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Pathogenic Variants in the Longitudinal Early-Onset Alzheimer's Disease Study Cohort

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ABSTRACT

INTRODUCTION: One goal of the Longitudinal Early Onset Alzheimer's Disease Study (LEADS) is to investigate the genetic etiology of early onset (40-64 years) cognitive impairment. Towards this goal, LEADS participants are screened for known pathogenic variants.

METHODS: LEADS amyloid positive (EOAD) or negative (EOnonAD) cases were whole exome sequenced (*N*=299). Pathogenic variant frequency in *APP, PSEN1, PSEN2, GRN, MAPT,* and *C9ORF72* was assessed for EOAD and EOnonAD. Gene burden testing was performed in cases compared to similar-age cognitively normal controls in the Parkinson's Progression Markers Initiative Study.

RESULTS: Previously reported pathogenic variants in the six genes were identified in 1.35% of EOAD (3/223) and 6.58% of EOnonAD (5/76). No genes showed enrichment for carriers of rare functional variants in LEADS cases.

DISCUSSION: Results suggest that LEADS is enriched for novel genetic causative variants, as previously reported variants are not observed in most cases.

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1 Introduction

Although early onset Alzheimer's disease (EOAD) has been estimated to be highly heritable (>90%), only ~5-10% of individuals with EOAD carry a known autosomal dominant pathogenic variant in the *APP*, *PSEN1,* or *PSEN2* genes [1]. Similarly, while about 30% of frontotemporal dementia (FTD) incidence is attributed to pathogenic variants in the *GRN* and *MAPT* genes, and expansion of a hexanucleotide repeat in the *C9ORF72* gene, with a small amount accounted for by rare pathogenic variants in several additional genes, a large portion of genetic etiology for this disease has not yet been identified [2].

The Longitudinal Early Onset Alzheimer's Disease Study (LEADS) targets enrollment of individuals with early onset (age 40-64 years) cognitive impairment who lack a strong family history of EOAD (study excludes individuals with >1 immediate relative with EOAD) and who do not have a known genetic etiology such as a pathogenic *PSEN1* variant [3]. LEADS is designed to fill a gap in the research of EOAD, by recruiting individuals who do not qualify for studies of Mendelian EOAD such as the Dominantly Inherited Alzheimer Network (DIAN); LEADS data is being utilized to investigate longitudinal cognitive impairments, fluid and neuroimaging biomarkers, and genetic causes of EOAD. Enrolled patients are screened for brain amyloid positivity (EOAD) or negativity (EOnonAD) using positron emission tomography (PET) neuroimaging. Cognitive impairment at any age can be caused by a host of etiologies, the most common being AD. Thus, while the majority of individuals screened for LEADS are amyloid positive, it is not surprising that some of the cognitively impaired LEADS participants are amyloid negative. These participants are also followed in LEADS, and their clinical profile is identical to SNAP (suspected

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non-AD pathophysiology) in older individuals [4]. An exploratory aim of LEADS is to investigate the genetic etiology of EOAD and EOnonAD, with the goal to identify novel genetic variants causal or contributing to risk for EOAD and EOnonAD. LEADS includes a genetic testing pipeline, wherein all participants are screened for previously reported pathogenic variants in *APP*, *PSEN1*, *PSEN2, GRN,* or *MAPT,* and pathogenic repeat expansions in *C9ORF72.* The objective of this report, including patients enrolled during the first half of LEADS, is to investigate the frequency of these identified pathogenic variants, as well as the potential contribution of other rare functional variants in *APP*, *PSEN1*, *PSEN2, GRN,* and *MAPT* to disease.

The goal of this analysis is to confirm that variants in screened genes *APP*, *PSEN1*, *PSEN2, GRN, MAPT,* and *C9ORF72* are not contributing to the genetic etiology of most LEADS EOAD/EOnonAD patients, who are selected based on lack of extensive family history of disease.

2 Methods

2.1 Participants

This study includes 299 individuals with early onset cognitive impairment enrolled in LEADS; cognitively normal controls were not submitted for sequencing and are not included in the analysis. Affected individuals had biospecimens including DNA collected at baseline and were assessed with a neurocognitive battery as well as neuroimaging including PET amyloid and tau imaging. Collected data included demographics such as age at enrollment and age of symptom onset for cognitive impairment, sex, race, ethnicity, and family history of AD in parents and siblings, as well as results of neurocognitive examinations including the Mini-Mental State Exam

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(MMSE) [5]. Study protocols have been extensively described in Apostolova et al. (2021) [3]. More information on LEADS leadership, resources, and data sharing policies are available on the LEADS website (https://leads-study.medicine.iu.edu/).

2.2 Data Availability Statement

LEADS data are available by request; proposals will be reviewed by the LEADS Data Sharing Committee (see Apostolova et al., 2021 [3] for more information).

