The Early-Onset Alzheimer's Disease Whole-genome Sequencing Project: study design and methodology

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ABSTRACT

INTRODUCTION: Sequencing efforts to identify genetic variants and pathways underlying Alzheimer's Disease (AD) have largely focused on late-onset AD although early-onset AD (EOAD) accounting for ~10% of cases is largely unexplained by known mutations, resulting in a lack of understanding of its molecular etiology.

METHODS: Whole-genome sequencing and harmonization of clinical, neuropathological, and biomarker data of over 5,000 EOAD cases of diverse ancestries.

RESULTS: A publicly available genomics resource for EOAD with extensive harmonized phenotypes. Primary analysis will (1) identify novel EOAD risk loci and druggable targets; (2) assess local-ancestry effects; (3) create EOAD prediction models; and (4) assess genetic overlap with cardiovascular and other traits.

DISCUSSION: This novel resource complements over 50,000 control and late-onset AD samples generated through the Alzheimer's Disease Sequencing Project (ADSP). The harmonized EOAD/ADSP joint call will be available through upcoming ADSP data releases and will allow for additional analyses across the full onset range.

1. BACKGROUND

Although aging is the predominant biological risk factor for developing Alzheimer's Disease (AD), about 5-10% of cases (e.g. ~250,000 individuals) in the US alone [1], show symptom onset before 65 years and are classified as early-onset Alzheimer's Disease (EOAD). A subset of EOAD cases are clinically similar to late-onset AD, with predominant cognitive impairment in the memory domain [2]. However, EOAD tends to be more aggressive in its course [3, 4] and shows a higher prevalence of atypical clinical features and impairment in other cognitive domains including impairment in executive dysfunction, apraxia, dyscalculia, visual dysfunction, and aphasia (fluent and non-fluent) [5-7]. In line with these differences in clinical presentation, individuals with EOAD often show different profiles on brain imaging and neuropathological assessment, even at similar stage of clinical impairment. EOAD tends to show less atrophy and neuropathological changes in medial temporal lobe structures (i.e. hippocampus and entorhinal cortex) but more widespread and faster progressing cortical atrophy and hypometabolism, and a higher degree of tau pathology in neocortical regions[8-13].

The early onset and clinical heterogeneity result in particularly detrimental medical, emotional, social, and financial consequences for patients and their families. Individuals with EOAD often receive a significantly delayed diagnosis [14], are misdiagnosed with other psychiatric/ neurodegenerative diseases such as Frontotemporal Dementia [15-20], and are often excluded from clinical research trials [21] resulting in stigmatization and inadequate access to treatment, disease education, patient and caregiver support resources.. The disease onset during the prime earning years frequently results in significant deprivation of income, loss of employment, health insurance and retirement benefits.

While EOAD has a considerable genetic basis with a heritability of over 90% [22], variation in known EOAD genes (including *APP, PSEN1, PSEN2*) accounts for only 5-10% of cases [23, 24]. Most cases are either sporadic or follow a non-Mendelian pattern of inheritance but are expected to be enriched for causative genetic factors.

Identifying this missing heritability is essential to understand the molecular mechanisms underlying this devastating form of the disease and identify more effective targets for screening, prevention, and treatment. However, individuals with EOAD have been significantly underrepresented in the major genomic efforts of AD. The leading national effort, the Alzheimer's Disease Sequencing Project (ADSP) [25] and its follow up study (Alzheimer's Disease Sequencing Project – Follow Up Study; ADSP-FUS)[26], focus mostly on the late-onset form of disease. The Dominantly Inherited Alzheimer Network is restricted to autosomal dominant EOAD accounting for only 2-5% of EOAD cases [27]. The Alzheimer's Disease Neuroimaging Initiative [28] and the Longitudinal Early-onset Alzheimer's Disease Study [29] are designed to track the progression of AD across disease stages with clinical, imaging, and biospecimen biomarkers. They are not, however, necessarily designed for gene discovery using large sample sizes.

