RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Mutations Modifying Sporadic Alzheimer's Disease Age of Onset

Jorge I. Vélez,[1,2] Francisco Lopera,[2] Hardip R. Patel,[1] Angad S. Johar,[1] Yeping Cai,[1] Dora Rivera,[2] Carlos Tobón,[2] Andrés Villegas,[2] Diego Sepulveda-Falla,[2,3] Shaun G. Lehmann,[4] Simon Easteal,[4] Claudio A. Mastronardi,[1] and Mauricio Arcos-Burgos[1,2]*

[1]Genomics and Predictive Medicine Group, Department of Genome Sciences, John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory, Australia

[2]Neuroscience Research Group, University of Antioquia, Medellín, Colombia

[3]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[4]Genome Diversity and Health Group, Department of Genome Sciences, John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory, Australia

Manuscript Received: 14 June 2015; Manuscript Accepted: 15 August 2016

The identification of mutations modifying the age of onset (AOO) in Alzheimer's disease (AD) is crucial for understanding the natural history of AD and, therefore, for early interventions. Patients with sporadic AD (*s*AD) from a genetic isolate in the extremes of the AOO distribution were whole-exome genotyped. Single- and multi-locus linear mixed-effects models were used to identify functional variants modifying AOO. A *posteriori* enrichment and bioinformatic analyses were applied to evaluate the non-random clustering of the associate variants to physiopathological pathways involved in AD. We identified more than 20 pathogenic, genome-wide statistically significant mutations of major modifier effect on the AOO. These variants are harbored in genes implicated in neuron apoptosis, neurogenesis, inflammatory processes linked to AD, oligodendrocyte differentiation, and memory processes. This set of new genes harboring these mutations could be of importance for prediction, follow-up and eventually as therapeutical targets of AD.

Key words: Alzheimer's disease; age of onset; extreme phenotypes; E280A mutation; genetic isolates; G protein-coupled receptors; modifier genes; *PSEN1*; whole exome analysis

How to Cite this Article:
Vélez JI, Lopera F, Patel HR, Johar AS, Cai Y, Rivera D, Tobón C, Villegas A, Sepulveda-Falla D, Lehmann SG, Easteal S, Mastronardi CA, Arcos-Burgos M. 2016. Mutations Modifying Sporadic Alzheimer's Disease Age of Onset.
Am J Med Genet Part B 171B:1116–1130.

INTRODUCTION

In the study of complex genetic disorders, a new comprehensive alternative approach has recently emerged to overcome the limitations of the common disease-common allele hypothesis [Cirulli and Goldstein, 2010]. This approach assesses genetic risk in terms of nonlinear interactions among genetic variants of major effect (i.e., exonic mutations) [Fearnhead et al., 2004; Bodmer and Bonilla, 2008; Bhatia et al., 2010; Liu and Leal, 2010a; Zuk et al., 2012] by studying patients with extreme phenotypes [Li et al., 2011;

Present address of Jorge I. Vélez is Department of Industrial Engineering, Universidad del Norte, Barranquilla, Colombia.

Conflicts of interest: None.
Grant sponsor: COLCIENCIAS; Grant sponsor: University of Antioquia; Grant number: 1115-408-20543.

Abbreviations: AD, Alzheimer's disease; AOO, age of onset; ADAOO, Alzheimer's disease age of onset; CEFV, common exonic functional variant; CERAD, Consortium to Establish a Registry for AD; *e*BIC, extended Bayes Information Criteria; EOAD, early-onset AD; FDR, false discovery rate; GPCR, protein-coupled receptor; GWAS, genome-wide association study; IBD, identical by descent; LMEM, linear mixed-effect model; LOAD, late-onset AD; MLMM, multi-locus mixed model; MAF, minor allele frequency; MCI, mild cognitive impairment; *m*BIC, modified Bayes Information Criteria; MMSE, Mini-mental State Exam; *m*PPA, Multiple Posterior Probability of Association; PSEN1, Presenilin 1; *s*AD, sporadic AD; SNP, single nucleotide polymorphism.

*Correspondence to:
Mauricio Arcos-Burgos, M.D., Ph.D., Genomics and Predictive Medicine Group, John Curtin School of Medical Research, The Australian National University, Building 131 Garran Road, Canberra, ACT, Australia.
E-mail: mauricio.arcos-burgos@anu.edu.au

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 30 August 2016
DOI 10.1002/ajmg.b.32493

Barnett et al., 2013; Johar et al., 2014] from extended and/or multigenerational pedigrees, or homogeneous cohorts from genetic isolates [Arcos-Burgos and Muenke, 2002; Arcos-Burgos et al., 2010; Jonsson et al., 2012; Velez et al., 2013].

Alzheimer's disease (AD) (OMIM 104300) is a neurodegenerative disorder that accounts for ~60–80% of dementia cases, especially on people over 65 years [Alzheimer's Association, 2014]. Prevalence estimates indicate that ~27 million people are affected with AD, and 1 in 85 individuals will be affected by 2050 worldwide [Brookmeyer et al., 2007]. In contrast with familial AD (*f*AD), sporadic AD (*s*AD) represents vastly most of AD cases and defines a subtype of AD where explicit affected relatives are either absent or cryptic to the clinical anamnesis.

Although Mendelian inheritance and major mutant causal genes are present in *f*AD [Goate et al., 1991; Levy-Lahad et al., 1995; Sherrington et al., 1995; Giraldo et al., 2013; Guerreiro et al., 2013; Cruchaga et al., 2014], only susceptibility loci of minor effect have been identified in *s*AD, with variants in the *Apolipoprotein E* (*APOE*) gene being the major genetic risk factor in late-onset cases. Recent genome-wide association studies (GWASs) have successfully identified over 20 loci of minor effect associated with late-onset AD (LOAD) outside the *APOE* locus [Chouraki and Seshadri, 2014; Logue et al., 2014].

Genetic isolates have shown to be a powerful tool for the genetic mapping of inherited diseases [Arcos-Burgos and Muenke, 2002]. During the last 30 years, we have studied the "Paisa" community from Antioquia, Colombia. This community is genetically homogeneous, exhibits high degrees of endogamy, and a number of sibs that is larger when compared to that of families from other areas of the country [Bravo et al., 1996]. Within the *Paisa* community, our group has studied the world's largest multigenerational pedigree in which the p.Glu280Ala E280A mutation in the *Presenilin-1* (*PSEN1*) gene co-segregates with early-onset AD (EOAD) [Lopera et al., 1994].

One of the most intriguing aspects outlined in this pedigree is the broad spectrum of the AD age of onset (ADAOO) that ranges from the earliest 30's to the 70's [Acosta-Baena et al., 2011]. In this pedigree, we have identified ADAOO modifier loci [Velez et al., 2015, 2016]. Following this approach, we performed whole-exome screening of functional variants in patients with *s*AD ascertained from the Paisa community who exhibited an extreme AOO. Several pathogenic exonic variants of major effect with remarkable genome-wide significance were found, some of them harbored in both previously reported and novel ADAOO modifier genes. Pathway, network and process enrichment analyses converge to support the role of these pathogenic variants in the physiopathology of *s*AD, opening new roads toward the understanding of the genetic basis of this complex form of AD.

METHODS

Subjects

Fifty-four individuals with *s*AD were included in this study. These patients inhabit in the Metropolitan Area of Medellin in Antioquia, Colombia. Genetic studies have shown that this community has not been subject to microdifferentiation, and shares the same genetic background and genealogy of the E280A pedigree [Bravo et al., 1996; Arcos-Burgos and Muenke, 2002].

Clinical Assessment

Clinical, neurological, and neuropsychological assessment of patients was performed at the Group of Neurosciences AD Clinic, University of Antioquia in Medellin, Colombia, using a Spanish version of The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) evaluation battery [Morris et al., 1989] adapted for the cultural and linguistic characteristics specific to this population (Supplementary Material) [Acosta-Baena et al., 2011; Fleisher et al., 2012]. Patients were defined as affected by mild cognitive impairment (MCI) based on Petersen's criteria [Petersen et al., 1999], and by AD if they met DSM-IV criteria [American Psychiatric Association, 2000]. Informed consent was obtained from all participants.

For the set of analyses presented below, only patients with defined AD were included (i.e., patients with MCI were not included for the genetic comparisons). Furthermore, the AD and MCI ages of onset were determined during anamnesis with information provided by the patients or their families, and looking for confirmation by several sources. Because some patients started their follow-up during MCI, their dementia age of onset was defined during the follow-up stage [Acosta-Baena et al., 2011]. This strategy was recently proven to be highly accurate [Aguirre-Acevedo et al., 2016].

Whole-Exome Genotyping

Genomic DNA was whole-genome amplified, fragmented, hybridized, fluorescently tagged, and scanned by the Australian Genome Facility (Melbourne, VIC, Australia) using the Infinium assay [Gunderson et al., 2005]. Whole-exome genotyping was conducted using Illumina HumanExome 12v1_A BeadChip, which covers regions with putative functional exonic variants selected from exome- and whole-genome sequences of >12,000 individuals. The exonic content consists of >250,000 markers representing diverse populations (including European, African, Chinese, and Hispanic individuals) in addition to common conditions such as type 2 diabetes, cancer, and metabolic and psychiatric disorders. In addition to pure exonic variation, the chip covers single nucleotide polymorphisms (SNPs) in splice sites, stop variants, promoter regions, and GWAS tag markers, among other potentially functional variation. Samples with calls below Illumina's expected 99% SNP call rates were excluded. In addition, the presence of *PSEN1* functional mutations in the group of sporadic patients has been discarded.

