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DATA REPORT

Excess of rare coding variants in *PLD3* in late- but not early-onset Alzheimer's diseaseEva C Schulte^{1,2}, Alexander Kurz³, Panagiotis Alexopoulos³, Harald Hampel^{4,5}, Annette Peters⁶, Christian Gieger⁷, Dan Rujescu⁸, Janine Diehl-Schmid³ and Juliane Winkelmann^{1,2,9,10,11}

Recently, mutations in phospholipase D3 (*PLD3*) were reported in late-onset Alzheimer's disease (AD). By screening the coding regions of *PLD3* for variants in a European cohort of 1,089 AD cases, 182 individuals with frontotemporal lobar degeneration and 1,456 controls, we identified 32 variants with a minor allele frequency < 5% and observed an excess of rare variants in individuals with late- but not early-onset AD ($P=0.034$, χ^2 -test; odds ratio = 1.46).

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Genome-wide association studies and linkage analyses have identified at least 25 genes associated with sporadic and familial Alzheimer's disease (AD).¹ These genes include classical genetic factors contributing to familial, early-onset forms of AD, such as the β -amyloid precursor protein and the presenilins (PSEN1 and PSEN2),^{2–5} as well as several more recently discovered genes that harbor common variants associated with increased risk of late-onset AD (LOAD).^{6–9} Together, these genes explain ~61% of the population attributable risk of AD,^{8,10} and novel genetic factors continue to be revealed. Most recently, rare variants in phospholipase D3 (*PLD3*) were implicated in late-onset familial and sporadic AD both by family-based whole-exome sequencing and by genotyping and gene-based resequencing.¹¹ Here we assessed the role of *PLD3* variants in central European AD and frontotemporal lobar degeneration (FTLD) patients, particularly investigating the role of *PLD3* variants in early-onset AD (EOAD).

Using Idaho LightScanner high-resolution melting curve analysis (Biofire Diagnostics, Inc., Salt Lake City, UT, USA), we screened the 13 coding exons and exon–intron boundaries (± 10 bp) of *PLD3* in 1089 German AD case subjects (75.6 \pm 18.6 years, 59.3% female, including 139 cases with an age of onset younger than 65 (61.5 \pm 5.5 years, 53.2% female)), 138 German FTLD cases (63.7 \pm 8.1 years, 42.0% female) and 1,456 general population controls belonging to the KORA general population cohort¹² (58.3 \pm 12.0 years, 48.2% female) based in southern Germany. When altered melting patterns suggested variants, Sanger sequencing ensued to identify the underlying genetic alteration. Gene-based burden tests (cohort allelic sum test) and single-variant association tests were performed using χ^2 analysis.

All subjects were diagnosed according to the NINCDS-ADRDA criteria or the revised Neary *et al.*¹³ criteria as appropriate by a senior psychiatrist specializing in dementias. Ethics review board approval and participants' written informed consent were obtained before the initiation of the study.

We observed a total of 32 variants with minor allele frequency (MAF) < 5% within the coding regions ± 10 bp, including: 3 near-splice variants, 1 deletion, 9 synonymous and 16 non-synonymous variants and 3 newly introduced stop codons (Figure 1, Table 1). Eight of the coding variants (27.5%) had been observed previously.¹¹ Notably, we found the variant most significantly associated with the AD phenotype in the previous study,¹¹ *PLD3* p.Val232Met (rs145999145), more frequently in controls ($n=6$; MAF = 0.20%; 55.6 \pm 12.6 years; 33% older than 65 years) than in AD patients ($n=1$; MAF = 0.05%; 66 years). The single AD individual harboring the p.Val232Met variant presented with AD at age 64 and reported that her mother had also suffered from dementia. Moreover, the synonymous variant (*PLD3* p.Ala442Ala; rs4819) that had been significantly associated with LOAD in the original publication¹¹ did not show association with the AD phenotype in our sample in either LOAD or EOAD cases (Table 1). However, we did identify significant associations between two different variants in *PLD3* and AD in our sample: *PLD3* p.Ile364Ile (rs51787324; $P_{\text{nominal}}=3.0 \times 10^{-8}$; $P_{\text{corrected}}=9.6 \times 10^{-7}$; χ^2 test; odds ratio (OR) = 63.50 (95% confidence interval (CI): 3.85–1,046.15)) in both the LOAD-only and combined AD sample and *PLD3* p.Asn284Ser (rs200274020; $P_{\text{nominal}}=5.0 \times 10^{-6}$; $P_{\text{corrected}}=1.6 \times 10^{-5}$; χ^2 test; OR = 52.66 (95% CI: 2.52–1,099.83)). To date, these variants have only been found in individuals with either LOAD¹¹ or EOAD (this study).