To facilitate EOAD variant discovery we have implemented the *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* (R01AG064614), a collaborative initiative to generate and analyze a large-scale genomics resource for EOAD comprising several thousand EOAD cases of diverse ancestry. These case-control data will be complemented by whole-genome sequencing (WGS) data generated on over 200 multiplex families loaded for EOAD through complementary efforts (RF1AG054080, U24AG056270). Application of ADSP pipelines for processing and harmonization of genomic and phenotype data across all datasets

ensures compatibility with ADSP and ADSP-FUS efforts. Inclusion of diverse ancestries will allow us to identify EOAD variants not detectable in individuals of European ancestry, providing critical information on mechanisms underlying EOAD subtypes and observed health disparities. Primary specific goals are to (1) create a publicly available large-scale genomics resource for EOAD with WGS data generated and processed using ADSP pipelines and extensive harmonized phenotype data; (2) identify novel genomic EOAD risk loci and loci modulating age at onset and decline in specific cognitive domains; (3) assess the role of polygenic and local-ancestry effects in EOAD etiology; (4) create EOAD-specific prediction models; (5) assess genetic overlap with cardiovascular and other potentially associated traits; and (6) identify druggable targets.

2. METHODS

2.1 Study design

The *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* is a collaborative large-scale WGS effort on EOAD led by the Taub Institute for Research on the Aging Brain at Columbia University, The Hussman Institute for Human Genomics (HIHG) at the University of Miami, and the NeuroGenomics and Informatics Center at Washington University School of Medicine in St. Louis in collaboration with the Alzheimer's Disease Genetics Consortium (ADGC). The project leverages existing sample ascertainment, sample processing, and data generation and processing pipelines by major AD research centers. EOAD samples and extensive phenotype data were obtained from the NIH-funded Alzheimer's Disease Research centers (ADCs) via the National Centralized Repository for Alzheimer's Disease and Related Dementias and the National Alzheimer's Coordinating Center (NACC), with AD research centers at Columbia University, University of Miami, Washington University School of Medicine in St. Louis, the Adult Changes of Thought Study [30], and other sites providing additional samples. Descriptions of the individual cohorts can be found in the Supplemental Material. WGS data

were generated at The American Genome Center (TAGC) at the Uniformed Services University of the Health Sciences (USUHS). Sequence data are being QCed, harmonized, and jointly called through the Genome Center for Alzheimer's Disease (GCAD) employing bioinformatics protocols implemented through the ADSP. Where missing, genome-wide association study (GWAS) data on the same samples are being generated, QCed, and imputed to the latest ancestry-specific reference panels.

2.2 Inclusion/exclusion criteria

Included in the *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* are cognitively healthy individuals, and individuals with EOAD or early-onset mild cognitive impairment (MCI) of diverse ancestries with an age of onset between <65 years meeting National Institute on Aging (NIA) criteria for AD or MCI [31, 32]. While baseline age required for controls is 60 years, 96% of the control samples are 70 years or older, and mean age at last evaluation is 85 years. Both EOAD cases with predominant amnestic impairment as well as cases with predominant impairment in other cognitive domains (i.e non amnestic presentation) and atypical presentation are included, allowing to identify the genetic variation underlying EOAD subtypes. A subset of individuals have a definite AD diagnosis through brain autopsy based on Braak and the Consortium to Establish a Registry for Alzheimer's Disease's criteria, or have CSF, plasma, or imaging biomarkers [33, 34]. Cases with competing diagnoses (Parkinson's disease, Huntington's disease, frontotemporal dementia, vascular dementia, depression, etc.) or with a known mutation in *APP*, *PSEN1*, and *PSEN2* are excluded from the effort. For all samples selected and whole-genome sequenced for this project sequence data in these genes are scrutinized ahead of any further downstream analyses to identify any additional samples potentially carrying pathogenic variants in these genes. All participants have provided

informed consent according to the Declaration of Helsinki and the policies of the respective institutional review boards at the contributing centers.

