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Decreased Platelet APP Isoform Ratios in Autosomal Dominant Alzheimer's Disease: Baseline Data from a DIAN Cohort Subset

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Abstract

Introduction—This study examines platelet amyloid precursor protein (APP) isoform ratios of 120KDa to 110KDa (APPr) between mutation carriers (MC) carrying a mutation for autosomal dominant Alzheimer's disease (ADAD) and non-carriers (NC). Two previous studies reported no significant difference in APPr between ADAD MC and NC, which may have been due to the small sample size in both studies. The current study examines APPr in MC versus NC in a larger sample. In addition, it investigated whether APPr correlate with neuroimaging data, neuropsychological data and cerebrospinal fluid biomarkers in a cohort subset derived from the Dominantly Inherited Alzheimer Network (DIAN) study.

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CONFLICT OF INTEREST

The authors confirm that this article contains no conflict of interest.

SUPPLEMENTARY MATERIALS

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Methods—APPr were quantified by western blotting. Fifteen MC (symptomatic and asymptomatic) were compared against twelve NC using univariate general linear model. All participants underwent neuroimaging and neuropsychological testing which were correlated with APPr using Pearson's correlation coefficient (r).

Results—APPr were lower in MC compared to NC ($p=0.003$) while Mini-Mental State Examination (MMSE) scores were not significantly different ($p>0.1$). Furthermore, APPr inversely correlated with amyloid imaging in the Caudate Nucleus ($r=-0.505$; $p<0.05$) and Precuneus ($r=-0.510$; $p<0.05$).

Conclusion—APPr are lower in ADAD MC compared to NC, and inversely correlated with brain amyloid load prior to significant differences in cognitive health. However, the use of APPr as a biomarker needs to be explored further.

Keywords

Alzheimer's disease; amyloid precursor protein; biomarkers; neuropsychological tests; Pittsburgh compound B; platelets

INTRODUCTION

Autosomal dominant Alzheimer's disease (ADAD) accounts for approximately 1% of Alzheimer's disease (AD) cases. It occurs in the presence of a mutation in *APP*, *PSEN1* or *PSEN2*. Mutations in these genes disturb the natural balance between amyloidogenic and non-amyloidogenic processing of Amyloid Precursor Protein (APP) leading to abnormal production of Amyloid- β ($A\beta$), the major protein component of senile plaques, an AD hallmark. $A\beta$ is derived when APP is processed by the amyloidogenic pathway, with proteolytic action of β and γ -secretase on APP. Alternatively, the non-amyloidogenic pathway employs α -secretase to cleave within the $A\beta$ segment, precluding its formation.

APP is expressed abundantly in the brain and platelets. The 695 amino acid isoform is abundant in the brain while the Kunitz Protease Inhibitor (KPI) domain containing 751 and 770 amino acid isoforms are predominant in the platelets [1, 2]. Platelets, like neurons, are equipped with APP processing machinery, α -secretase (ADAM 10) and β -secretase (BACE) [3, 4]. Altered secretase activities [3–5] along with various biochemical differences [6–10] in AD platelets compared to age-matched controls have been previously reported.

Several studies have shown significantly lower platelet APP isoform ratios (APPr; 120–130 KDa to 106–110 KDa) in sporadic AD cases compared to healthy controls [11–14]. Padovani and colleagues found an overall sensitivity of 88.2% and specificity of 89.4% with a cut off APPr score of 0.57 when AD ($n=85$) were compared to controls ($n=95$) [15]. However, two previous studies conducted on ADAD platelets did not find a significant difference in APPr between ADAD MC and NC [16, 17], which may have been due to the small sample size of both studies. The upper band at 120–130 KDa is thought to represent the mature form while the lower band at 106–110 KDa may represent the immature form of APP [13, 14]. Since mature APP is subjected to amyloidogenic enzymatic processing [18] and altered APP cleavage activity has been consistently reported in AD, we hypothesized that platelet APPr (120KDa:110KDa) are lower in mutation carriers (MC) compared to non-

carriers (NC). Further, this study also investigated whether platelet APPr correlate with neuropsychological changes and brain imaging data.

