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Common Variants in *PLD3* and Correlation to Amyloid-Related Phenotypes in Alzheimer's Disease

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Abstract

The phospholipase D3 (*PLD3*) gene has shown association with Alzheimer's disease (AD). However, the role of *PLD3* common variants in amyloid-β (Aβ) pathology remains unclear. We examined the association of thirteen common single nucleotide polymorphisms (SNPs) with cerebrospinal fluid (CSF) $A\beta_{1–42}$ levels and florbetapir retention on florbetapir ^{18}F amyloid positron emission tomography (AV45-PET) in a large population. We found that one SNP (rs11667768) was significantly associated with CSF $A\beta_{1–42}$ levels in the normal cognition group. We did not observe an association of any SNP with florbetapir retention. Our study predicted the potential role of *PLD3* variants in Aβ pathology.

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150110.

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Keywords

Alzheimer's disease; amyloid-β; association; *PLD3*

INTRODUCTION

Amyloid-β (Aβ) peptide deposition is considered to be the major pathological feature of Alzheimer's disease (AD), and the pathophysiological process related to brain amyloidosis is ongoing years prior to any clinical manifestations [1]. This has placed a great emphasis on identifying factors and mechanisms that promote brain amyloidosis. To date, several genetic variants in *APOE, CALHM1*, and *PICALM* have been reported to show association with Aβ deposition [2, 3]. Phospholipase D3 (*PLD3*) gene, coding for the PLD3 protein, is a new candidate gene for AD. Recent work has demonstrated that PLD3 was involved in the processing of the amyloid-β protein precursor (AβPP) and might play an important role in Aβ deposition [4]. Given that *PLD3* has been shown to correlate with AD, the role of *PLD3* common variants in Aβ pathology is still unclear.

The brain amyloid burden could be sensitively detected by some amyloid-related phenotypes. Cerebrospinal fluid (CSF) $A\beta_{1-42}$ and florbetapir ^{18}F amyloid positron emission tomography (AV45-PET) have both shown high specificity in reflecting brain amyloid burden, and are proposed as established endophenotypes for AD [5, 6]. Moreover, AD pathology could be found in the brains of nondemented elderly and mild cognitive impairment (MCI) patients, and genetic factors might play different roles at different levels of disease severity. Therefore, the goal of our study is to investigate a possible association between *PLD3* variants and Aβ-related phenotypes in normal cognition (NC), MCI, and AD patients separately, as well as combined diagnosis subjects.

METHODS

Participants

All participants included in this study were enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, a multicenter publicly funded longitudinal study of individuals with AD, MCI, and NC. Here, we restricted the present analysis to participants whose genotype data of *PLD3* single nucleotide polymorphisms (SNPs) were available. Furthermore, we selected only non-Hispanic (Caucasian) participants in order to avoid the population stratification effects which can lead to spurious findings. Finally, 812 individuals including 281 NC, 483 MCI (including 63 who converted to AD and 420 who did not), and 48 AD were included in our study.

Genotyping

We extracted the SNP genotypes of *PLD3* from the genome wide association study of ADNI for all included participants. Details of the genotyping methods were presented previously [7]. After quality control procedures, thirteen tag SNPs which captured the greatest amount of common variations in *PLD3* (rs7249146, rs11667768, rs10422343, rs4490097,

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rs11666860, rs12151243, rs45441197, rs11672825, rs10407447, rs4254419, rs4803330, rs62107640, rs112703240) remained for data analysis.

CSF $A\beta_{1-42}$ measures

Methods for CSF acquisition and biomarker measurement using the ADNI cohort have been reported previously [8]. The CSF concentrations of $A\beta_{1-42}$ were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit–based reagents. CSF measures for this article were cross-sectional from the baseline evaluation. The final samples for CSF analyses included 609 individuals (201 NC, 367 MCI, and 41 AD) with baseline CSF and corresponding genetic data.

AV45-PET measures

The AV45-PET phenotypic data were processed by the Jagust Lab, the University of Berkeley. A detailed description of PET image acquisition and processing can be found at http://adni.loni.usc.edu/data-samples/pet/. Briefly, we extracted the mean florbetapir uptake of four cortical grey matter regions (frontal, angerior/posterior cingulate, lateral parietal, lateral temporal) and cortical standard uptake value ratios (SUVR). SUVR were calculated by averaging across the four cortical regions and dividing this average by whole cerebellum. Each mean florbetapir uptake of the four main regions and cortical SUVR were used for analysis. Of the 812 participants, we included 707 participants (including 237 NC, 423 MCI, and 47 AD) in AV45-PET analysis.