2.3 Ancestral diversity

The study sample specifically includes individuals of diverse ancestry – African American (AA), Hispanic (HI), non-Hispanic White, Asian. It is clear that genetic ancestry plays a critical role in complex diseases and observed health disparities in AD [35-38]. AA and HI have up to twice the incidence of AD as Non-Hispanic Whites (NHWs) [39] and heritability of AD differs between ethnic groups [37, 40]. Alleles in known AD genes, (e.g., *APOE* and *ABCA7,* among others) account for some disease risk variability. African ancestry-specific AD risk variants in *ABCA7*, *TREM2* and other genes have been described by our group and others [35, 41, 42], along with loci specific to HI individuals [43-47]. For *ABCA7* in particular, a 44bp deletion is strongly associated with AD in AA [35] and is also present in HI with a high proportion of African global ancestry (41.8%) [48]; while other rare truncating and splice altering variants confer risk in NHW [49-51]. This suggests that some AD risk/protective variants will have European origins while others will have African or Native American origins, and still other variants may be rare, recent in origin, and unique to individual populations. These findings underscore the importance of investigating diverse populations for ancestry-specific AD risk variants, and the sample included in this project will allow to assess the ancestral background at identified genetic loci associated with EOAD.

3. RESULTS

3.1 Whole-genome Sequencing and Downstream Bioinformatics Processing

In total, the *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* has sequenced 4,097 EOAD and early-onset MCI samples meeting our minimum inclusion criteria (affection

status, age at onset, sex, and adequate DNA). In addition, samples from 1,109 elderly cognitive controls have been sequenced through this effort, selected to match case samples by sex and ancestry yielding the largest EOAD genomics resource to-date. These samples complement over 50,000 control and late-onset AD (LOAD) samples generated with similar protocols through the ADSP Discovery and Follow-up Studies and related efforts all with harmonized phenotype and genomics data allowing for additional analyses across the full range of onset, including analyses of factors modulating age of onset and shared genetic heritability across age at onset groups. This harmonized EOAD/ADSP joint call will be available through the upcoming fifth ADSP data release.

3.1.1 Sequencing library preparation and whole-genome sequencing. Sequencing library preparation and WGS of samples missing WGS data was performed through The American Genome Center at the Uniformed Services University of the Health Sciences (USUHS). USUHS has extensive experience in large-scale WGS workflows, including several large consortia-based sequencing efforts (NIA Alzheimer's Disease Sequencing Program, National Institute of Mental Health Army Study to Assess Risk and Resilience in Servicemembers – Longitudinal Study, the National Institute of Neurological Disorders and Stroke Dementia Resolution Study, Applied Proteogenomics Organizational Learning and Outcomes, etc). Samples were assessed for quantity (Quant-iT PicoGreen dsDNA assay) by concentration. Sequencing libraries were prepared using the TruSeq PCR-free Library Prep kit (Illumina) with unique dual index adapters and quantified using qPCR (KAPA Library Quant Kit). Libraries were normalized to 4 nM into a 24-26 sample pools. Pool concentration was quantified using qPCR (KAPA Library Quant Kit) and clustered onboard the NovaSeq 6000 platform (Illumina) with sequencing runs conducted on a S4 flowcell with paired-end 150 bp read length. After sequencing de-multiplexing was performed (bcl2fastq v2.20) and resequencing analysis on a QA workflow (Illumina HAS2.2);

data were reviewed for yield, read alignment percentage, bases greater than Q30, percent read duplicates, Picard mean coverage and contamination (FREEMIX < 0.05 by VerifyBamID). QA passing genomes were inventoried for data transfer of FASTQ sets to the Genome Center for Alzheimer's Disease (GCAD).

3.1.2 Bioinformatics Processing of WGS data. Sequence data generated by USUHS were processed and joint called by GCAD using the VCPA [52] pipeline developed by GCAD for ADSP-related projects. The approach uses Genome Analysis Toolkit (GATK) [53, 54] for single nucleotide variant (SNV)/Indel calling. The workflow includes mapping reads to hg38, BAM sorting, duplicate marking, quality scores, and local read realignments around known indels. GATK HaplotypeCaller is then applied to generate individual genotype calls in genomic and project-level VCF formats.

