

## Original Investigation

# Association of Long Runs of Homozygosity With Alzheimer Disease Among African American Individuals

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**IMPORTANCE** Mutations in known causal Alzheimer disease (AD) genes account for only 1% to 3% of patients and almost all are dominantly inherited. Recessive inheritance of complex phenotypes can be linked to long (>1-megabase [Mb]) runs of homozygosity (ROHs) detectable by single-nucleotide polymorphism (SNP) arrays.

**OBJECTIVE** To evaluate the association between ROHs and AD in an African American population known to have a risk for AD up to 3 times higher than white individuals.

**DESIGN, SETTING, AND PARTICIPANTS** Case-control study of a large African American data set previously genotyped on different genome-wide SNP arrays conducted from December 2013 to January 2015. Global and locus-based ROH measurements were analyzed using raw or imputed genotype data. We studied the raw genotypes from 2 case-control subsets grouped based on SNP array: Alzheimer's Disease Genetics Consortium data set (871 cases and 1620 control individuals) and Chicago Health and Aging Project-Indianapolis Ibadan Dementia Study data set (279 cases and 1367 control individuals). We then examined the entire data set using imputed genotypes from 1917 cases and 3858 control individuals.

**MAIN OUTCOMES AND MEASURES** The ROHs larger than 1 Mb, 2 Mb, or 3 Mb were investigated separately for global burden evaluation, consensus regions, and gene-based analyses.

**RESULTS** The African American cohort had a low degree of inbreeding ( $F = 0.006$ ). In the Alzheimer's Disease Genetics Consortium data set, we detected a significantly higher proportion of cases with ROHs greater than 2 Mb ( $P = .004$ ) or greater than 3 Mb ( $P = .02$ ), as well as a significant 114-kilobase consensus region on chr4q31.3 (empirical  $P$  value  $2 = .04$ ; ROHs >2 Mb). In the Chicago Health and Aging Project-Indianapolis Ibadan Dementia Study data set, we identified a significant 202-kilobase consensus region on Chr15q24.1 (empirical  $P$  value  $2 = .02$ ; ROHs >1 Mb) and a cluster of 13 significant genes on Chr3p21.31 (empirical  $P$  value  $2 = .03$ ; ROHs >3 Mb). A total of 43 of 49 nominally significant genes common for both data sets also mapped to Chr3p21.31. Analyses of imputed SNP data from the entire data set confirmed the association of AD with global ROH measurements (12.38 ROHs >1 Mb in cases vs 12.11 in controls; 2.986 Mb average size of ROHs >2 Mb in cases vs 2.889 Mb in controls; and 22% of cases with ROHs >3 Mb vs 19% of controls) and a gene-cluster on Chr3p21.31 (empirical  $P$  value  $2 = .006-.04$ ; ROHs >3 Mb). Also, we detected a significant association between AD and *CLDN17* (empirical  $P$  value  $2 = .01$ ; ROHs >1 Mb), encoding a protein from the Claudin family, members of which were previously suggested as AD biomarkers.

**CONCLUSIONS AND RELEVANCE** To our knowledge, we discovered the first evidence of increased burden of ROHs among patients with AD from an outbred African American population, which could reflect either the cumulative effect of multiple ROHs to AD or the contribution of specific loci harboring recessive mutations and risk haplotypes in a subset of patients. Sequencing is required to uncover AD variants in these individuals.

*JAMA Neurol.* 2015;72(11):1313-1323. doi:10.1001/jamaneurol.2015.1700  
Published online September 14, 2015.

 Supplemental content at  
[jamaneurology.com](http://jamaneurology.com)

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In addition to the causal early-onset Alzheimer disease (AD) genes (*APP*, *PSEN1*, and *PSEN2*) accounting for only 1% to 3% of patients,<sup>1</sup> variations of modest effect in more than 25 loci have been found to be significantly associated with late-onset AD (age >65 years), among them *APOE* has the largest effect.<sup>2</sup> These loci were mainly detected by genome-wide association studies (GWASs) using common single-nucleotide polymorphisms (SNPs) with a minor allele frequency greater than 5%, while the search for rare pathogenic mutations among them is still ongoing.<sup>3</sup> Notably, except for the 2 rare recessive mutations in *APP* (p.A673V<sup>4</sup> and E693Δ<sup>5</sup>), approximately 200 mutations in the 3 causal AD genes all cause a dominant early-onset form of the disease,<sup>6</sup> which is in contrast to a previous suggestion of up to approximately 90% recessive inheritance for early-onset AD.<sup>7</sup>

Recessive inheritance of complex phenotypes (eg, late-onset AD) can be linked to the presence of long runs of homozygosity (ROHs) detectable by SNP arrays used in GWASs. Runs of homozygosity could be the result of enhanced inbreeding in previous generations<sup>7-9</sup> or suppressed recombination by a large inversion leading to an extended haplotype (eg, at the *MAPT* locus<sup>10</sup>). Based on whole-exome data, long ROHs were reported to be significantly enriched for potentially deleterious homozygous mutations.<sup>11,12</sup> Because small ROHs are too frequent and less likely to harbor rare recessive variants, most studies have investigated ROHs greater than 1 megabase (Mb) or several cutoffs (eg, ROH >2 Mb or >3 Mb)<sup>13</sup> that could reveal hidden associations by excluding outliers.

