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Comprehensive Screening for Disease Risk Variants in Early-Onset Alzheimer's Disease Genes in African Americans Identifies Novel *PSEN* Variants

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Abstract

We conducted a comprehensive screening of rare coding variants in an African American cohort to identify novel pathogenic mutations within the early-onset Alzheimer's disease (EOAD) genes (*APP PSEN1,* and *PSEN2)* in this understudied population. Whole-exome sequencing of 238 African American subjects identified 6 rare missense variants within the EOAD genes, which were observed in AD cases but never among controls. These variants were analyzed in an independent cohort of 300 African American subjects in which *PSEN2*:NM_000447:exon5:c.T331C:p.Phe111Leu and *PSEN1-minilin* rs777923890 variants were again not observed, indicating that these novel rare variants, may contribute to AD risk in this population.

Keywords

African Americans; Alzheimer's disease; early onset; genetics; presenilins; whole exome sequencing

INTRODUCTION

Genome-wide association studies have identified genetic variants with modest effect sizes associated with late-onset Alzheimer's disease (LOAD) [1–3]; however, a large portion of LOAD heritability remains to be discovered. The AD risk variants identified to date

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SUPPLEMENTARY MATERIAL

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collectively account for only less than 40% of AD heritability [4, 5]. *APP, PSEN1*, and *PSEN2* can harbor autosomal dominant mutations that lead to early-onset familial AD (EOAD) [6]. Increasing evidence suggests that rare coding variants in these EOAD genes may play a role in LOAD genetics and represent a portion of the missing heritability [7–10]. Notably, the *APP*:NM_000484.3:c.2017G>A: p.Ala673Thr variant has been reported to be protective against LOAD in the Icelandic population [11], but assessment of this variant in other Caucasian populations indicated that its protective effect might be restricted to specific ethnic groups, underscoring the importance of studying EOAD gene variants in different populations [11–13].

While over 200 mutations have been identified in the EOAD genes in Caucasians [14], only 3 have been identified in African Americans: one *APP* mutation observed in a single family (p.Thr714Ile, rs63750973) [15] and two *PSEN1* mutations: p.Met139Val (rs63751037) observed in a single African American family [16] and p.Ile238Met identified in an African American woman with a family history consistent with familial EOAD [17]. To date, no *PSEN2* mutations have been discovered in African Americans. The contribution of EOAD genetic variants to risk for LOAD remains to be determined in this population, which is known to be at greater risk of AD compared to Caucasians [18–20].

This study aims to identify novel coding variants in the EOAD genes, *APP, PSEN1*, and *PSEN2*, that may influence LOAD risk in this population, through analysis of whole exome sequence (WES) data in an African American cohort.

MATERIALS AND METHODS

Study cohorts

Five hundred fifty unrelated African American individuals were recruited from the Mayo Clinic in Jacksonville Florida (201 AD and 345 controls) and the Wien Center for Alzheimer's Disease and Memory Disorders, Mount Sinai, Miami, Florida (3 AD and 1 control). Two hundred fifty samples were selected for the WES discovery study (137 AD and 113 controls) and 300 (67 AD and 233 controls) for the replication study. All subjects were evaluated by neurologists. Control subjects had a Clinical Dementia Rating scale (CDR) score of 0 at last examination, and cases had a diagnosis of possible or probable AD made following the NINCDS- ADRDA criteria [21]. Informed consent was obtained for all subjects and all studies were approved by the Institutional Review Boards of all participating institutions.

Whole exome sequencing

The Agilent Sure Select V4 + UTR exome capture kit [22] was used for exome isolation and sequencing was performed on the Illumina HiSeq 2000 platform. Sequence data quality control and filtering is described in the Supplementary Material.

Replication

Genotyping was performed using TaqMan® SNP Genotyping Assays on the QuantStudio™ 7 Flex Real-Time PCR system and analysis software (Applied Biosystems, California,

USA). Sanger sequencing was performed on the ABI 3730 auto-mated sequencer (Applied Biosystems, California, USA) using custom primers and data were analyzed using the Sequencher® v.5.0 software (Gene Codes Corporation, Michigan, USA).