3.1.3 Quality Control of WGS data. In line with ADSP efforts, variant-level quality metrics include VQSR quality tranches, call rates, average read depths, excessive read depths [>500 reads], and excess heterozygosity or departure from HWE [25, 55]. Sample-level QC includes within-sample genotype call rate, Ti/Tv ratio for SNVs, heterozygosity/homozygosity ratio, and excess burden of singleton/doubleton variants. The joint-genotyped called pVCF will be annotated using the pipeline which generates variant-level assessments of functional impact on genes and genetic regulation. Our pipeline is based upon the Ensembl Variant Effect Predictor, which overlays exon, transcript, and regulatory element information from the Ensembl database to generate all possible consequences (missense, frameshift, splicing, etc) a variant may have. Variant consequences relative to Ensembl/GENCODE transcripts are assigned an impact category (high, moderate, low, etc), and multiple variant scoring approaches are incorporated (CADD, REVEL, CATO, etc).

3.2 Clinical and cognitive assessment and phenotype data harmonization

All individuals from all contributing sites have completed standard clinical assessments that include self-reporting, informant reporting, medical records, and direct assessment information. Additionally, past medical history, family history, and detailed neurological data has been obtained. A total of 3,868 of the 5,206 cases and controls are from an ADC and have NACC Uniform Data Set assessment. Study personnel at the contributing sites conduct the clinical assessment, including an interview to assess subjective neuropsychiatric symptoms that pertain to activities of daily living, cognition, and mood. Disease history is collected, a Clinical Dementia Rating (CDR) is calculated to assign degree of severity, a neurological examination is conducted, and extensive cognitive test batteries are employed.

To ensure compatibility across datasets and with the leading LOAD sequencing efforts such as the ADSP Discovery and ADSP-FUS, we will compile, harmonize, and generate phenotypes, subphenotypes (AD diagnosis), cognitive measures, demographics, stage, age-at-onset (AAO; case) or age-at-examination (AAE; control), sex, race/ethnicity, and genomic data across all datasets. All phenotype data were checked for quality, integrity, and consistency, and we have developed a common coding scheme to match covariates and value formats (e.g. range and precision for continuous values, and codes for categorical data) from the different studies. We will recode the data using standardized measures whenever possible. We will compare summary statistics/distributions across studies and will conduct outlier studies to identify any potential coding errors or data collection bias.

3.2.1 Diagnosis and AAO/AAE. We will utilize established criteria for the diagnosis of AD which are available in all cohorts. The diagnoses of mild cognitive impairment and probable/possible

AD will be made using the NIA Alzheimer's Association workgroup diagnostic guidelines [31, 32] based on the in-person assessment by the study staff and norms based on age, education, and ethnic group. To assess AAO, we require information from a knowledgeable caregiver or family member concerning when the person manifested constant forgetfulness resulting in an inability to manage his schedule or daily activities. For normal controls without cognitive impairment, AAE will be the age when the individual was last examined.

3.2.2 Harmonization of neuropsychological data. Harmonization of neuropsychological data will be done in collaboration with the ADSP Phenotype Harmonization Consortium (U24AG074855). Each individual dataset has an extensive cognitive battery examining a variety of cognitive functions including memory, visuospatial awareness, language, and executive function. We will evaluate the internal consistency of each study's battery using Cronbach's α [56]. To derive harmonized composite scores for cognitive domains across cohorts (memory, visuospatial awareness, language, executive function), we will employ modern psychometric methods to the pooled sample, which tend to have better validity than scores derived from standard approaches, and are specifically recommended for genetic analyses [57-62]. Using information from time of first diagnosis for cases and last visit for cognitively healthy controls, we will recode observed item responses to avoid sparse response categories, preserving variability at the extremes of the distribution. Separately for cases only and the total sample, we will then fit, for each domain, factor analyses (single factor models assuming no residual relationships, and bifactor models assuming covariance by cognitive subdomains or methods effects) [63]. To determine which model is superior, we will compare single factor and bi-factor models for both sets of samples assessing the correlation between factor scores, compare the loadings for each indicator on the overall domain factor with and without the secondary domain

structure, and use fit statistics [64]. Missing data will be handled using full information maximum likelihood estimation [65].