Data analysis

Demographic characteristics of our subjects were presented using means and standard deviations (SD) for continuous variables and proportions for categorical variables. Demographics and genotypic frequencies were compared using one-way analysis of variance (ANOVA) or chi-square tests. Each of the thirteen SNPs was examined for associations with amyloid-related phenotypes for each diagnostic group separately (NC, MCI, and AD) and the entire cohort. Additionally, we also stratified the MCI group into MCI converting to dementia (MCI converter) and MCI not converting to dementia (MCI nonconverter). We used a multiple linear regression model to test for the independent associations and the dosage effect of each minor allele, controlling for age, gender, education, and number of *APOE ε*4 allele. To account for multiple testing, we performed a Bonferroni correction (p_C) for the number of tests applied in each analysis. All results were reported as statistically significant if $p < 0.05$.

RESULTS

The demographics, clinical data, and SNP distributions are summarized in Table 1. No statistical differences were observed among NC, MCI, and AD patients when comparing the distribution of all the tested SNPs allele frequencies in our study.

After Bonferroni correction, only rs11667768 was significant associated with CSF $A\beta_{1-42}$ level in the NC group ($p = 0.00048$, $p_c = 0.006$) (Fig. 1). For rs11667768, minor allele

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carriers showed higher CSF $A\beta_{1–42}$ levels in a dose-dependent manner (CC < TC<TT), suggesting that the minor T allele was associated with less amyloid burden. Moreover, we observed some nominal associations between three SNPs (rs11667768, rs11666860, and rs62107640) and CSF $A\beta_{1–42}$ levels in the MCI group ($p = 0.037$, $p = 0.045$, and $p = 0.035$, respectively). However, no association remained statistically significant after Bonferroni correction (Supplementary Table 1). There was no evidence for an effect of all SNPs on CSF $A\beta_{1–42}$ levels in the AD group, combined group, and two MCI subgroups.

Regarding the AV45-PET analysis, we did not observe any association of all SNPs with florbetapir retention in four main regions (frontal, angerior/posterior cingulate, lateral parietal, lateral temporal), as well as the cortical SUVR in all groups. Although we detected some nominal associations for three SNPs on florbetapir retention before Bonferroni correction, these associations were not significant after multiple testing correction (Supplementary Table 1).

DISCUSSION

PLD3 was a non-classical poorly characterized member of the PLD superfamily of phospholipases, playing an important role in myogenesis during myotube formation [9]. Recently, Cruchaga et al. first reported that PLD3 was implicated in AβPP trafficking. Overexpression of PLD3 led to a significant decrease in extracellular $A\beta_{1–42}$ and $A\beta_{1–40}$, and knockdown of PLD3 leaded to a significant increase in extracellular $A\beta_{1–42}$ and $A\beta_{1–40}$ [4]. Therefore, the role of *PLD3* in AD might be mediated by modifying the expression level of PLD3, and subsequently influencing the Aβ deposition. It also should be noted that rare coding variants of PLD3 had been identified as AD susceptibility loci, whereas the role of common variants in AD remains unknown. In the current study, we found an association between rs11667768 and Aβ-related phenotype (CSF $A\beta_{1–42}$), suggesting that this common SNP might play a role in Aβ pathology. To our knowledge, this is the first study to examine the role of *PLD3* SNPs using an intermediate phenotype approach in a large sample at different levels of disease severity.