MATERIALS AND METHODS

Study Design

The longitudinal multicentre Dominantly Inherited Alzheimer Network (DIAN) study enrolled adult offspring of a biological parent carrying a mutation responsible for ADAD. Offspring who inherited the mutation (mutation carrier; MC) and those who did not (non-carrier; NC) are both enrolled in the DIAN Study. The DIAN study inclusion criteria were fully followed [19]. Participants with a medical or psychiatric illness that would interfere in completing initial and follow-up assessments or requiring nursing home-level care were not included in the study. Further, no patient included in the present study was on anticoagulant medication. Enrolled participants who inherited the mutation have an expected time (observed in years) to onset of symptoms (EYO). EYO is an estimated parameter and is calculated from the difference of each participant's age and the parent's age at symptom onset (AAO). Participants who have EYO greater than three are followed up every three years, while participants with EYO less than three, as well as those displaying cognitive symptoms, are followed up every year [19]. The current study only includes a subset of the DIAN cohort from one site, at baseline (Perth, WA).

Participants—Participants belonged to the Western Australian subset of the DIAN cohort at baseline (<http://www.dian-info.org/institutions.htm>). The subset comprised 15 MC and 12 NC. Among 15 MC, two *PS1* MC were symptomatic based on the Clinical Dementia Rating (CDR) scale [20], where CDR=0 was defined as asymptomatic (Asym) and CDR>0 was defined as symptomatic (Sym). All participants or their caregivers gave informed consent, and the Ethics Committee of the Hollywood Private Hospital (WA), Edith Cowan University and the University of Western Australia approved the study. Participants underwent blood and cerebrospinal fluid collection after fasting overnight, neuroimaging, and a comprehensive battery of neuropsychological tests [19]. The neuropsychological data of one NC was not considered reliable and hence omitted from statistical analyses.

Blood collection and platelet processing: Blood collected in standard EDTA tubes with Prostaglandin E1 (PGE1), using standard serologic techniques was fractionated and used for genotyping analysis and platelet collection [21]. Genotyping analysis for presence or absence of known family mutations and *APOE* allele were determined using standard PCR methodology. The platelet fraction collected from the blood was resuspended in PBS-PGE1, to prevent platelet aggregation (33.3 ng/ml, Sapphire Biosciences®) and frozen immediately at -80°C [21].

All platelet samples underwent two freeze-thaw cycles for cell lysis. Protease inhibitor cocktail (cOmplete Mini, Roche®) in Phosphate Buffered Saline (PBS) was added to the platelet sample after the first thaw on ice. The platelets were sonicated for 30s after the second thaw.

Platelet APP Measure

A protein assay was carried out for each platelet sample using the Pierce Micro BCA Protein Assay Kit (Thermo Scientific®, linear working range of 2–40 µg/mL). Semi-quantitative wet western blotting technique was used to measure the platelet APP isoforms (120 KDa and 110 KDa bands). Total protein of 20 µg in sample buffer was heated at 95°C for 10 min prior to loading on 4–12% Bis-Tris mini gels (NuPAGE®) which was followed by incubation with antibody 22C11 (1:20,000, Millipore®). 22C11 binds to amino acid residues 66–81 of APP and several previous APPr studies have also used 22C11 [4, 11, 12, 14]. Samples were tested in duplicates and to avoid day-to-day variations, each method was carried out for all samples simultaneously. Platelet preparation and subsequent analyses were carried out by individuals who were blind to the mutation status of the participants.