3.2.3 Functional impairment categories for cognitive domains. Thresholds to define "substantial" relative impairments will be calculated as previously described [66]. This will create, for each subject, labels reflecting the predominant EOAD subtype (i.e., AD-Memory, AD-Executive, AD-Language, AD-Visuospatial, AD-No Domains, and AD-Multiple Domains). We will also analyze groups with a prominent or neutral memory impairment vs. those with relatively intact memory (I.e. AD-Memory, AD-No Domains, and AD-Multiple Domains, vs. the other three subtypes). These constructed categorical variables will provide a set of harmonized measures differently capturing cognitive impairment that can be readily used in genomic [67] and clinical [68] analyses.

3.2.4 AD Biomarkers normalization. A subset of participants recruited on this study cerebrospinal fluid (CSF) or plasma (Aβ, tau, ptau, TREM2, NFL, SNAP25) or amyloid imaging (see Table 1), and we expect this number to further increase through complementary efforts. As these biomarkers have been generated in different centers using different platforms it is not possible to simply combine the data across studies. We have developed robust approaches to harmonize AD biomarkers across datasets [69, 70]. Briefly, normalized z-scores are calculated by using the mean and standard deviation units across each cohort and applied to the entire endophenotype in order to account for within cohort variation. Then, we used a mixture modeling, which is a statistical method for estimating subpopulations within an overall group, to determine the biomarker positive and negative individuals. We assume that there are two normally distributed subgroups within each dataset. Using an expectation-maximization algorithm in the R package mixtools v1.0.4 [71], we can calculate estimated means, standard

deviations, and subgroup proportions for each study. We can calculate the intersection of the estimated Gaussian curves. Based on the assumption of two univariate normal distributions within each study we will obtain two estimated means (μ1 and μ2), two estimated standard deviations (σ1 and σ2), and two estimated mixing proportions. From these models we can determine biomarker status for each of the specific analytes, and perform further analyses [69, 70].

4. DISCUSSION

Besides creating a publicly available large-scale genomics resource for genetic research on EOAD and AAO with extensive harmonized phenotype and biomarker data, the *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* has several immediate analysis goals. Harmonized WGS data will be scrutinized with a wide array of computational tools and statistical approaches to identify novel genomic risk and protective loci for EOAD subtypes, decline in specific cognitive domains, and loci modulating AAO. These analyses include single variant, gene-based, and sliding window analyses and are expected to identify novel genes, pathways, and etiologic mechanisms that are shared or specific to a particular ethnic group (see Figure 1). Comparison with WGS data on multiplex EOAD families generated by our groups will allow us to determine which variants are associated with familial vs. sporadic disease. Incorporation of LOAD genomic data will allow us to identify loci, genes, and mechanistic pathways modulating AAO, map genomic loci shared between the early- and late-onset forms, and calculate the extent of shared EOAD/LOAD heritability. These findings will be pivotal steps in disentangling the genetic and mechanistic overlap with the late-onset form and clarifying whether both forms are in fact distinct disease entities. A wide array of computational approaches such as Linkage Disequilibrium Score Regression, Genome-wide Complex Trait Analysis, colocalization, and Mendelian randomization approaches coupled with extensive multi-tissue multi-omics data available to us will be employed