Genetic Statistical Analysis

Quality control, filtering, and classification of exonic variants. Genotypes were extracted using the Genotyping module of Illumina's GenomeStudio v2010.3 and the Illumina® HumanExome 12v1_A manifest cluster file. Genotype files were processed in Golden Helix® SNP Variation Suit (SVS) 8.2.1 (Golden Helix, Inc. Bozeman, MT). Marker exclusion criteria

included (i) deviations from Hardy–Weinberg equilibrium with $P < 2 \times 10^{-7}$ (0.05/250,000 markers); (ii) a minimum genotype call rate of 90%; (iii) the presence of one or more than two alleles; and (iv) a minor allele frequency (MAF) < 1% (i.e., excluding rare variants). Genotype and allelic frequencies were estimated by maximum likelihood. The identity by descent (IBD) matrix between all pairs of individuals was used for quality control.

Exonic variants with potential functional effect were determined using the functional prediction information available in the dbNSFP_NS_Functional_Predictions GRCh_37 annotation track implemented in the SVS Variant Classification Module. This module was also used to examine interactions between variants and gene transcripts to classify variants based on their potential effect on genes. Variants were classified according to their position in a gene transcript, and those in coding exons were further classified according to their effect on the gene's protein sequence.

GWAS of exonic variants. We studied the association of common exonic functional variants (CEFVs) to ADAOO using single- and multi-locus additive, dominant, and recessive linear mixed-effect models (LMEMs) with up to 20 steps in the backward/forward optimization algorithm [Segura et al., 2012]. The advantage of these models is the inclusion of both fixed (sex and years of education) and random effects, the latter to account for potential inbreeding by including the IBD matrix. A single-locus LMEM assumes that all loci have a small effect on the trait, while multi-locus LMEM assume that several loci have a large effect on the trait [Segura et al., 2012]. Both types of models are implemented in SVS 8.2.1. The optimal model was selected using a comprehensive exploration of multiple criteria including the Bayesian Information Criteria (*BIC*), the extended BIC (*eBIC*), the modified BIC (*mBIC*), the Multiple Posterior Probability of Association (*mPPA*), the log-likelihood, and the minimum pseudo-heritability. Correction for multiple testing was performed using Bonferroni's correction [Bonferroni, 1935], false discovery rate (FDR) [Benjamini and Hochberg, 1995], and a method based on extremes-value theory [Vélez et al., 2014].

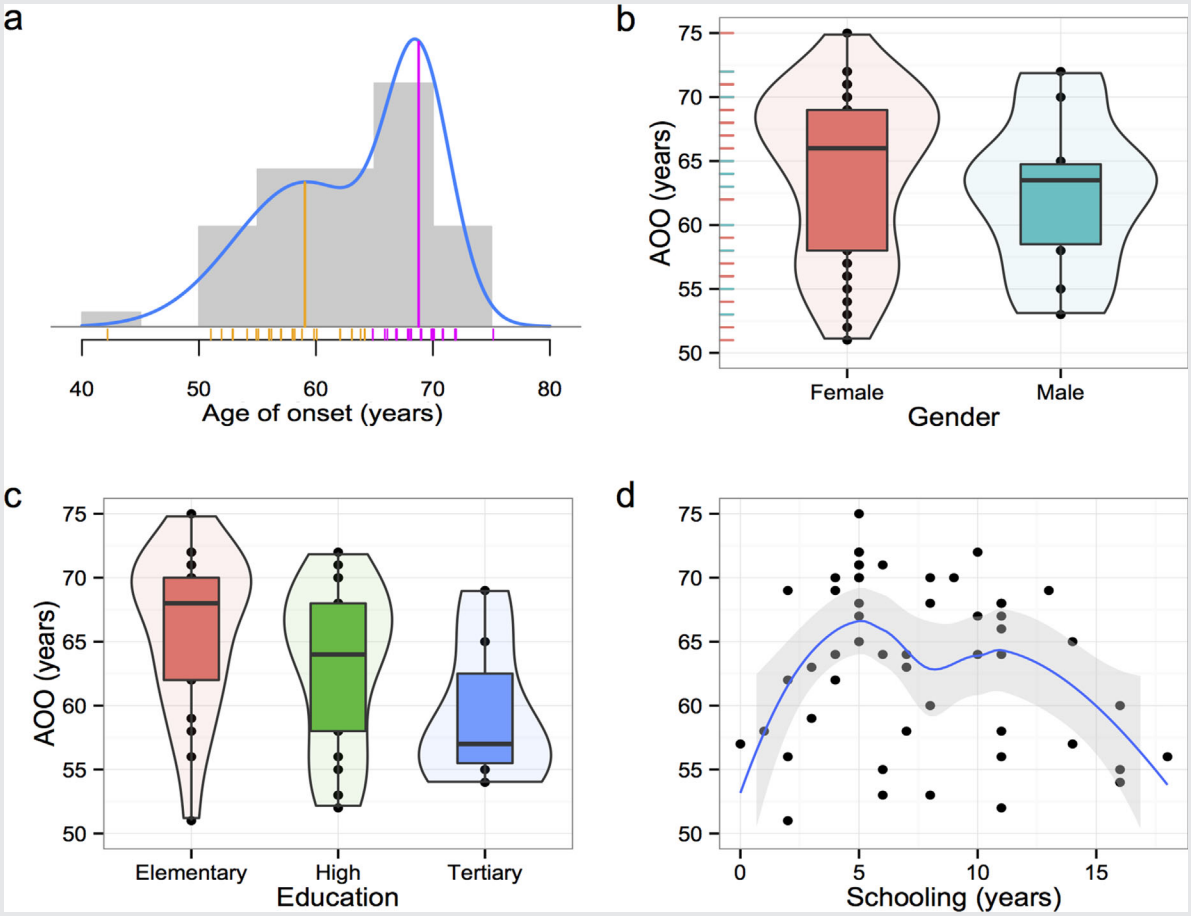
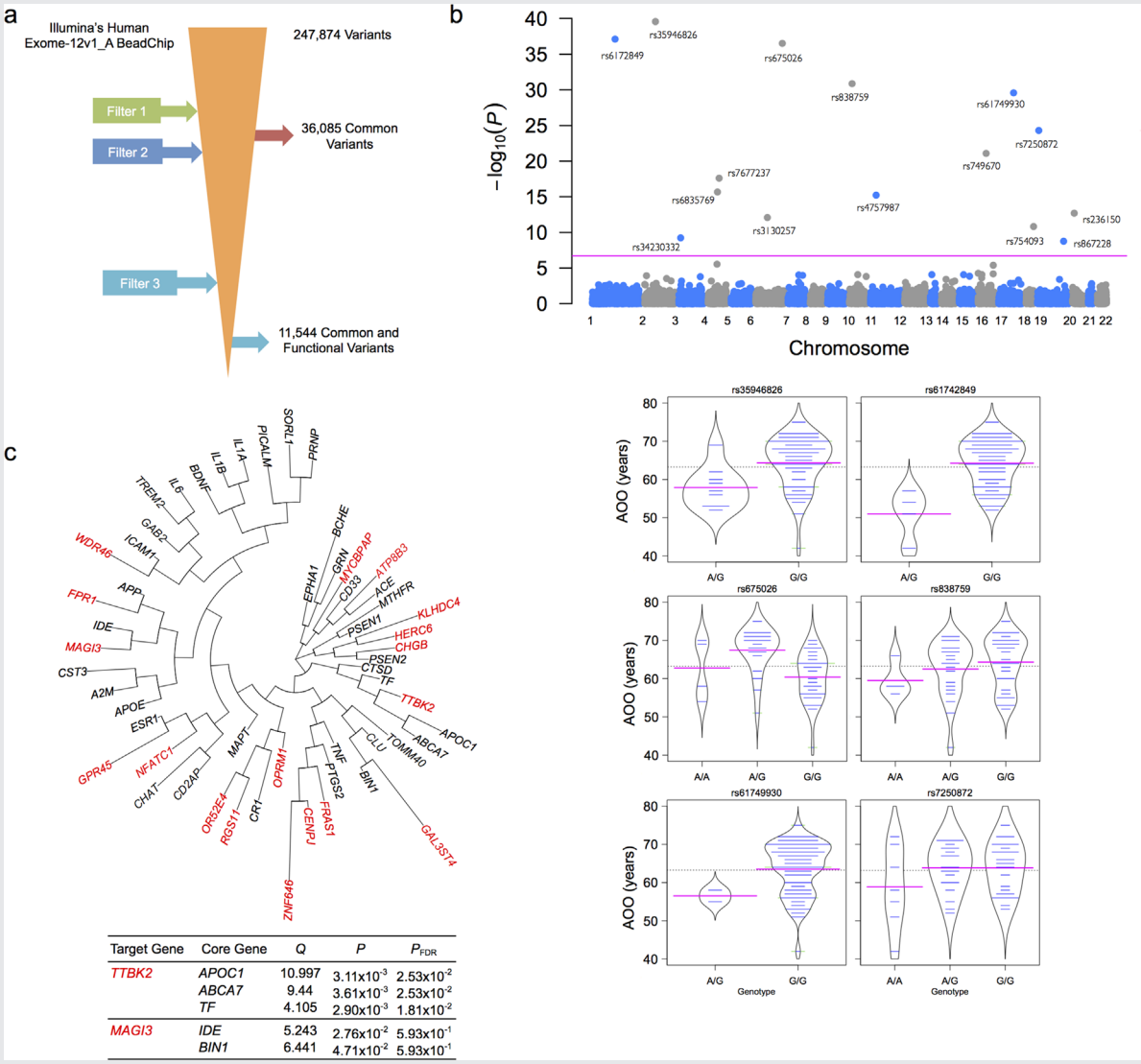


FIG. 1. (a) Histogram and probability density plots for the ADAOO in 54 patients with *s*AD. The disclosed the presence of two hidden groups with an average ADAOO of ~57 and ~69 years old, respectively. Box- and violin-plots for the ADAOO by (b) gender and (c) education group. No difference in the average ADAOO was found in either case. (d) ADAOO as a function of the years of education. Individuals with <3 or >13 years of education seem to have an earlier ADAOO. ADAOO, Alzheimer's disease age of onset; *s*AD, Sporadic Alzheimer's disease. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

For the analysis of rare exonic functional variants (REFVs), regression- and permutation-based Kernel-Based Adaptive Cluster (KBAC) methods were used [Liu and Leal, 2010b]. KBAC, implemented in SVS 8.3.0, catalogues rare variant data within each of a number of regions into multi-marker genotypes and, since variants are rare, only a relatively few different multi-marker genotypes are found in any given region. A special test is subsequently applied to determine their association with the (case/control) phenotype, weighting each multi-marker genotype by how often that genotype was expected to occur according to both the data and the null hypothesis that there is no association between that genotype and the case/control status of the sample [Liu and Leal, 2010b]. Thus, genotypes with high sample risks are given higher weights that can potentially separate causal from non-causal genotypes. Further, a one-sided test was applied due to the weighting procedure and the *P*-values were estimated using 10,000 permutations.