AD pathology is restricted to the brain, and hence CSF, which can reflect biochemical changes in the brain, is an optimal source of biomarkers (i.e., $A\beta_{1–42}$, total tau protein, and phosphorylated tau protein) for AD [10]. Of these biomarkers, $A\beta_{1–42}$ appears to be the core biochemical marker for the amyloidogenic process in AD. Several studies have reported a relationship between decreased CSF $A\beta_{1–42}$ and amyloid load in the brain [11, 12]. The present study demonstrated that one SNP (rs11667768) was strongly associated with $A\beta_{1–42}$ levels in normal population, independent of *APOE*. T allele carriers had significantly higher CSF $A\beta_{1–42}$ levels and a linear trend was observed. The most widely accepted explanation for the decreased CSF $A\beta_{1–42}$ in AD is that aggregation of Aβ into plaques results in reduced availability of Aβ to diffuse into the CSF [13]. Therefore, it may be that genetic variants in rs11667768 influenced pathways related to the aggregation of Aβ in the brain. This common SNP identified here might lie within the same linkage dis-equilibrium (LD) block with rare functional variants and have a potential effect on the expression or function of PLD3. It is worth noting that we did not find an association of rs11667768 with CSF $A\beta_{1–42}$ levels in MCI, AD, and the combined group. This might be explained because

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rs11667768 functions differently in individuals with normal cognition verses those with MCI and AD. Thence, rs11667768 contributes to brain amyloid burden in a way that can only be detected in normal populations. Minor T allele correlated with higher $A\beta_{1–42}$ levels was predicted to be protective for Aβ pathology in healthy adults.

None of the presently investigated SNPs showed significant association with florbetapir retention detected by AV45-PET. Currently, AV45-PET has generated increasing interest as a novel imaging agent to detect Aβ plaques [14]. In our study, AV45 uptake was evaluated in four target brain regions, which might not reflect the amyloid load of whole brain. Therefore, the lack of association might be a result of low statistical power, sample sizes, or more probably, not appropriate target brain regions (i.e., hippocampus, precuneus). Nevertheless, more association studies with larger sample sizes and more brain regions are still needed to confirm the present findings.

In conclusion, we have found one *PLD3* variant (rs11667768) associated with amyloid burden detected by CSF in normal individuals, suggesting the potential role of *PLD3* in Aβ pathology. Although this variant might specifically influence healthy individuals, it still could provide new insights into the underlying association between *PLD3* and AD. Further studies are now warranted to disentangle the detailed molecular mechanisms underlying the influence of *PLD3* on $A\beta_{1–42}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

[1]. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7, 280–292. [PubMed: 21514248]

[2]. Elias-Sonnenschein LS, Bertram L, Visser PJ (2012) Relationship between genetic risk factors and markers for Alzheimer's disease pathology. Biomark Med 6, 477–495. [PubMed: 22917148]

[3]. Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, Pasquier F, Galimberti D, Scarpini E, Mann D, Lendon C, Campion D, Amouyel P, Davies P, Foskett JK, Campagne F, Marambaud P (2008) A polymorphism in CALHM1 influences Ca2+homeostasis, Abeta levels, and Alzheimer's disease risk. Cell 133, 1149–1161. [PubMed: 18585350]

[4]. Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R, Harari O, Norton J, Budde J, Bertelsen S, Jeng AT, Cooper B, Skorupa T, Carrell D, Levitch D, Hsu S, Choi J, Ryten M, Hardy

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J, Trabzuni D, Weale ME, Ramasamy A, Smith C, Sassi C, Bras J, Gibbs JR, Hernandez DG, Lupton MK, Powell J, Forabosco P, Ridge PG, Corcoran CD, Tschanz JT, Norton MC, Munger RG, Schmutz C, Leary M, Demirci FY, Bamne MN, Wang X, Lopez OL, Ganguli M, Medway C, Turton J, Lord J, Braae A, Barber I, Brown K, Passmore P, Craig D, Johnston J, McGuinness B, Todd S, Heun R, Kolsch H, Kehoe PG, Hooper NM, Vardy ER, Mann DM, Pickering-Brown S, Kalsheker N, Lowe J, Morgan K, David Smith A, Wilcock G, Warden D, Holmes C, Pastor P, Lorenzo-Betancor O, Brkanac Z, Scott E, Topol E, Rogaeva E, Singleton AB, Kamboh MI, St George-Hyslop P, Cairns N, Morris JC, Kauwe JS, Goate AM (2014) Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 505, 550–554. [PubMed: 24336208]

[5]. Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, Pontecorvo MJ, Hefti F, Carpenter AP, Flitter ML, Krautkramer MJ, Kung HF, Coleman RE, Doraiswamy PM, Fleisher AS, Sabbagh MN, Sadowsky CH, Reiman EP, Zehntner SP, Skovronsky DM (2011) Use of florbetapir-PET for imaging beta-amyloid pathology. JAMA 305, 275–283. [PubMed: 21245183]