2.3 Ethics Statement

Written informed consent was obtained from all participants or their authorized representatives prior to study inclusion. A central Institutional Review Board (IRB) at Indiana University approved this study, which was conducted according to the ethical standards of the Helsinki Declaration of 1975.

2.4 Genetic Assays

Biospecimens are sent to the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) for processing and storage. As part of the standard DNA quality control pipeline, extracted DNA is run in-house on a custom 96-SNP microarray using Standard Biotools (formerly Fluidigm) microfluidic technology (Standard Biotools, San Francisco, CA). This assay generates a genetic fingerprint that NCRAD employs to check DNA quality and identity prior to distributions. This array includes SNPs rs7412 and rs429358, encoding *Apolipoprotein E* (*APOE*) *e2/e3/e4* alleles. This *APOE* allele data was returned to LEADS investigators and used in this analysis.

DNA is transferred to the Indiana University Genetics Testing Laboratories for *C9ORF74* hexanucleotide repeat expansion testing. This test was performed using the Asuragen AmplideX PCR/CE *C9ORF72* Kit to assess repeat number of the

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GGGGCC sequence located between noncoding exons 1a and 1b of the *C9ORF72* gene. PCR was performed on extracted DNA targeting this sequence using an Applied Biosystems thermal cycler, followed by capillary electrophoresis with an Applied Biosystems genetic analyzer. This kit generates numeric values for repeats up to 200, and categorical values for >200 repeats. Individuals with >30 repeats were reported as pathogenic repeat expansion carriers [6].

Genomic DNA was transferred to the Indiana University Center for Medical Genomics for library preparation and whole exome sequencing (WES). DNA was evaluated for quantity/quality using an Agilent TapeStation 4200. Next, 200 ng DNA was fragmented with a Covaris ME220 AFA sonicator, generating fragments averaging 300 bp in length. Subsequently, fragment end-repair, dA tailing, ligation of index adaptors, and amplification was performed. Libraries were then hybridized, captured, and amplified with the Agilent Human All-Exon V7 probe set (48Mb, hg38) using the Agilent SureSelect XTHS Target Enrichment System for Illumina Paired-End Multiplexed Sequencing protocol. Captured libraries were assessed for quantity/quality with a Qubit and an Agilent Bioanalyzer. Libraries were then pooled in equal molarity and sequenced using Illumina NovaSeq 6000 sequencers to generate 150 bp paired-end reads with 30X coverage.

2.5 Genetic Data Processing

LEADS WES data were processed following GATK Best Practices using Sentieon Genomics software (Sentieon, Inc., San Jose, CA) [7]. Briefly, paired-end FastQ files were aligned to Genome Reference Consortium Human Build 38 (hg38) using the recommend pipeline for Sentieon's proprietary BWA-MEM function fused with a process to account for the many alternate contigs included in hg38. BWA-MEM

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typically assigns reads that map to more than one location a mapping quality score of zero, leading to highly divergent genome regions being excluded from down-steam analyses. The alternate-contig-aware process adjusted read tags and de-coupled paired-end mates to prevent MAPQ dead zones, allowing the variant caller to include reads with mates mapping to different contigs [7].

Initially three Picard functions were implemented; RevertSam to produce unmapped BAM files, AddOrReplaceGroups to assign all reads in individual files to a single new read-group, and MergeBamAlignment to merge all aligned and unaligned reads per sample. Files were then sorted with Sentieon Util sort function. Duplicates were removed with Sentieon functions LocusCollector and Dedup. Resulting BAM files were realigned, and scores recalibrated using Sentieon Realigner and QualCal. Picard SortSam sorted recalibrated files. Then, NM, MD, and UQ tags were calculated by Picard SetNmMdAndUqTags, and the 0x1 paired flag was removed from reads with a piped gawk/samtools command. Files were finally indexed using Sentieon Util index.

Processed BAM files were used to generate gVCFs with Sentieon Haplotyper. Next, gVCFs were joint-called using Sentieon GVCFtyper. Resulting data was annotated with Annovar [8].

Copy Number Variants (CNVs) were detected from LEADS sequencing data using two programs, CANOES, implemented in R v4.1.1, and Copy Number Inference from Exome Reads (CoNIFER), implemented using Python v3.9 [9-11]. CANOES uses a negative binomial distribution to model whole exome sequencing read counts, with variance estimated using a regression-based approach (https://github.com/ShenLab/CANOES). CoNIFER employs a complimentary

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approach, using singular value decomposition normalization to calculate CNVs (https://conifer.sourceforge.net/index.html).

2.6 Variant Review

Data were reviewed for all affected participants. For genes *APP*, *PSEN1*, or *PSEN2*, genetic variants were considered pathogenic if they were included in the list of variants qualifying individuals for the Dominantly Inherited Alzheimer Network Trials Unit (DIAN-TU) [12, 13]. For genes *GRN* and *MAPT*, variants were manually reviewed and identified as pathogenic based on previous reports in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/), and the Leiden Open Variation Database (LOVD, https://www.lovd.nl/); annotations and were also checked using the Varsome software (https://varsome.com/) [14-19]. Patients were not informed about variants not meeting criteria for pathogenicity.