Hence, genome-wide study of ROHs could identify cases with a higher probability of disease-associated rare recessive mutations or risk haplotypes. We previously showed that the global burden measurements of ROHs are significantly associated with AD in an inbred population of Caribbean Hispanic individuals, in which the average length of ROHs was significantly larger in cases than control participants ( $P = .004$ ), and this association was stronger with familial AD ( $P < .001$ ).<sup>8</sup> Although inbred populations are more powerful for ROH study, in some outbred populations, ROHs were associated with several neurological disorders including Parkinson disease,<sup>14</sup> amyotrophic lateral sclerosis,<sup>15</sup> and schizophrenia.<sup>16</sup>

Because studies of 2 outbred AD data sets of North American and European origin did not detect an association between AD and ROHs,<sup>13,17</sup> we focused our investigation on African American individuals, who have a risk for AD up to 3 times higher than in white individuals<sup>18</sup> and their first-degree relatives with AD have a higher risk for dementia than those of white individuals with AD.<sup>19</sup> As a result, AD is the fourth leading cause of death among African American individuals.<sup>18</sup> Our investigation was also motivated by significant findings in a Caribbean Hispanic population that has substantial West African heritage.<sup>8</sup> However, a large data set is needed because studies of African American individuals is complicated by a high level of genetic divergence owing to their multiple sites of origin, mainly from West or Central Africa.<sup>20</sup>

Therefore, we conducted an ROH study of a large data set of African American patients with late-onset AD, consisting of 10 case-control cohorts previously genotyped on 6 different SNP arrays. The entire data set was previously evaluated by

the Alzheimer's Disease Genetics Consortium (ADGC) in an SNP-based GWAS, which replicated several AD loci (eg, *ABCA7*, *CRI*, *BINI*, *EPHA1*, and *CD33*).<sup>21</sup> We evaluated global and locus-based ROH measurements by analyzing raw genotypes from 2 independent African American cohorts that were grouped based on their genotyping arrays. To maximize the statistical power of our study that is dependent on both sample size and SNP density, we also investigated the entire data set (1917 cases and 3858 control individuals) using imputed SNP data from different genotyping arrays. Notably, SNP imputation has been suggested to be a reliable approach for ROH studies.<sup>9</sup>

## Methods

### Genotyping Data

Details of the African American data sets, genotyping arrays, and quality-control steps were reported previously.<sup>21</sup> The data sets for the study were approved for analysis by the institutional review board at the University of Pennsylvania, Philadelphia, and all participants provided written informed consent.

STRUCTURE<sup>22</sup> analysis was performed to identify hidden population substructure and remove outliers. We studied nonimputed data from 2 cohorts that were grouped based on their genotyping platforms. The first data set (called ADGC) was genotyped at Children's Hospital of Philadelphia (72.8% female; 36.5% *APOE* ε4 carriers) using the Human 1M Duo Bead Chip (Illumina Inc) that provided genotypes for 965 226 SNPs used for the ROH analyses. After removing 90 population outliers from the ADGC data set, 871 cases and 1620 control individuals were included in the study (eFigure 1A in the Supplement). The second data set consisted of merged data from the Chicago Health and Aging Project (CHAP) (65.8% female; 38.4% *APOE* ε4 carriers) and the Indianapolis Ibadan Dementia Study (IIDS) (65.6% female; 36.3% *APOE* ε4 carriers). All samples in the CHAP-IIDS data set were genotyped on the Illumina 1M platform (Illumina Inc) that provided genotypes for 787 726 SNPs for the ROH analyses. After removing 76 population outliers, 279 cases and 1367 control individuals were included in the study (eFigure 1B in the Supplement).

The ROH analyses were also conducted for the entire data set using imputed SNP data from all 10 cohorts. Genome-wide imputation of allele dosages to select the final SNP set for analyses ( $R^2 \geq 0.50$ ) was previously done using the June 2011 panel from the 1000 Genomes build 37.<sup>21</sup> IMPUTE<sup>23</sup> files were converted to PLINK<sup>24</sup> input files using the GTOOL program (<http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html>). We excluded SNPs and individuals with more than 2% missing genotypes, as well as SNPs with a minor allele frequency of 5% or less in the entire data set. After removal of population outliers,<sup>21</sup> we analyzed ROHs among 1917 cases and 3858 control individuals, with a total genotyping rate of more than 99% for 2 498 646 SNPs. The degree of inbreeding ( $F$ ) was estimated by the genetic relationship matrix implemented in the GCTA program.<sup>25</sup> Linkage disequilibrium structure was estimated using Haploview<sup>26</sup> and based on the control genotype data of each group.

Table 1. Global Burden Measurements of ROHs Using 3 Different-Sized Cutoffs

Measurement	1 Mb			2 Mb			3 Mb		
	Affected	Unaffected	P Value	Affected	Unaffected	P Value	Affected	Unaffected	P Value
<b>ADGC Data Set</b>									
Total No.	7178	12 993	...	1032	1700	...	479	755	...
ROH segments per genome/individual, No.	8.24	8.02	.07	1.19	1.05	.05	0.55	0.47	.12
Proportion	0.99	1	>.99	0.66	0.59	.004 <sup>a</sup>	0.32	0.28	.02
Total size of ROH, kb	14 850	13 910	.21	8884	8392	.38	13 490	12 910	.43
Average size of ROH, kb	1624	1579	.12	3274	3264	.46	4751	4739	.48
<b>CHAP-IIDS Data Set</b>									
Total No.	1919	9442	...	196	1087	...	66	351	...
ROH segments per genome/individual, No.	6.88	6.91	.56	0.70	0.79	.91	0.24	0.26	.66
Proportion	0.99	0.99	.93	0.48	0.52	.90	0.18	0.18	.50
Total size of ROH, kb	10 480	10 850	.69	5139	5633	.64	7453	9443	.73
Average size of ROH, kb	1487	1506	.67	3133	2993	.19	4997	4818	.36
<b>Imputed Data From the Entire African American Data Set (All 10 Cohorts)</b>									
Total No.	23 742	46 715	...	2199	4107	...	824	1412	...
ROH segments per genome/individual, No.	12.38	12.11	.02	1.15	1.07	.09	0.43	0.37	.11
Proportion	1	1	>.99	0.61	0.58	.06	0.22	0.19	.006 <sup>a</sup>
Total size of ROH, kb	18 790	18 080	.10	7401	6828	.25	12 650	12 210	.42
Average size of ROH, kb	1447	1431	.10	2986	2889	.03	4734	4599	.18