A priori power calculation based on published variant frequencies and effect sizes¹¹ using the Purcell power calculator¹⁴ suggested that a sample size of 1,089 AD cases and an equal number of general population controls would be sufficient to reach 100% power to detect an excess of rare variants with a MAF < 5% under an autosomal dominant model and 36% power for recessive effects. Accordingly, we also performed gene-based burden tests for variants with a KORA-derived MAF < 5%. Interestingly, although we observed an excess (Figure 1,

¹Neurologische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; ²Institut für Humangenetik, Helmholtz Zentrum München, Neuherberg, Germany; ³Klinik und Poliklinik für Psychiatrie und Psychotherapie, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; ⁴AXA Research Fund; ⁵Sorbonne Universités, Université Pierre et Marie Curie, Paris 06, Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A) & Institut du Cerveau et de la Moelle épinière (ICM), Département de Neurologie, Hôpital de la Pitié-Salpêtrière, Paris, France; Université Pierre et Marie Curie-Paris 6, AP-HP, Hôpital de la Salpêtrière, Paris, France; ⁶Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, Germany; ⁷Institute of Genetic Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; ⁸Klinik für Psychiatrie, Universität Halle, Halle, Germany; ⁹Institut für Humangenetik, Technische Universität München, Munich, Germany; ¹⁰Munich Cluster for Systems Neurology (SyNergy), Munich, Germany and ¹¹Department of Neurology and Neurosciences, Stanford University, Palo Alto, CA, USA.

Correspondence: J Winkelmann (winkelmann@lrz.tum.de or winkelmann@stanford.edu)

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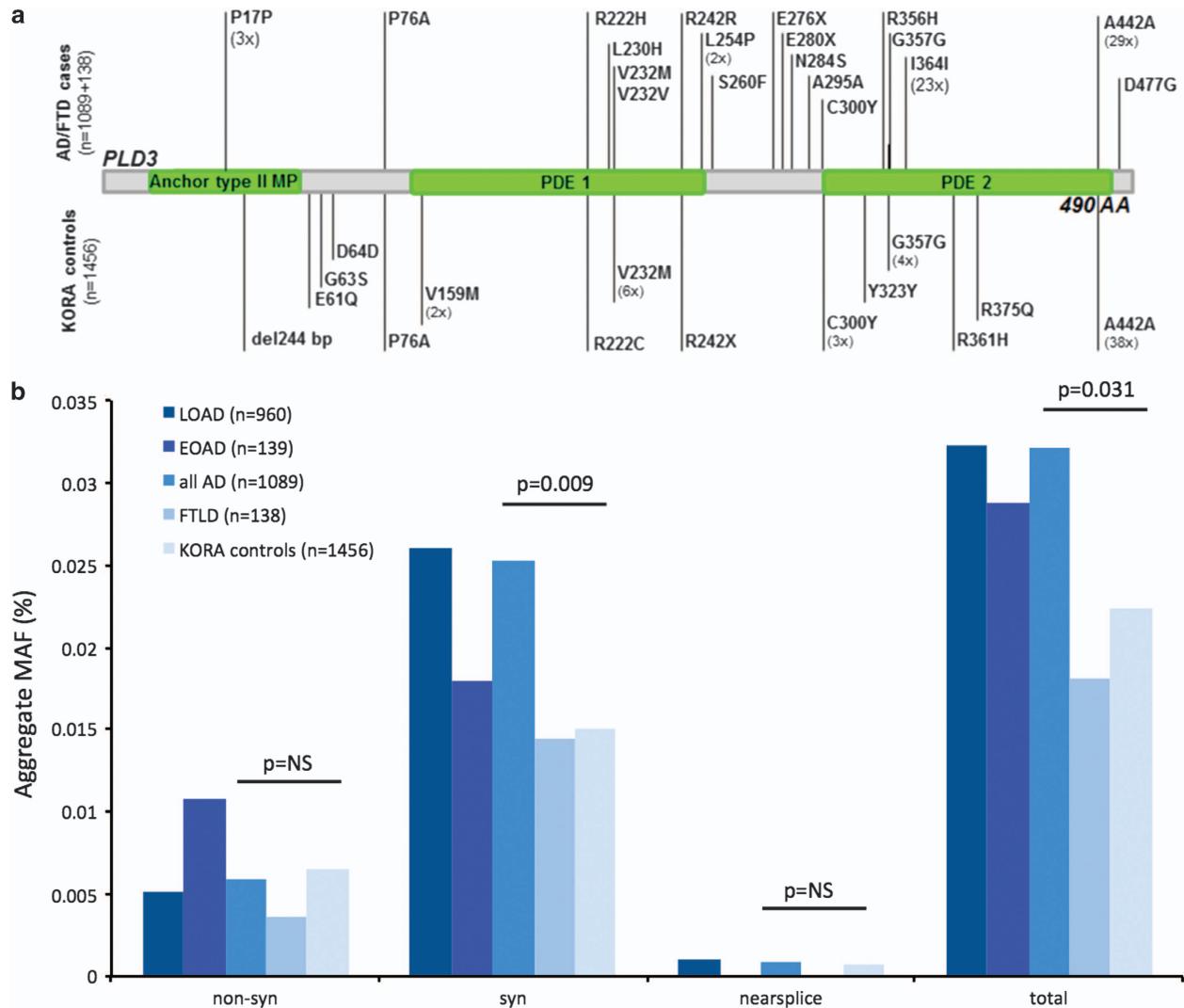