Neuropsychological Assessment and Neuroimaging

Participants underwent a comprehensive battery of neuropsychological measures as per the DIAN study protocol [19]. Within the battery of tests, Mini Mental State Examination (MMSE), range 0 (severe impairment) –30 (no impairment), as a measure of general cognitive function, and the Wechsler Memory Scale-Revised (WMS-III) Logical Memory (LM) subtest, Story A, Part-I (Immediate), range 0 (no recall) –25 (complete recall) and Part-D (Delayed), range 0 (no recall) –25 (complete recall) scores were considered for analysis in this study, as both tests are widely used in AD research [19]. Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) using ligands, ¹¹C Pittsburgh compound B (PiB) (for amyloid load) and ¹⁸F Fluorodeoxyglucose (FDG) (for glucose utilization) were performed for each participant according to the Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol, as previously described [19, 22]. For PET, standardized uptake value (SUV) data acquired 30 and 40 minutes post FDG and PiB injection, respectively, were normalized to the Pons SUV [23, 24], resulting in a region to Pons ratio termed the SUV ratio (SUVr).

Statistical Analysis

APP_r, neuroimaging and neuropsychological variables of interest were compared using univariate General Linear Model (GLM) within IBM® SPSS® version 20 between MC and NC, with and without adjustment for covariates age, EYO and APOE ε4. Due to the low number of males in the study cohort, we did not control for gender in the analysis. The MC group was subdivided into *PSI* MC and *APP* MC, and the *PSI* MC group was subdivided into *SymPSI* MC and *AsymPSI* MC for analyses. No further analysis was required for *APP* MC as all of them were asymptomatic. Pairwise comparison of group means were only conducted when the overall group effect within the Analysis of Variance table was significant. Residual analyses were used to assess whether the assumptions of the analyses conducted were satisfied. Variables of interest were log-transformed as required, in order for the normality and constant variance assumption of the GLM to hold, given that the central limit theorem cannot be invoked with such a small sample size. Fisher's exact test was performed for gender and APOE ε4 between MC and NC (Table 1 and Supplementary Table 1). APP_r were correlated with variables of interest with adjustment for covariates age and APOE ε4, using the Pearson's correlation coefficient (r) for all participants and separately

for MC, *PSI* MC, *AsymPSI* MC, *APP* MC and NC by partial correlations in IBM® SPSS® version 20 (Table 2).

RESULTS

Baseline demographic data, *APOE* genotype, major mutation type carried, MMSE, LM scores, SUVR for PiB and FDG PET, and hippocampal volume from MRI for the Western Australian subset of the DIAN cohort are presented in (Table 1 and Supplementary Table 1).

When participants were categorised into MC and NC, lower APPr ($p = 0.01$, Fig. 1) and higher brain amyloid load represented by PiB-SUVR (Caudate Nucleus (CN): $p = 0.01$; Precuneus (PC): $p < 0.01$) were observed in the MC group. On the other hand, no difference between MC and NC in brain glucose metabolism represented by FDG-SUVR, hippocampal atrophy represented by MRI volumes, cognitive functioning as assessed by MMSE and LM scores were observed. Controlling for age and *APOE* $\epsilon 4$ status also led to similar observations. However, when controlled for EYO and *APOE* $\epsilon 4$ status, APPr ($p < 0.05$) and PiB-PET SUVR in the CN ($p < 0.05$) only, remained different between NC and MC.

Categorizing MC based on the mutation (namely, *PSI* or *APP* mutations) we continued to find lower APPr in MC (*PSI* MC ($p < 0.01$) and *APP* MC ($p < 0.05$)) compared to NC. PiB-PET SUVR was higher in *PSI* MC ($p = 0.001$) compared to NC in both PC and CN, and in *APP* MC compared to NC in the CN ($p < 0.05$). No difference in brain glucose metabolism, hippocampal atrophy ($p = 0.070$), MMSE ($p = 0.059$) and LM scores were observed between NC and both *APP/PSI* MC. Controlling for age and *APOE* $\epsilon 4$ status resulted in similar observations, except that no difference was found in APPr between NC and *APP* MC ($p = 0.081$). In addition, a lower hippocampal volume ($p < 0.05$) was observed in *PSI* MC compared to NC. After controlling for EYO and *APOE* $\epsilon 4$, only PiB-PET SUVR was higher in *PSI* MC versus NC, both in PC and CN ($p = 0.01$). Additionally, the adjusted analyses recorded higher brain amyloid load and hippocampal atrophy, and lower glucose metabolism in *PSI* MC versus *APP* MC. Table 1 and Supplementary Table 1 present results when *PSI* MC were stratified based on disease stage into Sym ($n = 2$) and Asym ($n = 5$) *PSI* MC, however the numbers were very low.