Abbreviations: ADGC, Alzheimer's Disease Genetics; CHAP-IIDS, Chicago Health and Aging Project-Indianapolis Ibadan Dementia Study; ROHs, runs of homozygosity; ellipses, no comparison for pure number of ROHs.

<sup>a</sup> Results that remain significant even after Bonferroni correction ( $P < .02$ ) calculated based on the 3 ROH cutoffs.

### Runs of Homozygosity Analyses

Runs of homozygosity for the nonimputed data were analyzed as previously described,<sup>8</sup> while for the imputed data with many more SNPs, we used 100 (vs 50) SNPs in the PLINK sliding window and allowed 2 (vs 1) heterozygous SNPs in the window. The number, as well as the total and average length of ROHs, was calculated for each sample. Runs of homozygosity larger than 1 Mb, 2 Mb, or 3 Mb were investigated separately<sup>13</sup> in 3 types of analyses: (1) global burden evaluation; (2) analysis of consensus regions (>100 kilobase [Kb]; >3 SNPs), which were segments shared by all individuals carrying ROHs greater than 1 Mb at each given locus; and (3) gene-based analysis to estimate which genes were intersected by ROHs more frequently in cases vs control individuals.

We obtained  $P$  values uncorrected (empirical  $P$  value 1) and corrected (empirical  $P$  value 2) for multiple testing using PLINK. All nominally significant genes were checked if they belonged to the 77 genes reported to be associated with the 4 most common neurodegenerative disorders, keeping in mind their essential overlap at the clinical, neuropathological, and genetic levels.<sup>27</sup>

Global burden measurements among autosomal chromosomes were investigated with a 1-tailed test (10 000 permutations) for the number of ROHs, their total and average length per individual, and the proportions of cases and control individuals with ROHs. A 1-tailed test was used because African American individuals have a high incidence of AD<sup>18</sup> and such a population is more suitable for the detection of risk but not protective alleles.

