April 15, 2013

Note:

This is an update of a summary of a plan that is still being developed for the Alzheimer’s Disease Genome Sequencing Project (ADSP), an initiative jointly funded by the National Human Genome research Institute (NHGRI) and the National Institute on Aging (NIA). Readers should understand that, because this plan is still under development and has not been formally approved or announced, some important details may be subject to change.

This summary was extracted from the unfinished ADSP plan by NIH staff. The non-NIH ADSP participants that are working on the ADSP plan are not responsible for any omissions that may have resulted.

NIA is posting this summary to aid applicants to program announcement http://grants.nih.gov/grants/guide/pa-files/PAR-12-183.html “National Institute on Aging Analysis of Alzheimer’s Disease Genome Sequencing Project Data [U19]” in developing their research plan. Applicants are encouraged to contact the NIA program director for additional details:

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Overview and Summary of the Alzheimer’s Disease Sequencing Project Proposal

Overall objectives:

- Objective 1: Identify novel risk raising genes and alleles for late-onset AD.
- Objective 2: Identify novel protective genes and alleles for late-onset AD.

Four components:

1. **Family-based** sequencing: whole genome sequencing (WGS) of 582 family members from 111 informative multiplex families to identify genomic regions associated with increased risk of AD. When available the ADSP will include one WGS “unaffected” individual per pedigree in order to improve available information on vertical transmission, allow imputation, enhance phase information, and provide a contrast of unaffected chromosomes or individuals for analysis of protective alleles. Subsets of families include 229 Caucasian and 353 Hispanic (Dominican) individuals.
   
   Timeline for data production: March 2013-January 2014

2. **Case-Control** sequencing: whole exome capture sequencing (WES) of 5,000 cases / 5,000 controls for both risk raising and protective loci.
   
   **Enriched case** sequencing: in addition to the cases above, WES from an additional case group made-up of one individual from 1,000 additional AD families to identify regions associated with increased risk or protection from AD.
   
   Timeline for data production: June 2013- August 2014

3. **Replication and validation** of regions identified from case-control and family sequencing in a large number of samples from well phenotyped individuals by targeted sequencing and/or genotyping. 50,000 samples are targeted, but the final number may be smaller.
   
   Timeline for data production January 2014-March 2015

4. Deep **targeted** sequencing of candidate AD regions identified by previous linkage and chip-based association (GWAS and exome chip) and ADSP sequenced-based, analyses described above to identify potential functional variants beyond the exomes in regions implicated in and validated for AD risk and protection.
   
   Timeline for data production: December 2014- summer 2015
Over View and Summary of the Alzheimer’s Disease Sequencing Project Proposal

Samples:

• Samples for the family studies will be drawn from NIA-Late Onset of Alzheimer’s Disease (NIA LOAD) family study, National Cell Repository for Alzheimer’s Disease (NCRAD) families and families contributed by: Columbia University, University of Miami, University of Washington, University of Pennsylvania, Vanderbilt University, and Erasmus University.

• Samples for the Case-Control study will be drawn from the Alzheimer’s Disease Genetics Consortium (ADGC) and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortia.

Data:

All project sequence and other data will be made available through the NIAGADS website https://www.niagads.org/ including pre-existing phenotype, GWAS and gene chip data for the samples to be used.

Additional details:

Family study: Whole genome sequencing (WGS) for select subjects from large multigenerational extended families with late onset Alzheimer disease to identify novel genes and alleles associated with the occurrence of late-onset AD. Existing qualifying multiplex families from diverse race/ethnic backgrounds (N=111) have been identified for inclusion.

These families represent several different cohorts including the NIA-Late Onset of Alzheimer’s Disease (LOAD) family study, the National Cell Repository for Alzheimer’s Disease (NCRAD), Caribbean Hispanic Families collected by Dr. Richard Mayeux, and families from cohorts collected by investigators at the University of Miami, University of Washington, University of Pennsylvania, Vanderbilt University and Erasmus University.

Families were given priority status based on the number of affected individuals and generations affected, absence of known mutations, age, and common risk variants (APOEε4). Five tiers were created to rank the largest families. Initial sequencing will focus on tier 1 and 2 families. Briefly, tier 1 families were identified as having multiple affected individuals with DNA samples (>4 individuals) in two branches (e.g. cousins) who are not “clustered” for the APOEε4 allele, and ≥5 individuals if they are a sibship. In addition, tier 2 was defined as families that still have a high density of affected individuals and have at least one affected individual without an APOEε4 allele but also may have one or more with the APOEε4 allele so long as the age of onset is less than 72 years.

Over 1500 families were reviewed yielding 256 pedigrees containing four or more affected individuals without known mutations or risk genes. 111 multiplex families (62 tier 1 and 38 tier 2 and 11 tier 3-5) have been vetted for initial sequencing.
There are at least 1,000 additional families which are available but smaller with three or fewer affected family members but with strong family histories. The family selection committee has also been asked to select one individual from each of the remaining families for inclusion in the Case/Control project. 1000 affected individuals from these families have been selected for the Enriched Case sample set and whole exome capture sequencing (WES).