Figure 1. Rare coding variants in *PLD3* in AD, FTLD and control subjects. **(a)** Schematic representation of rare coding variants identified in *PLD3* in AD and FTLD case subjects (above the gene) and KORA general population controls (below the gene). Numbers in parentheses indicate variant counts. If no number is given, variants were identified only once. **(b)** Aggregate minor allele frequencies (MAFs) for variants of different classes in the different subsamples. *P*-values represent gene-based burden tests for rare variants in *PLD3* (MAF < 5%) based on variant counts (Supplementary Table S1) and calculated using χ^2 test statistics. AA, amino acids; AD, Alzheimer's disease; EOAD, EOAD, early-onset AD; FTLD, frontotemporal lobar degeneration; NS, not significant.

Supplementary Tables S1–S4) of rare variants of all classes in LOAD ($P=0.034$, χ^2 test; OR = 1.46 (95% CI: 1.02–2.07)) and LOAD+EOAD combined ($P=0.031$, χ^2 test; OR = 1.45 (95% CI: 1.03–2.05)), the same was not observed for EOAD alone ($P=0.54$, χ^2 test; OR = 1.28 (95% CI: 0.60–2.73)). This excess of rare variants included mainly synonymous variants (LOAD: $P=0.007$, χ^2 test; OR = 1.74 (95% CI: 1.15–2.62); LOAD+EOAD combined: $P=0.009$, χ^2 test; OR = 1.68 (95% CI: 1.13–2.52)). In the EOAD cases, however, non-synonymous variants were encountered at a MAF = 1.1%, which was almost twice as frequent as in either controls (MAF = 0.7%) or LOAD (MAF = 0.5%; Figure 1, Supplementary Table S2). This result fell short of statistical significance, possibly because of the small number of EOAD cases ($n=139$) in our sample.

Because an overlap in the genetic architecture of different dementia syndromes as well as neurodegenerative conditions^{15–17} has been described, we also assessed the contributions of rare variants in *PLD3* to the genetic framework of our FTLD samples. Rare variants in *PLD3* were found at an equal or lower frequency in FTLD case subjects relative to controls (Figure 1, Supplementary

Tables S1–S3), making a large-scale contribution of rare genetic variants in *PLD3* to the genetics of FTLD unlikely.

Both our data as well as previously published data¹¹ point to a significant contribution of a number of different rare synonymous variants in *PLD3* to the LOAD phenotype. This is especially interesting in light of the fact that although over 50 human diseases associated with synonymous mutations have been reported to date, few examples exist with regard to neuropsychiatric disorders.¹⁸ Functional assays have demonstrated that *PLD3* can directly alter β -amyloid precursor protein processing and β -amyloid formation,¹¹ and the apparent lack of contribution of rare *PLD3* variants in another neurodegenerative phenotype (that is, FTLD) indirectly supports this notion. It is known that *PLD3* p. Ala442Ala is associated with lower expression of total *PLD3* mRNA as well as lower levels of exon 11-containing transcripts. Whether a similar mechanism could be implicated in the association between *PLD3* p.Ile364Ile and, to a much lesser extent, p.Pro17Pro remains to be elucidated. Human Splicing Finder¹⁹ predicts that p.Ile364Ile ablates an enhancer, whereas p.Pro17Pro generates a

Table 1. Rare variants (MAF < 5%) identified by high-resolution melting curve analysis in 1,089 AD case subjects, 138 FTD case subjects and 1,456 KORA general population controls