### Results

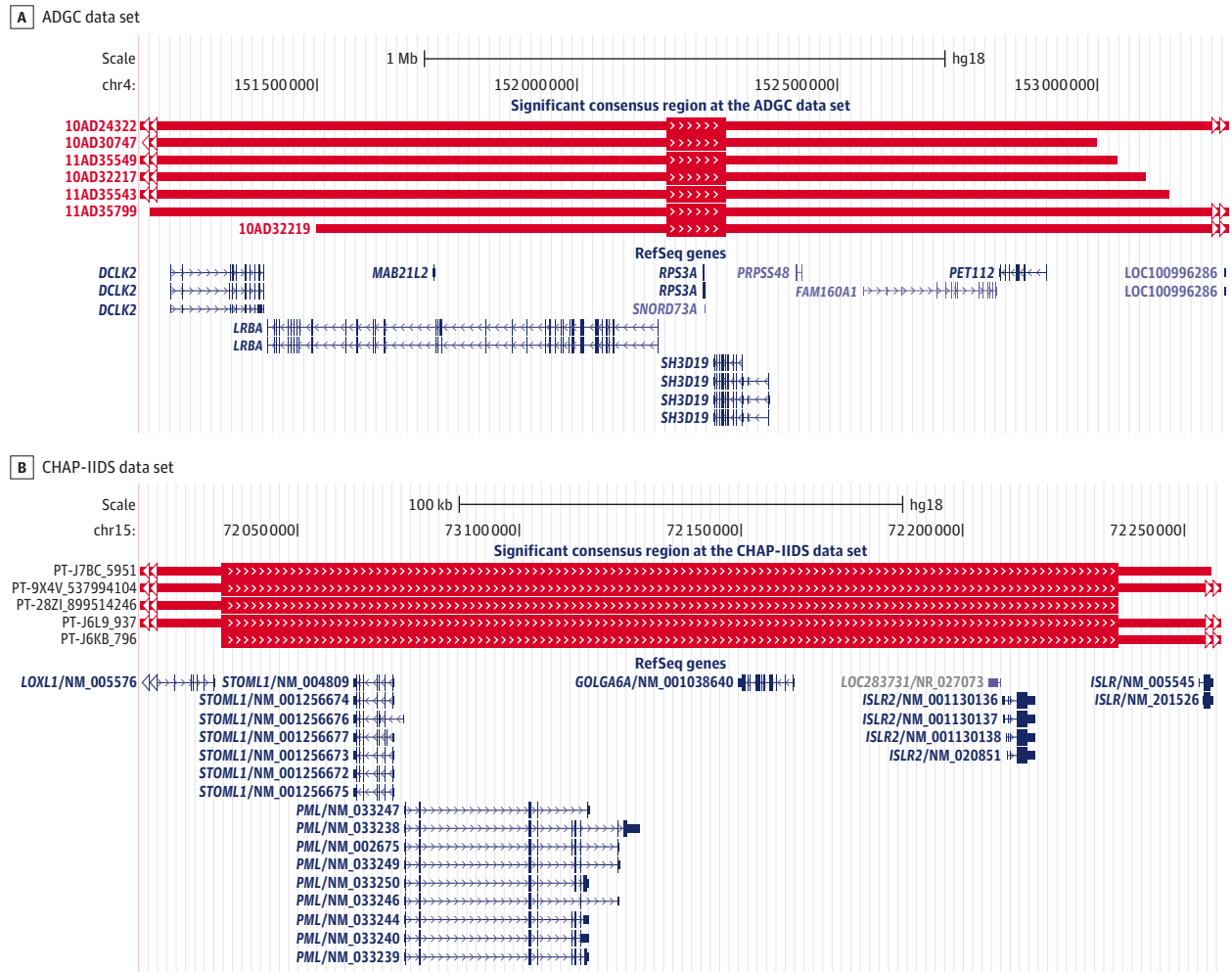
#### Analyses of the ADGC Data Set

Results of the global burden ROH analysis of the ADGC data set (871 cases and 1620 control individuals) are presented in **Table 1**. We detected a significantly higher proportion of cases with ROHs greater than 2 Mb ( $P = .004$ ) or greater than 3 Mb ( $P = .02$ ) compared with control individuals. In addition, the global rate of ROHs greater than 2 Mb per person was marginally higher in cases than control individuals ( $P = .05$ ). Analysis of ROH consensus regions detected a significant association (empirical  $P$  value 1 < .001; empirical  $P$  value 2 = .04) between AD and a 114-kb locus on chr4q31.3 containing the *SH3D19* and *RPS3A* genes (Chr4: 152172448-152286356/hg18 flanked by rs6817611 and rs7669180). This consensus region was overlapped by ROHs greater than 2 Mb in 7 cases and no control individuals (**Figure 1A**; eTable 1 in the **Supplement**) and belongs to a single linkage disequilibrium block based on Haploview investigation of the ADGC control genotypes (eFigure 2 in the **Supplement**). Gene-based analysis revealed only nominally significant loci, including *PSEN2* (empirical  $P$  value 1 = .003) overlapped by ROHs greater than 1 Mb in 1.26% of cases ( $n = 11$ ) vs 0.25% of control individuals ( $n = 4$ ) and *SIGMAR1* (empirical  $P$  value 1 < .001) overlapped by ROHs greater than 1 Mb in 1.61% of cases ( $n = 14$ ) vs 0.25% of control individuals ( $n = 4$ ) (**Table 2**).

#### Analyses of CHAP-IIDS Data Set

The global burden analyses of ROHs did not reveal significant results in the CHAP-IIDS data set, likely owing to the limited

Figure 1. Significant Results Obtained by Analyses of Consensus Regions



Consensus regions are indicated by red bars containing white arrowheads. A, The consensus region detected in the Alzheimer’s Disease Genetics Consortium (ADGC) data set contains the *SH3D19* and *RPS3A* genes intersected by runs of homozygosity greater than 2 Mb in 7 cases (samples 10AD24322, 10AD30747, 11AD35799, 11AD35549, 10AD32217, 10AD32219, and 11AD35543) and no control individuals. B, The consensus region detected in the Chicago

Health and Aging Project (CHAP)–Indianapolis Ibadan Dementia Study (IIDS) data set contains the *STOML1*, *PML*, *GOLGA6A*, and *ISLR2* genes intersected by runs of homozygosity greater than 1 Mb in 5 cases (samples PT-J6KB\_796, PT-J6L9\_937, PT-28ZI\_899514246, PT-9X4V\_537994104, and PT-J7BC\_5951) and no control individuals.

number of patients (279 cases and 1367 control individuals) (Table 1). However, analysis of consensus regions detected a significant association between AD and a 202-kb region on Chr15q24.1, which was overlapped by ROHs greater than 1 Mb in 5 cases and no control individuals (empirical *P* value 1 < .001; empirical *P* value 2 = .02). This region is flanked by SNPs rs12442211 and rs11635599 (chr15:72032728-72235049/hg18) and contains the *STOML1*, *PML*, *GOLGA6A*, and *ISLR2* genes (Figure 1B; eFigure 3 and eTable 2 in the Supplement). The ROH grouping function of PLINK revealed that 4 of 5 cases with this consensus region have a shared haplotype (eTable 2 in the Supplement). Notably, in the gene-based analysis of ROHs greater than 1 Mb, the genes located at this consensus region generated the top nominally significant results (empirical *P* value 1 < .001), while in the analysis of ROHs greater than 2 Mb, the top nominally significant gene was *CD2AP* (the AD gene

detected by GWAS<sup>28</sup>), which was intersected in 3 cases (1%) but no control individuals (empirical *P* value 1 = .005).

After correction for multiple testing, the only association with AD in the gene-based analysis was observed for 13 genes within a 3-Mb region on Chr3p21.31 (*PFKFB4*, *UCN2*, *COL7A1*, *UQCRC1*, *TMEM89*, *C3orf18*, *HEMK1*, *CISH*, *MAPKAPK3*, *DOCK3*, *MANF*, *RBM15B*, and *VPRBP*) that were intersected by ROHs greater than 3 Mb more frequently in cases (*n* = 8; 2.9%) vs control individuals (*n* = 5-6; 0.4%) (empirical *P* value 1 < .001; empirical *P* value 2 = .03) (Figure 2).