RESULTS

Discovery study

WES was performed on 250 African American subjects. After quality control, 12 subjects were excluded (3 subjects were excluded due to poor sequence quality, 5 due to gender ambiguity, and 4 due to cryptic relatedness with other samples of the cohort). A total of 238 subjects (131 AD cases and 107 controls) remained for analysis (Table 1). Out of the 735,555 bi-allelic variants analyzed, 60,513 variants mapping to more than one genomic region (BLAT-score>1), and 558,666 with no effect on protein structure or function (based on SNPeff annotations [23]) were excluded. Of the remaining 116,376 non-synonymous coding variants predicted to have functional impact, 14 were within the EOAD genes: 7 in *APP,* 3 in *PSEN1,* and 4 in *PSEN2* (Table 2).

Of these 14 SNPs, two have been previously reported on the Alzheimer Disease & Frontotemporal Dementia Mutation Database [14]. *PSEN2* variant rs58973334 (p.Arg62His) has been observed in 7 Caucasian families but has an unclear pathogenicity [14]. This variant was observed in 9 control subjects (MAF = 4.2%) and 8 AD cases (MAF = 3.1%) of our discovery cohort. The *PSEN2* variant rs138836272 (p.Ala252Thr) previously reported in two African control subjects [24] was observed in 1 control subject (MAF = 0.47%) and 1 AD case (MAF = 0.38%). None of the APP variants observed are in the mutation “hotspot” of the protein from amino acid 670 to 717; however, four had predicted deleterious CADD scores > 20 [25]. Among the 14 variants, 6 were observed in at least one AD case but never in the controls (Table 2).

Replication study

The 6 EOAD gene variants observed only among the African American AD cases of the discovery cohort were confirmed and further investigated in an independent cohort of 300 African American subjects (67 AD and 233 controls) (Table 1). Of the 6 variants studied, *PSEN2* :NM_ 000447:exon5:c.T331C:p.Phe111Leu and *PSEN1:* uc001xnq. 4:exon5:c.C500T:p.Ser167Phe (rs77792 3890) were not observed in the controls of this replication cohort (Table 2, Supplementary Figure 1), further suggesting that these rare variants might be implicated in AD risk in this population. Notably, *PSEN1* rs777923890, while intronic in the full length transcript, leads to an amino acid changep.Ser167Phe in isoform 4 of this protein, also known as minilin (http://www.uniprot.org/blast/?about=P49768–4).

Clinical description of the PSEN2:NM_000447:exon5:c.T331C:p.Phe111Leu carrier

This patient is an African American female recruited at the Mayo Clinic. She has a college degree and had been a teacher for over 30 years. She started experiencing memory issues at the age of 77, and was diagnosed with AD at the age of 78. She had a number of motor vehicle accidents and was advised not to drive. She declined and was admitted to a nursing

home 4 years after her diagnosis. This patient had no family history of memory impairment. Her mother died of a gastrointestinal malignancy, her father of prostate cancer, and she has no siblings. At the time of diagnosis, her Mini-Mental State Examination (MMSE) score was 22/30 and brain magnetic resonance imaging (MRI) showed mild atrophy.

This patient carries a second coding *PSEN2* variant, rs111567390 which is observed more frequently among the control subjects (MAF = 25.7%) than the AD cases (MAF = 21.4%) of our discovery cohort, and thus unlikely to contribute to the disease risk in this patient. She has *APOE* ε3/ε3 genotype.