A negative correlation was found between APPr and PiB-PET SUVR scores (Fig. 2), after adjusting for covariates age and *APOE* $\epsilon 4$, in all participants (PC: $r = -0.510$, $p < 0.05$). A trend continued when the cohort was further stratified into, MC (PC: $r = -0.512$, $p = 0.074$) and became significant in *PSI* MC (PC: $r = -0.969$, $p < 0.01$). The correlation between platelet APPr and FDG-PET was found to be significant in *PSI* MC (PC: $r = 0.894$, $p < 0.05$). Hippocampal atrophy represented by MRI was not found to correlate with APPr in any of the categories into which the cohort was stratified.

Additionally, no correlation was observed between APPr and MMSE in the total sample and after stratification into categories. APPr correlated with LM-I in MC ($r = 0.555$, $p < 0.05$) and *PSI* MC ($r = 0.931$, $p < 0.05$) but did not correlate across other categories into which the cohort was categorised (Refer to Table 2 for all correlations).

We also correlated APPr with plasma A β ₄₀, A β ₄₂ and CSF A β ₄₂, tau and phosphorylated-tau181 (data obtained from DIAN database; levels of plasma A β species, and CSF A β and tau species were measured by xMAP technology INNO-BIA Plasma A β Forms Multiplex Assay™, Innogenetics, Ghent, Belgium and INNO-BIA AlzBio3™, Innogenetics, Ghent, Belgium, respectively). We observed that plasma A β ₄₀ ($r = -.434$, $p < 0.05$) and A β ₄₂ ($r = -.394$, $p < 0.05$) inversely correlated with APPr while CSF A β ₄₂ ($r = .422$, $p < 0.05$) positively correlated with APPr in all participants. CSF tau ($r = -.534$, $p = 0.007$) and CSF phosphorylated-tau181 ($r = -.414$, $p = 0.044$) inversely correlated with APPr in all participants.

DISCUSSION

The DIAN cohort is a very unique and powerful cohort as it comprises both symptomatic and asymptomatic individuals carrying a mutation responsible for ADAD. This enables tracking biochemical changes that culminate in disease pathology in individuals predestined to be affected by the disease. Another unique aspect of this study was the investigation of a correlation between APPr and brain amyloid load, brain glucose metabolism, hippocampal atrophy and cerebrospinal fluid tau and A β concentrations which to our knowledge has not been addressed previously.

The present study shows that APPr are significantly lower in MC compared to NC before cognitive dysfunction (as assessed using neuropsychological measures) or hippocampal atrophy (represented by MRI data) can be detected between MC versus NC. This decreased APPr in MC may suggest that altered APP processing in ADAD pathology occurs prior to its clinical manifestations, such as cognitive decline and hippocampal atrophy.

We observed that APPr significantly correlate with PiB-SUVr in all participants, where APPr decrease with increasing PiB-SUVr. SUVr of the CN and PC regions of the brain were chosen as they have early A β deposition in ADAD pathology [19, 25]. Interestingly we did not find a correlation between APPr and PiB-SUVr in the *APP* MC group and MC group as a whole, while a strong correlation was observed in *PSI* MC. This is because the *APP* MC in this study carried the hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D) mutation (*APP* E693Q). In contrast to *PSI* mutation carriers who develop very aggressive pathology very early in life, individuals carrying the Dutch type (HCHWA-D) mutation (*APP* E693Q) belong to the other end of the spectrum where they develop pathology much later in life with the majority of the amyloid deposition in the blood vessels rather than as amyloid plaques, and hence not captured by PiB-PET. This may explain the lack of statistically significant correlation between APPr and PiB in *APP* MC and all MC (All MC = *PSI*MC + *APP* MC).