Calls for each CNV for each subject from both CANOES and CoNIFER results were aligned and compared for overlap to identify high-confidence calls. Identified overlapping CNVs were reviewed to investigate if any occurred within genes *APP*, *PSEN1*, *PSEN2, GRN,* or *MAPT*.

2.7 Pathogenic Variant Confirmation

For participants with an identified, previously-reported pathogenic variant in one of the six screened genes who signed an informed consent to have genetic test results returned, a separate 6 ml tube of blood was transferred to the Indiana University Genetics Testing Laboratories, or to GeneDx (GeneDx, LLC, Gaithersburg, MD), for DNA extraction and PCR-based genotype confirmation in a CLIA-certified laboratory.

2.8 Controls for Statistical Analyses

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Since only LEADS cases have been sequenced, whole genome sequencing data from healthy controls (HC) in the Parkinson's Progression Markers Initiative (PPMI) (N=195) were obtained from the Laboratory for Neuroimaging (LONI), for use in statistical analyses (see Section 2.9). The PPMI data were selected for comparison because these controls, while older than LEADS, were more closely matched in age to LEADS cases than those available in other late onset Alzheimer's disease data sets with sequencing available, such as the Alzheimer's Disease Neuroimaging Initiative. Data for PPMI participants are available from LONI (https://www.loni.usc.edu/) to approved investigators. Participants enrolled in this study and collected measures have been extensively described in previous publications [20].

LEADS sequencing data were merged with PPMI HC VCFs using bcftools. The analysis data set included 193 HC with sequencing data and demographic and clinical information. Merged genetic data were filtered to include rare (<1% minor allele frequency) coding and splicing variants for *APP*, *PSEN1*, *PSEN2, GRN,* and *MAPT.*

2.9 Statistical Analyses

Statistical analyses of participant demographics - including age at enrollment and symptom onset, sex, race/ethnicity, and clinical and genetic variables including family history of AD, *APOE e4* allele carrier status, and MMSE score - were performed using SPSS Statistics software (IBM Corp., Armonk, NY). Demographic variables were tested for significant differences between diagnostic groups using ANOVA or Fisher's Exact tests.

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Gene burden testing was performed using the SNP-set (Sequence) Kernel Association Test (SKAT) in R [21]. Merged VCF data including LEADS cases and PPMI HC were subset to including *APP*, *PSEN1*, *PSEN2*, *GRN*, and *MAPT* (39 variants total); data were then converted to binary PLINK format. Prior to analysis, eight LEADS cases with previously reported pathogenic variants were removed, leaving a total sample size of 291 LEADS cases for analysis. Data were analyzed for gene variant burden in all LEADS cases compared to PPMI controls, with *post*-hoc testing of EOAD/HC (220 cases/193 controls) and EOnonAD/HC (71 cases/193 controls). The SKAT-O method was used to test each gene as a set for enrichment of minor alleles for rare (minor allele frequency < 1% in GnomAD) coding and splicing variants in cases compared to controls. All tests covaried for age at enrollment, sex, and *APOE e4* carrier status. For SNPs included in gene burden analyses, minor allele frequency and Hardy-Weinberg equilibrium statistics were calculated in PLINK [22, 23]. Population frequencies for analyzed SNPs were obtained from GnomAD [24].

Post-hoc testing was also performed to investigate the contribution of individual variants to enrichment analysis results via logistic association testing in PLINK, covarying for age, sex, and *APOE e4* carrier status; testing was limited to SNPs with at least one minor allele in cases and controls.

3 Results

3.1 Pathogenic Variants Identified

Of the 299 LEADS EOAD and EOnonAD with sequencing data, a total of eight pathogenic variant or repeat carriers were identified (carrier frequency of 2.68%), including three EOAD heterozygous for *PSEN1* variants, 2 EOnonAD heterozygous

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for *GRN* variants, two EOnonAD with heterozygous *C9ORF72* pathogenic expansion repeats, and one EOnonAD heterozygous for a *MAPT* variant (Table 1). The two heterozygous *C9ORF72* repeat expansion carriers both had full (beyond assay quantifiable detection limit) repeat expansions. The rate of previously-reported pathogenic variants is 1.35% in EOAD (3/223), and 6.58% in EOnonAD(5/76) (Figure 1).

Table 2 presents summary information for EOAD and EOnonAD carriers of previously reported pathogenic variants, as well as summary statistics for non-carriers. Examining family history of AD, 33% of EOAD pathogenic variant carriers and 60% of EOnonAD carriers had a reported first degree relative with AD, though for EOAD and EOnonAD non-carriers, <40% of each group had a first degree relative with AD. The majority of variant carriers were male, and there was a greater percentage of *APOE e4* allele carriers in the EOAD variant carriers than in EOnonAD carriers (Figure 2).