Target Gene	Core Gene	Q	P	P_{FDR}
TTBK2	*APOC1*	10.997	3.11×10^{-3}	2.53×10^{-2}
	ABCA7	9.44	3.61×10^{-3}	2.53×10^{-2}
	TF	4.105	2.90×10^{-3}	1.81×10^{-2}
MAGI3	*IDE*	5.243	2.76×10^{-2}	5.93×10^{-1}
	BIN1	6.441	4.71×10^{-2}	5.93×10^{-1}

FIG. 2. (a) Filtering process applied to exonic variants. *Filter 1* excludes variants with a genotype call rate <90%, in Hardy–Weinberg disequilibrium and with one or more than two alleles. *Filters 2* excludes variants with MAF < 1% and *Filter 3* those not functional. A total of 11,544 common functional variants remained for analysis at the end of this process. (b) Manhattan plot for the 11,544 common functional variants included for analysis. Markers with $-\log_{10}(P) > 3.98$ (in red) were significant after FDR correction. Beanplots for the ADAOO in the top six FDR significant variants are also shown. Pink, blue, and dotted horizontal lines, respectively, correspond to the within genotype average ADAOO, the individuals' ADAOO and the global average ADAOO in our 54 patients with *s*AD. (c) Dendrogram showing the biological relatedness between ADAOO modifier genes (Table I, shown in red) and the top 10 percentile genes with functional relevance in AD. Genes with statistically significant biological relatedness are presented at the bottom. Abbreviations as in Figure 1. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

TABLE I. Results of the Association Analysis for AOO in 54 Patients With Sporadic Alzheimer's Disease From the Paisa Genetic Isolate

				Marker information				Multi-locus linear mixed-effects model		
Chr	SNP	Position[a]	Gene	R/A	MA (Freq)	CR	Change	β ($SE_β$)	*P*	P_{FDR}
2	rs35946826	105,859,249	*GPR45*	C/T	C (0.915)	1.000	p.Leu312Phe	−12.668 (0.148)	2.66×10^{-40}	3.08×10^{-36}
1	rs61742849	114,226,143	*MAGI3*	G/A	G (0.962)	1.000	p.Gly1318Asp	−14.322 (0.199)	7.59×10^{-38}	4.38×10^{-34}
6	rs675026	154,414,563	*OPRM1*	A/G	C (0.731)	0.981	[b]	5.424 (0.079)	2.99×10^{-37}	1.15×10^{-33}
10	rs838759	22,498,468	*EBLN1*	C/T	C (0.745)	1.000	p.Gly149Arg	−4.259 (0.092)	1.35×10^{-31}	3.90×10^{-28}
17	rs61749930	48,594,691	*MYCBPAP*	G/A	G (0.981)	1.000	p.Arg124His	−12.077 (0.286)	2.63×10^{-30}	6.06×10^{-27}
19	rs7250872	1,811,603	*ATP8B3*	C/T	C (0.673)	0.981	p.Gly45Arg	−2.543 (0.088)	4.98×10^{-25}	9.57×10^{-22}
16	rs749670	31,088,625	*ZNF646*	A/G	A (0.627)	0.962	p.Glu327Gly	−1.525 (0.067)	8.21×10^{-22}	1.35×10^{-18}
4	rs7677237	89,306,659	*HERC6*	T/C	T (0.877)	1.000	p.Met123Thr	2.142 (0.122)	2.48×10^{-18}	3.58×10^{-15}
4	rs6835769	79,284,694	*FRAS1*	C/T	T (0.519)	0.981	p.Ala817Val	−1.112 (0.074)	2.14×10^{-16}	2.74×10^{-13}
11	rs4757987	5,906,205	*OR52E4*	G/A	G (0.663)	0.981	p.Arg228His	1.023 (0.07)	5.94×10^{-16}	6.86×10^{-13}
20	rs236150	5,903,141	*CHGB*	G/C	G (0.934)	1.000	p.Lys117Asn	−2.145 (0.181)	2.02×10^{-13}	2.12×10^{-10}
6	rs3130257	33,256,471	*WDR46*	T/C	C (0.962)	1.000	p.Thr40Ala	−2.349 (0.209)	8.23×10^{-13}	7.92×10^{-10}
18	rs754093	77,246,406	*NFATC1*	T/G	T (0.558)	0.981	p.Cys751Gly	−0.941 (0.094)	1.51×10^{-11}	1.34×10^{-8}
3	rs34230332	14,725,878	*C3orf20*	A/G	A (0.943)	1.000	p.Gln83Arg	1.593 (0.185)	5.83×10^{-10}	4.81×10^{-7}
19	rs867228	52,249,211	*FPR1*	T/G	G (0.827)	0.981	p.Glu346Ala	-0.944 (0.115)	1.73×10^{-9}	1.34×10^{-6}
4	rs3733251	77,192,838	*FAM47E*	A/C	A (0.894)	0.981	p.Lys165Gln	−0.713 (0.127)	2.87×10^{-6}	2.07×10^{-3}
16	rs2303772	87,795,580	*KLHDC4*	G/C	G (0.943)	1.000	p.Leu56Val	0.748 (0.135)	4.05×10^{-6}	2.75×10^{-3}
16	rs739999	319,511	*RGS11*	A/G	A (0.808)	0.981	p.Met416Thr	0.35 (0.075)	5.43×10^{-5}	3.48×10^{-2}
16	rs34779002	87,782,396	*KLHDC4*	C/A	C (0.953)	1.000	p.Gly73Val	0.787 (0.172)	6.58×10^{-5}	4.00×10^{-2}
15	rs6493068	43,170,793	*TTBK2*	A/G	A (0.736)	1.000	p.Leu8Pro	−0.479 (0.107)	8.50×10^{-5}	4.27×10^{-2}
16	rs17137138	4,606,743	*C16orf96*	G/C	G (0.981)	1.000	p.Val85Leu	1.002 (0.223)	8.39×10^{-5}	4.40×10^{-2}
7	rs3823646	99,757,612	*GAL3ST4*	G/A	G (0.566)	1.000	p.Ala467Val	−0.307 (0.069)	9.29×10^{-5}	4.47×10^{-2}
13	rs17081389	25,487,001	*CENPJ*	G/C	G (0.981)	1.000	p.Pro55Ala	1.002 (0.223)	8.39×10^{-5}	4.61×10^{-2}
10	rs78334417	75,071,618	*TTC18*	G/A	G (0.981)	1.000	p.Pro450Ser	1.002 (0.223)	8.39×10^{-5}	4.84×10^{-2}
7	rs186048202	134,678,273	*AGBL3*	C/T	C (0.951)	0.962	p.Arg52Trp	0.614 (0.139)	1.06×10^{-4}	4.91×10^{-2}

AOO, Age of onset; AD, Alzheimer's disease; Chr, Chromosome; SNP, Single nucleotide polymorphism, R/A, Reference/alternative; MA, Major allele; Freq, Frequency; CR, Call rate; β, Regression coefficient; $SE_β$, Standard error of β; *P*, *P*-value; FDR, False discovery rate.

Highlighted variants increase ADAOO (i.e., have a protective effect).

[a]UCSC GRCh37/hg19 coordinates.

[b]DNA methylation by reduced representation bisulfite seq from ENCODE, and UCSF brain DNA methylation site.

Network enrichment analysis. We performed network and pathway analyses in order to identify key physiological pathways and networks harboring the candidate genes considered as ADAOO modifiers. The identification of these networks allows the acquisition of rich ontologies for biological processes at the protein and molecular level with potential importance in AD. Genes with potential functional effect were examined in MetaCore version 6.20 build 66481 (Thomson Reuters, NY) using the "Analyse Network," "Process Networks," "Shortest Paths," and "Direct Interactions" algorithms, which provide a heuristic interpretation of maps and networks and rich ontologies for diseases based on the biological role of candidate genes. To minimize artefacts in the statistical analysis, only nodes with direct physical interactions between the encoded proteins in the database were included.

Biological relatedness between candidate and core genes. We used the Human Gene Connectome (HGC) database [Itan et al., 2014] to quantify the biological relatedness between ADAOO modifier genes in our patients with *s*AD, and genes previously reported in AD. Similar to MetaCore, the rationale of the HGC is to prioritize candidate genes on the basis of their functional relevance to the *s*AD phenotype. Candidate genes were chosen on the basis of their quantitative relatedness or biological distance to genes already established as having functional importance in AD. Biological distances [Itan et al., 2014] were calculated between genes identified in our association analyses and those previously identified in AD. The list of genes with known functional/physiological relevance and/or association to AD was obtained from the Gene Prospector database [Yu et al., 2008]. Only genes in the top 10 percentile were selected for the HGC-based analysis. To evaluate the significance of these distances, *P*-values were estimated via random permutation of pairwise gene interactions in the HGC database, and subsequently corrected using FDR.

It is worth mentioning that the Human Genome Connectome (HGC) is more effectively applied when seeking to identify Mendelian disease-causing genes. Given that the rational of this study is the detection of major effect gene variation we have assumed that the HGC could disclose some relevant information pointing cryptic networks out from the genes reported by the genetic screening.

