

Gary W. Beecham, PhD
J.C. Bis, PhD
E.R. Martin, PhD
S.-H. Choi, PhD
A. L. DeStefano, PhD
C.M. van Duijn, PhD
M. Fornage, PhD
S.B. Gabriel, PhD
D.C. Kibbi, PhD
D.E. Larson, PhD
A.C. Naj, PhD
B.M. Psaty, PhD
W. Salerno, PhD
W.S. Bush, PhD
T.M. Foroud, PhD
E. Wijsman, PhD
L.A. Farrer, PhD
A. Goate, PhD
J.L. Haines, PhD
Margaret A. Pericak-
Vance, PhD
E. Boerwinkle, PhD
R. Mayeux, MD
S. Seshadri, MD
G. Schellenberg, PhD

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THE ALZHEIMER'S DISEASE SEQUENCING PROJECT: STUDY DESIGN AND SAMPLE SELECTION

OPEN

Late-onset Alzheimer disease (LOAD) is the leading cause of dementia worldwide, with substantial economic and public health implications.¹ LOAD is a neurodegenerative disease characterized by progressive dementia typically manifesting in the seventh to ninth decades. Neuropathological changes precede clinical symptoms by 10–20 years, resulting in clinically asymptomatic individuals carrying neuropathologic features of LOAD.² Much of the heritability of LOAD remains unexplained, despite LOAD having a high heritability (60%–80%) and despite the identification of the *APOE* locus, a major genetic determinant for LOAD.³ Genetic analyses have identified more than 25 other variants associated with smaller individual effects on disease risk.⁴

To identify novel genetic variation influencing AD risk and protection, the Alzheimer's Disease Sequencing Project (ADSP) was implemented as a collaborative effort of the National Institutes on Aging, the National Human Genome Research Institute, and the Alzheimer disease research community. Individual contributors include the Alzheimer's Disease Genetics Consortium, the Neurology Phenotype Working Group of the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium, and the Large Scale Sequencing and Analysis Centers at Baylor University, the Broad Institute, and Washington University.

Study design and sample selection were conducted to address issues of phenotypic heterogeneity and maximize statistical power. The study design includes 2 primary phases: a whole-genome sequencing (WGS) family-based study and a whole-exome sequencing (WES) case-control study. The WGS study was designed to target rarer variation through allelic segregation and linkage analyses in multiplex AD families. The WES case-control study was designed to target low-frequency coding variation in genes that contribute to AD risk or protection.

ADSP family study design. Approximately 1,400 multiplex LOAD families were reviewed for inclusion. Families were required to have multiple members with LOAD, genomic DNA, and available *APOE* genotypes.

Families meeting initial criteria were assigned a priority rank based on number and age at onset of affected individuals, number of generations affected, and presence of *APOE* $\epsilon 4$ alleles. Priority was given to families heavily loaded for AD (≥ 4 affected members with DNA available) with minimal *APOE* $\epsilon 4$ alleles. Cases met National Institute of Neurological Diseases–Alzheimer's NINCDS-ADRD (National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and related Disorders Association; now, Alzheimer's Association) criteria for possible, probable, or definite AD. Controls were free of clinical AD on cognitive assessment. A detailed description of the family design is in Appendix 1 at Neurology.org/ng.

In total, we selected 582 individuals (498 affected and 84 unaffected) from 111 families for WGS to identify genomic regions associated with increased risk of LOAD. Selected individuals include 229 European ancestry and 353 Caribbean Hispanic (CH) individuals (table). The European ancestry families included 2 large Dutch families from the Erasmus Rucphen Family study.⁵ Most of these families were recently analyzed for genetic linkage, an analysis that will be used in the analysis of the sequence data.^{6,7} By design, no $\epsilon 4/\epsilon 4$ individuals were selected for sequencing, and we prioritized $\epsilon 3/\epsilon 4$ individuals with earlier disease onset. Twenty-seven percent of families had at least 1 case with autopsy confirmation.

ADSP case-control design. Over 30,000 samples were considered for inclusion in the case-control design. All cases met NINCDS-ADRD criteria for possible, probable, or definite AD, had documented age at onset or age at death (for pathologically verified cases), and *APOE* genotyping. All controls were at least 60 years old and were free of dementia by direct, documented cognitive assessment. Three primary case-control selection strategies were evaluated, and ultimately, a design was chosen that targeted cases with minimal risk as predicted by known risk factors (age, sex, and *APOE*) and targeted controls with the least probability of conversion to AD by age 85 years. The details and rationale of the case-control selection process and the evaluation of alternate study designs are described in detail in Appendix 2.