All cases had similar mean age of symptom onset; EOAD pathogenic variant carriers (mean age 55.67) and non-carriers (mean age 55.31) as well as EOnonAD carriers (mean age 56.20) and non-carriers (mean age 54.24) had average onset in their mid-fifties.

There were no identified CNVs overlapping genes *APP, PSEN1, PSEN2, GRN,* or *MAPT*.

3.2 Gene Burden Results

Assessment of participant demographics for the LEADS cases (excluding previously reported pathogenic variant carriers) and PPMI controls identified significant

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diagnostic group differences for sex, enrollment age, and *APOE e4* carrier status (Table 3).

Comparing both diagnostic groups and controls, enrichment of *APOE e4* heterozygotes was observed in both EOAD (39.1%) and EOnonAD (39.4%) compared to PPMI controls (23.3%). Interestingly, while rates of heterozygotes were similar for EOAD/EOnonAD, enrichment of *APOE e4* homozygotes was observed in 15.5% of EOAD compared to similar rates of homozygous carriers in EOnonAD (2.8%) and controls (2.1%).

SKAT-O testing results for rare functional variant enrichment in *APP*, *PSEN2*, *GRN*, and *C9ORF72*, covarying for age, sex, and *APOE e4* carrier status within all cases, EOAD, or EOnonAD cases compared to controls are reported in Table 4. *PSEN1* gene burden testing was not performed since there was only one variant meeting inclusion criteria, with <3 minor alleles in the data set. Rare functional variants in *PSEN2* showed significant variant enrichment (p=0.0121, Table 4); however, *post-hoc* association analysis of SNPs in plink showed that this result was driven by rs140501902, which was more common in controls than in cases (p=0.04; Table 5), rather than enrichment of rare variants in cases compared to controls. There were no genes showing significant enrichment in only EOAD or only EOnonAD compared to controls, though in EOAD, there was a trend for enrichment of variants in *PSEN2* (p=0.059), again driven by rs140501902 minor allele enrichment in controls. This SNP has also been reported as Benign/likely-benign in ClinVar.

4 Discussion

Screening indicates that the frequency of previously reported pathogenic variants in *APP, PSEN1, PSEN2, GRN, MAPT, or C9ORF72* is low for both EOAD and

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EOnonAD LEADS participants, though variants are more frequent in EOnonAD than EOAD. Results from the gene burden analysis of rare functional variants in these genes also supports this conclusion, showing that unidentified rare variants in these genes are also not responsible for a significant portion of EOAD or EOnonAD cases. This highlights the importance of future studies to investigate other genetic factors and genes that may play roles in genetic risk or etiology of early onset cognitive impairment in the LEADS study. Preliminary results from genetic screening of LEADS participants also indicates that study exclusion criteria for individuals with extensive AD family history have been successful in avoiding enrichment of autosomal dominantly inherited pathogenic variants for Alzheimer's disease and frontotemporal dementia.

We observed that both EOAD and EOnonAD diagnostic groups include more *APOE* e4 heterozygotes compared to controls, and participants with EOAD had more *APOE e4* homozygotes compared to controls. This supports the role of *APOE* in both early- and late-onset AD, as identified by previous studies [25-27].

While autosomal dominant pathogenic variants in *APP*, *PSEN1*, and *PSEN2* are estimated to account for ~10-15% of EOAD, the observed frequency of these variants is lower in LEADS, showing that, as expected, variants in these genes do not account for most disease risk in this cohort [28]. Up to 70% of EOAD following a Mendelian inheritance pattern is attributed to pathogenic variants in the *PSEN1* gene in the literature (https://www.alzforum.org/alzgene). In LEADS, similarly, the three EOAD cases with pathogenic variants all occurred in *PSEN1*; we did not identify any pathogenic variants in *APP* or *PSEN2* in SNP or CNV results. While there were not sufficient variant carriers to statistically compare demographic or clinical

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characteristics of carriers and non-carriers, observation of summary statistics for each group shows that age of symptom onset is similar. It was also observed that MMSE scores are much lower in non-carriers (mean 21.54) compared to carriers (mean 25), though participants in each group were of similar age. Surprisingly, only one of the three AD variant carriers reported a family history of disease, supporting the importance of genetic screening to ensure that these participants are excluded from further analysis.

Heritability of frontotemporal dementia is largely attributed to autosomal dominant pathogenic hexanucleotide repeat expansion in *C9ORF72* and pathogenic variants in *GRN* and *MAPT*, with variants in these three genes accounting for up to 30% of frontotemporal dementia [2, 29]. While we observe a slightly higher frequency of EOnonAD pathogenic variant carriers compared to EOAD pathogenic variant carriers, it is still well below the frequency observed in patients with frontotemporal dementia and a strong family history of disease, showing that variants in these genes do not account for a significant portion of the genetic etiology of EOnonAD cases in LEADS. Interestingly, participant age of symptom onset is not lower for pathogenic variant carriers compared to non-carriers, and most other demographic and clinical characteristics reviewed appear similar as well. Though the sample size is currently too small to make any inferences based on this observation, it is interesting to note that EOnonAD pathogenic variant carriers had a higher frequency of AD family history (60%) than EOAD pathogenic variant carriers (30%), which were similar to EOAD (38%) and EOnonAD (33%) non-carriers in frequency of AD family history.

