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Featured Article

Linkage analyses in Caribbean Hispanic families identify novel loci associated with familial late-onset Alzheimer's disease

Sandra Barral[a,b,c], Rong Cheng[a,b], Christiane Reitz[a,b,c,d], Badri Vardarajan[a,b,c], Joseph Lee[a,b,d], Brian Kunkle[f], Gary Beecham[f], Laura S. Cantwell[g], Margaret A. Pericak-Vance[f,h], Lindsay A. Farrer[i,j,k,l,m], Jonathan L. Haines[n], Alison M. Goate[o], Tatiana Foroud[p], Eric Boerwinkle[e,q], Gerard D. Schellenberg[g], Richard Mayeux[a,b,c,d,*]

[a] *Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, New York, NY, USA*
[b] *Gertrude H. Sergievsky Center, Columbia University Medical Center, New York, NY, USA*
[c] *Department of Neurology, Columbia University Medical Center and New York Presbyterian Hospital, New York, NY, USA*
[d] *Department of Epidemiology, School of Public Health, Columbia University, New York, NY, USA*
[e] *Department of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, Houston, TX, USA*
[f] *The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA*
[g] *Perelman School of Medicine University of Pennsylvania, Philadelphia, PA, USA*
[h] *Dr. John T. Macdonald Foundation Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, FL, USA*
[i] *Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA*
[j] *Department of Medicine (Biomedical Genetics), Boston University School of Medicine and Public Health, Boston, MA, USA*
[k] *Department of Neurology, Boston University School of Medicine and Public Health, Boston, MA, USA*
[l] *Department of Ophthalmology, Boston University School of Medicine and Public Health, Boston, MA, USA*
[m] *Department of Epidemiology, Boston University School of Medicine and Public Health, Boston, MA, USA*
[n] *Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH, USA*
[o] *Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[p] *Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA*
[q] *Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX, USA*

Abstract

Introduction: We performed linkage analyses in Caribbean Hispanic families with multiple late-onset Alzheimer's disease (LOAD) cases to identify regions that may contain disease causative variants.
Methods: We selected 67 LOAD families to perform genome-wide linkage scan. Analysis of the linked regions was repeated using the entire sample of 282 families. Validated chromosomal regions were analyzed using joint linkage and association.
Results: We identified 26 regions linked to LOAD (HLOD $\geq$3.6). We validated 13 of the regions (HLOD $\geq$2.5) using the entire family sample. The strongest signal was at 11q12.3 (rs2232932: $\text{HLOD}_{max} = 4.7$, $P_{joint} = 6.6 \times 10^{-6}$), a locus located $\sim$2 Mb upstream of the membrane-spanning 4A gene cluster. We additionally identified a locus at 7p14.3 (rs10255835: $\text{HLOD}_{max} = 4.9$, $P_{joint} = 1.2 \times 10^{-5}$), a region harboring genes associated with the nervous system (*GARS*, *GHRHR*, and *NEUROD6*).
Discussion: Future sequencing efforts should focus on these regions because they may harbor familial LOAD causative mutations.

Keywords: Caribbean Hispanic families; Late-onset Alzheimer's disease; Linkage analysis; Joint linkage and association

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None of the authors has a conflict of interest.

*Corresponding author. Tel.: +1-212-305-2391; Fax: +1-212-305-2518.

E-mail address: rpm2@cumc.columbia.edu

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1. Introduction

Late-onset Alzheimer's disease (LOAD) is a highly prevalent, progressive, neurodegenerative disorder characterized by a slow transition from mild memory impairment to severe cognitive loss. Genetic variants in the amyloid precursor protein (*APP*) gene [1] and presenilin 1 (*PSEN1*) [2] and 2 (*PSEN2*) [3,4] genes cause early onset Alzheimer's disease (AD) and enhance generation or aggregation of the amyloid ß peptide [5,6]. Simultaneously, the apolipoprotein E (*APOE*) ε4 allele decreases age of onset and increases disease susceptibility [7]. Several large-scale genome-wide association studies (GWAS) using dense single-nucleotide polymorphism (SNP) arrays identified additional susceptibility loci for LOAD: *CLU*, *PICALM*, *CR1*, *BIN1*, membrane-spanning *4A (MS4A4A)*, *ABCA7*, *CD2AP*, *CD33*, *EPHA1*, *HLA-DRB5/HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4*, *DSG2*, *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2*, and *CASS4*. As is the case for other complex genetic disorders, the effect sizes of these loci are small (odds ratios, 1.0–1.2). In large multiplex pedigrees with LOAD, targeted exome sequencing identified missense, nonsense, and splice-site variants in *APP, PSEN1, PSEN2, APOE, GRN,* and *MAPT9*. Other genes with rare variants (RVs) for LOAD include *SORL1* [8], *ADAM10* [9], *PICALM* [10], *PLD3* [11], and *TREM2* [12]. Overall, most genes identified to date cluster in four pathways, namely inflammation and immune response, lipid metabolism, *APP* generation and metabolism, and endocytosis and intracellular vesicle trafficking.