Despite previous studies on APPr in sporadic AD showing a positive correlation of APPr with MMSE scores [26, 27], we did not observe any correlation between APPr and MMSE scores in our study. This may have been attributed to most MC individuals in this study having a MMSE score in the normal range (i.e. ≥ 28) and perhaps due to APPr decline occurring prior to cognitive deterioration in ADAD. Consistent with our findings, a recent study on a Thai cohort comprising sporadic AD and healthy individuals, did not observe a

correlation between APPr and the Thai Mental State Examination (TMSE) [28]. Moreover, another study reported a significant positive correlation between APPr and MMSE at baseline, but at a one-year follow up when divided into decliners and non-decliners, the study did not see a correlation between the decline in APPr and the decline in MMSE in the decliner group [29]. Nonetheless, we did observe a significant correlation between APPr and LM-I scores in *PS1* MC. This, along with previous findings, implies that there may be an indirect association between cognition and APPr.

The exact biological meaning of lower platelet APPr is yet to be confirmed. However, altered α and β secretase levels [4], altered membrane fluidity [13], increased cleavage of mature APP and decreased cleavage of immature APP [30] are some of the hypotheses posited previously. Additionally, lower APPr observed in ADAD may be due to elevated cleavage of mature APP by secretases [18] responsible for A β production. We observed a negative correlation between APPr and plasma A β_{40} and A β_{42} . This can further be corroborated by the increased plasma A β_{42} levels reported in ADAD MC [31], since platelets have been reported to be the main source of APP and A β in the plasma [2, 32].

Additionally, Vignini *et al* [33] observed increased levels of total APP mRNA in sporadic AD patients compared to controls, while Pastorino *et al* [16] did not observe a significant difference in total APP mRNA between *PS2* MC and NC. These reports led us to compare the total amount of 120 KDa and 110 KDa APP species levels (total APP) between MC and NC. We did not find a significant difference between the two groups. However, when MC were split into *APP* MC and *PS1* MC, the total APP decreased in *APP* MC compared to NC ($p < 0.05$), while no significant difference in total APP was observed between *PS1* MC and NC. This decrease in total APP in *APP* MC could either reflect a decline in APP production, but more likely could represent enhanced degradation.

Across all studies on APPr where AD patients have been compared against control individuals, some have observed a denser upper band (~120 KDa) [4, 11, 14, 26, 28] while others reported a denser lower band (~110 KDa) [5, 12, 13, 15, 27]. These differences may be due to different rates of platelet activation during blood collection and platelet extraction, for instance by the use of a tourniquet or different anticoagulants between laboratories [34]. Additionally, while Rosenberg *et al.* [11] found a single band at 110 KDa, we and other studies found a double band [12, 27]. However, the basic consensus of lower APP isoform ratios in AD platelets compared to healthy control platelets remained the same in all studies.

To the best of our knowledge, only two studies have previously compared platelet APPr in ADAD MC with NC [16, 17]. Contrary to our results, neither found a significant difference in APPr between MC and NC. This difference in observation may be attributed to the small sample size of MC ($n = 4$) in the above mentioned studies, due to the rare occurrence of ADAD. Furthermore, we did not have any *PS2* MC in our study, in contrast to another [16]. Previous research conducted on platelet APP isoform ratios in sporadic AD corroborate our findings of lower APP ratios in AD [4, 5, 14, 35], emphasizing a similarity in the pathobiology between the more common sporadic AD and the rare autosomal dominant form of AD. Previously, a significant difference in APPr between AD patients and healthy

controls was reported, where the AD group comprised 56% early onset AD patients (satisfied NINCDS-ADRDA diagnostic criteria at age < 60 years) [13].

