The landscape of autosomal-dominant Alzheimer's disease: global distribution and age of onset

Haiyan Liu,[1] Thomas W. Marsh,[2] Xinyu Shi,[3] Alan E. Renton,[4] Kevin M. Bowling,[5] Ellen Ziegemeier,[1] Guoqiao Wang,[3] Yuchen Cao,[3] Alisha Aristel,[4] Jessie Li,[6] Alexa Dickson,[5] Richard J. Perrin,[1,5] Alison M. Goate,[4] Victoria Fernández,[7] Gregory S. Day,[8] Michelle Doering,[9] Alisha Daniels,[1] Brian A. Gordon,[10] Tammie L.S. Benzinger,[10] Jason Hassenstab,[1] Laura Ibanez,[11] Charlene Supnet-Bell,[1],Chengjie Xiong,[3] Ricardo Allegri,[12] Sarah B. Berman,[13] Nick C. Fox,[14] Natalie Ryan,[14] Edward D. Huey,[15] Jonathan Vöglein,[16,17] James M. Noble,[18] Jee Hoon Roh,[19] Mathias Jucker,[20,21] Christoph Laske,[20] Takeshi Ikeuchi,[22] Raquel Sanchez-Valle,[23] Peter R. Schofield,[24,25] Patricio Chrem Mendez,[26] Jasmeer P. Chhatwal,[27] Martin Farlow,[28] Jae-Hong Lee,[29] Allan I. Levey,[30] Johannes Levin,[16,17] Francisco Lopera,[31,†] Ralph Martins,[32] Yoshiki Niimi,[33] Pedro Rosa-Neto,[34] John C. Morris,[1] Randall J. Bateman,[1] Celeste M. Karch,[2] Carlos Cruchaga,[2] Eric McDade[1] and Jorge J. Llibre-Guerra[1] for the Dominantly Inherited Alzheimer Network

[†]**Deceased.**

Abstract

We present a comprehensive global analysis of genetic variants associated with autosomal-dominant Alzheimer's disease (ADAD). A total of 550 variants in the *APP*, *PSEN1*, and *PSEN2* genes were identified, of which 279 were classified as pathogenic or likely pathogenic based on ACMG-AMP criteria, utilizing data from the Dominantly Inherited Alzheimer Network (DIAN), literature, and public databases. Symptomatic age at onset (AAO) data was estimated for 227 of these variants, allowing detailed characterization of their frequency, pathogenicity, and AAO. Importantly, 226 variants meet eligibility criteria for inclusion in disease-modifying clinical trials. Furthermore, we demonstrate the predictive value of mean variant AAO and parental AAO in predicting symptomatic AAO, validated against converters who became symptomatic during

follow-up in the DIAN Observational Study (DIAN-OBS). This dataset provides critical insights into the global landscape of ADAD and reveals the genetic and AAO heterogeneity of ADAD variants while refining variant trial eligibility criteria.

Author affiliations:

1 Department of Neurology, Washington University School of Medicine, St Louis, MO, 63110, USA

2 Department of Psychiatry, Washington University School of Medicine, St Louis, MO, 63110, USA

3 Division of Biostatistics, Washington University School of Medicine, St Louis, MO, 63110, USA

4 Ronald M. Loeb Center for Alzheimer's Disease, Department of Genetics and Genomic Sciences, and Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA

5 Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, 63110, USA

6 Department of Medicine, University of Missouri, Columbia, MO, 65212, USA

7 Fundacio ACE. Institut Catala de Neurosciencies Aplicades, Barcelona, 08028, Spain

8 Department of Neurology, Mayo Clinic in Florida, Jacksonville, FL, 32224, USA

9 Bernard Becker Medical Library, Washington University School of Medicine, St Louis, MO, 63110, USA

10 Department of Radiology, Washington University School of Medicine, St. Louis, MO, 63110, USA

11 Departments of Psychiatry and Neurology, Washington University School of Medicine, St. Louis, MO, 63110, USA

12 Department of Cognitive Neurology, Instituto Neurológico Fleni, Buenos Aires, C1428AQK, Argentina

13 Department of Neurology and Clinical & Translational Science, University of Pittsburgh, Pittsburgh PA, 15213, USA

14 Department of Neurodegenerative Disease and UK Dementia Research Institute at Queen Square Institute of Neurology, University College London, London, WC1N 3BG, UK

15 Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University, Providence, RI, 02903, USA

16 Department of Neurology, LMU University Hospital, LMU Munich, Munich, 81377, Germany

17 German Center for Neurodegenerative Diseases (DZNE), Munich, 81377, Germany

18 Taub Institute for Research on Alzheimer's disease and the Aging Brain, GH Sergievsky Center, Department of Neurology, Columbia University Irving Medical Center, NY, 10032, USA

19 Departments of Neurology and Physiology, Korea University Anam Hospital, Korea University College of Medicine, Seoul, 02841, South Korea

20 German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076, Germany

21 Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, 72076, Germany

22 Brain Research Institute, Niigata University, Niigata, 951-8585, Japan

23 Raquel Sánchez-Valle. Alzheimer's and other cognitive disorders unit. Hospital Clínic de Barcelona, FRCB-IDIBAPS, University of Barcelona, Barcelona, 08036, Spain

24 Neuroscience Research Australia, Sydney, NSW, 2031, Australia

25 School of Biomedical Sciences, Faculty of Health and Medicine, University of New South Wales, Sydney, NSW, 2052, Australia

26 Department of Neurology, Institute for Neurological Research Fleni, Buenos Aires, C1428AQK, Argentina

27 Massachusetts General Hospital, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02114, USA

28 Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, 46202, USA

29 Asian Medical Center, Seoul, 05278, South Korea

30 Goizueta Alzheimer's Disease Research Center, Emory University, Atlanta, GA, 30329, USA

31 Group of Neuroscience of Antioquia, GNA, Medical School, University of Antioquia, Medellin, 050010, Colombia

32 Ageing and Alzheimer's Disease Center, Edith Cowan University, Perth, Western Australia, 6027, Australia

33 Unit for Early and Exploratory Clinical Development, University of Tokyo Hospital, Tokyo, 113-8655, Japan.

34 McGill University Research Centre for Studies in Aging, Department of Neurology and Neurosurgery, Psychiatry and Pharmacology and Therapeutics, McGill University, Montreal, QC H4H 1R3, Canada

Correspondence to: Jorge J. Llibre-Guerra

Department of Neurology

Washington University School of Medicine in St.Louis

4488 Forest Park 00328

St. Louis MO63108, USA

E-mail: jllibre-guerra@wustl.edu

Correspondence may also be addressed to: Eric McDade

E-mail: ericmcdade@wustl.edu

Keywords: presenilin-1; presenilin-2; amyloid precursor protein; prevalence; pathogenicity

Introduction

Alzheimer's disease (AD), is a complex neurodegenerative disorder characterized by the accumulation of amyloid-beta and tau protein aggregates in the brain, leading to neurodegeneration and progressive cognitive and functional decline.[1,2] While the majority of AD cases are sporadic, a fraction (<1%) of cases are caused by autosomal dominant mutations in three key genes: *APP*, *PSEN1*, and *PSEN2*.[1,3]These variants result in altered processing of amyloid precursor protein, accelerating the pathological aggregation of amyloid-beta peptides.[3–7]Unlike sporadic AD, patients with autosomal-dominant Alzheimer's disease (ADAD) typically experience an earlier symptomatic age at onset (AAO) that can be reasonably predicted using the specific variant AAO, enabling the estimation of years to symptom onset (EYO) in asymptomatic family members.[8,9] The study of patients with ADAD provides a valuable model for elucidating the early pathogenic events in AD progression, offering a crucial window for intervention that could inform therapeutic strategies targeting familial and sporadic forms of the disease.