**Case / Control:** The objective is to use a case-control design and WES capture in a sample of European-Americans to identify novel genes and alleles associated with the increased risk of or protection from late-onset AD. Using sex, age and APOE genotype to calculate risk 5,000 case samples have been identified having definite or probable AD who were at lowest risk for AD. Control samples (N=5,000) have been identified as those free of disease having the least amount of expected misclassification. Based on previous simulations and power calculations under a variety of scenarios, the cases and controls for the risk raising and protective analyses are the same.

Samples for the case-control design will be drawn from two Alzheimer’s Disease Genetics consortia:

**ADGC.** This consortium currently has available for sequencing ~10,000 cases and ~10,000 controls. The ADGC is continually expanding this sample by collecting new cases and controls from the 29 NIA-funded Alzheimer Disease Centers (ADCs) and by establishing new collaborations with US and foreign researchers.

**CHARGE.** The CHARGE consortium and collaborating cohorts currently have 6,941 cases and 43,207 controls of European American ancestry.

For both the power calculations and the actual selection of samples, investigators calculated a “risk score” that included APOE genotype, sex and either onset age for cases or age at last exam for controls (age at death for neuropathology controls). For AD cases, the risk score is the incidence of AD at the sample’s age at onset for their sex and APOE genotype. For controls, the risk score is the probability of developing AD between the age at last exam and age 85, accounting for their known APOE genotypes, sex, age at last exam, and availability of neuropathology information. The risk score was computed in both controls and cases that have readily available genomic DNA (no cell line samples). The risk profile is based on direct genotyping of APOE. Investigators did not consider the risk alleles at other AD loci as these have small effects and incorporating these into our sample selection process would limit our ability to detect rare causal variants at these recently identified loci. Details of the risk score calculation are provided below.

Genome-wide searches for rare variants protecting against AD are complicated by misclassification of controls who may have a genetic liability that has not yet manifested clinically. To mitigate this uncertainty, one can select controls based on a risk score that accounts for the probability of developing AD in the future based on population incidence.
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data for sex and age, and the known genetic risk factors for AD. The major determinants of this risk score are age and APOE genotype with a comparatively smaller effect from allelic data for the other 10 genes. Thus, controls who have the lowest probability of converting would be a subgroup of persons who are ages 85 and above and who possess the least genetic liability (i.e., combination of the APOE ε2/ε2 genotype and no risk alleles at other AD loci). Among these older controls are also persons who have survived a high genetic risk (based on genotypes at APOE and other risk loci) but have escaped the disease, and these persons would be enriched for protective loci.

Power analyses suggest that the gain in power attenuates beyond a sample size of 5,000 AD cases and 5,000 controls for detecting association with rare variants assessed individually or collectively by a gene-based test. This number of AD cases is also the minimum required to confidently detect rare variants with frequencies as small as 0.1%. All controls will be at least 60 years old and have either clinical assessment for dementia or absence of Alzheimer features upon neuropathology examination. All cases meet criteria for probable or definite AD based on clinical assessment, or had presence of AD features upon neuropathology examination. The control samples are selected as those having the least amount of expected misclassification (misclassification was defined using age at last exam, sex, and APOE genotype specific incidence measures). The case samples are selected to be of lowest risk for AD, based on their age, sex, and APOE status (also calculated using measures of AD incidence).

Using the same control group and a new case group consisting of one individual selected from families aggregating for late onset AD, it is possible to identify novel genes and alleles associated with the increased risk of AD. This “enriched case set” consists of one member of each European-American family. It is anticipated that approximately 1,000 individuals will be in this “enriched” case set, of which 100 will have already been sequenced. A previous study selected the earliest onset case with the most definitive diagnosis of AD from each of 867 NIA-LOAD families. These individuals were then sequenced for pathogenic mutations in the familial AD and frontotemporal dementia genes: APP, PSEN1, PSEN2, MAPT and GRN (Cruchaga et al., 2012). All individuals were also screened for expansion of the intronic repeat in C9orf72 that is associated with FTD/ALS (Harms et al., 2012). Sixty-five families were removed based on the presence of a pathogenic mutation in one of these genes. It is anticipated that the remaining mutation negative families will be enriched for novel AD risk alleles.

Replication: Replicate and validate the discoveries made in the above studies in independent samples of European-American AD cases and controls (n~>50,000 samples). In addition, evaluate the generalizability of these discoveries in European-Americans in other race/ethnic groups: African-American, Hispanic and Afro-Caribbean, East Asians and genetically isolated populations including the Amish, Icelanders and Israeli-Arabs.

Samples will be drawn from the above CHARGE and ADGC consortia, as well as other consortia.