We acknowledge that this study has some limitations. Platelet collection is not part of the DIAN blood fractionation protocol and hence we only had access to platelets from the Western Australian study site. In addition, recruiting families for each mutation was difficult, and low numbers were expected as ADAD is rare. Due to the small number of subjects and varying AAO within each family, family effects could not be adjusted for in the analysis. Also, being a hypothesis based study with a modest sample size no corrections for multiple testing were made. Although both *PSI* and *APP* mutation carriers have been combined as MC for sample size purposes in this study, it should be noted that mutations in *PSI* responsible for ADAD are more aggressive than mutations in *APP* and thus exhibit disease pathology at an earlier age [36]. Further, although statistically significant results were observed between symptomatic and asymptomatic *PSI* MC, it is important to highlight the small sample size of these subgroups. Lastly, since antibody 22C11 also binds to amyloid precursor like protein (APLP), in addition to probing with antibody 22C11, we also probed with antibody 6E10, which binds to the A β region of APP that APLP is devoid of, and obtained similar bands, confirming the bands measured are APP (data not shown).

Currently an autopsy is the only definitive diagnosis for Alzheimer's disease (AD), while pre-mortem biomarkers in the cerebrospinal fluid and neuroimaging are invasive and uneconomical. This study shows differences in APP_r in individuals destined to develop AD (MC) compared to those who are non-carriers of the mutations (NC) through an easily accessible medium, the blood.

CONCLUSION

We reported here that APP_r are significantly lower in MC compared to NC. However the use of APP_r as a potential early biomarker (also, within a panel of biomarkers) needs to be explored further. The findings from this study will have to be validated in a larger sample size, such that the study could be conducted in terms of estimated years from expected symptom onset [19]. This will reveal the number of years prior to symptom onset where differences in APP_r between MC and NC begin to occur.

Finally, it is important to investigate platelet biomarkers for AD [2, 37–39]. Platelets from AD patients are biochemically different from platelets in healthy individuals [7, 40]. Furthermore, unlike plasma and serum, which most bio-marker studies focus on, platelets are mostly nucleic acid free and devoid of abundant concentrations of proteins such as albumin and IgG, which hamper the search for novel bio-markers deeper into the proteome [41]. The findings from previous studies and the present study should motivate bio-marker research to be extended to blood cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ADAD	Autosomal dominant Alzheimer's disease
MC	Mutation carriers
NC	Non-carriers
DIAN	Dominantly inherited Alzheimer network
APP	Amyloid Precursor Protein
APP_r	Amyloid Precursor Protein isoform ratio (120–130 KDa to 106–110 KDa)
Aβ	Amyloid- β
AAO	Age at symptom onset
EYO	Expected years to onset
Asym	Asymptomatic
Sym	Symptomatic
CDR	Clinical dementia rating
APOE	Apolipoprotein gene
MMSE	Mini mental state examination
LM-I	Wechsler Memory Scale-Revised, Logical Memory subtest, Story A, Immediate
LM-D	Wechsler Memory Scale-Revised, Logical Memory subtest, Story A, Delayed
PET	Positron emission tomography
PiB	¹¹ C Pittsburgh compound B
FDG	¹⁸ F Fluorodeoxyglucose
SUV_R	Standardized uptake value ratio
CN	Caudate nucleus
PC	Precuneus

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Fig. 1. Comparison of platelet APP isoform ratios in mutation carriers versus non-carriers

A) Representative western blot depicting APP isoforms in platelets of mutation carriers (MC) and platelets of non-carriers (NC) using the monoclonal antibody 22C11. Beta-actin (β actin) was used as a loading control. Box plots represent APP isoform ratios (120–130 KDa to 106–110 KDa) in NC versus in B) MC ($p = 0.01$), C) *PS1* MC ($p < 0.01$) and D) *APP* MC ($p < 0.05$) respectively. The line segment within each box plot represents the median of APP ratios and error bars represent the range of APP isoform ratios for each group.

Fig. 2. Correlation between APP isoform ratios and Pittsburgh compound B Standardized Uptake Value Ratio (SUVR)

APP isoform ratios (120–130 KDa to 106–110 KDa) correlated with Standardized Uptake Value Ratio of the Caudate Nucleus ($r=-0.510$; $p=0.013$) and Precuneus ($r=-0.505$; $p=0.014$) in all participants. The correlation coefficient was calculated using Pearson's correlation coefficient (r).