### Analyses of the Entire Data Set

Global burden ROH analyses of the entire data set using imputed SNP data from 1917 cases and 3858 control individuals revealed a significantly higher rate of ROHs greater than 1 Mb in cases vs control individuals (*P* = .02). Also, the average size

Table 2. Nominally Significant Results Obtained in Gene-Based ROH Analyses for the Genes Known to Be Linked With Neurodegenerative Disorders

ROH Minimum Size	Gene	Transcript	Associated Disease	Empirical P Value		Frequency, %	
				1	2	Cases	Controls
<b>ADGC Data Set</b>							
1 Mb	<i>HIP1R</i>	NM_003959	PD	.002	.93	2.30	0.80
	<i>PSEN2</i>	NM_000447	AD	.003	.90	1.26	0.25
	<i>SIGMAR1</i>	NM_001282209	ALS/FTD	<.001	.23	1.61	0.25
	<i>VCP</i>	NM_007126	ALS/FTD	.03	>.99	0.92	0.25
2 Mb	<i>SIGMAR1</i>	NM_001282209	ALS/FTD	.01	.81	0.69	0.06
	<i>VCP</i>	NM_007126	ALS/FTD	.01	.81	0.69	0.06
3 Mb	<i>SIGMAR1</i>	NM_001282209	ALS/FTD	.046	.99	0.34	0
	<i>VCP</i>	NM_007126	ALS/FTD	.046	.99	0.34	0
<b>CHAP-IIDS Data Set</b>							
1 Mb	<i>ATXN2</i>	NM_002973	ALS/FTD	<.001	.99	10.75	5.27
	<i>CD2AP</i>	NM_012120	AD	.02	>.99	1.08	0.07
2 Mb	<i>CD2AP</i>	NM_012120	AD	.004	.54	1.08	0
3 Mb	<i>MEF2C</i>	NM_001193350	AD	.03	.88	0.72	0

Abbreviations: AD, Alzheimer disease; ADGC, Alzheimer's Disease Genetics Consortium; ALS, amyotrophic lateral sclerosis; CHAP-IIDS, Chicago Health and Aging Project-Indianapolis Ibadan Dementia Study; FTD, frontotemporal dementia; PD, Parkinson disease; ROH, run of homozygosity.

of ROHs greater than 2 Mb ( $P = .03$ ) and the proportion of ROHs greater than 3 Mb ( $P = .006$ ) were significantly higher in cases compared with control individuals (Table 1). Of note, analyses of imputed data for the ADGC data set confirmed a significantly higher global proportion of cases with ROHs greater than 2 Mb ( $P = .004$ ) or ROHs greater than 3 Mb ( $P = .002$ ) observed in the nonimputed ADGC data, indicating reliability of the ROH results generated based on imputed data.

Evaluation of relatedness revealed a low degree of inbreeding for both cases and control individuals ( $F \sim 0.006$ ). Thus, we also conducted the global burden analyses of smaller ROHs ( $>0.5$  Mb) that showed significant association of AD with ROH rate ( $P = .04$ ); however, the gene-based analysis did not reveal any significant results after correction for multiple testing. In contrast, gene-based analysis of ROHs greater than 1 Mb revealed a significant association between AD and the *CLDN17* gene on 21q22.11, which was intersected by ROHs in 11 cases (0.57%) but no control individuals (empirical  $P$  value 1  $< .001$ ; empirical  $P$  value 2 = .01) (Figure 3). We also observed a significant gene cluster on Chr3p21.31 (empirical  $P$  value 2 = .006-.04) that was intersected by ROHs greater than 3 Mb in approximately 2.4% of cases vs approximately 1% of control individuals (eTable 3 in the Supplement). This association was mainly driven by the CHAP-IIDS data set because genes from this locus were also significant in the analysis of raw genotypes from the CHAP-IIDS data set (*C3orf18*, *CISH*, *COL7A1*, *DOCK3*, *HEMK1*, *MAPKAPK3*, *PFKFB4*, and *UCN2*). Indeed, the genes at the Chr3p21.31 locus became insignificant after the CHAP-IIDS data set was removed from the entire data set, although a global proportion of ROHs greater than 3 Mb remained significantly higher in cases vs control individuals ( $P = .004$ ).

