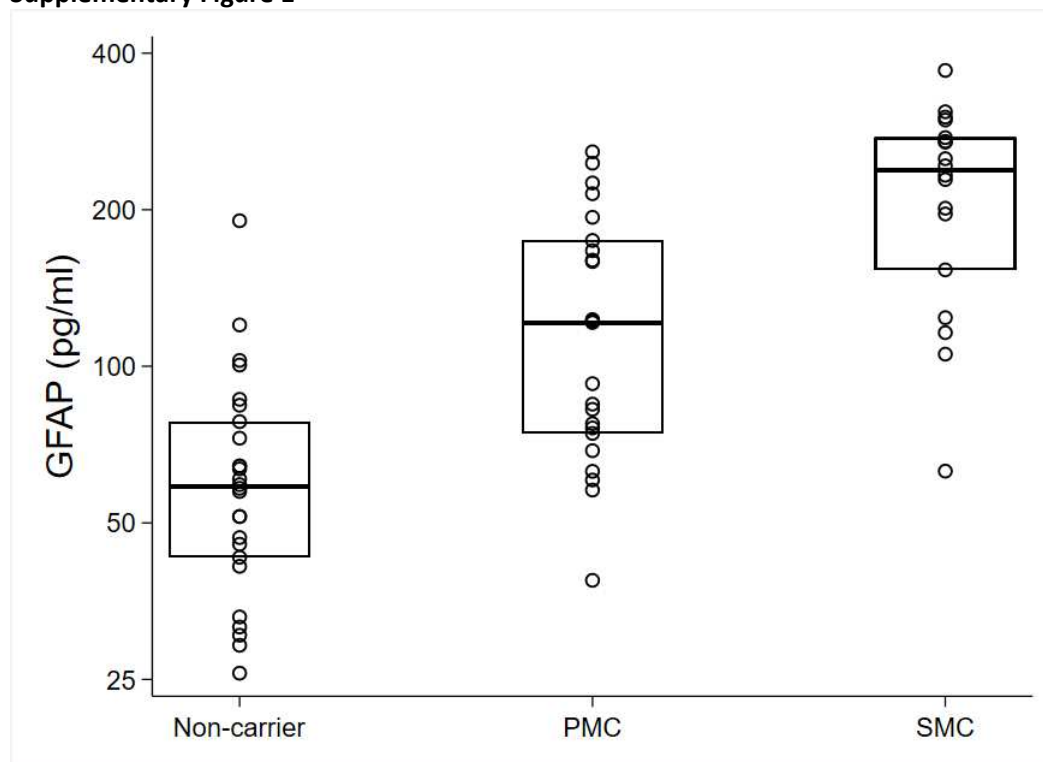
	<b>Non-carrier</b>	<b>PMC</b>	<b>SMC</b>
<b>N</b>	27	23	19
<b>Sex, n (%)</b>			
<b>Women</b>	16 (59%)	11 (48%)	7 (37%)
<b>Men</b>	11 (41%)	12 (52%)	12 (63%)
<b>Age, years (mean (SD))</b>	38.1 (10.7)	35.7 (5.8)	51.3 (9.5)
<b>EYO, years (mean (SD))</b>	N/A	-10.4 (7.6)	4.2 (3.8)
<b>MMSE (Median [IQR])</b>	30 [30, 30]	30 [29, 30]	23 [16, 25] <sup>#</sup>
<b>CDR Global (Median [IQR])</b>	0 [0, 0]	0 [0, 0]	0.5 [0.5, 0.75] (n = 16)
<b>CDR SOB (Median [IQR])</b>	0 [0, 0]	0 [0, 0]	3.5 [1.75, 4.25] (n = 16)
<b>GFAP follow-up duration, years (mean, (SD), for participants with more than one visit)</b>	3.0 (2.0) (n=17)	3.1 (1.8) (n=17)	3.5 (2.0) (n=6)
<b>Samples per participant (mean)*</b>	2.1	2.4	1.8

<b>GFAP, pg/ml (mean (SD))</b>	80.2 (85.7)	127.9 (66.2)	287.4 (293.3)
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\* Forty of the overall 69 participants had at least two visits.

#Of the symptomatic group, two had severe AD (MMSE <10), five had moderate AD (MMSE 10-20), and 12 had mild AD (MMSE > 20).

### Supplementary Figure 1



**Box plot for observed baseline plasma GFAP concentrations with outliers removed.** The measured plasma GFAP concentrations at baseline (first visit) are shown. Mutation carriers have been divided into those who are symptomatic (SMC) and those who are presymptomatic (PMC). Two high outliers (one SMC, one non-carrier; each with one observation) have been removed. After adjusting for age and sex, geometric mean GFAP is estimated to be 218% higher (95%CI: 137%, 328%) in SMC compared to non-carriers ( $p < 0.001$ ) and is estimated to be 104% higher (95%CI: 60%, 160%) in PMC compared to non-carriers ( $p < 0.001$ ). Additionally after adjusting for age and sex, geometric mean GFAP is an estimated 56% higher (95%CI: 17%, 108%) in SMC compared to PMC ( $p = 0.002$ ). Boxes show the median and first and third quartiles. Dots represent individual observations. The y-axis scale is logarithmic.

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