Understanding the genetic mechanisms that underpin ADAD and influence AAO, and the global distribution of ADAD variants is vital for clinical genetics, supporting affected families, and informing public health strategies.[10] Insights into population-level ADAD variants can facilitate early diagnosis, prompt intervention, and optimize resource allocation, potentially improving outcomes. [11] Furthermore, identifying novel ADAD variants can propel the development of treatments for both ADAD and sporadic AD by enabling the identification of molecular targets and pathways that may be amenable to pharmacological intervention. This approach serves as a critical bridge in translating genetic discoveries into actionable medical practices that could lead to more effective, personalized therapies, significantly delaying or even preventing the clinical onset of Alzheimer's disease in susceptible populations.[12,13,14,15,]

While previous research has shed light on AAO of participants with ADAD and clinical characteristics, ongoing efforts by the Dominantly Inherited Alzheimer Network Observational Study (DIAN OBS), DIAN Trials Unit (DIAN-TU), and DIAN Expanded Registry (DIAN EXR) are uncovering new variants and evaluating their clinical significance and AAO. [16,17,18,19]. This study aims to provide a comprehensive analysis of ADAD by reviewing variant reports from the literature, public databases, and DIAN prospective studies. We examined the global distribution of ADAD, including pathogenic variants and variants of uncertain significance (VUS), and

estimated mean variant AAO. We further assessed the ability of variant and parental AAO to accurately predict symptomatic AAO.

Materials and methods

Data sources

To assess ADAD global distribution, AAO, and variant pathogenicity, we reviewed data from 1) a systematic literature review, 2) existing DIAN data (an international research effort focused on ADAD), and 3) the Alzheimer Research Forum Database (Alzforum)[20, 21, 22] Details regarding data sources are provided below.

Systematic Review and Alzforum

The published literature was searched for ADAD variants, clinical symptoms, and reported AAO. The search strategies were created by a committee composed of a medical librarian, DIAN researchers, and other stakeholders with expertise in AD genetics. The search strategies were established using a combination of standardized terms and keywords, including but not limited to (Alzheimer's disease) AND (autosomal dominant inheritance, OR presenilin 1 OR presenilin 2 OR amyloid precursor protein) AND (age of onset OR middle-aged). The search was run in December 2023 using a librarian-created filter for non-animal studies and a date restriction from 2014-current (date of previous review by our group)[22] in the databases Ovid Medline 1946-, Embase 1947-, Scopus 1960-, Cochrane Central, and Clinicaltrials.gov. The final search yielded 4,528 citations, which were imported into EndNote. 3,343 duplicates were identified and excluded, leaving 1,185 citations for further examination. These were assessed for relevance based on predetermined inclusion and exclusion criteria, as detailed in Figure 1. Specifically, studies reporting on the clinical characteristics of ADAD families with variants in *PSEN1*, *PSEN2*, or *APP* were considered for inclusion. Following an initial screening of abstracts, 202 studies qualified for a full-text review to verify their relevance and validity. This detailed evaluation resulted in the selection of 184 peer-reviewed articles for comprehensive analysis (refer to Figure 1). In addition to database searches, an exploratory review of Alzforum contributed five additional peer-reviewed studies to our final dataset.

DIAN-OBS

The DIAN OBS is a longitudinal, observational study of individuals and families who carry genetic variants associated with ADAD. The DIAN OBS was established to inform ADAD natural history, biomarkers, and clinical outcomes, and to identify intervention strategies.[20] The study began in 2008 and has enrolled more than 600 participants across multiple sites in North America, Europe, Latin America, Australia, and Asia.

DIAN-TU

The DIAN-TU is a global research effort established in 2012 to design and conduct clinical trials for the prevention or treatment of ADAD. The present analysis only included DIAN-TU participants enrolled in the placebo groups.

DIAN-EXR

The DIAN EXR is an international registry that comprises more than 700 individuals from families affected by ADAD. The DIAN EXR was established in 2011 as a collaborative research effort to facilitate study referral to DIAN OBS and DIAN-TU, and to support educational and outreach activities with ADAD family members (https://dian.wustl.edu/our-research/registry/).

DIAN OBS and DIAN-TU study assessments include detailed information on parental and participant AAO documented across partner sites using a standardized AAO form. In addition, DIAN-TU and DIAN OBS collect extensive data from participants, including clinical-cognitive data obtained through the Clinical Dementia Rating® score (CDR®)as well as cerebrospinal fluid (CSF), plasma, and neuroimaging biomarkers. Details about DIAN OBS and DIAN-TU protocols have been described previously.[18, 21–23]

Information on sociodemographic characteristics, country reports, and evidence of variant pathogenicity was extracted from each data source. Information on clinical features (AAO, age of death, disease duration, clinical presentation, atypical manifestations, and neurological findings) was obtained when available. We considered each symptom or sign as present or absent when clearly stated in the reports. To avoid potential double reporting across DIAN studies and published literature, pedigrees for each ADAD variant were manually examined to identify and remove possible duplicates. The combined dataset included 387 pedigrees, including 3,275

individuals, of whom 2,110 had cognitive impairment attributed to ADAD with known AAO. Key definitions about AAO used in our study are provided in Box 1.

Variant pathogenicity assessment and DIAN trial eligible list

For the purpose of this review, we assessed variants using two approaches. First, we categorized variants as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign according to the ACMG-AMP guidelines. This classification was informed by data from literature reports, Alzforum records, and findings from DIAN studies. Second, we determined trial eligibility based on the DIAN-TU trial eligibility criteria (see Figure 2), which evaluate variants for inclusion in disease-modifying clinical trials. Below, we describe the DIAN-TU eligibility algorithm and relevant measures in the study.

The inclusion of ADAD individuals in the DIAN-TU clinical trials requires rigorously validated evidence for variant pathogenicity. As a result, DIAN-TU utilizes stringent criteria for enrolling patients with pathogenic ADAD variants, as delineated by the DIAN-TU Clinical-Genetics Core. ADAD variants eligible for trial inclusion must meet rigorous standards for evidence of pathogenicity. The DIAN-TU eligibility list is dynamic and regularly updated by assessing new variants as scientific evidence—such as new families, AD-related biomarkers, results from segregation, and functional analyses—becomes available. This ensures that new variants are evaluated and added to the trial list.

The DIAN-TU eligibility algorithm integrates three key streams of information: 1) Family history of dementia, 2) evidence of AD, and 3) genetic data suggesting a causal relationship between variant and AD. Corresponding supportive findings include 1) a multi-generational family history of dementia suggesting an autosomal dominant pattern of inheritance; 2) clinical documentation of amnestic-predominant, progressive cognitive impairment leading to dementia due to AD, ideally with fluid or imaging biomarkers supportive of AD, and/or neuropathological confirmation of AD in at least one family member; and, 3) predictors of variant pathogenicity, including low frequency in a large population series (Genome Aggregation Database [gnomAD], v4.1.0), and 4-evidence of variant segregation or functional analysis exploring Aβ isoform levels in vitro (Aβ42 and Aβ40 levels, or the Aβ42/40 ratio) relative to wild type and known pathogenic variants.[30] Additional criteria supportive of variant eligibility include conservation of the variant amino acid residue between *PSEN1* and *PSEN2*, presence of other ADAD pathogenic variants at the residue,

and *in silico* prediction of damaging effects. While the DIAN-TU pathogenicity algorithm incorporates several *in silico* predictions of damaging effects (Combined Annotation Dependent Depletion [CADD] (GRCh37-v1.6), rare exome variant ensemble learner [REVEL][29], Sorting Intolerant From Tolerant [SIFT] (Ensembl 66), PolyPhen-2 (V13-3)), it predominantly relies on the CADD score. This score is an integrative approach used in genomics to assess the deleteriousness of single nucleotide variants (SNVs) and insertion-deletion variants (indels) across the human genome. Developed to prioritize functional, deleterious, and disease causal variants in clinical genomics and genetic research, CADD integrates multiple annotations into a single metric by contrasting variants that survived natural selection with simulated variants to derive a score for each variant, reflecting its potential pathogenic impact. [30,31] As a result in this study, we provide additional evidence on the utility of CADD scores to predict pathogenicity. DIAN-TU algorithm to assess eligibility for trial inclusion is shown in Figure 2.