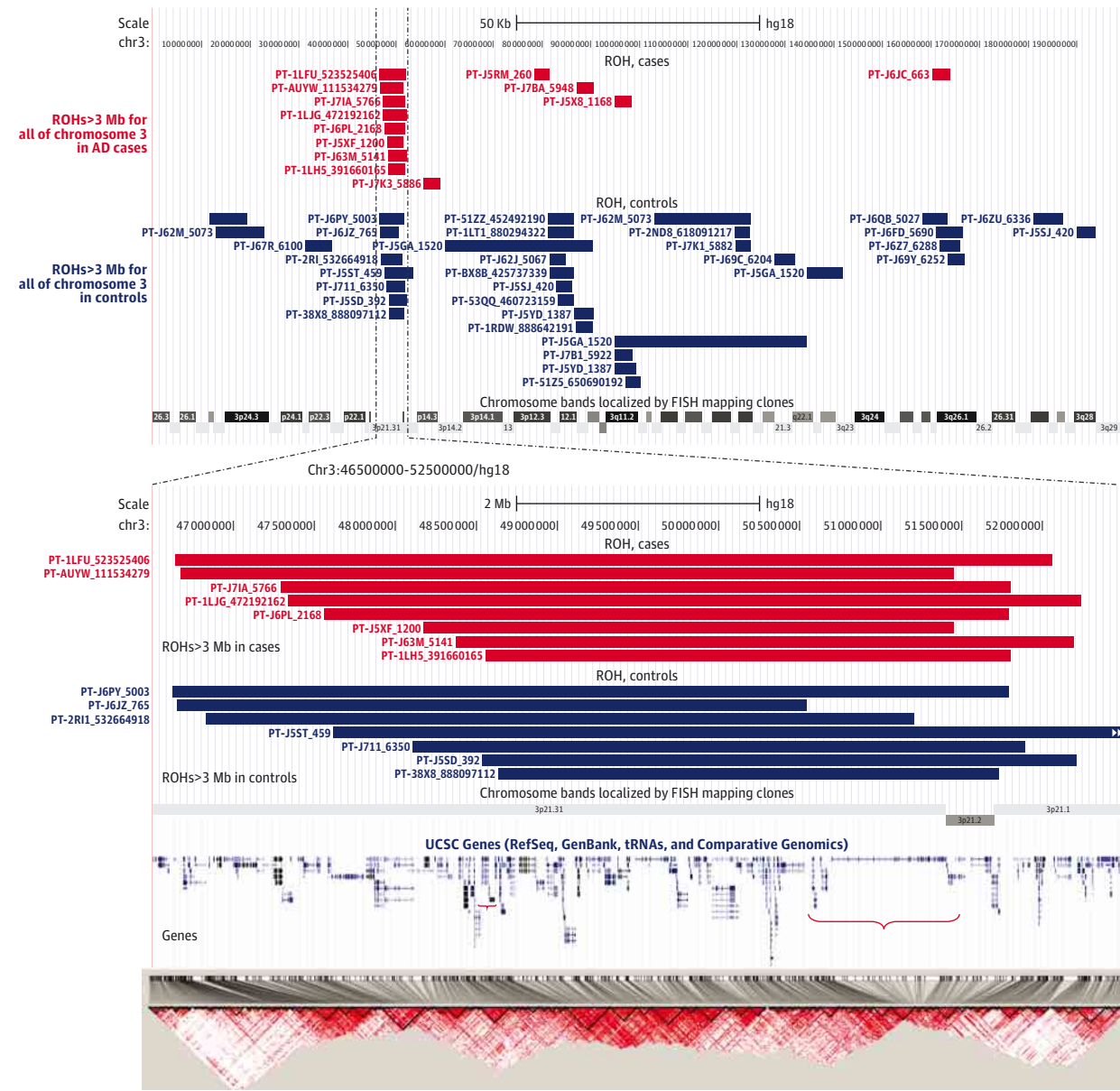
## Discussion

Our results suggest the existence of recessive AD loci among African American individuals. A greater global burden of ROH

measurements was detected in the entire (imputed) data set and ADGC cohort but not in the much smaller CHAP-IIDS data set (Table 1). To our knowledge, this is the first report of an association between AD and ROHs in an outbred population ( $F \sim 0.006$ ), in contrast to the report of Caribbean Hispanic individuals with a level of inbreeding similar to second cousins ( $F \sim 0.02$ ).<sup>8,29</sup> The mean total length of ROHs among African American individuals from both the ADGC (15 Mb) and CHAP-IIDS (10 Mb) data sets was comparable with that in Caribbean Hispanic individuals of African origin (19 Mb),<sup>8</sup> but much less than in Caribbean Hispanic individuals of European origin (40 Mb) who have a very high degree of inbreeding ( $F \sim 0.06$ ),<sup>8</sup> likely owing to an increase in consanguineous marriages after settlement in the Dominican Republic and Puerto Rico. Likewise, the average ROH size for the Caribbean Hispanic individuals of European origin was larger (2.1 Mb)<sup>8</sup> than for the African American individuals (1.5 Mb), reflecting more recombination events in an older African American population.

Locus-based ROH analyses could reveal only a small proportion of the genetic variance contributing to AD because we analyzed very rare and sparse ROHs (7-12 per genome; Table 1). The significant results observed in the locus-based investigation were unique to our African American data set; only 12 nominal genes were detected in both the Caribbean Hispanic<sup>8</sup> and African American cohorts: *NKTR*, *SEC22C*, *SS18L2*, *ZBTB47*, *SCN5A*, and *RBMS3* (Chr3p22-24); *PAX5*, *ZCCHC7*, *NFX1*, and *AQP7* (Chr9p13); and *INSR* and *ZNF557* (Chr19p13.2) (eTable 4 in the Supplement). Also, no significant loci were common between the ADGC and CHAP-IIDS data sets, which could in part be explained by the difference in data set size and the sparse overlap of SNPs between the 2 genotyping arrays. In general, replication of the association is expected for common variations (eg, SNPs in GWASs with frequency of  $>5\%$ ); however, rare genetic variations (eg, ROHs) with a frequency of less than 1% could be unique founder events that might not be observed in other data sets.<sup>30</sup> Nevertheless, the locus-based analyses detected 61 nominally significant genes common to both data sets

Figure 2. Significant Results Obtained by Gene-Based Analyses of the Chicago Health and Aging Project (CHAP)–Indianapolis Ibadan Dementia Study (IIDS) Data Set