[6]. Kauwe JS, Wang J, Mayo K, Morris JC, Fagan AM, Holtzman DM, Goate AM (2009) Alzheimer's disease risk variants show association with cerebrospinal fluid amyloid beta. Neurogenetics 10, 13–17. [PubMed: 18813964]

[7]. Saykin AJ, Shen L, Foroud TM, Potkin SG, Swaminathan S, Kim S, Risacher SL, Nho K, Huentelman MJ, Craig DW, Thompson PM, Stein JL, Moore JH, Farrer LA, Green RC, Bertram L, Jack CR, Jr, Weiner MW (2010) Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. Alzheimers Dement 6, 265–273. [PubMed: 20451875]

[8]. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter W, Lee VM, Trojanowski JQ (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 65, 403–413. [PubMed: 19296504]

[9]. Osisami M, Ali W, Frohman MA (2012) A role for phospholipase D3 in myotube formation. PLoS One 7, e33341. [PubMed: 22428023]

[10]. Blennow K, Hampel H, Weiner M, Zetterberg H (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 6, 131–144. [PubMed: 20157306]

[11]. Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM (2006) Inverse relation between *in vivo* amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 59, 512–519. [PubMed: 16372280]

[12]. Strozyk D, Blennow K, White LR, Launer LJ (2003) CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 60, 652–656. [PubMed: 12601108]

[13]. Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, Chang L, Miller B, Clark C, Green R et al. (1995) Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. Ann Neurol 38, 643–648. [PubMed: 7574461]

[14]. Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, Green RC, Harvey D, Jack CR, Jagust W, Liu E, Morris JC, Petersen RC, Saykin AJ, Schmidt ME, Shaw L, Shen L, Siuciak JA, Soares H, Toga AW, Trojanowski JQ (2013) The Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception. Alzheimers Dement 9, e111–e194. [PubMed: 23932184]

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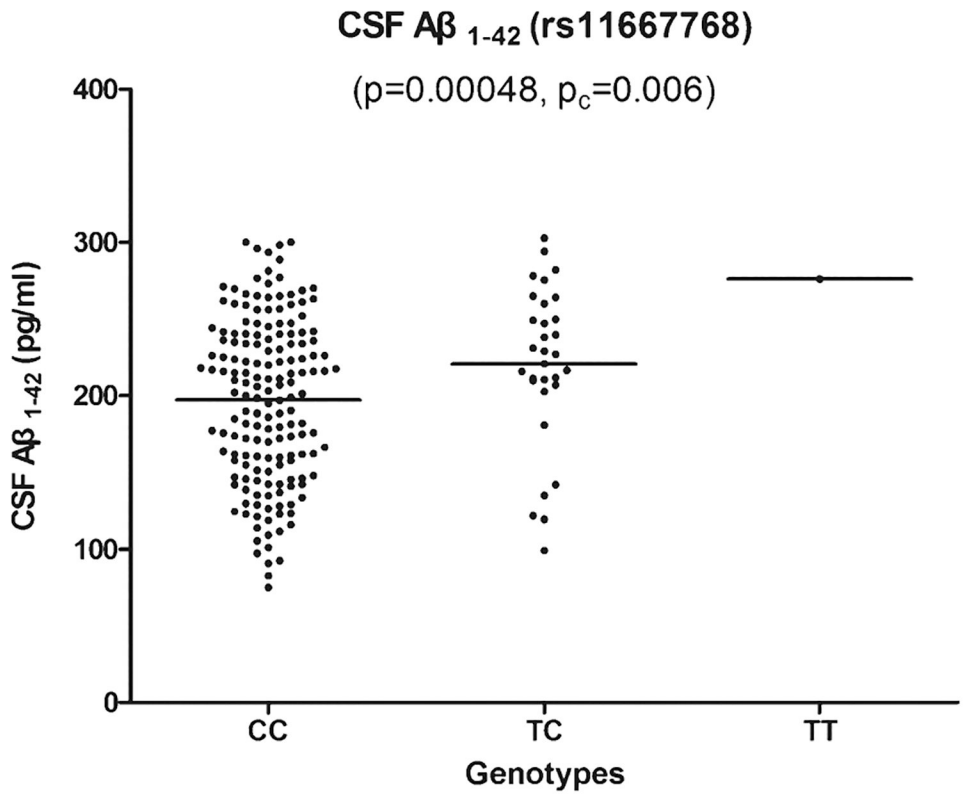

Fig. 1.
Cerebrospinal fluid (CSF) $A\beta_{1-42}$ levels in relation to rs11667768 in the NC group.