Gene	Exon	Genome position	Variant	AA subst.	LOAD (n = 960)	EOAD (n = 139)	FTD (n = 138)	Controls (n = 1456) (dbSNP139)	Reference cases/controls	MAF AD	P _{nominal}	Class	PolyPhen2 (MAF)	1000G (MAF)	ESP (cases/ controls) ¹¹	Cruchaga et al. ¹¹
<i>PLD3</i>	Int 4	chr19:40872671	c.103-9T>A				1	Novel	0.00%/0.03%	NS	NS	Nearsplice	N/A	N/F	N/A	N/A
<i>PLD3</i>	Int 5	chr19:40873599	c.246-4 C>G				1	Novel	0.00%/0.03%	NS	NS	Nearsplice	N/A	N/F	N/A	N/A
<i>PLD3</i>	Int 9	chr19:40877785	c.879+5G>A				1	Novel	0.05%/0.00%	NS	NS	Nearsplice	N/A	N/F	N/A	N/A
<i>PLD3</i>	4	chr19:40872540	c.51 C>T	P17P	1 (homo)		1	rs200094590	0.13%/0.00%	0.03	0.03	Syn	N/A	0.04%	0.02%	N/F
<i>PLD3</i>	4/5	chr19:40872502_745	del 244bp	del exons 4 & 5	3		1	novel	0.00%/0.03%	NS	NS	Deletion	N/A	N/F	N/F	N/F
<i>PLD3</i>	5	chr19:40872758	c.181G>C				1	Novel	0.00%/0.03%	NS	NS	Non-syn	Benign	N/F	N/F	N/F
<i>PLD3</i>	5	chr19:40872764	c.187G>A				1	rs142070038	0.00%/0.03%	NS	NS	Non-syn	Benign	0.09%	0.16%	0.08%/0.12%
<i>PLD3</i>	5	chr19:40872769	c.192 C>T				1	Novel	0.00%/0.03%	NS	NS	Syn	N/A	N/F	N/F	N/F
<i>PLD3</i>	5	chr19:40872803	c.226 C>G				1	rs138674695	0.05%/0.03%	NS	NS	Non-syn	Benign	0.03%	0.03%	0.08%/0.00%
<i>PLD3</i>	7	chr19:40875860	c.475G>A				2	rs374184677	0.00%/0.06%	NS	NS	Non-syn	Deleterious	N/F	0.02%	N/F
<i>PLD3</i>	8	chr19:40876130	c.664 C>T				2	rs200077325	0.00%/0.03%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	8	chr19:40876131	c.665G>A				1	Novel	0.00%/0.00%	0.001	0.001	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877590	c.689T>A				1	Novel	0.05%/0.00%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877595	c.694T>A				6	rs145999145	0.05%/0.20%	NS	NS	Non-syn	Deleterious	0.30%	0.48%	1.36%/0.79%
<i>PLD3</i>	9	chr19:40877597	c.696G>T				1	Novel	0.05%/0.00%	NS	NS	Syn	N/A	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877625	c.724 C>T				1	Novel	0.00%/0.03%	NS	NS	Stop	N/A	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877627	c.726 AA>GG				1	Novel	0.05%/0.00%	NS	NS	Syn	N/A	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877662	c.761TT>CC				1 (homo)	Novel	0.09%/0.00%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877680	c.779 C>T				1	Novel	0.05%/0.00%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877727	c.826G>T				1	Novel	0.05%/0.00%	NS	NS	Stop	N/A	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877739	c.838G>T				1	Novel	0.05%/0.00%	NS	NS	Stop	N/A	N/F	N/F	N/F
<i>PLD3</i>	9	chr19:40877752	c.851A>G				2	rs200274020	0.09%/0.00%	5.0 × 10 ⁻⁶	5.0 × 10 ⁻⁶	non-syn	Deleterious	0.06%	N/F	N/F
<i>PLD3</i>	10	chr19:40880393	c.885G>A				1	Novel	0.05%/0.00%	NS	NS	Syn	N/A	N/F	N/F	N/F
<i>PLD3</i>	10	chr19:40880475	c.899G>A				3	rs146083475	0.05%/0.1%	NS	NS	Non-syn	Deleterious	N/F	0.09%	0.10%/0.04%
<i>PLD3</i>	10	chr19:40880477	c.969 C>T				1	rs369989744	0.00%/0.03%	NS	NS	Syn	N/A	N/F	0.02%	N/F
<i>PLD3</i>	11	chr19:40882563	c.1067G>A				1	Novel	0.05%/0.00%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	11	chr19:40882536	c.1071 C>T				4	rs147721393	0.05%/0.14%	NS	NS	Syn	N/A	N/F	0.10%	N/F
<i>PLD3</i>	11	chr19:40882578	c.1082G>A				1	Novel	0.00%/0.03%	NS	NS	Non-syn	Deleterious	N/F	N/F	N/F
<i>PLD3</i>	11	chr19:40882588	c.1092 C>T		15 (het)/4 (homo)		1	rs57187324	1.05%/0.00%	3.0 × 10 ⁻⁸	3.0 × 10 ⁻⁸	Syn	N/A	N/F	0.96%	4.62%/2.33% ^a
<i>PLD3</i>	11	chr19:40882620	c.1124G>A				1	rs374352480	0.00%/0.03%	NS	NS	Non-syn	Deleterious	N/F	0.00%	N/F
<i>PLD3</i>	13	chr19:40883933	c.1326G>A		20		39	rs4819	1.45%/1.30%	NS	NS	Syn	N/A	0.95%	1.59%	2.09%/0.90%
<i>PLD3</i>	13	chr19:40884037	c.1430G>A		1		1	rs14721330	0.05%/0.00%	NS	NS	Non-syn	Benign	0.04%	0.02%	0.02%/0.02%