Clinical description of the PSEN1:uc001xnq.4:exon5:c.C500T:p.Ser167Phe (rs777923890) carrier

This patient was an African American male recruited at the Mayo Clinic. This patient had 12 years of formal education. At the time of diagnosis at age 77, he had a 2-year history of worsening cognitive function with difficulties in memory, visuospatial skills, comprehension, and word-finding. It was unclear whether the onset was abrupt or the progression step-wise. There was no history to suggest a past stroke. He had irritability which improved after commencement of donepezil and memantine 3 and 2 years prior to presenting at the Mayo Clinic, respectively. The patient had 6 siblings, 3 of whom were deceased. According to the patient, no one in his family suffered from memory deficits. The patient also had a history of untreated obstructive sleep apnea, vitamin B12 deficiency on injection supplement, hypertension, and hyperlipidemia. At the time of diagnosis, he had a MMSE score of 14/30, short Boston naming test 13/15, mild right nasolabial fold flattening, and bradycardia at 36–55 bpm and blood pressure 160/70 mmHg. No brain imaging data was available. The patient died 4 years after diagnosis. He had an *APOE* ε3/ε4 genotype.

It should be noted that for both of these patients, given lack of age of death of the parents or age of siblings, a potential censoring effect on parental status and family history of AD cannot be ruled out. Additionally, lack of detailed family history and DNA samples from family members limit confirmation of this status.

DISCUSSION

Comprehensive screening of the EOAD genes by WES in our AD case-control series of 238 African Americans identified two rare *PSEN* coding variants observed only in African American AD patients but never in the controls of our discovery cohort or in any of the 300 subjects from our replication cohort. The presence of these variants was confirmed either by Sanger sequencing or Taqman genotyping. In review of the 1000 Genomes Project, HapMap, the Exome Sequencing Project or ExAC, which together represent more than 60,000 subjects [26–29], we did not observe *PSEN*2:NM_000447:exon5:c.T331C:p.Phe111Leu at all, and detected the rs777923890 in 1 of the 11565 Latinos of the ExAC dataset [26–29]. It should be noted that demographic information is not available in the ExAC database, hence there is a possibility that this Latino carrier may have the disease or may be in the pre-symptomatic stages of the disease.

These non-synonymous variants result in a change of the amino acid sequence of the protein encoded by these genes and are predicted to alter protein function. PSEN2:NM_000447:exon5:c.T331C:p.Phe111Leu occurs in a luminal domain of the protein between transmembrane domains I and II. This domain harbors other EOAD mutations [14]. *PSEN2:* NM_000447:exon5:c.T331C:p.Phe111Leu is predicted to be possibly damaging based on the Polymorphism Phenotyping v2 (PolyPhen-2) software [30], has a CADD score of 25.2, although SIFT prediction is “Tolerated”. Based on these collective data, this mutation meets “likely pathogenic” classification according to the American College of Medical Genetics and Genomics (ACMG) guidelines [31].

PSEN1 :uc001xnq.4:exon5:c.C500T:p.Ser167Phe (rs777923890) leads to a coding change in minilin, an alternative protein encoded by the *PSEN1* gene that differs from the canonical PSEN1 protein by a different sequence from amino acid (a.a.) 162–185, and the truncation of a.a. 185–467. This rare mutation is predicted to be deleterious to the structure and function of the protein using the SIFT algorithm [32], although the PolyPhen-2 prediction is benign. This mutation is also “likely pathogenic” according to ACMG guidelines [31]. The function of the minilin protein remains unclear. Hence, the role of p.Ser167Phe in conferring AD risk remains to be established.

To our knowledge, this study is the first comprehensive genetic screening of coding variants in the known EOAD genes conducted in the African American population using whole exome sequencing. Moreover, this study reports the first *PSEN2* likely pathogenic variant identified in an African American AD subject. Despite these novel aspects, our study has several shortcomings, including lack of detailed clinical information or blood samples on the family members and limited power. Further, beyond any algorithmic predictions regarding pathogenicity, the actual impact of any variant needs to be determined through well-powered family-based and case-control studies, as well as biological investigations of functional outcome. Given the rarity and predicted pathogenic impact of the PSEN2 and minilin variants, which are only observed in two AD patients, they merit further investigations as novel rare potential AD risk variants by seeking well- powered replications in other cohorts as well as through functional analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographics of the African American cohorts