The top section shows the runs of homozygosity (ROHs) greater than 3 Mb on chromosome 3 among cases ( $n = 279$ ) and control individuals ( $n = 1367$ ). Owing to an unbalanced distribution of cases and control individuals, fewer ROHs were observed among cases compared with control individuals, except at the Chr3p21.31 locus (section within the dashed lines), which was affected by ROHs greater than 3 Mb significantly more frequently in cases (2.9%, red bars)

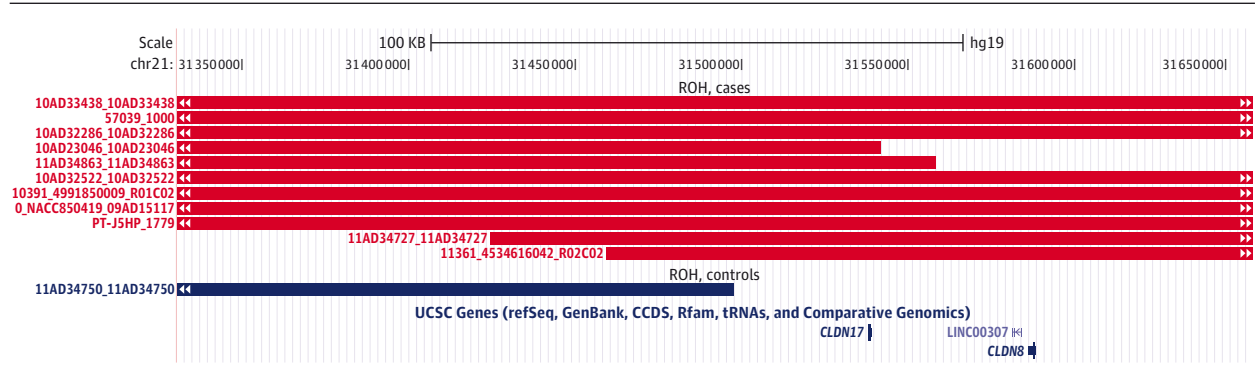
compared with control individuals (0.4%, blue bars). The middle section shows 2 down-brackets pointing to the significantly overlapped genes. The bottom section shows the linkage disequilibrium structure of the Chr3:46500000–52500000/hg18 region estimated based on control genotypes from the CHAP-IIDS data set. tRNA indicates transfer ribonucleic acid; UCSC, University of California–Santa Cruz.

(eTable 5 in the Supplement), including 49 coding genes, with 43 of them located at an approximate 2-Mb region within Chr3p21.31, where genes that survived correction for multiple testing were detected in the CHAP-IIDS data set. The functional significance of the Chr3p21.31 locus is also supported by its epigenomic architecture with a high density of gene regulatory elements according to the map of histone modifications obtained by ChIP sequencing of the IMR90 cell line (eFig-

ure 4 in the Supplement). Importantly, such loci are enriched in disease-associated genetic variants,<sup>31,32</sup> further encouraging the targeted sequencing of the Chr3p21.31 locus.

Most GWASs' significant loci (SNP or ROH based) remain to be explained by follow-up studies. The molecular basis of genetic association is usually investigated in 3 steps: detection of the disease loci followed by its sequencing and functional studies of potentially damaging variations. Our study

Figure 3. Significant Results Obtained by Gene-Based Analyses of the Entire Data Set



The *CLDN17* gene was intersected by runs of homozygosity (ROH) in 11 cases (red bars) but no control individuals (blue bar). CCDS indicates consensus coding sequence; tRNA, transfer ribonucleic acid; UCSC, University of California–Santa Cruz.

represents the first step that revealed the patients with a higher probability of having rare recessive mutations at certain ROH locus, and these individuals will be included in the sequencing step. There is also a possibility of a more complicated mechanism underlying the observed association, such as the action of risk haplotypes or a cumulative effect of ROHs on AD risk, making it more challenging to dissect the molecular basis of the association with ROHs.

Yet, it is essential to conduct follow-up sequencing studies because long ROHs are likely to harbor deleterious mutations.<sup>11,12</sup> The first priority should be given to significant loci in each investigated data set. In addition to the gene cluster on Chr3p21.31, a consensus region significantly associated with AD was detected on Chr15q24.1 in the CHAP-IIDS data set (empirical  $P$  value 2 = .02) and on Chr4q31.3 in the ADGC data set (empirical  $P$  value 2 = .04). Both loci contain good functional gene candidates. For instance, the locus on Chr15q24.1 includes *PML*, which is involved in the pathway of presenilin-APP-PML-p53 and overexpressed in AD brain,<sup>33</sup> while the Chr4q31.3 region includes *SH3D19*, which is implicated in the regulation of the ADAM family of metalloproteins responsible for  $\alpha$ -secretase activity in the amyloid pathway.<sup>34–37</sup> Potentially damaging variations reported in public databases within both consensus regions are presented in eTable 6 in the Supplement. Although the Database of Genomic Variants does not indicate that any large (>1-Mb) deletions affect the significant loci identified in our study, gene dosage analyses should be included in the follow-up study because, in some instances, ROHs could be the result of hemizygous deletions. Notably, recurrent microdeletions at 15q24 could not be responsible for the association with AD because such deletions cause a syndrome accompanied by major dysmorphic features (OMIM 613406).<sup>38,39</sup>