Statistical analysis

Participant symptomatic AAO prediction analysis

We extracted the AAO for each ADAD-affected family member and determined the parental/family proxy AAO and variant AAO for those with the same ADAD variant. Next, we assessed the accuracy of the variant AAO and parental AAO in predicting participant symptomatic AAO. We identified participants who developed cognitive impairment. (i.e, progressed from cognitively normal state [CDR=0] to impaired [CDR>0]) during the DIAN study and classified them as "converters". Converters' symptomatic AAO was considered the gold standard and compared with the predicted AAO according to the variant AAO present in the family and the parental AAO. If there were insufficient data to assess variant AAO (<3 known affected variant carriers), only parental AAO was used to calculate the EYO. Linear regression was used to explore the relationship between variant AAO, parental AAO, and participant symptomatic AAO.

Pathogenicity prediction analysis

We further explored the validity and predictive ability of the DIAN-TU eligibility algorithm by producing a Receiver Operating Characteristic (ROC) curve using the pROC R package. The results from the functional analysis exploring Aβ isoform levels in vitro relative to wild type and known pathogenic variants were considered the gold standard. The DIAN-TU algorithm was

validated using 71 variants encompassing diverse parameters such as gene and variant, protein-level effect, gnomAD exome/genome frequency, CADD score, SIFT prediction/score, PolyPhen-2 prediction/score, clinical phenotype, mean differences of Aβ42, Aβ40, and Aβ42/40 relative to wild type variants and known pathogenic variants, and mean AAO. Our primary analytical focus was on assessing the potential of the AAO, AD biomarkers, population frequency, variant segregation, and CADD scores to predict pathogenicity. Subsequent statistical analyses included Maximum Likelihood Estimates with emphasis on the Wald Chi-Square test to deduce the association between the AAO and CADD score and the variant Aβ42/40 *in vitro* results. To measure the strength and direction of this association, we computed odds ratios. To validate further the diagnostic utility of the AAO and CADD scores in predicting pathogenicity, we conducted a ROC analysis.

All statistical analyses were conducted using either SAS software (version 9.4, SAS Institute Inc., Cary, NC) or R (Version 4.3.1). A p-value threshold of <0.05 was considered statistically significant.

Results

Global distribution of variants

Global analysis of ADAD variant distribution revealed 550 variants across patients in 55 countries: 67.7% (372) in *PSEN1*, 16.5% (91) in *PSEN2* and 15.8% (87) in *APP* (which *APP* duplications). Variants were classified following ACMG-AMP criteria, using data derived from literature reports, Alzforum records, and DIAN studies. Using ACMG-AMP criteria 279/550 ADAD variants were classified as pathogenic/likely pathogenic, 27 as variants of uncertain significance (VUS), 50 variants as benign/likely benign (including one [*APP* p.A673T] as protective). In addition, 194 variants were not classified due to insufficient evidence to support a pathogenic or benign designation. Additionally, variants co-occurring in individuals with other known pathogenic mutations or those with ambiguous application of ACMG-AMP criteria could not be conclusively classified.

The highest number of reported pathogenic variants were from the United States (74 variants; *PSEN1*=65, *PSEN2*=1, *APP*=8), France (67 variants; *PSEN1*=54, *PSEN2*=4, *APP*=9), and the United Kingdom (42 variants; *PSEN1*=39, *APP*=3). Figure 3 provides a breakdown of pathogenic and likely pathogenic variants by gene, number of variants, and country.

Age at onset and estimated years to symptom onset:

Data on AAO were available in 2,110 individuals with 227 unique variants: 176 in *PSEN1*, 20 in *PSEN2*, and 31 in *APP*. The combined dataset revealed a mean AAO of 47.3 years (SD=10.1), ranging from 21 to 90 years. The mean variant AAO was 44.9 years (SD=9.4, range from 22 to 90 years) for *PSEN1* variants, 59.5 years (SD=6.8, ranging from 21 to 82 years) for *PSEN2* variants, and 52.1 years (SD=8.1, ranging from 30 to 88 years) for *APP* variants. (Table 1). *PSEN2* variants had a later AAO compared to other groups (PSEN2vsPSEN1 P<0.001, PSEN2vsAPP p=0.01). Table 1 provides a summary of AAO for each gene and according to variant pathogenicity. In addition, supplemental tables 1 and 2 provide AAO for each independent variant. Figure 4 depicts the relationship between individual participants' symptomatic AAO and variant AAO by variant and gene (*APP, PSEN1*, or *PSEN2*). Figure 4A's scatter plot shows the association between individual AAO (y-axis) and mean variant AAO (x-axis). The strong association between variant AAO and observed AAO (R^2=0.56) suggests that specific variants account for a substantial proportion (56%) of variability in individual AAO overall. The degree of association varied by specific genes (Figure 4B), being strongest for *PSEN1* (R^2=0.60), modest for *APP* (R^2=0.30), and weak for *PSEN2* (R^2=0.13). In addition, we explored whether there is a greater degree of variability in AAO across different genes (APP, PSEN1, PSEN2) and according to variant-level factors, including the codon location (PSEN1 < 200 vs. > 200) and the affected protein domain (cytoplasmic vs. transmembrane), as shown in Supplemental Tables 3 and 4. First, we examined potential variability in AAO at the individual level (Supplemental Table 3); despite significant differences in mean AAO across genes—PSEN1 (45.7 years), APP (50.8 years), and PSEN2 (55.8 years), we found a similar degree of individual-level variability in AAO across genes (p=0.13). Similarly, variant level factors like protein domain location (cytoplasmic vs. transmembrane) and PSEN1, variant locations pre- and post-codon 200, showed no significant effect in AAO variability (p = 0.07 and p = 0.19, respectively). In summary, our analysis suggests that gene or variant-level

factors do not significantly influence the degree of AAO variability at the individual or family level.

To assess the accuracy of variant and parental AAO in predicting individual symptomatic AAO, we performed a comparative analysis of estimated and participant symptomatic AAO in 53 participants within the DIAN-TU placebo arm and DIAN OBS study who developed cognitive impairment during follow-up ("converters"). Table 1 shows the distribution of the converter AAO relative to mean variant AAO and parental AAO. The analysis revealed an average discrepancy between participant symptomatic AAO and expected parental AAO of -1.1 years (SD=5.6; 95% CI: -2.6 to 0.5), with an R^2 of 0.52; meaning that observed AAO occurred, on average, 1.1 years earlier than reported in a participant's parent. Similarly, the difference between participant symptomatic AAO and variant-specific AAO, was -0.9 years (SD=5.2; 95% CI: -2.3 to 0.5), with an R^2 of 0.56 ($p<0.0001$). Figures 4C and D demonstrate the predictive validity of both parental and variant-specific AAO for observed AAO, highlighting the substantial association between observed AAO and EYO in ADAD.

DIAN-TU trial eligibility

Application of the DIAN-TU eligibility algorithm (Figure 2) determined that 226 of the 551 identified ADAD variants qualified for inclusion in the DIAN-TU (see supplemental table 1). Remarkably, 35 eligible variants were previously categorized as VUS based on literature reports, with an additional 21 not documented in existing variant databases. The eligibility of VUS for inclusion in the DIAN-TU was re-assessed based on AD-specific biomarkers and neuropathology reports, which were primarily obtained through the DIAN-OBS and contributions from DIAN site investigators.