RESULTS

Patients

Of the 54 individuals included in this study, 43 (80%) were women and 11 (20%) men. The average ADAOO was 63.26 ± 6.94 years in these patients. Analysis of the ADAOO distribution suggests that patients may be clustered in two different groups; the first group consists of 30 (57%) individuals with an average ADAOO of 57.86 ± 5.13 years (Fig. 1a, orange line), while 24 (43%) individuals belong to the second group and have an average ADAOO of 69.08 ± 2.41 years (Fig. 1a, pink line). As intended, statistically significant differences in ADAOO were found between groups ($P = 2.92 \times 10^{-10}$). No difference in ADAOO was found by gender (Females: 63.95 ± 6.44; Males: 60.54 ± 8.42, $P = 0.232$) (Fig. 1b). In those individuals with available information ($n = 53$), years of education ranged from 0 to 18 years: one patient (2%) never attended school; 22 (42%) completed primary school (grades 1–5); 23 (43%) completed high school (grades 6–11, inclusive); and seven (13%) attended tertiary education. Average ADAOO did not differ significantly across education groups education ($F_{2,49} = 1.362$, $P = 0.265$) (Fig. 1c). Although not statistically significant, ADAOO slightly increases for individuals with <3 and >13 years of education (Fig. 1d).

Common Functional Exonic Variants (CFEVs) Modifying ADAOO

After quality control and filtering, a total of 11,544 CEFVs remained for analysis (Fig. 2a). A recessive multi-locus LMEM with 17 steps in the forward selection algorithm was chosen based on the lowest *eBIC*, the highest *m*PPA and the lowest pseudo-heritability criteria. After FDR correction, a total of 25 variants were significantly associated as ADAOO modifiers in our patients with *s*AD (Table I and Fig. 2b). Based on the estimated regression coefficient $\hat{\beta}$ of the multi-locus LMEM, these CEFVs can be classified as accelerators (group 1, $\hat{\beta} < 0$, $n = 14$, Table I), or decelerators (group 2, $\hat{\beta} > 0$, $n = 11$, Table I) of the ADAOO. Variants rs35946826 (*GPR45*, $\hat{\beta} = -12.7$, $P_{FDR} = 3.08 \times 10^{-36}$), rs61742849 (*MAGI3*, $\hat{\beta} = -14.3$, $P_{FDR} = 4.38 \times 10^{-34}$), and rs61749930 (*MYCBPAP*, $\hat{\beta} = -12.1$, $P_{FDR} = 6.06 \times 10^{-27}$) in the first group, and variants rs675026 (*OPRM1*, $\hat{\beta} = 5.4$, $P_{FDR} = 1.15 \times 10^{-33}$), rs7677237 (*HERC6*, $\hat{\beta} = 2.1$, $P_{FDR} = 3.58 \times 10^{-15}$), and rs34230332 (*C3orf20*, $\hat{\beta} = 1.6$, $P_{FDR} = 4.81 \times 10^{-7}$) in the second group are of particular interest because of their ADAOO modifier effect. In particular, having two copies of the A allele in rs35946826 (*GPR45*) accelerates ADAOO by ~13 years compared to having zero or one copies of the allele, and having two copies of the A allele in rs675026 (*OPRM1*) delays ADAOO by ~5.5 years compared to having zero or one copies of it (Table I and Fig. 2b). There were no qualitative differences in the number of loci selected by the linear mixed model when either the FDR or the Bonferroni correction was used. In addition, no significant rare variant was found to be associated with the ADAOO using KBAC.

HGC-Based Biological Relatedness

Figure 2c shows the dendrogram constructed from the biological relatedness similarity between the top 10 percentile of core AD genes and the genes reported herein. We successfully identified statistically significant biological relatedness between *TTBK2* and *APOC1*, *ABCA7* and *TF* (three of the core AD genes), while both *MAGI3* and *IDE*, and *MAGI3* and *BIN1* were nominally significant (Fig. 2c, bottom). Of particular importance is the pairwise comparison between *TTBK2* and *TF* as this pair of genes had the most significant biological relatedness (Fig. 2c). Additionally, *TTBK2* is biologically related to *APOC1* and *ABCA7* (Fig. 2c) (additional interpretations are provided in the Supplementary Material).

Pathway Enrichment Analysis

Apoptosis-related and neurological signaling-related processes. We identified key physiological processes involving some of our ADAOO modifier genes (Table I). Indeed, *MAGI3*, *FPR1*, *OPRM1*, and *NFATC1* are involved in physiological

TABLE II. Apoptosis- and Neurological Signaling-Related Processes, and Disease Ontological Descriptions of Candidate Genes From the Pathway, Network, and Enrichment Analyses

		Network				
Gene	**Network algorithm**	**Name**	***P*-value**	**FDR**	**Process, disease annotations, and disease ontology (*P*-value)**	**Biological process node in network**
MAGI3	Shortest paths	[a]	[a]	[a]	Regulation of neuron apoptosis (2.24×10^{-4})	Binding of β-adrenergic receptor to *MAGI3*
		[a]	[a]	[a]	Regulation of apoptotic processes (1.67×10^{-4})	Binding of β-adrenergic receptor to *MAGI3*, and cleavage of *MAGI3* by cathepsin K
		[a]	[a]	[a]	Regulation of MAP kinase activity (1.31×10^{-6})	Binding of β-adrenergic receptor to *MAGI3*
FPR1	Process networks	NPS	7.64×10^{-4} **(3/155)**	1.23×10^{-2}	Negative regulation of synaptic transmission (2.04×10^{-7})	Binding of G protein α-q 11 to *FPR1*
					Alzheimer's Disease (4.84×10^{-20})	
					EOAD (4.63×10^{-3})	
					LOAD (5.76×10^{-10})[b]	
OPRM1	Process networks	NPS	7.64×10^{-4} **(3/155)**	1.23×10^{-2}	Positive regulation of IL1β production (6.23×10^{-3})	Binding of G protein α-q 11 to *FPR1*
					Regulate NFkB activity (5.78×10^{-3})	
					Neurogenesis (7.73×10^{-7})	
					Dopamine receptor signaling (5.27×10^{-4})	
NFATC1	Process networks	InS	7.07×10^{-3} **(2/104)**	4.28×10^{-2}	Memory (4.22×10^{-4})	Dephosphorylation of *NFATC1* by calcineurin, ad phosphorylation of *NFATC1* by ERK1/2 kinase
					Alzheimer's Disease (4.52×10^{-23}) LOAD (8.37×10^{-19})	
					TLR3 signaling (2.74×10^{-2})	
					Oligodendrocyte differentiation (1.69×10^{-2})	
FPR1/ OPRM1	Process networks	NPS	7.64×10^{-4} **(3/155)**	1.23×10^{-2}	Positive regulation of I-kappaB kinase (4.62×10^{-2})	GTP hydrolysis of GPCRs (encoded by *FPR1* and *OPRM1*), leading to activation of G-protein α-I family
					Positive regulation of neurogenesis (1.34×10^{-3})	
					Dopamine receptor signaling (1.22×10^{-7})	
	Direct interactions[c]	DIs	[d]	[d]	Positive regulation of cytosolic Ca^{2+} ion concentration (2.54×10^{-2})	Aβ protein binding G protein coupled receptors (encoded by *FPR1* and *OPRM1*)
					Neuron projection development (1.22×10^{-4})	
					Neuron recognition (6.38×10^{-3})	
					Regulation of Ca^{2+} transport (5.40×10^{-3})	Cleaved Aβ protein which binds GPCRs
					Positive regulation of neuron differentiation (4.48×10^{-2})	Aβ binding to GPCRs (encoded by *FPR1* and *OPRM1*)
					Regulation of apoptotic signaling (4.69×10^{-4})	
					Neuron generation (1.88×10^{-6})	*ESR1* transcriptionally regulates *ACE1*, which cleaves Aβ proteins (inhibitory effect)
					Alzheimer's Disease (2.76×10^{-14})	
					LOAD (7.14×10^{-11})	Cleaved protein binds to GPCRs (encoded by *FPR1* and *OPRM1*)

Enrichment scores are shown in bold.
DIs, Direct interactions; EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; InS, Inflammation/IL2 signaling; NPS, Neuropeptide signaling; FDR, False discovery rate.
The *P*-values associated to each network give the probability of getting a certain number of genes obtained from a given network algorithm from the input list by chance. Enrichment scores are similarly interpreted.
[a] *P*-value is not included because the shortest path algorithm only builds a single network.
[b] Includes all nodes only involving *FPR1* and *OPRM1*.
[c] Contains genes from Table I and the top two percentile of AD-related genes from the Gene Prospector database.
[d] *P*-values not included because only a single network is produced.

processes such as neuron apoptosis, neurogenesis, and memory, all containing statistically significant ontological process ($P < 0.05$, Table II). Specifically, the network node showing the interaction of the β-adrenergic receptor with *MAGI3* (Fig. 3a) suggests that this gene is involved in neuron apoptosis ($P = 2.24 \times 10^{-4}$), apoptotic processes ($P = 1.67 \times 10^{-4}$), and regulation of the MAP kinase activity ($P = 1.31 \times 10^{-6}$).