Much work lies ahead to determine the specific roles these loci play in LOAD and if so, which mutations or variants are truly causal. In addition, the identified loci only explain a fraction of the population attributable risk leaving additional genetic variants to be identified. Because large families enriched with AD cases are more likely to harbor causal variants, we performed parametric and nonparametric linkage analyses in 67 Caribbean Hispanic LOAD families with five or more affecteds genotyped individuals per family, with low frequency of APOE ε4 (defined as families where less than half of its members were APOE ε4 carriers), without mutations in known genes and with available genome-wide genotype data. We subsequently evaluated the identified loci in the entire sample of 282 LOAD families.

2. Materials and methods

2.1. Ethics statement

Study participants were recruited as part of the Estudio Familiar de Influencia Genetica de Alzheimer (EFIGA) study. Written informed consent for the study was obtained from all subjects and/or authorized representatives and study partners. The EFIGA study was approved by the Institutional Review Board of the New York State Psychiatric Institute.

2.1.1. Study samples

A set of 67 families with 469 genotyped members was selected from a total sample of 282 families with 1060 members, to perform the initial linkage analysis (Table 1). The selection criteria were based on there being a large number of affecteds in the family (an average of five or more affected individuals per family), a low APOE ε4 allele frequency (17.6%), absence of known mutations in *PSEN1, PSEN2,* or *APP*, and the availability of GWAS data. The 282 additional families (591 subjects) had lower density of affecteds per family but similar phenotyping, low APOE ε4 allele frequency (26%), and GWAS data were available. These 282 Caribbean Hispanic families were from a family-based and case-control study of LOAD, the EFIGA [13].

EFIGA participants have been recruited since January 1998 from clinics in the Dominican Republic and Puerto Rico, as well as the Alzheimer Disease Research Center Memory Disorders Clinic at Columbia University in New York City. Participants are followed up every 18 months. Each participant completed a standardized assessment at approximately 18-month intervals, including medical history, physical and neurologic examination, and an extensive neuropsychological battery for evaluation of cognitive impairment [14]. This battery measured cognitive function in key domains affected by aging and dementia, including memory, visuospatial ability, psychomotor speed, and executive function. The measures include the selective reminding test [15], the Benton visual retention test recognition and matching trials [16], the Rosen drawing test [17], the Boston naming test [18], the controlled oral word association test [19], the category fluency test [20], the color trails test [21], the similarities subtest from the Wechsler adult intelligence scale [22], and the orientation items from the mini-mental state examination [23]. Brief tests of writing and

Table 1
Late-onset Caribbean Hispanics family sample characteristics

Characteristics	67 families	Additional 215 families
Families, n	67	215
Subjects, n	469	591
Affection status, n (%)		
Affected	354 (75.5)	434 (73.4)
Average (SD) of affected individuals per family	5.1 (1.8)	2.1 (0.8)
Unaffected	114 (24.3)	156 (26.4)
Average (SD) of unaffected individuals per family	2.2 (1.5)	1.5 (0.9)
Unknown	1 (0.2)	1 (0.2)
Females, n (%)	276 (5.9)	385 (65.1)
Age at onset of affecteds, mean (SD)	74 (9.6)	74.1 (9.5)
Age at last examination of unaffecteds, mean (SD)	68 (9.7)	69.6 (8.3)
APOE allele frequency, n (%)		
ε4	165 (17.6)	309 (26.1)
ε3	735 (78.4)	821 (69.5)
ε2	38 (4.0)	52 (4.4)

Abbreviation: SD, standard deviation.