4.1 Limitations

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Though given the rarity of EOAD in the general population, the sample size of the LEADS cohort is impressive, it is still small in terms of a genetics study. LEADS study size and diagnostic heterogeneity currently limits the ability to perform discovery-based genetic analyses; however, the use of gene burden testing allows us to leverage summary-level data to investigate the contribution of rare functional variants in screened genes to case status. Given the small sample size, we did not remove individuals with diverse race or ethnicity. However, we did perform gene burden testing in only white non-Hispanic individuals to check sensitivity; results were not significantly different from results including all participants. An important limitation to note is that we did not have WES data for LEADS controls for this analysis. It is possible that merging data for LEADS cases and PPMI controls may introduce batch effects to the gene burden analysis; it will be important to future work to conduct gene burden testing including LEADS control sequencing data once available, to verify and expand these results. Additionally, this report focused on the genetic screening pipeline, which includes six genes accounting for the majority of known pathogenic variants in AD and FTD; however, it is possible that rare variants in other neurodegenerative disease-related genes could account for some portion of the genetic etiology of the LEADS cohort. Future work as enrollment continues will expand to encompass additional genes. Finally, current CNV results are based on whole exome sequencing data; it is possible that whole genome sequencing will identify additional CNVs not detectable with the data currently available.

4.2 Future Directions

It will be important to expand these analyses to the entire LEADS cohort once enrolled, to validate preliminary findings regarding pathogenic variant frequency in

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APP, *PSEN1*, *PSEN2*, *GRN*, *MAPT,* and *C9ORF72* in LEADS cases, as well as to expand analyses to include additional neurodegenerative disease-related genes. Future plans also include performing whole genome sequencing on all participants, which will enable assessment of the contribution of non-coding variants in genes of interest, as well as a more complete assessment of CNVs. It will additionally be important for future studies to leverage planned enrollment of more heterogeneous individuals to investigate the contribution of genetic ancestry and genetic background in diverse geographic and racial/ethnic cohorts to disease risk and progression.

4.3 Conclusions

These initial findings highlight the LEADS cohort as an excellent source of early onset cognitive impairment cases for future analyses of novel genetic etiology for EOAD and EOnonAD and support the important complimentary role of LEADS compared to studies such as the Dominantly Inherited Alzheimer Network (DIAN) in AD research and future clinical trials.

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References

[1] Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. Arch Neurol. 2012;69:59-64.

[2] Greaves CV, Rohrer JD. An update on genetic frontotemporal dementia. J Neurol. 2019;266:2075-86.

[3] Apostolova LG, Aisen P, Eloyan A, Fagan A, Fargo KN, Foroud T, et al. The Longitudinal Early-onset Alzheimer's Disease Study (LEADS): Framework and methodology. Alzheimers Dement. 2021;17:2043-55.

[4] Jack CR, Jr., Knopman DS, Chetelat G, Dickson D, Fagan AM, Frisoni GB, et al. Suspected non-Alzheimer disease pathophysiology--concept and controversy. Nat Rev Neurol. 2016;12:117-24.

[5] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189-98.

[6] Cruts M, Gijselinck I, Van Langenhove T, van der Zee J, Van Broeckhoven C. Current insights into the C9orf72 repeat expansion diseases of the FTLD/ALS spectrum. Trends Neurosci. 2013;36:450-9.

[7] Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics. 2013;43:11 0 1-0 33.

[8] Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.

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Author Manuscript

[9] Backenroth D, Homsy J, Murillo LR, Glessner J, Lin E, Brueckner M, et al. CANOES: detecting rare copy number variants from whole exome sequencing data. Nucleic Acids Res. 2014;42:e97.

[10] Krumm N, Sudmant PH, Ko A, O'Roak BJ, Malig M, Coe BP, et al. Copy number variation detection and genotyping from exome sequence data. Genome Res. 2012;22:1525-32.

[11] O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. 2012;485:246-50.

[12] Mills SM, Mallmann J, Santacruz AM, Fuqua A, Carril M, Aisen PS, et al. Preclinical trials in autosomal dominant AD: implementation of the DIAN-TU trial. Rev Neurol (Paris). 2013;169:737-43.

[13] Moulder KL, Snider BJ, Mills SL, Buckles VD, Santacruz AM, Bateman RJ, et al. Dominantly Inherited Alzheimer Network: facilitating research and clinical trials. Alzheimers Res Ther. 2013;5:48.

[14] Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J, et al. ClinVar: improvements to accessing data. Nucleic Acids Res. 2020;48:D835-D44.

[15] Landrum MJ, Kattman BL. ClinVar at five years: Delivering on the promise. Hum Mutat. 2018;39:1623-30.