195/226 of DIAN-TU eligible variants were in *PSEN1*, 23 in *APP*, and 8 in *PSEN2*. The mean AAO for DIAN-TU eligible variants was 43.3 years (SD=8.3, range 26-63), and the average CADD score was 27.3 (SD=2.7). Gene-specific analyses revealed *PSEN1* variants with an AAO of 43.2 years (SD=8.2, range 26-60) and a CADD score of 27.4 (SD=2.8), *PSEN2* variants had an AAO of 53.5 years (SD=7.0, range 46-56) and a CADD score of 26.0 (SD=1.5), and *APP* variants were noted for an AAO of 47.5 years (SD=7.2, range 34-58) and a CADD score of 27.1 (SD=2.3).

For an in-depth review of each variant, including country report, mean AAO, clinical phenotype, and other details, see Supplemental Tables 1 and 2.

CADD score and predictor of pathogenicity

CADD scores are widely used to interpret whole-genome sequencing data, providing a high-resolution view of pathogenicity across the human genome.[33] In this study, we also evaluated the CADD score as a predictor of clinical classifications, specifically targeting variants likely to be detrimental and potentially pathogenic. CADD scores were available for 520 out of 551 variants. The analysis showed that the average CADD score for variants classified as pathogenic or likely pathogenic was 26.9 and 25 for variants of uncertain significance (VUS). Variants identified as benign had an average CADD score of 14.4. There was a significant difference in the average CADD score among different groups (Pathogenic vs VUS, Pathogenic vs Benign, VUS vs Benign; $p<0.0001$). All 226 DIAN-TU trial eligible variants with an available CADD score, registered scores exceeding 21.

We also evaluated the utility of the CADD score to predict variant functional analysis results. Variants that led to a significant rise in Aβ isoform levels (or Aβ42/40 ratio) had higher CADD scores (mean = 26.2; SD = 3.0) than variants that did not alter Aβ40 or Aβ42 levels (mean = 22.1; SD = 5.1) ($p = 0.002$). In vitro functional assays revealed that a one-unit increase in the CADD score was associated with a 25% increase in the odds of changes in Aβ isoform levels (OR=1.25; 95% CI= 1.1-1.5). The predictive utility of the CADD score is further substantiated by the ROC analyses, with AUC of 0.68 (Supplementary Figure 4). The use of the CADD score to predict variant functional analysis results was further enriched by including the variant genomAD frequency in the model (AUC=0.75). Additional analysis on the utility of CADD scores in identifying potential damaging variants is provided in supplemental information 1 including supplemental figures 1-3 and supplemental table 5.

Discussion

This study represents a substantial advancement in our understanding of ADAD epidemiology through the systematic analysis of 550 genetic variants across *APP*, *PSEN1*, and *PSEN2*. We have demonstrated a notable global distribution of these variants, provided variant-specific AAO, and validated the predictive accuracy of variant and parental AAO in forecasting symptomatic onset (EYO). Our investigations identified 550 ADAD variants, with 226 considered eligible for the DIAN-TU trial eligibility. The DIAN OBS and DIAN-TU studies offered deep phenotype data, which included clinical, cognitive, fluid biomarkers, and neuroimaging biomarkers for 109 variants spanning *PSEN1*, *PSEN2*, and *APP*, which supported the classification of several variants of uncertain significance or not previously in the literature. Overall, our findings not only enhance the genetic and clinical understanding of ADAD, but also will inform future ADAD prevention trials.

The occurrence of ADAD varied across different countries, with United States, France, and the United Kingdom documenting the highest number of ADAD variants. This observation is consistent with previous studies that have reported a higher frequency of ADAD in developed countries.[29–32] However, the observed variation in ADAD frequency is in part due to unequal access to diagnostic testing, including genetic testing. Similarly, countries lacking reports of ADAD variants might be related to limited access to health care, lack of awareness, and resource limitations. DIAN is attempting to mitigate these issues through comprehensive outreach programs and support of genetic counseling and testing via a growing number of increasingly distributed study sites in regions with fewer resources and by supporting genetic counseling and testing.[14,15.]

Relative to previous analysis [22], we have now expanded on the number of ADAD variants with variant AAO and included a larger number of mutation carriers with known age at symptom onset (n=2,110), which will enhance the accuracy of the predicted AAO in ADAD patients. Our study also supports using variant AAO and parental AAO to predict an individual's AAO (EYO). In assessing the predictive accuracy of AAO in DIAN converters, a comparison between the observed AAO and the expected AAO derived from parental data revealed an average discrepancy of -1.1 years (95% CI -2.6 to 0.5), indicating the strength of parental AAO to predict symptomatic AAO

at preclinical stages. A similar analysis comparing observed AAO to variant AAO showed a slightly smaller discrepancy of -0.9 years, (95% CI: -2.3 to 0.5). Despite the high correlation between variant AAO and participant symptomatic AAO, our findings also highlight variability in AAO across individuals within-families and within-variants. This variability suggests that additional factors may either confer resilience or pose risks, thereby significantly influencing the clinical presentation and progression of the disease.[38] Variability across different variants is likely explained by the differential effects of each variant on γ-secretase activity and amyloid β production. Previous analysis in the DIAN Obs study indicates that variants in the 3rd transmembrane domain of PSEN1 were associated with pronounced PIB accumulation and steep cognitive declines. In contrast, variants in the 8th transmembrane domain showed only modest PIB accumulation. Distinctions were more pronounced when comparing variants based on their location relative to codon 200.[19] In addition, carriers of transmembrane-affecting variants exhibited more severe cognitive impairment, reduced hippocampal volume, and higher phosphorylated tau levels compared to cytoplasmic variant carriers and non-carriers.[39]

These variant-level effects primarily capture the initial stage in the AD cascade, leading to tau aggregation, neurodegeneration, and cognitive decline. Consequently, variability in the AAO within families carrying the same variant is likely due to differences in additional genetic, environmental, or lifestyle factors that modulate these downstream processes.

Variability in this study and others underscores the need to further refine predictive models to enhance clinical utility.[36, 37] Current models are based only familial data, which may not account for environmental factors or interactions between multiple genetic loci that could influence AAO in a broader population. It is also important to recognize that study findings apply to participants at the group level. For individuals carrying such variants, it is crucial to understand that the development of the disease within 1 year of EYO is not assured. Lifestyle factors and other risk factors can significantly influence the onset and progression of the disease.[42,43] Such insights emphasize the importance of a current comprehensive dataset in improving the accuracy of predictive models for AAO in ADAD patients.

In our evaluation, 226 of the 550 analyzed ADAD variants met the eligibility criteria for the DIAN-TU trial, the robustness and reliability of the DIAN-TU algorithm are enriched by the integration of clinical, cognitive, and biomarker data from the DIAN studies, which allowed the inclusion of

variants previously categorized as VUS and the identification of 21 novel pathogenic variants to be eligible for trial inclusion. These outcomes support the need for ongoing updates and reassessments in genetic variant databases to enhance their clinical relevance and accuracy. Moreover, the demonstrated precision of the DIAN-TU algorithm in evaluating trial-worthy variants suggests its potential for wider application in clinical and research settings.

Recent advancements in the study of ADAD underscore the need to understand the pre-symptomatic stages of AD and implement early disease-modifying trials.[44,45] Knowledge concerning the duration of the pre-symptomatic stages of AD and the ability to accurately define EYO are central to efforts to improve early detection and management of AD, and to the design of clinical trials aimed at preventing the symptomatic onset of AD.[46] Our findings highlight the utility of using AAO and EYO as screening tools to enhance selection of participants with ADAD for clinical trials, minimize confounding variables, and improve the likelihood of successful outcomes through more accurate EYO estimation. These measures can inform the design of prevention trials and the implementation of stringent inclusion and exclusion criteria, ensuring that suitable candidates are enrolled at appropriate ages, and enhancing trial reliability. Furthermore, our study extends beyond conventional uses of AAO and EYO by incorporating a globally representative dataset, which allows for the discovery of novel ADAD variants. This broadens our understanding of ADAD's genetic and clinical diversity, enabling healthcare professionals to utilize our comprehensive data for more precise outcome forecasting, informed treatment planning, and prognostication in patients with ADAD. Collectively, these findings have the potential to impact patient care and advance the development of treatments for AD.