Table 1

Comparison of demographic characteristics, *APOE* $\epsilon 4$ status, APP ratios (120–130 KDa to 106–110 KDa), neuropsychological scores from Mini mental state examination (MMSE) and neuroimaging data from Pittsburgh compound B-PET (PiB) between mutation carriers (MC) and non-carriers (NC) using General Linear Model or two sided Fisher's exact test as appropriate. The MC group has been subdivided into *APP* MC and *PSI* MC and the *PSI* MC group has been subdivided into asymptomatic (Asym) *PSI* MC and symptomatic (Sym) *PSI* MC. Non adjusted analyses with Dunnett's post hoc test and adjusted analyses with covariates age and *APOE* $\epsilon 4$ are represented. SUVR is the standardized uptake value ratio of the region of interest to the Pons. Regions of interest include the Caudate Nucleus (CN) and Precuneus (PC). ‘*’, ‘**’, ‘***’, ‘***’ represent $p < 0.05$, ‘**’, ‘***’, ‘***’ and ‘***’ represent $p < 0.01$, and ‘***’ and ‘***’ represent $p < 0.001$. ‘*’ represents comparison between MC and NC groups, while ‘#’ represents comparison within MC groups. ‘†’ indicates p-values obtained from variables transformed to the logarithmic scale for analyses. The adjusted mean $\pm$ SE have been represented in logarithmic scale for variables transformed to the logarithmic scale for analyses. $P > 0.1$ have been represented as non-significant (NS).

MC vs NC	Non-Adjusted Mean $\pm$ SD			Adjusted Mean $\pm$ SE for Covariates Age and <i>APOE</i> $\epsilon 4$		
	MC	NC	p-value	MC	NC	p-value
Number F/M	13/2	9/3	NS	-	-	-
Age (years)	38.800 $\pm$ 8.858	36.000 $\pm$ 10.331	NS	-	-	-
EYO	6.200 $\pm$ 5.943	14.250 $\pm$ 8.390	0.007**	-	-	-
<i>APOE</i> $\epsilon 4$ carrier %	33	16	NS	-	-	-
APP ratio	0.22 $\pm$ 0.181	0.55 $\pm$ 0.370	0.003***†	-1.688 $\pm$ 0.184	-0.887 $\pm$ 0.206	0.009***†
MMSE	27.47 $\pm$ 2.696	28.18 $\pm$ 1.991	NS	27.074 $\pm$ 0.970	28.114 $\pm$ 0.603	NS
PiB-PC SUVR	0.84 $\pm$ 0.280	0.61 $\pm$ 0.044	0.005***†	-0.223 $\pm$ 0.060	-0.490 $\pm$ 0.071	0.011*†
PiB-CN SUVR	0.80 $\pm$ 0.216	0.57 $\pm$ 0.056	0.001***†	-0.253 $\pm$ 0.052	-0.544 $\pm$ 0.062	0.002***†
<i>APPMC</i> , <i>PSI/MC</i> vs NC						
Number F/M	6/2	7/0	NS	-	-	-
Age (years)	45.000 $\pm$ 5.806*	31.714 $\pm$ 5.823	0.012	-	-	-
EYO	6.250 $\pm$ 5.751*	6.143 $\pm$ 6.619*	0.030	-	-	-
<i>APOE</i> $\epsilon 4$ carrier %	25	43	NS	-	-	-
APP ratio	0.253 $\pm$ 0.238*	0.182 $\pm$ 0.084**	0.013†	-1.558 $\pm$ 0.284	-1.826 $\pm$ 0.294*	0.030†
MMSE	28.750 $\pm$ 1.389	26.00 $\pm$ 3.162	0.059	28.301 $\pm$ 0.766	25.729 $\pm$ 1.685	NS
PiB-PC SUVR	0.704 $\pm$ 0.141#	0.995 $\pm$ 0.328***	0.000†	-0.388 $\pm$ 0.081#	-0.053 $\pm$ 0.083***	0.002†
PiB-CN SUVR	0.692 $\pm$ 0.086*	0.935 $\pm$ 0.253***	0.000†	-0.382 $\pm$ 0.072#	-0.119 $\pm$ 0.074***	0.001†
<i>AsymPSI</i> , <i>SymPSI</i> vs NC						
	<i>Asym PSI</i>	<i>Sym PSI</i>	NC	<i>Asym PSI</i>	<i>Sym PSI</i>	NC
			p-value			p-value