[16] Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018;46:D1062-D7.

[17] Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, et al. Human Gene Mutation Database (HGMD): 2003 update. Hum Mutat. 2003;21:577-81.

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Author Manuscript

[18] Fokkema I, Kroon M, Lopez Hernandez JA, Asscheman D, Lugtenburg I, Hoogenboom J, et al. The LOVD3 platform: efficient genome-wide sharing of genetic variants. Eur J Hum Genet. 2021;29:1796-803.

[19] Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, et al. VarSome: the human genomic variant search engine. Bioinformatics. 2019;35:1978-80.

[20] Marek K, Chowdhury S, Siderowf A, Lasch S, Coffey CS, Caspell-Garcia C, et al. The Parkinson's progression markers initiative (PPMI) - establishing a PD biomarker cohort. Ann Clin Transl Neurol. 2018;5:1460-77.

[21] Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, et al. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. Am J Hum Genet. 2012;91:224-37.

[22] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559-75.

[23] Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7.

[24] Gudmundsson S, Singer-Berk M, Watts NA, Phu W, Goodrich JK, Solomonson M, et al. Variant interpretation using population databases: Lessons from gnomAD. Hum Mutat. 2022;43:1012-30.

[25] Payami H, Kaye J, Heston LL, Bird TD, Schellenberg GD. Apolipoprotein E genotype and Alzheimer's disease. Lancet. 1993;342:738.

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Author Manuscript

[26] Rademakers R, Cruts M, Van Broeckhoven C. Genetics of early-onset Alzheimer dementia. ScientificWorldJournal. 2003;3:497-519.

[27] Sorbi S, Nacmias B, Forleo P, Piacentini S, Latorraca S, Amaducci L. Epistatic effect of APP717 mutation and apolipoprotein E genotype in familial Alzheimer's disease. Ann Neurol. 1995;38:124-7.

[28] Ayodele T, Rogaeva E, Kurup JT, Beecham G, Reitz C. Early-Onset Alzheimer's Disease: What Is Missing in Research? Curr Neurol Neurosci Rep. 2021;21:4.

[29] Kirola L, Mukherjee A, Mutsuddi M. Recent Updates on the Genetics of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. Mol Neurobiol. 2022;59:5673-94.

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Conflicts of Interest

Dr. Dage is an inventor on patents or patent applications of Eli Lilly and Company relating to the assays, methods, reagents and / or compositions of matter related to measurement of P-tau217. Dr. Dage has served as a consultant for Abbvie, Genotix Biotechnologies Inc, Gates Ventures, Karuna Therapeutics, AlzPath Inc, Cognito Therapeutics, Inc., and received research support from ADx Neurosciences, Fujirebio, AlzPath Inc, Roche Diagnostics and Eli Lilly and Company in the past two years. Dr. Dage is serving on a scientific advisory board for Eisai. Dr. Dage has received speaker fees from Eli Lilly and Company.

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KEYWORDS: Alzheimer's disease; dementia; genetics; early onset; sequencing; *APP; PSEN1; PSEN2; GRN; MAPT; C9ORF7*

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Figure Legends

Figure 1. Pie Chart of EOAD and EOnonAD Pathogenic Variant Carriers. Percentages of LEADS EOAD participants carrying a *PSEN1* previously reported pathogenic variant and non-carriers (left) and LEADS EOnonAD participants carrying a *GRN*, *C9ORF72,* or *MAPT* previously reported pathogenic variant and non-carriers (right). Screening did not identify any *APP* or *PSEN2* pathogenic variant carriers.

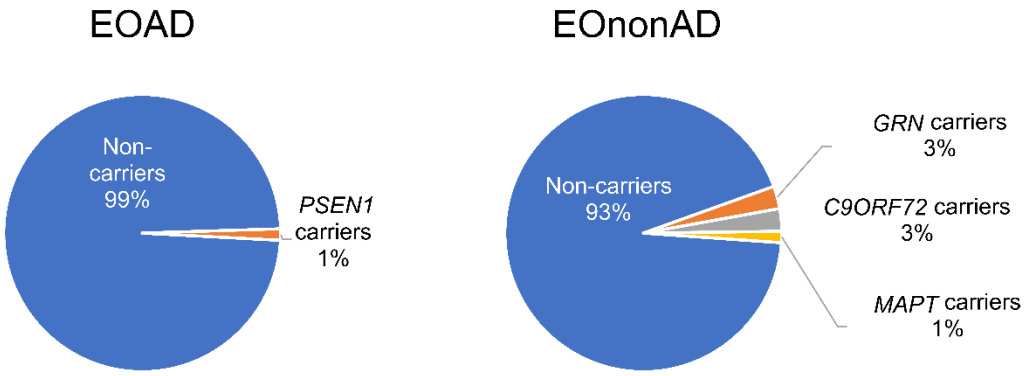

Figure 2. Pie Chart of *APOE e4* Allele Carriers and Non-Carriers. Participant counts and percentages of LEADS EOAD and EOnonAD pathogenic variant carriers and non-carriers, as well as PPMI controls, carrying one or two *APOE e4* alleles (blue) compared to individuals with no *APOE e4* alleles (orange).