The use of the CADD score in this study underscores its value as a robust tool for predicting the pathogenicity of genetic variants in ADAD. Higher CADD scores were associated with variants classified as pathogenic, affirming the score's effectiveness in distinguishing likely pathogenic variants from benign variations in a clinical context. However, the reliance on CADD scores also presents certain limitations. CADD has limitations in classifying large structural or splice-site variants and is influenced by reported clinical observations, allele frequencies, and molecular data. While the CADD approach is evolving, factors such as variant spectrum, penetrance, resilience mechanisms, genetic background, disorder heterogeneity, variant classification quality, and inheritance patterns must be considered to enhance its clinical utility. Therefore, while CADD scores are instrumental in enhancing the predictive accuracy of our pathogenicity assessments,

they should be interpreted with caution and supplemented with disease-specific functional assays and clinical data when available.

This manuscript presents several distinct strengths that contribute to the field of neurogenetics and the ongoing study of ADAD. First, we offer a comprehensive analysis of 550 genetic variants in the *PSEN1*, *PSEN2* and *APP* genes, which allows for a detailed examination of the genetic landscape of ADAD, offering a richer understanding of its global prevalence and genetic diversity that may inform public health strategies and resource allocation for AD care and prevention globally. Second, a substantial contribution of this study, is the innovative use of the DIAN-TU pathogenicity algorithm, which integrates clinical, biomarker, and genetic data to enhance the accuracy of variant eligibility for clinical trials. Additionally, our approach validates the predictive accuracy of the estimated AAO models and supports its application in clinical trials aimed at prevention. Overall, these findings have direct implications for the design and execution of clinical trials aimed at preventing or delaying the onset of AD symptoms in at-risk populations.

However, this study is also subject to certain limitations. First, it is possible that pertinent studies may have been overlooked despite a systematic literature review. Additionally, our systemic review was limited to variants with sufficient information to accurately determine an AAO (227/550). Second, the use of AAO instead of age at diagnosis was intended to account for differences in delay to diagnosis. However, this method is subject to its own limitations. In many cases, the AAO is reported by participants with mild cognitive impairment or their caregivers, which may result in recall bias. Furthermore, the estimation of AAO relied on clinical data that may have variability in diagnostic accuracy and criteria across different research sites, which could affect the consistency of the symptomatic AAO data and the classification of variants. Third, although the study covers a large number of genetic variants, the geographic distribution of the studied population may not fully represent global diversity. Certain regions are underrepresented due to limited access to genetic testing, potentially impeding the generalization of study findings across ethnic and racial groups. Additionally, reports of new variants are more likely to occur in regions with active ADAD research programs. While healthcare institutions with clinical genetic diagnostic capabilities might conduct testing for *APP*, *PSEN1*, and *PSEN2*, routine clinical findings might not be documented in the scientific literature without an active research program.

Finally, the field of genetic research in AD is rapidly advancing. As the landscape of genomic data expands and new ADAD variants and families are described, interpretations and conclusions drawn in this study will need to be revisited and revised.

Data availability

Data supporting the findings of this study are available on request according to the policies of the DIAN (https://dian.wustl.edu), which comply with the guidelines established by the Collaboration for Alzheimer's Prevention.[32] To protect the privacy of participants some data are not publicly accessible.

Acknowledgements

We acknowledge the altruism of the participants and their families and the contributions of the DIAN research and support staff at each of the participating sites for their contributions to this study.

Funding

Data collection and sharing for this project was supported by The Dominantly Inherited Alzheimer Network (DIAN, U19AG032438) funded by the National Institute on Aging (NIA),the Alzheimer's Association (SG-20-690363-DIAN), the German Center for Neurodegenerative Diseases (DZNE), Raul Carrea Institute for Neurological Research (FLENI), Partial support by the Research and Development Grants for Dementia from Japan Agency for Medical Research and Development, AMED, and the Korea Dementia Research Project through the Korea Dementia Research Center (KDRC), funded by the Ministry of Health & Welfare and Ministry of Science and ICT, Republic of Korea (RS-2024-00344521), Spanish Institute of Health Carlos III (ISCIII), Canadian Institutes of Health Research (CIHR), Canadian Consortium of Neurodegeneration and Aging, Brain Canada Foundation, and Fonds de Recherche du Québec – Santé. This manuscript has been reviewed by DIAN Study investigators for scientific content and consistency of data interpretation with previous DIAN Study publications. We acknowledge the altruism of the

participants and their families and contributions of the DIAN research and support staff at each of the participating sites for their contributions to this study.

Competing interests

JCM is the Friedman Distinguished Professor of Neurology, Director, Knight ADRC; Associate Director of DIAN and Founding Principal Investigator of DIAN. He is funded by NIH grants # P30 AG066444; P01AG003991; P01AG026276; U19 AG032438; and U19 AG024904. Neither Dr. Morris nor his family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company.

JH is a paid consultant for F. Hoffmann-La Roche, Ltd., Takeda, and Lundbeck, and is on the Data Safety and Monitoring Board for Eisai.

CC receives research support from: Biogen, EISAI, Alector and Parabon. The funders of the study had no role in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. Dr. Cruchaga is a member of the advisory board of Vivid genetics, Halia Therapeutics and Adx Healthcare.

RJB is the Director of the DIAN-TU and Principal Investigator of the DIAN-TU-001. He receives research support from the National Institute on Aging of the National Institutes of Health, DIAN-TU Trial Pharmaceutical Partners (Eli Lilly and Company, F. Hoffman-La Roche, Ltd., and Avid Radiopharmaceuticals), Alzheimer's Association, GHR Foundation, Anonymous Organization, DIAN-TU Pharma Consortium (Active: Biogen, Eisai, Eli Lilly and Company, Janssen, F. Hoffmann-La Roche, Ltd./Genentech, United Neuroscience. Previous: AbbVie, Amgen, AstraZeneca, Forum, Mithridion, Novartis, Pfizer, Sanofi). He has been an invited speaker and consultant for AC Immune, F. Hoffman La Roche, Ltd., and Janssen and a consultant for Amgen and Eisai.

AED reports no competing interests. He receives research support for this work from the National Institute on Aging (R01AG053267, U19AG032438).

TI reports no competing interests. He received research support for this work from AMED (JP23dk0207066 and JP23dk0207060).

GSD reports no competing interests that are directly relevant to this work. His research is supported by NIH (K23AG064029, U01AG057195, U01NS120901, U19AG032438) and the Chan Zuckerberg Initiative. He serves as a consultant for Parabon Nanolabs Inc and as a Topic Editor (Dementia) for DynaMed (EBSCO). He is the co-Project PI for a clinical trial in anti-NMDAR encephalitis, which receives support from Horizon Pharmaceuticals. He has developed educational materials for PeerView Media, Inc, and Continuing Education Inc. He owns stock in ANI pharmaceuticals. Dr. Day's institution has received support from Eli Lilly for development and participation in an educational event promoting early diagnosis of symptomatic Alzheimer disease, and in-kind contributions of radiotracer precursors for tau-PET neuroimaging in studies of memory and aging (via Avid Radiopharmaceuticals, a wholly-owned subsidiary of Eli Lilly).

CHvD receives research support from the National Institute on Aging of the National Institutes of Health, Biogen, Eisai, Eli Lilly and Company, Janssen, F. Hoffmann-La Roche, Ltd./Genentech, UCB, and Cerevel. He serves as a consultant for F. Hoffman La Roche, Ltd., Eisai, Ono, and Cerevel.