to infer causality of identified variants and genes, and identify potential druggable targets. Second, we will comprehensively assess the role of polygenic effects: to understand EOAD etiology and EOAD genetic risk, develop effective screening tools, and identify druggable targets. It is critical to determine whether EOAD and its subtypes are the result of rare genetic variation of strong effect, or if it is highly polygenic with weak effects of individual variants. Analyses of networks and polygenic effects in the late-onset form of AD have identified several specific gene-sets that seem to influence disease including immune response, inflammation, and endocytosis [72, 73]. To test these polygenic hypotheses in EOAD, we will perform in-depth network and pathway-based tests of association, and construct and assess the utility of genetic risk scores (calculated by summing an individual's genome-wide genotypes weighted by their corresponding z-scores) employing state of the art methods specifically developed for these analyses. Sub-analyses risk score analyses will include non-genetic factors as this can improve predictive power of polygenic scores significantly [74]. Third, we will comprehensively assess the role of ancestry in EOAD and its subtypes, capitalizing on the rich diversity of this dataset. All analyses will be conducted within and across ancestry groups, and we will utilize a wide array of tools to assess global ancestry, local ancestry, admixture, and the evolutionary history of identified risk and protective alleles. These analyses will determine variants, loci, and pathways that are shared across ethnic groups, as well as variants that are specific to a particular ethnic group. The results will provide pivotal information for development of personalized preventive and therapeutic measures, and disentangling observed health disparities. Fourth, we will assess genetic overlap with traits potentially sharing or impacting etiologic mechanisms such as cardiovascular disease by employing computational approaches developed to determine shared heritability. Finally, bioinformatics and phenotype harmonization protocols in line with the ADSP and ADSP-FUS studies will allow for joint examination across these efforts allowing an extensive array of additional critical hypotheses to disentangle EOAD etiology, including assessment of blood biomarkers, neuropathological

changes, and structural and functional brain imaging phenotypes across the full spectrum of AAO strata. The harmonized EOAD/ADSP joint call will be available through upcoming ADSP data releases via the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS; https://dss.niagads.org/).

5. Data Sharing and Publication Policy

The data have been deposited at NIAGADS (https://dss.niagads.org/) and are available as qualified access. To request access to the dataset, researchers can submit a data access request to NIAGADS for the ADSP dataset (ng00067; https://dss.niagads.org/datasets/ng00067/).

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CONFLICTS OF INTEREST
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CONSENT STATEMENT
All human subjects provided informed consent.

KEY WORDS
Alzheimer's disease, early-onset Alzheimer's disease, whole-genome sequencing, study design, genetics.

Figure 1. Project flow and primary aims of the Early-Onset Alzheimer's Disease Whole-genome Sequencing Project

The 5206 samples (4097 cases and 1109 controls) sequenced by the Early-Onset Alzheimer's Disease Whole-genome Sequencing Project will be integrated with over 50,000 whole-genomes collected by the Alzheimer's Disease Sequencing Project (ADSP) and 200 multiplex families loaded for early-onset Alzheimer's Disease (EOAD). Phenotype information will be harmonized using AD biomarkers, brain imaging, cognitive, neuropath and multi-omics data. First-pass analyses will be conducted to identify novel loci associated with EOAD, cognitive decline, and age-at-onset modulation. Follow-up analyses will be conducted to assess polygenic risk, mechanistic pathways, comparison of the genetic architecture between EOAD and late-onset Alzheimer's Disease (LOAD) and vascular traits, and to examine local ancestry.

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- Sequencing efforts to identify genetic variants and pathways underlying Alzheimer's Disease (AD) have largely focused on late-onset AD although early-onset AD (EOAD) accounting for ~10% of cases is largely unexplained by known mutations. This results in a significant lack of understanding of the molecular etiology of this devastating form of the disease.
- The Early-Onset Alzheimer's Disease Whole-genome Sequencing Project is a collaborative initiative to generate a large-scale genomics resource for early-onset Alzheimer's Disease with extensive harmonized phenotype data.
- Primary analyses are designed to (1) identify novel EOAD risk and protective loci and druggable targets; (2) assess local-ancestry effects; (3) create EOAD prediction models; and (4) assess genetic overlap with cardiovascular and other traits.
- The harmonized genomic and phenotypic data from this initiative will be available through NIAGADS.

Research In Context

Systematic review:

Relevant literature and related efforts were screened by reviewing PubMed, NIAGADS and dbGaP for efforts on early-onset Alzheimer's disease (EOAD).