NFATC1, *OPRM1*, and *FPR1* are involved in oligodendrocyte differentiation ($P = 1.69 \times 10^{-2}$), positive regulation of neurogenesis ($P = 1.34 \times 10^{-3}$), and negative regulation of synaptic transmission ($P = 2.04 \times 10^{-7}$), respectively, and have important implications in AD, EOAD, LOAD, and memory (Table II). *FPR1* and *NFATC1* are involved in immune related processes, which could also have implications for AD, while *FPR1* is involved in the release of IL1β, which is thought to contribute to neuroinflammation and neurodegeneration ($P = 6.23 \times 10^{-3}$), and *NFATC1* is a necessary activator of TLR3 signaling ($P = 2.74 \times 10^{-2}$) (Table II). This later process is seemingly facilitated by dephosphorylation of NFATC1 by calcineurin (Table II and Fig. 3b). *OPRM1* was also found implicated in the regulation of NF-κB activity ($P = 5.78 \times 10^{-3}$), neurogenesis ($P = 7.73 \times 10^{-7}$), and dopamine receptor signaling ($P = 7.73 \times 10^{-7}$) through the binding of G protein α-q/11 to FPR1 (Fig. 3c). Our findings also indicate that the ACE1 cleavage enzyme cleaves amyloid beta (Aβ), resulting in an inhibitory effect on the activity and accumulation (Fig. 3d). The cleaved protein then binds to and subsequently activates G protein-coupled receptors (GPCRs), which are important molecules involved in neuron recognition ($P = 6.38 \times 10^{-3}$), development ($P = 1.22 \times 10^{-4}$), differentiation ($P = 4.48 \times 10^{-2}$), and generation ($P = 1.87 \times 10^{-6}$), as well as in apoptotic signaling ($P = 4.68 \times 10^{-4}$) and calcium transport ($P = 5.40 \times 10^{-3}$) (Table II). Hence, a mutation in the GPCR encoding genes (*FPR1* and *OPRM1*) might change the regulation of these functions, which in turn could affect neurological function, potentially leading to symptoms in AD ($P = 2.76 \times 10^{-14}$) and LOAD ($P = 7.14 \times 10^{-11}$). This mechanism coincides with other previous

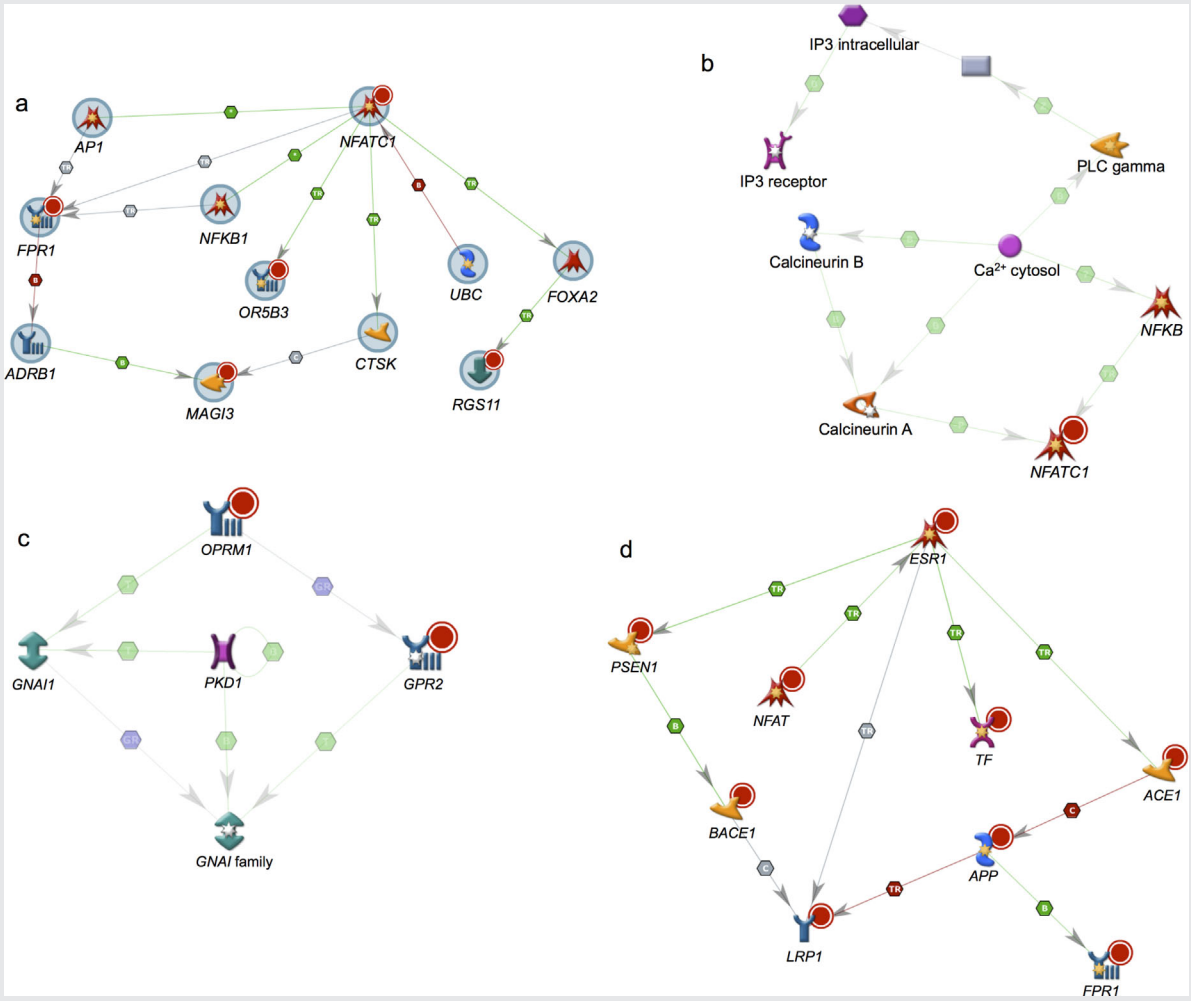


FIG. 3. Networks for (a) *MAGI3*, (b) *NFATC1*, (c) *OPRM1* and *FPR1*, and (d) reflecting direct interactions involving *FPR1* and *OPRM1*. Additional information in Table II. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

findings showing that Aβ accumulation can disrupt GPCR function and, therefore, the aforementioned processes [Thathiah and De Strooper, 2011]. However, a mutation in the GPCR encoding proteins would also prevent these essential processes, thereby favoring AD pathogenesis.

Wnt signaling-related processes. *FRP1* and *OPRM1* were also identified to have key roles of potential relevance in *Wnt* signaling via various mechanisms (Table III and Fig. 4). Figure 4a suggests the initiation of *Wnt* signaling process through the transcriptional regulation of GPCRs (encoded by *OPRM* and *FPR1*) by the *c*-myc transcription factor and/or the binding to and activation of Phospholipase C (PLC) γ. Gene ontology analysis shows that this mechanism might be important in positive regulation of apoptosis ($P = 4.01 \times 10^{-5}$, Table III). GPCRs can also impact *Wnt* signaling via alternate pathways and processes by binding and subsequently activating G Protein α I family members (Fig. 4b), which might result in the negative regulation of *Wnt* protein secretion ($P = 1.28 \times 10^{-2}$, Table III). The NPFF receptor 2 and the Nociceptin receptor bind the μ-type opioid receptor encoded by *OPRM1* (Fig. 4c). This binding has an inhibitory antagonistic effect on *OPRM1* and thereby restricts the regulatory capabilities of this protein in important processes such as calcium ion transport and homeostasis ($P = 1.39 \times 10^{-5}$), and PLC signaling ($P = 4.29 \times 10^{-3}$) (Table III).

The network and enrichment analysis further indicated that *Wnt* signaling is not only influenced by activation effects through proteins binding to receptors, but could also be regulated by antagonistic effects such as the binding of Nociceptin receptors, SSTR2, substance P receptor, and NPFF receptor 2 to the μ-type opioid receptor (encoded by *OPRM1*) (Fig. 4c,d). Both networks suggest that this mechanism inhibits the activity of the μ-type opioid receptor, which consequently results in the negative regulation of *Wnt* protein secretion. This regulatory cascade seems to continue as a consequence of the μ-type opioid receptor binding to and activating the δ-type opioid receptor. Furthermore, this process is also facilitated by activation of G Protein β/γ after it binds to GPCRs ($P = 1.66 \times 10^{-7}$ for Fig. 4c, $P = 4.98 \times 10^{-6}$ for Fig. 4d, respectively).

DISCUSSION

Despite the clinical, neuropsychological and neuropathological proven similarities between familial and sporadic AD [Duara et al., 1993; Lehtovirta et al., 1996; Israel et al., 2012], the identification of causative genetic variants has only been successful in the former [Piaceri et al., 2013; Alzheimer's Association, 2014]. Here, we report 25 CFEVs that modify ADAOO in patients with *s*AD ascertained from a genetic isolate. In addition to their potential functional implications, the effect sizes of these mutations is genome-wide significant and stand after correction for multiple testing. Furthermore, some of these pathogenic mutations point toward conspicuous genes that have been either previously implicated in the physiopathology of AD, or significantly linked to pathways, networks, or processes related to AD.

Some genes harboring these mutations deserve a more detailed report. Our network and pathway enrichment analysis showed that *MAGI3* interacts with the β-adrenergic receptor playing a role in neuron apoptosis. In addition, *MAGI3* interacts with *PTPRB* [Adamsky et al., 2003] and *PTEN* [Wu et al., 2000], modulators of the *AKT1* gene, which is a critical mediator of growth factor-induced neuronal survival, reported as disturbed in AD [Guan et al., 2000; Persad et al., 2001; Rickle et al., 2004].

GPCRs mediate most cellular responses to hormones and neurotransmitters and play important role in the physiopathology of AD (see Table II) [Rosenbaum et al., 2009; Thathiah and De Strooper, 2011; Ghanemi, 2015]. We found that GPCRs (encoded by *FPR1* and *OPRM1*) might also have a key role in apoptotic processes (Table III and Fig. 4). Thus, a mutation in either or both of these genes encoding GPCRs may disturb the apoptotic regulation process. Excessively high levels of apoptosis may lead to destruction of cells key to neurological processes. Conversely, if the mutation(s) lead(s) to a reduction in apoptotic activity, this may lead to the accumulation of cell contents and or proteins with potential neurotoxic effects. *GPR45* is expressed in the central nervous system, the periphery and neural cells [Marchese et al., 1999; Kawasawa et al., 2000]. To find that a CEFVs in *GPR45* (rs35946826, $\hat{\beta} = -12.7$, $P_{FDR} = 3.08 \times 10^{-36}$) accelerates AOO in our patients with *s*AD, strongly highlights the potential of GPCRs as a therapeutic target in *s*AD.