EMM receives Grant Funding from NIA; Institutional funding from Eli Lilly, Hoffmann-La Roche, Eisai. He is a DSMB member (paid directly) for Alector; Eli Lilly; a Scientific Advisory Board Member (paid directly to me) for Alzamend, Fondation Alzheimer. He acts as a Consultant/Advisor for Sage Therapeutics, Eli Lilly, Sanofi, AstraZeneca,Hoffmann La-Roche

NCF reports consulting fees from Biogen, Eisai, Ionis, Lilly, Roche/Genentech, and Siemens paid to UCL; he has served on a Data Safety Monitoring Board for Biogen. He acknowledges grant support from the Alzheimer's Society, Alzheimer's Research UK, Rosetrees Trust, the Sigrid Rausing Trust, the UK Dementia Research Institute and the UK NIHR UCLH Biomedical Research Centre.

SBB reports no competing interests relevant to this work. Her research is supported by the National Institutes of Health and the Michael J Fox Foundation.

JLlG's research is supported by NIH-NIA (K01AG073526), the Alzheimer's Association (AARFD-21-851415, SG-20-690363), the Michael J. Fox Foundation (MJFF-020770), the Foundation for Barnes-Jewish Hospital and the McDonnell Academy.

J.L. (Jae-Hong Lee) and J.H.R. (Jee Hoon Roh) report no competing interests. They receive research support for this work from the Korea Dementia Research Project through the Korea Dementia Research Center (KDRC), funded by the Ministry of Health & Welfare and Ministry of Science and ICT, Republic of Korea (RS-2024-00344521).

TLSB has investigator-initiated research funding from the NIH, the Alzheimer's Association, the Barnes-Jewish Hospital Foundation and Avid Radiopharmaceuticals. Dr. Benzinger participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly and Company, Biogen, Eisai, Jaansen, and F. Hoffmann-La Roche, Ltd. She also serves as an unpaid consultant to Eisai and Siemensand is on the Speaker's Bureau for Biogen.

RJP is Neuropathology Core Leader for the DIAN observational study and the DIAN Trials Unit. He receives research support for this work from the National Institute on Aging (U19 AG032438, U19AG032438-09S1, R01AG068319). His laboratory receives cost recovery funding from Biogen for tissue procurement and processing services related to ALS clinical trials. Neither he nor his family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company.

CX is supported by National Institute on Aging (NIA) grants R01 AG067505 and R01 AG053550.

All other authors have no disclosure.

Supplementary material

Supplementary material is available at *Brain* online.

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46. Public Workshop: EVALUATING INCLUSION AND EXCLUSION CRITERIA IN CLINICAL TRIALS.

Figure legends

Figure 1 Systematic review flow chart. Flowchart outlined the sequential steps in the systematic literature review. VUS:Variant with uncertain significance.

Figure 2 DIAN-TU algorithm to classify variant trial eligibility. Algorithm to assess eligibility for DIAN-TU trials. This model was modified from the algorithm previously proposed by

Guerreiro et al. in 2010 and Hsu et al. in 2018. gnomAD frequency is used to determine whether APP, PSEN1, and PSEN2 variants represented rare or common polymorphisms. ADAD pathogenic variant PSEN1 A79V allele frequency is used as a cut-off reference to define for rare variants. Additional supportive criteria include: a.Whether other variants at the same residue have been previously confirmed as pathogenic. b.Whether a given presenilin variant is at a residue conserved between PSEN1 and PSEN2. c.Number of unrelated families in which variant is present, at a consistent age at onset and evidence of AD biomarkers. d. Number of generations with EOAD (<65 years). e.In silico predictions (CADD, REVEL score or comparable computational score). ** The presence of multiple affected family members is considered supportive evidence but is not required for variant review or trial inclusion. APP, PSEN1, and PSEN2 de novo variants are also assessed for pathogenicity by the Clinical-Genetic Committee using the DIAN algorithm. Families that exhibit multigenerational incidence of biomarker-confirmed Alzheimer's dementia and age of onset under 40 years—are automatically deemed eligible for inclusion in the trial while additional evidence is systematically gathered. AD=Alzheimer's disease. AC=Allete count. EOAD: Early-onset Alzheimer's disease. CADD: Combined Annotation Dependent Depletion. REVEL: Rare exome variant ensemble learner. GnomAD: Genome Aggregation Database.

Figure 3 Global distribution of pathogenic ADAD variants. Number of pathogenic variants in APP, PSEN1, or PSEN2 by country. The map displays PSEN1 variants in green, APP variants in yellow, and PSEN2 variants in red. The colors indicate the presence of different genetic variants across various countries, but they don't depict the distribution within each country. The count of variants within individual genes is represented numerically.

Figure 4 Individual symptomatic AAO vs variant AAO and accuracy assessment of AAO. Panel A & B present each affected individual participant's symptomatic age at onset (AAO) (n=2110) on the y-axis, plotted against values predicted from variant age at onset on the x-axis. Panel A shows all individuals combined regardless of gene type. Plot points for everyone are colored/shaped according to each ADAD variant. Panel B Shows all individuals according to gene (PSEN1, PSEN2, APP). Panel C shows the relationship between converters and the variant AAO, while panel D shows the relationship between converters and parental AAO. Each different color

represents the different genes. Blue square represents PSEN1, red dots represent PSEN2, and green triangle represents the APP gene. Regression lines and adjusted R2 values showing the strength of the association between individual AAO and variant AAO are displayed. AAO: Age at Onset

Table 1 Distribution and characteristics of variants with available AAO across *PSEN1*, *PSEN2*, *APP*

	PSEN1	***PSEN2***	***APP***	**Total**
Affected family members, no. (%)	1591 (75.4%)	117 (5.5%)	402 (19.1%)	2110
All ADAD variants with known AAO, no. (%)	176 (77.5%)	20 (8.8%)	31 (13.7%)	227
Age at Onset, mean (SD) (year)	44.9 (9.4)	59.5(6.8)	52.1(8.1)	47.3 (10.1)
DIAN-TU Trial Eligible Variants				
DIAN-TU Trial Eligible Variants no. (%)	195 (86.3%)	8 (3.5%)	23 (10.2%)	226
DIAN-TU Trial Eligible variants with known AAO (year) Mean (SD)	43.2 (8.2)	53.5 (7.0)	47.5 (7.2)	43.9 (8.3)
Converters Analysis				
DIAN-Converters, mean (SD) (year)	42.8 (4.6)	48.6	48.2 (6.1)	43.4 (7.7)
Parental AAO, mean (SD)	43.6 (5.7)	47.0	48.1 (4.9)	44.5 (7.3)
Mean Variant AAO, mean (SD)[a] (year)	43.9 (4.7)	50.2	48.4 (4.6)	44.3 (6.5)
Difference between DIAN AAO (year) (Converter - parental AO), Mean (SD)	−1.3 (3.5)	1.6	−0.4 (7.0)	−1.1 (5.6)
Difference between DIAN AAO (year) (Converter - variant), Mean (SD)	−1.1 (2.8)	−1.6	−0.4 (7.1)	−0.9 (5.2)

This table presents the distribution of affected family members and pathogenic variants within the *PSEN1*, *PSEN2*, and *APP* genes and their respective ages at onset (AAO). Included are counts of affected individuals, pathogenic variant numbers, and eligibility for the DIAN-TU study. It details the mean AAO across genes, DIAN-TU trial eligibility, mean AAO for converters, parental AAO, and variant AAO. The table also compares the AAO differences between DIAN converters and both parental and known variant averages, with standard deviation (SD) indicating the precision of these mean values.

[a]All percentages are relative to the total numbers within each respective category.

Box 1 Definitions and methods for calculating variant age at onset (AAO) and estimated years to symptom onset (EYO) in ADAD participants

Participant symptomatic AAO: The age at which progressive symptoms attributed to AD [25] (e.g., cognitive, behavioral, or motor) were first noticed by someone who knew the participant well (i.e., their collateral source).