Interpretation:

EOAD has been largely excluded from major AD genomics efforts, resulting in an extensive lack of understanding of its underlying molecular etiology. Generation of a large-scale EOAD whole-genome resource will allow for identification of genetic variants, genes and molecular pathways underlying this form of AD.

Future directions:

Integration of the generated EOAD resource with the ADSP, ADSP-FUS, and related large-scale AD genomics, multi-omics and functional genomics efforts across a range of diverse ancestries will readily allow for examination of several additional critical hypotheses including mechanisms underlying changes in blood biomarkers, neuropathological measures, and structural and functional brain imaging phenotypes across the full spectrum of age at onset strata.

Supplemental Material

Description of datasets

The **Adult Changes in Thought (ACT) study** supports the conduct of scientific research on older adults, cognition, and brain aging via a longstanding cohort and data resources. The overall goal of ACT is to conduct research to understand factors that contribute to Alzheimer's Disease and Related Dementias, and to leverage a repository of carefully collected and curated data resources including self-report, electronic health records, biologic, and device data to deepen our understanding of the aging brain in a well characterized community-based longitudinal prospective cohort study. ACT recruits participants from random samples of Kaiser Permanente Washington (previously Group Health) health plan members, and since its inception in 1994 has recruited an Original Cohort (n=2,581), an Expansion Cohort (n=811), and a Replacement Cohort (n=2,371). The ongoing recruitment of new participants into the ACT Study as part of the Replacement Cohort is designed to maintain a study cohort of ~2,000 participants without dementia who are actively undergoing biennial follow-up. At baseline and then approximately every two years, ACT participants undergo clinical assessments including a cognitive screening test. If they score below a set threshold on the standard cognitive screening test participants are referred for additional cognitive and neurological testing, and a consensus committee then convenes to determine whether to assign a dementia diagnosis to the participant. Participants continue on the biennial track until dementia diagnosis, death, or study withdrawal.

The **Memory and Aging Project at the Knight-ADRC (Knight ADRC-MAP)** collects plasma, CSF, fibroblast, neuroimaging clinical and cognition data longitudinally and autopsied brain

samples. Knight-ADRC participants must be at least 45 years old and present no memory problems or mild dementia at the time of enrollment. There is no age at onset criteria for this cohort. Cases had to have a CDR >=0.5 whereas controls had to have a CDR=0 at last assessment. AD definition is based on a combination of both clinical and pathological information if available. Pathologic diagnosis will overrule clinical diagnosis. Autopsy information was provided if available, but it is not a requirement for enrollment. Participants recruited at the Knight-ADRC undertake annual interviews (every three years for <65 yo) to assess participant's memory and thinking; participants provide a blood sample every two years for genomic and other omic studies, lumbar puncture for CSF biomarkers and brain scans (MRI, PET) every three years. Participants are mostly Non-Hispanic white from North America (82.47%) and African American (13.3%). Since the inception of the MAP project in 1979, and as of 2022, the Knight ADRC has collected over 5,510 participants, including 2,426 AD cases, 148 FTD, 88 DLB and 2,156 cognitive normal healthy individuals. The Knight ADRC has collected over 5,121 cognitive assessments with some participants having as many as 10 data points (some participants have participated for over 30 years). As of 2022, the Knight-ADRC has over 822 active participants; there is DNA available for 3,532 individuals, CSF samples for 1,002 participants, and brain scans for 1,098 participants (2,168 MR sessions and 1,608 PET sessions). These participants also can contribute with brain donation for autopsy with neuropathology data on 1,768 participants.

The **Cardiff EOAD cohort** was ascertained using a standard clinical assessment, together with detailed family ascertainment. Our ascertainment protocol used the UK Medical Research Council -AD clinical collection scales[1], an assessment based on the Manchester structured

clinical interview for dementia[2], the Addenbrooke's Cognitive Examination[3], the NeuroPsychiatric Inventory[4], CAMDEX[5], Mattis Dementia Rating Scale[6] and the Mini-Mental state examination (MMSE)[7]. The protocol recognized the particular challenges in the diagnosis and assessment of EOAD patients including: i) distinguishing patients with anxiety/depression from AD ("pseudo-dementia"); ii) diagnosing behavioral variant frontotemporal dementia (FTD), progressive non-fluent aphasia and semantic dementia and distinguishing these conditions from AD; iii) defining AD variants such as the biparietal syndrome, and iv) providing care and support for patients and families with or at risk of Mendelian neurogenetic disease. All control samples were screened for dementia using the MMSE or ADAS-cog, were determined to be free from dementia at neuropathological examination or had a Braak score of 2.5 or lower.