Gene ontology annotations relate *FPR1* with *FPRL1*, a variant of the formyl peptide receptor (*FPR*). FPRL1 is activated by $A\beta_{42}$ and participates in its internalization in macrophages and subsequently in the cytotoxicity for neuronal cells, which suggests that is involved in inflammatory aspects of AD [Cui et al., 2002; Slowik et al., 2012]. Furthermore, our network and pathway analyses linked *FPR1* with the release of IL1β, which is thought to contribute to neuroinflammation and the neurodegeneration that follows [Shaftel et al., 2007].

OPRM1 belongs to the μ-Opioid receptor family, which has been implicated in learning and memory, as well as in locomotor activity, thermoregulation, hormone secretion, and immune functions. *OPRM1* was also involved in the regulation of NFkB activity, neurogenesis, and dopamine receptor signaling, through the binding of G protein α-q/11 to FPR1 (Fig. 3c), all fundamental mechanisms linked to the genesis of AD.

Wnt signaling regulates the structure and function of the nervous system in adults [Rosso and Inestrosa, 2013]. Recent studies suggest that perturbations of *Wnt* signaling may promote human neurodegenerative diseases [Logan and Nusse, 2004]. Because the loss of *Wnt*/β-catenin signaling function underlies the Aβ-dependent neurodegeneration observed in AD, the *Wnt* signaling pathway has been proposed as a therapeutic treatment for AD [Moon et al., 2004; Inestrosa and Toledo, 2008]. Our analysis showed a key role of *OPRM1* and *FPR1* in the negative regulation of *Wnt* protein secretion ($P = 1.28 \times 10^{-2}$) and the positive regulation of apoptosis via the canonical *Wnt* signaling pathway ($P = 4.09 \times 10^{-5}$) (Table III). A mutation in either one of them can restrict or modify these biological processes aforementioned in addition to severally disturb the regulation of $Ca2^{+}$ transport, and PLC activity [Mouledous et al., 2012].

NFATC1 encodes a protein that is a necessary activator of the TLR3 signaling, which is facilitated by the dephosphorylation of NFATC1 by calcineurin [Fric et al., 2014]. The TLR3 neuroinflammatory response and overexpression in the nervous system

TABLE III. *Wnt* Signaling-Related Processes and Disease Ontological Descriptions of Candidate Genes From the Pathway, Network, and Enrichment Analyses

	Network				
Network algorithm	**Name**	***P*-value**	**FDR**	**Process, disease annotations, and disease ontology (*P*-value)**	**Biological process node in network**
Analyse network[a]	μ-type opioid receptor	4.97×10^{-20} **(8/50)**	[b]	Regulation of neuron development (8.31×10^{-4}) Negative regulation of *Wnt* protein secretion (4.98×10^{-6}) Negative Regulation of Ca^{2+} cytosolic concentration (1.39×10^{-5}) Alzheimer's Disease (6.274×10^{-11}) LOAD (1.89×10^{-10})	SSTR2, NPFF, substance P, and/or nociceptin receptor binding to mu type opioid receptor (encoded by *OPRM1*). Also GPCRs (encoded by *FPR1* and *OPRM1*) hydrolysed by GTP, activates G protein β/γ
Analyse network from GO processes	μ-type Opioid receptor, δ-type opioid receptor[c]	8.76×10^{-7} **(6/50)**	3.5×10^{-4}	Negative regulation of *Wnt* protein secretion (1.67×10^{-7})	SSTR2, NPFF, substance P, and/or nociceptin receptor binding to mu-type opioid receptor (encoded by *OPRM1*), in turn binds to δ-type opioid receptor
Process networks[a]	Chemotaxis	5.32×10^{-4} **(3/137)**	1.23×10^{-2}	Negative regulation of *Wnt* protein secretion (1.28×10^{-2}) Phospholipase A2 Activity (1.92×10^{-2}) Phospholipase C activating G protein coupled receptor signaling (6.99×10^{-4}) Motor neuron axon guidance (8.22×10^{-5}) Alzheimer's disease (3.37×10^{-11}) LOAD (3.53×10^{-2})	GTP hydrolysis of GPCRs (transformation reaction), leading to activation of G protein α I family members
	Neuropeptide signaling	7.64×10^{-4} **(3/155)**	1.28×10^{-2}	Negative regulation of *Wnt* protein secretion (3.63×10^{-5}) Neuron differentiation (3.49×10^{-8}) Alzheimer's disease (4.84×10^{-20}) EOAD (4.63×10^{-3}) LOAD (5.76×10^{-10})	GTP hydrolysis (transformation) of GPCRs encoded by *FPR1* and *OPRM1* leading to activation of G protein α I family members
	Cholecystokinin signal transduction	7.33×10^{-3} **(2/106)**	4.28×10^{-2}	Canonical *Wnt* signaling pathway in positive regulation of apoptosis	*c*-myc transcription regulation of GPCRs (encoded by *FPR1* and *OPRM1*). GPCRs in turn hydrolysed, leading to activation of G protein α I family members.

(*Continued*)

TABLE III. (*Continued*)

Network					
Network algorithm	**Name**	***P*-value**	**FDR**	**Process, disease annotations, and disease ontology (*P*-value)**	**Biological process node in network**
				(4.01×10^{-5})	
				Regulation of neuron projection development (1.68×10^{-5})	Regulation of release of Ca^{2+} ion in cytosol by SR (4.29×10^{-3})
				GPCRs are involved in binding and activating PLC (Phospholipase C) γ	
				Neuron differentiation (3.49×10^{-8})	GPCRs activate G protein α-12 family proteins, which activate PKA-cat phosphatase, in turn phosphorylates PLC γ
				Alzheimer's disease (4.84×10^{-20})	
				LOAD (5.76×10^{-10})	
EOAD (4.63×10^{-3})					

Enrichment scores are shown in bold.
EOAD, Early-onset Alzheimer's disease; GPCR, G protein-coupled receptor; LOAD, Late-onset Alzheimer's disease; FDR, False discovery rate.
The *P*-values associated to each network give the probability of getting a certain number of genes obtained from a given network algorithm from the input list by chance. Enrichment scores are similarly interpreted.
[a]Results only include *OPRM1* and *FRP1* genes.

leads to inhibition of neuronal growth cones, and overall neuron development in mice. Conversely, mice with defects in TLR3 are less susceptible to neurodegenerative disorders [Cameron et al., 2007]. Recently, a mouse model of AD showed that inhibition of the NFAT pathway alleviates Aβ neurotoxicity of AD [Hudry et al., 2012], which confirms the previously reported association between NFAT/calcineurin signaling with AD [Abdul et al., 2010] and cognitive decline [Abdul et al., 2009]. Our discovery of a mutation in *NFATC1* would be crucial to better understand the implications of this pathway in causing AD.

TTBK2 is mildly expressed in the prefrontal cortex, encodes a serine-threonine kinase that acts as a key regulator of ciliogenesis, and putatively phosphorylates tau and tubulin proteins [Houlden et al., 2007]. *TTBK2* was shown to be biologically related to *APOC1*, *ABCA7*, and *TF*, all of them important AD genes (Fig. 2c, bottom). Mutations in *TTBK2* cause spinocerebellar ataxia type 11, a neurodegenerative disease characterized by progressive ataxia and atrophy of the cerebellum and brainstem [Houlden et al., 2007]. Mouse models of AD have also shown that the TTBK gene family plays an important role in neuronal and cognitive dysfunction in mammalian models in vivo [Sato et al., 2008]. Finding that a CEFV in *TTBK2* modifies ADAOO (rs6493068, $\hat{\beta} = -0.479$, $P_{FDR} = 4.27 \times 10^{-2}$) strongly implicate this gene in the natural history of *s*AD.

We targeted genes reported associated to either Alzheimer's (EOAD and LOAD), Alzheimer's age of onset, Alzheimer's cognitive decline, Alzheimer's survival time, and Alzheimer's biomarkers, as compiled by the gwasCatalog (UCSC genome browser). A window of 60 kb outlining the targeted genes allowed the inclusion of markers/genes in potential LD to the reported marker/gene (for the Paisa community, a whole genome average of ~60 kb of LD blocks has been published elsewhere [Arcos-Burgos et al., 2010]). A total of 195 markers from our functional chip where harbored in those regions. Mixed models applied to these markers were no significant after correction by multiple testing; the lowest *P*-value was found at the marker rs11935573 harbored in the *Homo sapiens dachsous cadherin-related 2* (*DCHS2*) gene ($P = 0.0015$, $P_{FDR} = 0.2667$).

We want to raise a flag of caution to account for the fundamental fact that the sample size small, the sample size for the exomechip genotyping array is small (with less genomic content compared with whole exome sequencing and other standard chips for GWAS). As with any analysis, a small sample size has power for very large effects. However, we have made power analyses of small samples to detect large effects (as defined by the Cohen's d parameter) that have been published in the Supplementary Material of Velez et al. [2015]. These power analyses remain useful provided homogeneity of the population. In summary, for a $k = 3$ group design, 50 individuals would be sufficient to detect up to ~70% true positives and a large effect (defined by the Cohen's d parameter; $d = 0.82$, Supplementary Fig. S5 [Velez et al., 2015]), when $m = 100{,}000$ variants are tested for association (a value that certainly overcomes the final number of variants used during the LMEM analyses). The selection of such effect is based on our hypothesis that variants of large effect (i.e., mutations) modify ADAOO (see also the $\widehat{\beta}$ coefficients in Table I of the manuscript); k is selected based on the maximum number of possible genotypes for a biallelic genetic marker; and m, as mentioned before, to be conservative.