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Table 1

Demographic and clinical characteristics

	n	NC	n	MCI	n	AD	*p*	*p*<0.05
Age (years)	281	74.51 ± 5.56	483	72.28 ± 7.44	48	75.51 ± 9.22	<0.0001	b, c
Gender (men: women)	281	136/145	483	282/201	48	30/18	0.035	–
Education (years)	281	16.41 ± 2.66	483	15.98 ± 2.82	48	15.72 ± 2.62	0.079	–
APOE ε4 (0/1/2)	275	198/70/7	480	260/179/41	47	14/24/9	<0.0001	
CDRSB	281	0.03 ± 0.13	483	1.44 ± 0.87	48	4.44 ± 1.69	<0.0001	a, b, c
ADAS11	281	5.79 ± 2.95	483	9.50 ± 4.41	48	19.34 ± 6.87	<0.0001	a, b, c
ADAS13	281	9.06 ± 4.23	483	15.1 ± 6.90	48	29.80 ± 8.44	<0.0001	a, b, c
MMSE	281	29.07 ± 1.15	483	27.89 ± 1.69	48	22.96 ± 2.03	<0.0001	a, b, c
RAVLT total	281	44.64 ± 10.10	483	36.16 ± 10.86	48	21.67 ± 8.98	<0.0001	a, b, c
FAQ	281	0.17 ± 0.66	483	2.80 ± 4.06	48	12.60 ± 7.14	<0.0001	a, b, c
Hippocampus (mm^3)	256	7341.85 ± 897.22	421	6998.67 ± 1127.14	38	5763.42 ± 960.07	<0.0001	a, b, c
Genotypes								
rs11667768(CC/TC/TT)	275	229/45/1	476	419/52/5	46	39/7/0	0.195	
rs7249146 (TT/CT/CC)	281	120/130/31	483	212/215/56	48	17/26/5	0.782	–
rs10422343 (TT/GT/GG)	281	189/83/9	483	331/134/18	48	37/11/0	0.540	
rs11666860 (TT/CT/CC)	281	232/48/1	482	325/53/5	48	39/9/0	0.437	
rs4490097(AA/CA/CC)	280	104/133/43	479	159/241/79	46	14/26/6	0.707	–
rs45441197 (CC/TC/TT)	281	209/70/2	483	386/93/4	47	34/13/0	0.318	
rs11672825 (TT/GT/GG)	281	98/125/58	483	167/238/78	48	17/26/5	0.313	
rs12151243(GG/TG/TT)	281	190/84/7	483	336/129/18	48	37/11/0	0.400	–
rs10407447(AA/GA/GG)	281	195/80/6	482	330/138/14	47	29/16/2	0.803	–
rs4254419 (GG/TG/TT)	281	189/86/6	483	339/132/12	48	35/11/2	0.702	
rs4803330 (AA/GA/GG)	280	95/134/51	483	163/232/88	48	13/26/9	0.913	
rs62107640 (GG/AG/AA)	266	184/77/5	454	322/103/29	45	28/16/1	0.120	
rs112703240 (GG/AG/AA)	281	191/82/8	480	320/140/20	48	37/9/2	0.512	

NC, normal cognition; MCI, mild cognitive impairment; AD, Alzheimer's disease; CDRSB, Clinical Dementia Rating scale sum of boxes; ADAS, Alzheimer's Disease Assessment Scale; MMSE, Mini-Mental State Exam; RAVLT, Rey Auditory Verbal Learning Test; FAQ, Functional Activities Questionnaire. Data are presented as means ± SD for continuous variable and proportions for categorical variables. *p* value indicates the value for the main effect of each group, as assessed with analyses of one-way analysis of variance (ANOVA) or chi-square tests. [a]AD versus CN; [b]MCI versus CN; [c]AD versus MCI.