In total, we selected 5,096 cases and 4,965 controls under the chosen design (table). We selected

Table	Sample demographics for family and case-control studies			
	Family study		Case-control study	
	Affected	Unaffected	Case	Control
N	498	84	5,778	5,136
Age at onset/examination (SD)	73.7 (9.4)	68.0 (11.0)	76.0 (9.2)	86.1 (5.2)
Male (%)	38.8	38.1	43.1	40.7
Hispanic/Latino (%)	60.6	59.5	3.7	3.4
Autopsy confirmation (%)	15.6	0.0	32.8	7.0
APOE genotype				
ε3ε3	268 (54%)	60 (71%)	2,915 (50%)	3,394 (66%)
ε3ε4	196 (39%)	9 (11%)	2,198 (38%)	679 (13%)
ε4ε4	0 (0%)	0 (0%)	161 (3%)	17 (<1%)
ε2ε2	1 (<1%)	1 (1%)	23 (<1%)	48 (1%)
ε2ε3	29 (6%)	11 (13%)	359 (6%)	925 (18%)
ε2ε4	4 (1%)	2 (2%)	122 (2%)	73 (1%)

682 additional unrelated cases from additional multiplex families that had a strong family history for LOAD. Because some of these 682 cases arose from CH multiplex families, we included 171 cognitively normal CH control samples in the WES.

The sequencing of the nearly 600 whole genomes and 11,000 whole exomes has been completed; the data sets are currently available to the research community through qualified access (dbGaP study phs000572.v7.p4). This data set will be used to identify genetic factors influencing AD risk and protection and will be a critical resource for the LOAD research community.

Standard protocol approvals, registrations, and patient consents. This study has the approval of the institutional review boards of participating institutions, and informed consent was obtained from all patients.

From the John P. Hussman Institute for Human Genomics (G.W.B., E.R.M., M.A.P.-V.) and Dr. John T. Macdonald Foundation Department of Human Genetics (G.W.B., E.R.M., M.A.P.-V.), Miller School of Medicine, University of Miami, FL; Cardiovascular Health Research Unit (J.C.B.), Department of Medicine, Cardiovascular Health Research Unit (B.M.P.), Departments of Medicine, Epidemiology, Health Services, Department of Biostatistics (E.W.), and Division of Medical Genetics (E.W.), Department of Medicine, University of Washington, Seattle; Department of Biostatistics (S.-H.C., A.D., L.A.F.), Boston University School of Public Health, MA; The Framingham Heart Study (A.D., S.S.), MA; Department of Neurology (A.D., L.A.F., S.S.), Boston University School of Medicine, MA; Department of Epidemiology (C.M.v.D.), Erasmus MC, Rotterdam, Netherlands; Brown Foundation Institute of Molecular Medicine (M.F.) and Human Genetics Center (M.F.), University of Texas Health Science Center, Houston; The Eli and Edythe L. Broad Institute of Massachusetts Institute of Technology (S.B.G.), Cambridge; Harvard University (S.B.G.), Cambridge, MA; The McDonnell Genome Institute (D.C.K., D.E.L.) and Department of Genetics (D.E.L.), Washington University, St. Louis, MO;

Department of Biostatistics and Epidemiology (A.C.N.) and Perelman School of Medicine (G.S.), University of Pennsylvania, Philadelphia; Group Health Research Institute (B.M.P.), Group Health Cooperative, Seattle, WA; Human Genome Sequencing Center (W.S., E.B.), Baylor College of Medicine, Houston, TX; Department of Epidemiology and Biostatistics (W.S.B., J.L.H.), Case Western Reserve University, Cleveland, OH; Department of Medical and Molecular Genetics (T.M.F.), Indiana University School of Medicine, Indianapolis; Department of Medicine (Biomedical Genetics) (L.A.F.), Department of Ophthalmology (L.A.F.), and Department of Epidemiology (L.A.F.), Boston University School of Medicine and Public Health, MA; Department of Neuroscience (A.G.), Icahn School of Medicine at Mount Sinai, New York, NY; Human Genetics Center (E.B.), UT Health School of Public Health, Houston, TX; Taub Institute for Research on Alzheimer's Disease and the Aging Brain (R.M.) and Gertrude H. Sergievsky Center (R.M.), Columbia University Medical Center, New York, NY; Department of Neurology (R.M.), Columbia University Medical Center and New York Presbyterian Hospital, NY; and Department of Epidemiology (R.M.), Mailman School of Public Health, Columbia University, New York, NY.

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Correspondence to Dr. Beecham: gbeechem@med.miami.edu

1. Prince M, Jackson J. Alzheimer's Disease International, World Alzheimer Report 2009: The Global Prevalence of Dementia; 2009. Available at: alz.co.uk/research/files/WorldAlzheimerReport.pdf. Accessed October, 2016.

2. Bateman RJ, Xiong C, Benzinger TL, et al; Dominantly Inherited Alzheimer Network. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 2012;367:795–804.
3. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261:921–923.
4. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013;45:1452–1458.
5. Liu F, Arias-Vásquez A, Sleegers K, et al. A genomewide screen for late-onset Alzheimer disease in a genetically isolated Dutch population. *Am J Hum Genet* 2007;81:17–31.
6. Barral S, Cheng R, Reitz C, et al. Linkage analyses in Caribbean Hispanic families identify novel loci associated with familial late-onset Alzheimer's disease. *Alzheimers Dement* 2015;11:1397–1406.
7. Kunkle BW, Jaworski J, Barral S, et al. Genome-wide linkage analyses of non-Hispanic white families identify novel loci for familial late-onset Alzheimer's disease. *Alzheimers Dement* 2016;12:2–10.

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