Abbreviations: AA, amino acid; AD, Alzheimer's disease; EOAD, early-onset AD; ESP, Exome Sequencing Project; FTD, frontotemporal dementia; subst, substitution; Int, intron; LOAD, late-onset AD; MAF, minor allele frequency; N/A, not available; N/F, not found; Non-syn, non-synonymous; NS, nonsignificant; PLD3, phospholipase D3; syn, synonymous. ^aMAFs derived from the African-American sample because the variant was not identified in individuals of European ancestry by Cruchaga et al.¹¹

novel enhancer site. However, experimental evidence is lacking to date. In this context, although we did not observe a similar role of rare synonymous variants in EOAD cases, it seems noteworthy that we identified several non-synonymous *PLD3* variants in our small EOAD sample. One could hypothesize that non-synonymous variants with possibly larger effects might also contribute to this comparatively more severe phenotype. However, given the dearth of statistical significance to support this assumption, it currently remains a hypothesis at best.

When considering the individual variants identified in our screening and their contribution to AD genetics, an additional caveat would have to be that association *P*-values and ORs appear inflated possibly due to the small total number of variants, the small sample sizes (for EOAD) or the possible existence of unaccounted population substructure. Conversely, because we used general population controls, we are unable to exclude the possibility that some of the controls have or will develop AD, thus underestimating the calculated effect sizes. ORs between 50 and 60 typically suggest (near) monogenic disease. However, OR estimates for the total rare genetic variation in *PLD3* and its contribution to the AD phenotype (that is, ORs of ~1.5–2.0) seem more realistic because it is likely that these variants contribute to AD risk but are not causal by themselves.

In summary, our data corroborate the role of rare variants in *PLD3* and further highlight the significant contribution of rare synonymous variants in this gene to the genetic architecture of LOAD. Interestingly, the association between *PLD3* and LOAD was largely driven by variants not significantly associated with the phenotype in the original study,¹¹ whereas the individual variants showing significant associations in the original study could be replicated directly. While rare variants overall or synonymous variants alone do not seem to play a large role in bringing about EOAD, the role for non-synonymous *PLD3* variants in EOAD remains open to debate.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.526>, <http://dx.doi.org/10.6084/m9.figshare.hgv.530>, <http://dx.doi.org/10.6084/m9.figshare.hgv.532>, <http://dx.doi.org/10.6084/m9.figshare.hgv.534>, <http://dx.doi.org/10.6084/m9.figshare.hgv.536>, <http://dx.doi.org/10.6084/m9.figshare.hgv.538>, <http://dx.doi.org/10.6084/m9.figshare.hgv.540>, <http://dx.doi.org/10.6084/m9.figshare.hgv.542>, <http://dx.doi.org/10.6084/m9.figshare.hgv.544>, <http://dx.doi.org/10.6084/m9.figshare.hgv.546>, <http://dx.doi.org/10.6084/m9.figshare.hgv.548>, <http://dx.doi.org/10.6084/m9.figshare.hgv.550>, <http://dx.doi.org/10.6084/m9.figshare.hgv.552>, <http://dx.doi.org/10.6084/m9.figshare.hgv.554>, <http://dx.doi.org/10.6084/m9.figshare.hgv.556>, <http://dx.doi.org/10.6084/m9.figshare.hgv.558>, <http://dx.doi.org/10.6084/m9.figshare.hgv.560>, <http://dx.doi.org/10.6084/m9.figshare.hgv.564>, <http://dx.doi.org/10.6084/m9.figshare.hgv.566>.

COMPETING INTERESTS

The authors declare no conflict of interest.

Supplemental Information for this article can be found on the *Human Genome Variation* website (<http://www.nature.com/hgv>)

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