The **NIA ADC** cohort includes subjects ascertained and evaluated by the clinical and neuropathology cores of the 32 NIA-funded ADCs. Data collection is coordinated by the National Alzheimer's Coordinating Center (NACC). NACC coordinates collection of phenotype data from the 32 ADCs, cleans all data, coordinates implementation of definitions of Alzheimer's disease cases and controls, and coordinates collection of samples. The ADC cohort consists of autopsy-confirmed and clinically-confirmed Alzheimer's disease cases, individuals with mild cognitive impairment (MCI), and cognitively normal elders (CNEs) evaluated using the Uniform dataset (UDS) protocol[8, 9]. Alzheimer's disease cases were demented according to NINCDS-ADRDA/DSMIV-V criteria[10, 11] or Clinical Dementia Rating (CDR)[12] ≥ 1. Controls do not meet NINCDS-ADRDA/DSMIV-V criteria for dementia, do not have a diagnosis of mild cognitive impairment (MCI), and have a CDR of 0.

The University of Miami/Case Western Reserve University/Mt. Sinai School of Medicine (**UM/CWR/MSSM**; formerly UM/VU/MSSM) cohort[13-16] contains cases and CNEs ascertained at the University of Miami, Case Western Reserve University and Mt. Sinai School of Medicine[17], including autopsy-confirmed cases and controls, primarily from the Mt. Sinai School of Medicine. Each affected individual met NINCDS-ADRDA criteria[10, 11] for probable or definite Alzheimer's disease with age-at-onset greater than 60 years as determined from specific probe questions within the clinical history provided by a reliable family informant or from documentation of significant cognitive impairment in the medical record. Cognitively healthy controls were unrelated individuals from the same catchment areas and frequency matched by age and gender, and had a documented MMSE or 3MS score in the normal range.

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Table 1. Demographic and clinical characteristics of *Early-Onset Alzheimer's Disease Whole-genome Sequencing Project* samples sequenced to date.

	Affected	Unaffected
Individuals, n	4097	1109
Early-onset MCI	541	-
Early-onset AD	3490	-
Early-onset other dementia	66	-
Female, n (%)	2178 (53.16)	695 (62.67)
Age at last evaluation (years), mean	69.46	84.90
Age at onset (years), mean	61.21	-
Early-onset MCI	64.57	-
Early-onset AD	60.73	-
Early-onset other dementia	58.98	-
Ethnicity		
NHW	3506	962
HI	310	77
AA	171	69
other	99	1
unknown	11	0
CDR		
0	37*	899
0.5	917	48
1	902	4
>=2	1803	2
% CSF biomarkers	6.66	8.39
% plasma biomarkers	6.47	12.17

* These individuals are affected with MCI and have a clinical judgment of impaired cognition.

Early-Onset Alzheimer's Disease Whole-genome Sequencing Project (n=5206)

ADSP Phenotype Harmonization Consortium
- AD biomarkers
- Brain imaging data
- Cognitive data
- Neuropath
- Multi-omics data

ADSP
(n>50,000 whole-genomes)

~200 Multiplex Families Loaded for EOAD

Identify Novel Genomic Loci
- EOAD subtypes
- Cognitive decline
- Age-at-onset modulation

Polygenic Risk

Functional Gene Mapping
- Functional Annotation
- Gene-based Analysis
- Pathway Analysis
- Architecture Comparison
  - EOAD/LOAD
  - Vascular traits
- Multi-tissue/ Multi-omics Mapping
- Mendelian Randomization
- eQTL Mapping
- Drug Repositioning

Local Ancestry