Analyses of the entire data set using imputed SNP data confirmed the significant contribution of recessive loci in the genetics of AD among African American individuals. We observed a higher rate of ROHs greater than 1 Mb per individual ( $P$  = .02), larger average size of ROHs greater than 2 Mb ( $P$  = .03), and a greater proportion of individuals with ROHs greater than 3 Mb ( $P$  = .006) in cases than control individuals (Table 1). Also,

gene-based analyses revealed significant association with *CLDN17* (empirical  $P$  value 2 = .01) that encodes claudin 17, a member of the claudin family. Claudins were suggested as AD biomarkers<sup>40</sup> and are important for the formation of tight junctions, particularly at the blood-brain barrier, where their expression is altered in AD and vascular dementia.<sup>41</sup> Our results encourage further investigation of genes responsible for the integrity of the blood-brain barrier, the disruption of which has been implicated in AD pathogenesis.<sup>42,43</sup>

Similar to the white population, the *APOE*  $\epsilon$ 4 allele contributes to AD risk in a dose-dependent manner in the African American population.<sup>44</sup> However, we and others<sup>8,13,17</sup> did not observe significant ROHs overlapping *APOE*, likely owing to frequent recombination events at this locus. Indeed, SNP-based GWASs have detected only small, approximately 70-kb extended *APOE* haplotypes.<sup>45</sup> Nevertheless, several genes associated with neurodegenerative diseases were nominally significant including AD genes (*PSEN2* and *CD2AP*) and *VCP* (Table 2). The overlap between different loci implicated in neurodegenerative disorders has to be systematically explored because there are many similarities that connect these disorders. For instance, *VCP* mutations have been shown to segregate with different disease phenotypes, including dementia (OMIM 601023), and *VCP* has been implicated in several cellular functions, including ubiquitin-dependent protein degradation highly relevant to neurodegeneration.<sup>46</sup>

## Conclusions

We observed a significant enrichment of ROHs among cases with AD, indicating the existence of recessive risk factors in African American individuals. So far, investigation of AD loci detected by the SNP-based studies have revealed only a few damaging variants (eg, in *ABCA7*<sup>47</sup> or *SORL1*<sup>48</sup>). Similarly, AD-associated ROH loci have to be examined by targeted sequencing for the presence of rare recessive mutations.<sup>11</sup> The complex genetics of late-onset AD might also be explained by the cumulative effect of multiple risk haplotypes underlying the association between AD and greater global burden of ROHs in our study.

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**Accepted for Publication:** June 4, 2015.

**Published Online:** September 14, 2015.

doi:10.1001/jamaneurol.2015.1700.

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**Obtained funding:** Naj, Larson, Evans, Barnes, Fallin, Manly, Kamboh, Bennett, Hall, Haines, Schellenberg, St. George-Hyslop, Mayeux, Rogaeva.

**Administrative, technical, or material support:** Ghani, Jun, Sato, Naj, Wang, Larson, Crane, Buxbaum, Ertekin-Taner, Logue, Barnes, Cantwell, Obisesan, Manly, Lopez, Hendrie, Byrd, Kukull,

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**Conflict of Interest Disclosures:** Support for this study was provided by GlaxoSmithKline. No other disclosures were reported.

**Funding/Support:** This study was supported by the Canadian Institutes of Health Research (Drs St. George-Hyslop and Rogava), Wellcome Trust, Medical Research Council, National Institutes of Health, National Institute of Health Research, Ontario Research Fund, and Alzheimer Society of Ontario (Dr St. George-Hyslop). The National Institute on Aging (NIA) supported this work through grants U01-AG032984, RC2-AG036528, and U01-AG016976 (Dr Kukull); U24 AG026395, U24 AG026390, R01AG037212, R37 AG015473, and P01-AG07232 (Dr Mayeux); U01 AG032984 (Dr Schellenberg); R01-AG25259, R01-AG 4048927, and P30-AG13846 (Dr Farrer); K23AG034550 (Dr Reitz); U24-AG021886 (Dr Foroud); R01AG009956 and RC2 AG036650 (Dr Hall); U01 AG06781 and U01 HG004610 (Dr Larson); R01 AG009029 (Dr Farrer); 5R01AG20688 (Dr Fallin); P50 AG005133 and AG030653 (Dr Kamboh); R01 AG019085 (Dr Haines); R01 AG11101 and R01 AG030146 (Dr Evans); P30AG10161, R01AG15819, R01AG30146, R01AG17917, and R01AG15819 (Dr Bennett); R01AG028786 (Dr Manly); R01AG22018 and P30AG10161 (Dr Barnes); P50AG16574 (Drs Ertekin-Taner and Graff-Radford); R01 AG032990, U01 AG046139, and R01 NS080820 (Dr Ertekin-Taner); R01 AG027944 and R01 AG028786 (Dr Pericak-Vance); P20 MD000546 and R01 AG28786-01A1 (Dr Byrd); AG005138 (Dr Buxbaum); P50 AG05681, P01 AG03991, and P01 AG026276 (Dr Goate); U24AG041689 (Dr Wang); and P30AG019610, P30AG13846, U01-AG10483, R01CA129769, R01MH080295, R01AG017173, R01AG025259, R01AG33193, P50AG008702, P30AG028377, AG05128, AG025688, P30AG10133, P50AG005146, P50AG005134, P01AG002219, P30AG08051, M01RR000096, UL1RR029893, P30AG013854, P30AG008017, R01AG026916, R01AG019085, P50AG016582, UL1RR02777, R01AG031581, P30AG010129, P50AG016573, P50AG016575, P50AG016576,

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**Role of the Funder/Sponsor:** The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Additional Contributions:** We thank Creighton Phelps, PhD, Stephen Synder, PhD, and Marilyn Miller, PhD, from the NIA, who are ex-officio members of the ADGC. They provided general guidance and support for this study, for which they did not receive compensation from a funding sponsor.

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