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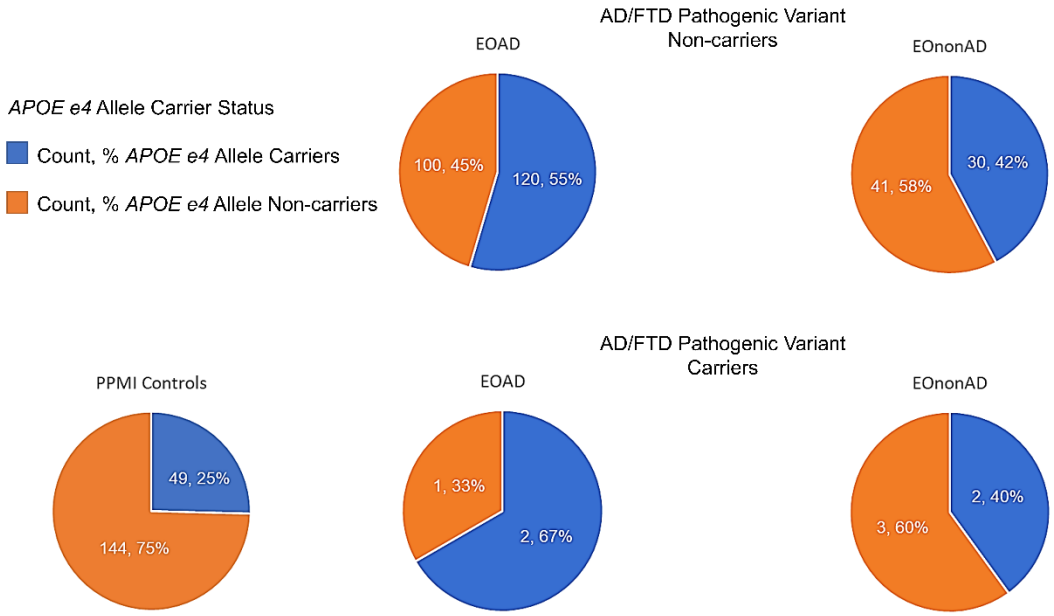

Table 1. EOAD and EOnonAD Pathogenic Variant Frequency

	EOAD	EOnonAD
Pathogenic variant frequency, AD genes	**1.35%**	**0%**
Pathogenic variant frequency, FTD genes	**0%**	**6.58%**

EOAD = early onset Alzheimer's Disease; EOnonAD = early onset non-Alzheimer's disease (cognitively impaired, amyloid negative); AD = Alzheimer's disease; FTD = frontotemporal dementia

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Table 2. LEADS Pathogenic Variant Carrier Demographics

Variable	EOAD Pathogenic Variant Carriers (N=3)	EOAD Non-carriers (N=220)*	EOnonAD Pathogenic Variant Carriers (N=5)	EOnonAD Non-carriers (N=71)*
Enrolled age mean (StDev)	59.33 (4.16)	58.83 (4.05)	61.00 (1.15)	57.68 (6.21)
Age of cognitive symptom Onset mean (StDev)	55.67 (4.04)	55.31 (4.24)	56.20 (2.17)	54.24 (6.76)
Count, % Male	3, 100%	103, 47%	4, 80%	46, 65%
Count, % *APOE e4* carriers	2, 67%	120, 55%	2, 40%	30, 42%
Count, % White non-Hispanic	3, 100%	202, 92%	5, 100%	60, 87%
Count, % with AD family history**	1, 33%	80, 38%	3, 60%	22, 33%
MMSE mean (StDev)	25.00 (2.65)	21.54 (5.17)	27.00 (1.87)	25.89 (3.67)

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EOAD = early onset Alzheimer's Disease; EOnonAD = early onset non-Alzheimer's disease (cognitively impaired, amyloid negative); StDev = standard deviation; MMSE = Mini-Mental State Exam score

*Some participants missing data: for EOAD, 1 missing race, 9 missing symptom onset age, 3 missing MMSE, and 10 missing family history; for EOnonAD, 2 missing race, 4 missing symptom onset age, and 4 missing family history. Percentages for variables with missing data were calculated based on non-missing group size.