Parental/family proxy AAO: The age at which progressive symptoms attributed to AD (e.g., cognitive, behavioral, or motor) were first noted in the participant's parent or relative.[25]

Variant AAO: The mean AAO for a specific ADAD variant calculated across all known symptomatic carriers with the same variant (e.g. mean age of onset for all participants known to carry *PSEN1 E184D*).[17, 22] For this analysis, variant AAO is reported only when the age at which first progressive symptoms attributed to AD was available for three or more carriers of the same variant.

Estimated years to symptom onset (EYO): Calculated by subtracting the variant AAO or parental AAO from the age of the participant at the study visit (e.g. a participant of 25 years with a parental AAO at 37 will be at EYO -12). EYO serves as a variable of time along the disease stages of ADAD, centered on the individual participant's estimated AAO (EYO=0). EYO<0 refers to participants who are younger than their estimated AAO (i.e., those who have not yet reached their EYO). EYO>0 refers to participants who are older than their estimated AAO (i.e., those who have exceeded their EYO). [16,26,27,28]

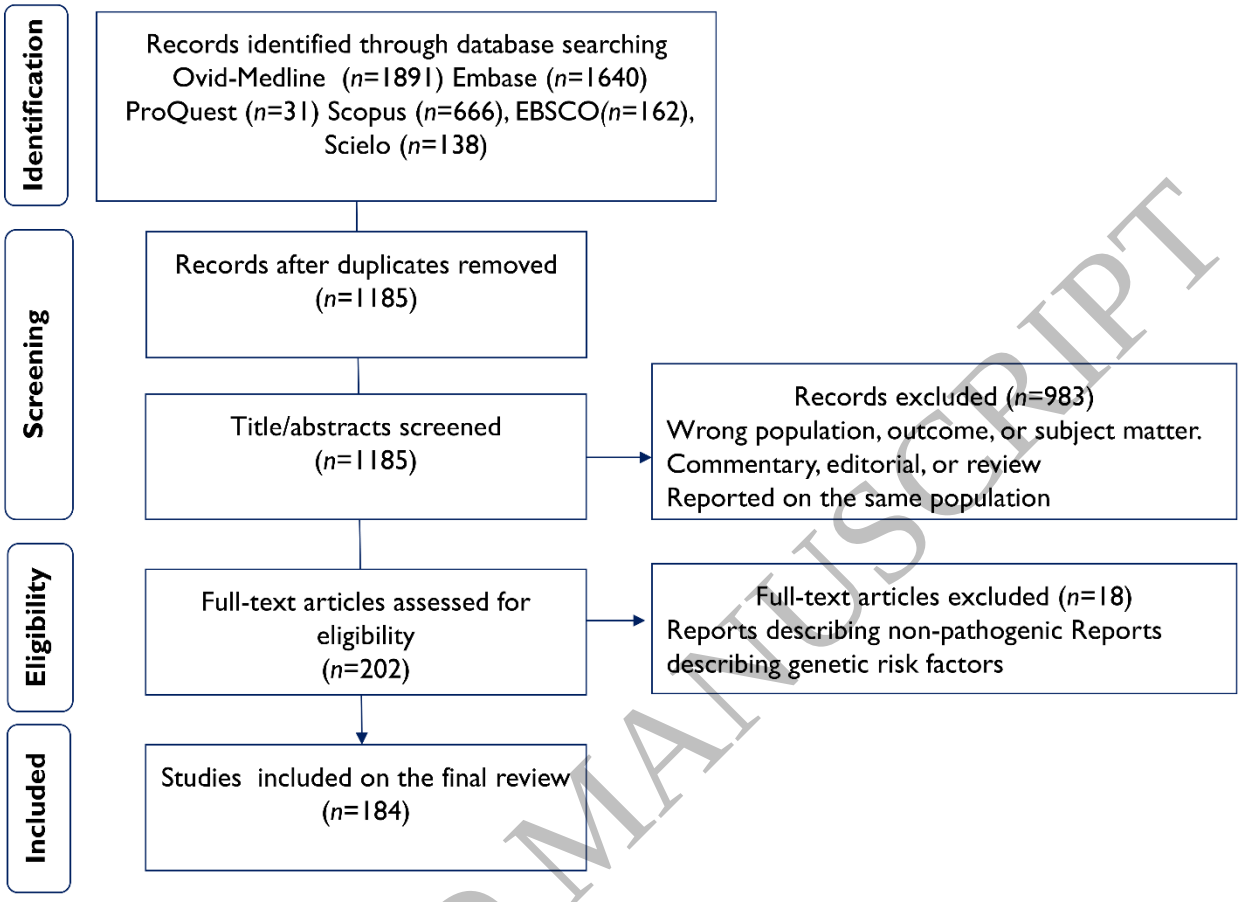

Figure 1
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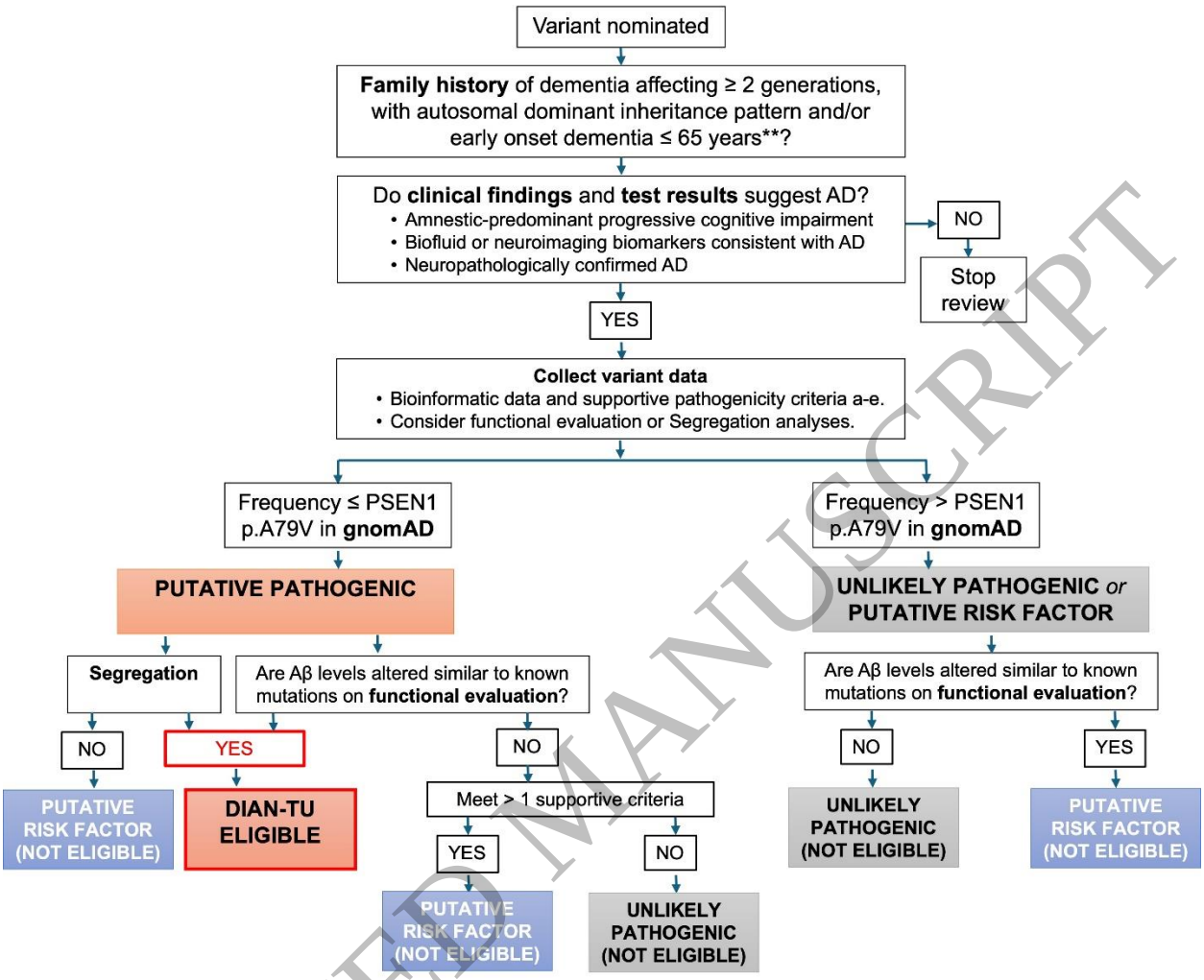