It is worth mentioning that we previously applied a chip built with intronic variation that suits the search for loci with minor

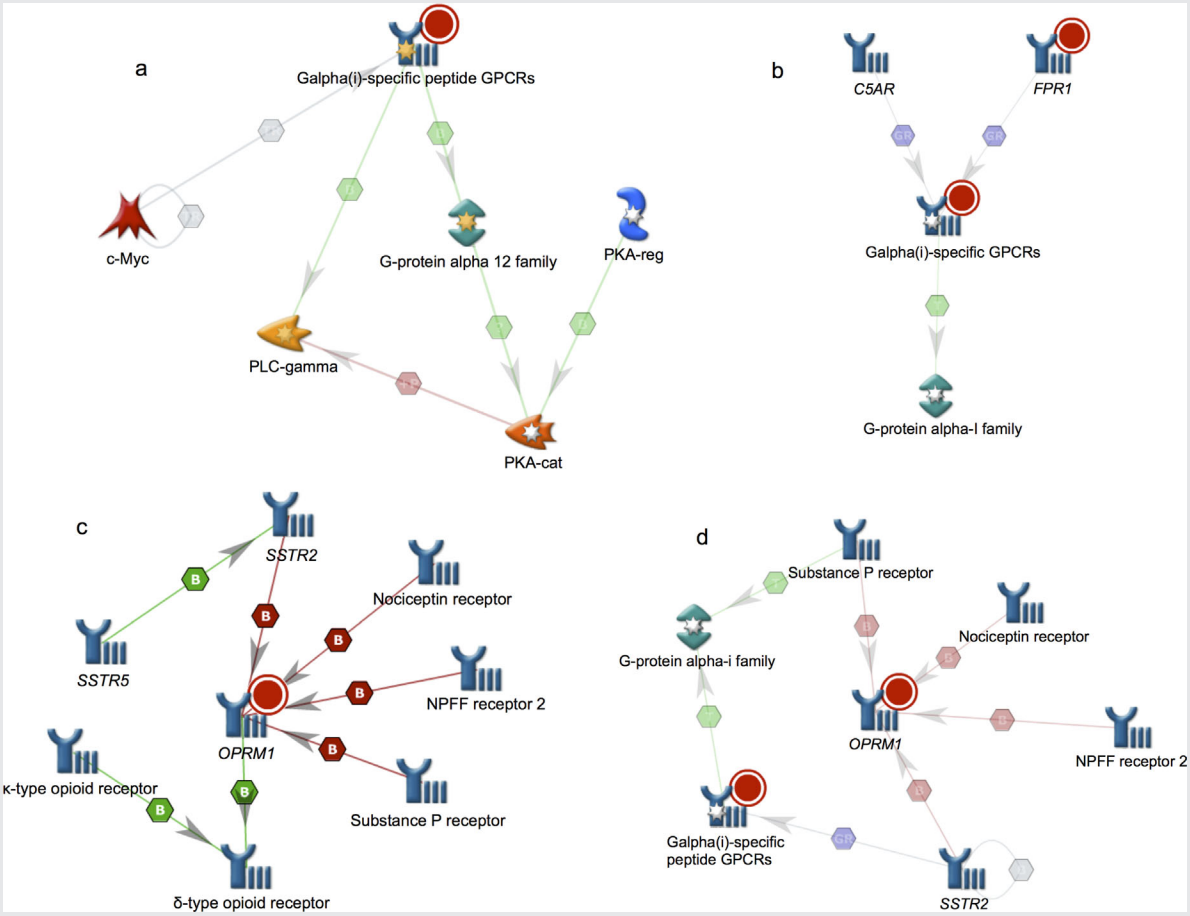


FIG. 4. ***Wnt*** **signaling-related networks showing processes involving (a) cholecystokinin signaling, (b) chemotaxis, (c) μ/δ opioid receptors, and (d) μ-type opioid receptor. Additional information in Table III. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].**

effect in *PSEN1* E280A AD [Velez et al., 2013]. In this scenario, the "functional" chip applied herein attempts to tackle the hypothesis of major effect variation. We have recently replicated one of these ADAOO modifiers loci in this sample of sporadic cases [Velez et al., 2015], and showed that loci of minor effect in *PSEN1* E280A AD also contain ADAOO modifier variants of large effect [Velez et al., 2016]. Although other reciprocal analyses are in the process of completion using extended and independent samples using ADAOO as a continuous phenotype, we are particularly interested in studying genetic associations of functional mutations with the AD diagnosis as a discrete trait in order to determine causal mutations.

Here, we report common pathogenic genetic variants significantly associated to ADAOO with remarkable accelerating/delaying effects after applying a comprehensive approach involving the whole-exome genotyping of functional variants in a group of patients ascertained from a genetic isolate, and exhibiting an extreme AOO phenotype. Genes harboring these mutations are involved in crucial pathways that might largely contribute to the pathophysiology of *s*AD, and provide an initial explanation for the wide range of ADAAO. Although the common mechanisms involved result in known AD pathological pathways such as Aβ or hyperphosphorylated tau deposition, the modulation or fine-tuning of this or other relevant pathways are determining of AD pathology onset. In future studies, we plan to model their functionality in mouse models of AD as a first step to evaluate the potential of these ADAOO modifier genes as therapeutical targets.

ACKNOWLEDGEMENTS

We express our highest sentiment of appreciation and gratitude to the patients from the Paisa community and their relatives enrolled in this study for more than 25 years. COLCIENCIAS and the University of Antioquia, grant 1115-408-20543, financed this study. JIV was supported by The Eccles Scholarship in Medical Sciences, the Fenner Merit Scholarship, and the Australian National University (ANU) High Degree Research scholarship. DSF was supported by the Hamburg State Ministry of Science and Research, Landesforschungsförderung "Molekulare mechanismen der netzwerkmodifizierung." JIV, ASJ, and SGL are doctoral

students at ANU. Some of this work is to be presented in partial fulfilment of the PhD degree requirements.

The sponsor of the study has no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. JIV, FL, and MAB have full access to all the data in the study. JIV and MAB are responsible for submitting this work for publication.

REFERENCES

Abdul HM, Furman JL, Sama MA, Mathis DM, Norris CM. 2010. NFATs and Alzheimer's disease. Mol Cell Pharmacol 2(1):7–14.

Abdul HM, Sama MA, Furman JL, Mathis DM, Beckett TL, Weidner AM, Patel ES, Baig I, Murphy MP, LeVine H III, et al. 2009. Cognitive decline in Alzheimer's disease is associated with selective changes in calcineurin/NFAT signaling. J Neurosci 29(41):12957–12969.

Acosta-Baena N, Sepulveda-Falla D, Lopera-Gomez CM, Jaramillo-Elorza MC, Moreno S, Aguirre-Acevedo DC, Saldarriaga A, Lopera F. 2011. Pre-dementia clinical stages in presenilin 1 E280A familial early-onset Alzheimer's disease: A retrospective cohort study. Lancet Neurol 10(3):213–220.

Adamsky K, Arnold K, Sabanay H, Peles E. 2003. Junctional protein MAGI-3 interacts with receptor tyrosine phosphatase beta (RPTP beta) and tyrosine-phosphorylated proteins. J Cell Sci 116(Pt 7):1279–1289.

Aguirre-Acevedo DC, Jaimes-Barragan F, Henao E, Tirado V, Munoz C, Reiman EM, Tariot PN, Langbaum JB, Lopera F. 2016. Diagnostic accuracy of CERAD total score in a Colombian cohort with mild cognitive impairment and Alzheimer's disease affected by E280A mutation on presenilin-1 gene. Int Psychogeriatr 28(3):503–510.

Alzheimer's Association. 2014. 2014 Alzheimer's disease facts and figures. Alzheimer's Dementia 10(2):1–75.

American Psychiatric Association. 2000. American Psychiatric Association: Diagnostic and statistical manual of mental disorders. Association AP, editor. Washington, DC.

Arcos-Burgos M, Jain M, Acosta MT, Shively S, Stanescu H, Wallis D, Domene S, Velez JI, Karkera JD, Balog J. 2010. A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. Mol Psychiatr 15(11):1053–1066.

Arcos-Burgos M, Muenke M. 2002. Genetics of population isolates. Clin Genet 61(4):233–247.

Barnett IJ, Lee S, Lin X. 2013. Detecting rare variant effects using extreme phenotype sampling in sequencing association studies. Genet Epidemiol 37(2):142–151.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J Royal Stat Soc Series B (Methodol) 57(1):289–300.

Bhatia G, Bansal V, Harismendy O, Schork NJ, Topol EJ, Frazer K, Bafna V. 2010. A covering method for detecting genetic associations between rare variants and common phenotypes. PLoS Comput Biol 6(10):1000954.

Bodmer W, Bonilla C. 2008. Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet 40(6):695–701.

Bonferroni CE. 1935. II calcolo delle assicurazioni su gruppi di teste. In Studi in Onore del Professore Salvatore Ortu Carboni 13–60.

Bravo ML, Valenzuela CY, Arcos-Burgos OM. 1996. Polymorphisms and phyletic relationships of the Paisa community from Antioquia (Colombia). Gene Geogr 10(1):11–17.

Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. 2007. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement 3(3):186–191.

Cameron JS, Alexopoulou L, Sloane JA, DiBernardo AB, Ma Y, Kosaras B, Flavell R, Strittmatter SM, Volpe J, Sidman R. 2007. Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals. J Neurosc 27(47):13033–13041.

Chouraki V, Seshadri S. 2014. Genetics of Alzheimer's disease. Adv Genet 87:245–294.

Cirulli ET, Goldstein DB. 2010. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 11(6):415–425.

Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R, Harari O, Norton J, Budde J, Bertelsen S, et al. 2014. Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 505(7484):550–554.

Cui Y, Le Y, Yazawa H, Gong W, Wang JM. 2002. Potential role of the formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer's disease. J Leukoc Biol 72(4):628–635.

Duara R, Lopez-Alberola RF, Barker WW, Loewenstein DA, Zatinsky M, Eisdorfer CE, Weinberg GB. 1993. A comparison of familial and sporadic Alzheimer's disease. Neurology 43(7):1377–1384.

