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PLD3-variants in population studies

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Cruchaga *et al*.[1] report that rare genetic variants in *PLD3*(phospholipase D3) are associated with increased Alzheimer's disease(AD) risk. They showed that *PLD3* is involved in amyloid-β precursor protein (APP) processing and overexpressed in brain tissue from AD patients. However, even the key variant *PLD3*-Val232Met, did not pass genome wide significance. This observation raises the question if the *genetic* association of *PLD3* with AD replicates. We associated *PLD3*-Val232Met with AD in 3 large population-based studies and 3 case-control studies. In total, we meta-analyzed results from 1914 AD cases and 8021 controls of European descent. Additionally we searched for other coding *PLD3*-variants in sequence data of 1067 AD cases and 1553 controls.

Carrier frequencies of *PLD3*-Val232Met in controls ranged from 0.34-1.42%, consistent with 0-1.17% reported by Cruchaga *et al*.[1] (Table 1). Likewise, the frequencies of *PLD3*-Val232Met in cases ranged from 0.66-2.19% compared to 0.7-2.6%.[1] We note that the range of carrier frequencies overlaps between cases and controls, such that in some population based cohorts, the carrier frequency in controls (e.g., 1.28% in FHS) is higher than that of cases in other cohorts (e.g., 0.68% in AGES). *Within* each cohort frequencies of *PLD3*-Val232Met were higher in cases than controls (Table 1), but in none of the populations the case carrier frequency for *PLD3*-Val232Met was significantly increased. However, pooled analyses showed a 1.94 fold increased risk of AD of carriers compared to non-carriers (Odds Ratio [OR] 1.94, adjusted for age and sex, 95% confidence interval=1.05 to 3.57) that was marginally significant (*p-value* = 0.03)(Table 1). Of note, the crude ORs often differed considerably from the age and sex adjusted estimates. With the exception of ADNI, the ORs were higher after adjustment for age and sex, suggesting that many a-symptomatic carriers were relatively young compared to cases and age needs to be controlled for in the analyses as a putative confounder.

We further associated other coding variants in *PLD3* with AD and performed a gene-based test using sequence data from two studies encompassing 1067 AD cases and 1553 controls. We meta-analyzed results of whole genome sequence data of the ADNI study, 499 AD cases and 293 controls, with results of a combined cohort of 568 Dutch AD cases and 1260 Dutch controls. We observed 21 rare polymorphic coding variants and 1 splice site variant. Of the 20 observed *PLD3*-variants detected by Cruchaga *et al*.[1], we observed 9 (S63G, P76A, V232M, N284S, C300Y, A442A, G452E, D447G and R488C). Five variants showed the same direction of effect as seen by Cruchaga *et al*.[1]. *PLD3*-A442A was one of the variants that showed a same direction of effect(Odds Ratio [OR] 1.24, 95% confidence interval = 0.74 to 2.06, *p-value* = 0.41). After correcting the p-value for multiple testing, none of the variants observed in our study conferred a significant increase in AD risk. Gene-based analysis also did not show significant association of *PLD3*-variants with AD risk (SKAT-O *p-value* = 0.61 and burden test OR 1.27 95% confidence interval [CI] =0.85 to 1.9, *p-value* = 0.24).

In conclusion, the carrier frequencies of *PLD3*-Val232Met in our data set are consistent with those reported by Cruchaga *et al*.[1] and we showed a nominally significant association of *PLD3*-Val232Met with AD. This is in contrast to findings presented in companion papers by Heilmann *et al*., Lambert *et al*. and Hooli *et al*. However, in contrast to Cruchaga *et al*.[1] we found no significant association of other *PLD3*-variants with AD in the single variant or gene based analyses. Therefore, in our analyses *PLD3* does not yet meet the criteria proposed by MacArthur et al.[2] to be implicated in AD. Hence, our data do not strongly support an important contribution of rare *PLD3*-variants in the etiology of AD. The most notable findings in our study are the need to control for age as a confounder in rare variant analyses and the high variability of the frequency of *PLD3*-Val232Met across populations. The latter finding highlights the need for careful matching of cases and controls for ethnic background when investigating rare variants.

Methods

RS[3], FHS[4] and AGES[5] were genotyped on the Illumina exome chip[6]. Amsterdam Dementia cohort[7], Alzheimer Center Erasmus MC and RS underwent whole exome sequencing at Center for Biomics, Rotterdam. RS exome sequence and exome chip data partially overlapped, genotypes were concordant and non-overlapping samples were used in *PLD3*-Val232Met analysis. As covariates gender and age at onset for AD cases or the date of last examination/censoring for cognitively healthy controls were used. GRIP[8] was imputed using the Dutch specific reference panel(Imputation quality [Rsq]=0.74)[9,10]. Subjects aged below 55 were excluded. The R-package “seqMeta”(version seqMeta_1.4) was used for meta-analysis of single variant score test and gene-based test.

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Table 1

Association of *PLD3*-Val232Met with Alzheimer's disease

Cohort	Cases			Controls			Overall carrier frequency (%)	Crude OR	OR (95% CI)	P-value
	Carriers (N)	Non-carriers (N)	Carrier frequency (%)	Carriers (N)	Non-carriers (N)	Carrier frequency (%)				
AGES	1	145	0.68	12	2371	0.50	0.51	1.36	3.18 (0.17 - 58.73)	0.44
Dutch Alzheimer centers	3	451	0.66	4	609	0.65	0.66	1.01	1.55 (0.25 - 9.38)	0.64
GRIP	2	109	1.80	14	975	1.42	1.45	1.28	1.58 (0.25 - 9.91)	0.62
RS	6	470	1.26	23	2389	0.95	1.00	1.33	1.43 (0.51 - 4.03)	0.49
ADNI	7	492	1.40	1	292	0.34	1.01	4.15	2.94 (0.63 - 13.8)	0.17
FHS	5	223	2.19	17	1314	1.28	1.41	1.73	2.63 (0.71 - 9.70)	0.15
Combined	24	1890	1.25	71	7950	0.89	0.96	1.53	1.94 (1.05 - 3.57)	0.03

Odds Ratios(OR) and *p-values* from score tests are shown adjusted for age and sex, based on a logistic regression model. Crude ORs were calculated using carrier frequencies of cases and controls. Combined crude OR is the *Mantel-Haenszel* estimate of the pooled crude ORs(95% CI 0.91 to 2.57 and two-sided *p-value*=0.11). Age, Gene/Environment Susceptibility-Reykjavik Study(AGES). Framingham Heart Study(FHS) and Rotterdam Study(RS) where genotyped on the Illumina exome chip version 1.0. Genetic Research in Isolated Populations(GRIP) subjects were imputed. Dutch Alzheimer centers; encompass whole exome sequence data of AD cases from Amsterdam Dementia Cohort, Alzheimer Center Rotterdam MC and controls from RS(not genotyped on the exome chip). Alzheimer's Disease Neuroimaging Initiative(ADNI) samples are whole genome sequenced.