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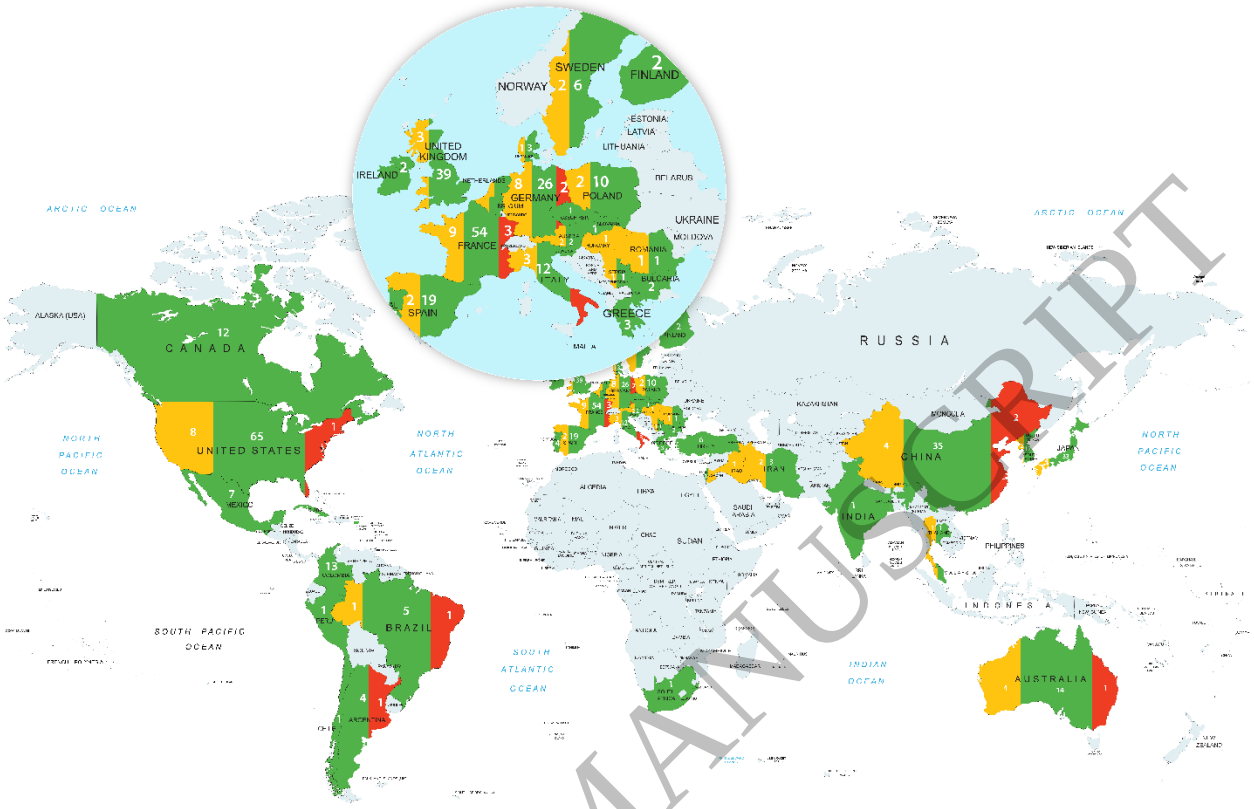

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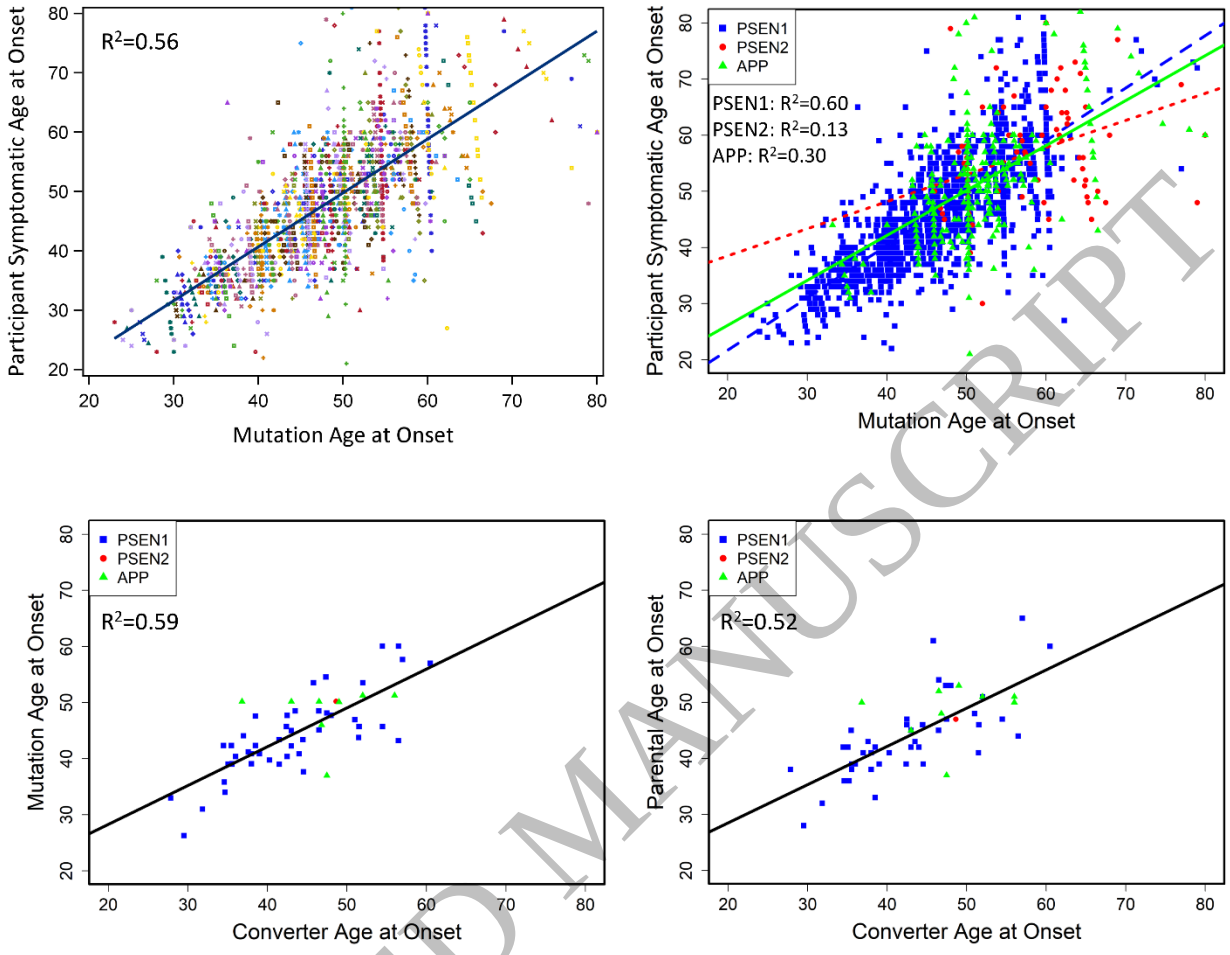

Figure 4
213x165 mm (x DPI)