Fearnhead NS, Wilding JL, Winney B, Tonks S, Bartlett S, Bicknell DC, Tomlinson IP, Mortensen NJ, Bodmer WF. 2004. Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas. Proc Natl Acad Sci U S A 101(45):15992–15997.

Fleisher AS, Chen K, Quiroz YT, Jakimovich LJ, Gomez MG, Langois CM, Langbaum JB, Ayutyanont N, Roontiva A, Thiyyagura P, et al. 2012. Florbetapir PET analysis of amyloid-beta deposition in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: A cross-sectional study. Lancet Neurol 11(12):1057–1065.

Fric J, Zelante T, Ricciardi-Castagnoli P. 2014. Phagocytosis of particulate antigens—All roads lead to calcineurin/NFAT signaling pathway. Front Immunol 4:513.

Ghanemi A. 2015. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. Saudi Pharm J 23(2):115–129.

Giraldo M, Lopera F, Siniard AL, Corneveaux JJ, Schrauwen I, Carvajal J, Munoz C, Ramirez-Restrepo M, Gaiteri C, Myers AJ. 2013. Variants in triggering receptor expressed on myeloid cells 2 are associated with both behavioral variant frontotemporal lobar degeneration and Alzheimer's disease. Neurobiol Aging 34(8):e11–e18.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349(6311):704–706.

Guan KL, Figueroa C, Brtva TR, Zhu T, Taylor J, Barber TD, Vojtek AB. 2000. Negative regulation of the serine/threonine kinase B-Raf by Akt. J Biol Chem 275(35):27354–27359.

Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S. 2013. TREM2 variants in Alzheimer's disease. New Engl J Med 368(2):117–127.

Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS. 2005. A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37(5):549–554.

Houlden H, Johnson J, Gardner-Thorpe C, Lashley T, Hernandez D, Worth P, Singleton AB, Hilton DA, Holton J, Revesz T. 2007. Mutations in TTBK2, encoding a kinase implicated in tau phosphorylation, segregate with spinocerebellar ataxia type 11. Nat Genet 39(12):1434–1436.

Hudry E, Wu HY, Arbel-Ornath M, Hashimoto T, Matsouaka R, Fan Z, Spires-Jones TL, Betensky RA, Bacskai BJ, Hyman BT. 2012. Inhibition of the NFAT pathway alleviates amyloid beta neurotoxicity in a mouse model of Alzheimer's disease. J Neurosci 32(9):3176–3192.

Inestrosa NC, Toledo EM. 2008. The role of Wnt signaling in neuronal dysfunction in Alzheimer's Disease. Mol Neurodegener 3:9.

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS. 2012. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482(7384):216–220.

Itan Y, Mazel M, Mazel B, Abhyankar A, Nitschke P, Quintana-Murci L, Boisson-Dupuis S, Boisson B, Abel L, Zhang SY. 2014. HGCS: An online tool for prioritizing disease-causing gene variants by biological distance. BMC Genom 15:256.

Johar AS, Anaya JM, Andrews D, Patel HR, Field M, Goodnow C, Arcos-Burgos M. 2014. Candidate gene discovery in autoimmunity by using extreme phenotypes, next generation sequencing and whole exome capture. Autoimmun Rev 14(3):204–209.

Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J. 2012. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 488(7409):96–99.

Kawasawa Y, Kume K, Nakade S, Haga H, Izumi T, Shimizu T. 2000. Brain-specific expression of novel G-protein-coupled receptors, with homologies to Xenopus PSP24 and human GPR45. Biochem Biophys Res Commun 276(3):952–956.

Lehtovirta M, Soininen H, Helisalmi S, Mannermaa A, Helkala EL, Hartikainen P, Hanninen T, Ryynanen M, Riekkinen PJ. 1996. Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease: Relation to apolipoprotein E polymorphism. Neurology 46(2):413–419.

Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Jondro PD, Schmidt SD, Wang K. 1995. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269(5226): 973–977.

Li D, Lewinger JP, Gauderman WJ, Murcray CE, Conti D. 2011. Using extreme phenotype sampling to identify the rare causal variants of quantitative traits in association studies. Genet Epidemiol 35(8): 790–799.

Liu DJ, Leal SM. 2010a. A novel adaptive method for the analysis of next-generation sequencing data to detect complex trait associations with rare variants due to gene main effects and interactions. PLoS Genet 6(10): e1001156.

Liu DJ, Leal SM. 2010b. Replication strategies for rare variant complex trait association studies via next-generation sequencing. Am J Hum Genet 87(6):790–801.

Logan CY, Nusse R. 2004. The Wnt signaling pathway in development and disease. Ann Rev Cell Dev Biol 20:781–810.

Logue MW, Schu M, Vardarajan BN, Farrell J, Bennett DA, Buxbaum JD, Byrd GS, Ertekin-Taner N, Evans D, Foroud T, et al. 2014. Two rare AKAP9 variants are associated with Alzheimer's disease in African Americans. Alzheimers Dement 10(6):609–618.

Lopera F, Arcos M, Madrigal L, Kosik KS, Cornejo W, Ossa J. 1994. Alzheimer-type dementia with familial aggregaton in Antioquia, Colombia. Acta Neurolgógica Colombiana 10:173–187.

Marchese A, Sawzdargo M, Nguyen T, Cheng R, Heng HH, Nowak T, Im DS, Lynch KR, George SR, O'Dowd BF. 1999. Discovery of three novel orphan G-protein-coupled receptors. Genomics 56(1):12–21.

Moon RT, Kohn AD, De Ferrari GV, Kaykas A. 2004. WNT and beta-catenin signalling: Diseases and therapies. Nat Rev Genet 5(9):691–701.

Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C. 1989. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39(9): 1159–1165.

Mouledous L, Froment C, Dauvillier S, Burlet-Schiltz O, Zajac JM, Mollereau C. 2012. GRK2 protein-mediated transphosphorylation contributes to loss of function of mu-opioid receptors induced by neuropeptide FF (NPFF2) receptors. J Biol Chem 287(16): 12736–12749.

Persad S, Attwell S, Gray V, Mawji N, Deng JT, Leung D, Yan J, Sanghera J, Walsh MP, Dedhar S. 2001. Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: Critical roles for kinase activity and amino acids arginine 211 and serine 343. J Biol Chem 276(29):27462–27469.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. 1999. Mild cognitive impairment: Clinical characterization and outcome. Arch Neurol 56(3):303–308.

Piaceri I, Nacmias B, Sorbi S. 2013. Genetics of familial and sporadic Alzheimer's disease. Front Biosci 5:167–177.

Rickle A, Bogdanovic N, Volkman I, Winblad B, Ravid R, Cowburn RF. 2004. Akt activity in Alzheimer's disease and other neurodegenerative disorders. Neuroreport 15(6):955–959.

Rosenbaum DM, Rasmussen SG, Kobilka BK. 2009. The structure and function of G-protein-coupled receptors. Nature 459(7245): 356–363.

Rosso SB, Inestrosa NC. 2013. WNT signaling in neuronal maturation and synaptogenesis. Front Cell Neurosci 7:103.

Sato S, Xu J, Okuyama S, Martinez LB, Walsh SM, Jacobsen MT, Swan RJ, Schlautman JD, Ciborowski P, Ikezu T. 2008. Spatial learning impairment, enhanced CDK5/p35 activity, and downregulation of NMDA receptor expression in transgenic mice expressing tau-tubulin kinase 1. J Neurosci 28(53):14511–14521.

Segura V, Vilhjalmsson BJ, Platt A, Korte A, Seren U, Long Q, Nordborg M. 2012. An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet 44(7):825–830.

Shaftel SS, Kyrkanides S, Olschowka JA, Miller JN, Johnson RE, O'Banion MK. 2007. Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J Clin Invest 117(6):1595–1604.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, et al. 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375(6534):754–760.

Slowik A, Merres J, Elfgen A, Jansen S, Mohr F, Wruck CJ, Pufe T, Brandenburg LO. 2012. Involvement of formyl peptide receptors in receptor for advanced glycation end products (RAGE)-and amyloid beta 1-42-induced signal transduction in glial cells. Mol Neurodegener 7:55.

Thathiah A, De Strooper B. 2011. The role of G protein-coupled receptors in the pathology of Alzheimer's disease. Nat Rev Neurosci 12(2):73–87.

Velez JI, Chandrasekharappa SC, Henao E, Martinez AF, Harper U, Jones M, Solomon BD, Lopez L, Garcia G, Aguirre-Acevedo DC. 2013. Pooling/bootstrap-based GWAS (pbGWAS) identifies new loci modifying the age of onset in PSEN1 p.Glu280Ala Alzheimer's disease. Mol Psychiatr 18(5):568–575.

Vélez JI, Correa JC, Arcos-Burgos M. 2014. A new method for detecting significant p-values with applications to genetic data. Revista Colombiana de Estadistica 37(1):67–76.

Velez JI, Lopera F, Sepulveda-Falla D, Patel HR, Johar AS, Chuah A, Tobon C, Rivera D, Villegas A, Cai Y. 2015. APOE*E2 allele delays age of onset in PSEN1 E280A Alzheimer's disease. Mol Psychiatry 21(7):916–924.

Velez JI, Rivera D, Mastronardi CA, Patel HR, Tobon C, Villegas A, Cai Y, Easteal S, Lopera F, Arcos-Burgos M. 2016. A mutation in DAOA modifies the age of onset in PSEN1 E280A Alzheimer's disease. Neural Plast 2016:9760314.

Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q, Lasky LA. 2000. Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. J Biol Chem 275(28):21477–21485.

Yu W, Wulf A, Liu T, Khoury MJ, Gwinn M. 2008. Gene Prospector: An evidence gateway for evaluating potential susceptibility genes and interacting risk factors for human diseases. BMC Bioinform 9:528.

Zuk O, Hechter E, Sunyaev SR, Lander ES. 2012. The mystery of missing heritability: Genetic interactions create phantom heritability. Proc Natl Acad Sci U S A 109(4):8.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.