MC vs NC	Non-Adjusted Mean±SD			Adjusted Mean±SE for Covariates Age and APOE ε4		
	MC	NC	p-value	MC	NC	p-value
Number F/M	5/0	2/0	9/3 NS	-	-	-
Age (years)	31.800±7.050	31.500±2.121	36.00±10.331 NS	-	-	-
EYO	7.600±7.503	2.500±0.707	14.250±8.390 0.066	-	-	-
APOEε4 carrier %	40	50	16 NS	-	-	-
APP ratio	0.221±0.063	0.085±0.029**	0.550±0.370 0.008 [†]	-1.558±0.292	-2.479±0.460**	-0.847±0.189 0.017 [†]
MMSE	27.400±0.548 [#]	22.500±4.950***	28.182±1.991 0.001 [†]	3.310±0.040 [#]	3.098±0.063***	3.337±0.027 0.002 [†]
PIB-PC SUVR	0.838±0.184**	1.388±0.277***	0.610±0.044 0.000 [†]	-0.204±0.065***	0.303±0.103***	-0.490±0.044 0.000 [†]
PIB-CN SUVR	0.825±0.201***	1.209±0.106***	0.579±0.056 0.000 [†]	-0.245±0.072 **	0.146±0.113***	-0.531±0.049 0.000 [†]

Table 2

Correlation of APP isoform ratios (120–130 KDa to 106–110 KDa) with Mini mental state examination (MMSE), logical memory story A; Immediate (LM-I) and Delayed (LM-D), Pittsburgh compound B-PET (PiB) SUVR, Fluorodeoxyglucose-PET (FDG) SUVR and MRI hippocampal volume (H) using Pearson's correlation coefficient (r) in all participants and separately in mutation carriers (MC), *PS1* MC, *APP* MC and non-carriers (NC) adjusting for covariates age and *APOE* ϵ 4 status. SUVR is the standardized uptake value ratio of the region of interest to the Pons. Regions of interest include the Caudate Nucleus (CN) and Precuneus (PC). $P > 0.1$ have been represented as non-significant (NS).

Category	MMSE	LM-I	LM-D	PiB SUVR-CN	PiB SUVR-PC	FDG SUVR-CN	FDG SUVR-PC	MRI-H
All participants N=27	$r=.289$ NS	$r=.356$ $p=.095$	$r=.248$ NS	$r=-.505$ $p=.014$	$r=-.510$ $p=.013$	$r=-.025$ NS	$r=.065$ NS	$r=.043$ NS
MC N=15	$r=.247$ NS	$r=.555$ $p=.049$	$r=.476$ NS	$r=-.497$ $p=.084$	$r=-.512$ $p=.074$	$r=-.133$ NS	$r=.245$ NS	$r=-.267$ NS
<i>PS1</i> MC N=7	$r=.571$ NS	$r=.931$ $p=.022$	$r=.794$ NS	$r=-.774$ NS	$r=-.969$ $p=.006$	$r=-.632$ NS	$r=.894$ $p=.041$	$r=-.159$ NS
<i>APP</i> MC N=8	$r=-.158$ NS	$r=.270$ NS	$r=.210$ NS	$r=-.672$ NS	$r=-.391$ NS	$r=-.573$ NS	$r=-.597$ NS	$r=-.710$ NS
NC N=12	$r=-.085$ NS	$r=-.029$ NS	$r=-.248$ NS	$r=.038$ NS	$r=-.509$ NS	$r=.077$ NS	$r=-.157$ NS	$r=.043$ NS