**% of participants with a first degree relative with Alzheimer's disease

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Table 3. LEADS and PPMI Participant Demographics for Gene Burden Analysis

Variable	PPMI Healthy Controls (HC; N=193)	LEADS EOAD (N=220)	Test Statistic* (p-value) for EOAD vs. HC	LEADS EOnonAD (N=71)	Test Statistic* (p-value) for EOnonAD vs. HC
Age (mean, StDev)	60.58 (11.268)	58.74 (4.035)	5.113 (0.024)	57.61 (6.200)	4.430 (0.036)
Sex (count, % male)	123 (63.7%)	103 (46.8%)	(0.001)	46 (64.8%)	(1.000)
APOE e4 heterozygotes (count, %)	45 (23.3%)	86 (39.1%)	*APOE* e4 carrier status: (0.000)	28 (39.4%)	*APOE* e4 carrier status: (0.010)
APOE e4 homozygotes (count, %)	4 (2.1%)	34 (15.5%)		2 (2.8%)	

EOAD = early onset Alzheimer's Disease; EOnonAD = early onset non-Alzheimer's disease (cognitively impaired, amyloid negative); StDev = standard deviation.

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*F Test statistic for ANOVA test for age, Fisher's Exact Test statistic for sex and *APOE* ε4 carriers (homozygotes and heterozygotes compared to non-carriers).

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Table 4. Gene Burden Analysis Results

Comparison	Gene*	SNPs Tested**	MAC	p-value
EOAD + EOnonAD / HC	*APP*	11	11	0.454
	PSEN2	10	15	0.0121
	GRN	9	14	0.551
	MAPT	8	12	0.879
EOAD / HC	*APP*	9	9	0.149
	PSEN2	9	14	0.059
	GRN	9	14	0.828
	MAPT	8	12	0.744
EOnonAD / HC	*APP*	7	7	0.981
	PSEN2	6	10	0.363
	GRN	6	7	0.174
	MAPT	4	4	0.416

EOAD = early onset Alzheimer's disease; EOnonAD = early onset non-Alzheimer's disease (cognitively impaired, amyloid negative); MAC = minor allele count

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*Genes tested did not include *PSEN1*, as this gene was under-powered for analysis, with only one variant meeting criteria and with <3 MAC in the data set.

**Analyzed SNPs included coding or splicing variants with minor allele frequency<1% and at least one minor allele in the data set.

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Table 5. SNP Summary from Gene Burden Analysis of All Cases

Gene	CHR	SNP	BP	A1	NMISS	OR	STAT	P	O(HET)	E(HET)	AFF	UNAFF
PSEN2	1	rs20003777 1	226881996	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	1	rs14350187 0	226883712	G	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	1	chr1:226883763:G:A	226883763	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	1	rs14050190 2	226883774	T	484	0.087	-2.033	0.042	0.01240	0.01232	0/1/290	0/5/188
	1	rs75689946 3	226883855	C	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	1	rs63750197	226885570	T	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	1	rs20093124 4	226885668	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	1	rs61757781	226888112	G	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	1	rs14354926 6	226894097	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	1	rs14269022 5	226894111	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
GRN	17	rs63750742	44349263	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	17	rs63750742	44349263	T	484	1.219	0.209	0.835	0.00207	0.00206	0/3/288	0/2/191
	17	chr17:44349674C,G	44349674	G	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193

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	17	rs63750043	44350237	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	17	rs63750479	44350524	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	17	chr17:44351106:T:C	44351106	C	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	17	rs63750541	44351586	A	484	0.823	-0.137	0.891	0.00413	0.00412	0/1/290	0/1/192
	17	rs63750412	44352132	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
	17	rs25647	44352471	C	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	17	rs3775973733	45983354	A	469	NA	NA	NA	0.00213	0.00213	0/1/275	0/0/193
	17	rs14112047475	45983475	G	469	0.436	-0.578	0.564	0.00426	0.00426	0/1/275	0/1/192
	17	rs11571033590	45983590	C	469	NA	NA	NA	0.00213	0.00213	0/1/275	0/0/193
MAPT	17	rs14395688 2	45989975	T	470	1.584	0.444	0.657	0.00213	0.00636	1/0/276	0/1/192
	17	rs14362451 9	45991484	A	470	NA	NA	NA	0.00426	0.00425	0/2/275	0/0/193
	17	rs18776048 3	45993950	T	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
	17	rs26760492 1	45993953	G	469	NA	NA	NA	0.00213	0.00213	0/1/275	0/0/193
	17	rs63750096	45996557	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
APP	21	rs752361848	25897617	G	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193

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21	rs76133991 4	25954626	T	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
21	rs77979292 9	25954665	G	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
21	rs14379456 0	25975093	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
21	rs89081530 6	25997377	A	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
21	rs76228801 3	26021865	T	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
21	chr21:26021913A,T	26021913	A	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
21	rs13981900 6	26022001	A	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
21	rs14999557 9	26051060	A	484	NA	NA	NA	0.00207	0.00206	0/1/290	0/0/193
21	rs14508170 8	26051070	G	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192
21	rs14510507 85	26053291	C	484	NA	NA	NA	0.00207	0.00206	0/0/291	0/1/192

CHR = chromosome; BP = base pair (hg38); NMISS = number non-missing; OR = odds ratio; STAT = test statistic; p = p-value; O(HET) = observed; E(HET) = expected; AFF = affected; UNAFF = unaffected (controls); NA = not available

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