	DISCOVERY COHORT		REPLICATION COHORT	
	CONTROLS	AD	CONTROLS	AD
N	107	131	233	67
% Female	78.5	68.7	76.4	74.6
Mean age (range)	80.9(51 to 96)	76.2 (51 to 99)	75.5 (33 to 98)	80.2 (61 to 96)
APOE ε4 frequency (%)	17.7	40.8	19.3	38.8

Discovery cohort, subjects included in the analysis, following WES data QC. The replication cohort does not overlap with the discovery cohort.

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Table 2

Analysis of the EOAD genes coding variants identified by WES

							DISCOVERY STUDY						REPLICATION STUDY					
							CONTROL			AD			CONTROL			AD		
chr:position (dbSNP142)*	**Gene**	**Maj/ Min allele**	**Amino Acid change**	**CADD_PHRED**	**SIFT Prediction**	**Polyphen Prediction**	**N Maj**	**N Min**	**MAF (%)**	**N Maj**	**N Min**	**MAF (%)**	**N Maj**	**N Min**	**MAF (%)**	**N Maj**	**N Min**	**MAF (%)**
1:227071595 (c.331T>C)	*PSEN2*	T/C	PSEN2:NM_000447:exon5:c.T331C:p.PhelllLeu	25.2	Tolerated	Possibility damaging	214	0	0	261	1	0.4	462	0	0	130	0	0
14:73640435 (rs777923890)	*PSEN1*	C/T	PSEN1 :uc001xnq.4:exon5:c.C500T:p.Serl67Phe[a,b]	10.89	Deleterious	Benign	214	0	0	261	1	0.4	444	0	0	132	0	0
21:27284211 (rsl45371658)	*APP*	G/C	APP:NM_001204301:exonl4:c.C1751G:p.Pro584Arg	23.7	Deleterious	Possibility damaging	214	0	0	261	1	0.4	461	1	0.22	134	0	0
21:27369675 (c.922C>T)	*APP*	G/A	APP:NM_001136130:exon7:c.C922T :p.Leu308Phe[a]	21.7	Tolerated	Benign	214	0	0	261	1	0.4	413	1	0.24	124	0	0
21:27394195 (rs202198008)	*APP*	T/A	APP:NM_000484:exon6:c.A826T:p.Thr276Ser	20.6	Tolerated	Unknown	214	0	0	261	1	0.4	459	3	0.65	131	1	0.76
21:27462388 (rs151188448)	*APP*	C/T	APP:NM_000484:exon3:c.G226A: p.Val76Ile	23.4	Tolerated	Probably damaging	214	0	0	260	2	0.8	465	1	0.21	134	0	0
1:227068398 (rs111567390)	PSEN2	G/T	PSEN2: ENST00000391872:c.G52T:p.Alal8Ser[c]	0.955	Tolerated	Benign	159	55	25.7	206	56	21.4						
1:227071449 (rs58973334)	PSEN2	G/A	PSEN2:NM_000447:exon5:c.G185A: p.Arg62His	16.22	Tolerated	Possibly damaging	205	9	4.2	254	8	3.1						
1:227076717 (rs138836272)	PSEN2	G/A	PSEN2:NM_000447:exon8:c.G754A:p.Ala252Thr	23	Tolerated	Possibly damaging	213	1	0.5	261	1	0.4						
14:73626743 (rs114944042)	PSEN1	G/A	PSEN1: ENST00000553447: p.Ala34Thr[c]	–	–	–	201	13	6.1	254	8	3.1						
14:73673178 (rsl7125721)	PSEN1	A/G	PSEN 1: NM.000021 :exon9: c.A953G:p.Glu318Gly	16.92	Deleterious	Possibly damaging	213	1	0.5	260	2	0.8						
21:27284122 (rs112263157)	APP	T/C	APP:NM_001136129:exonll:c.A1447G:p.Ser483Gly	18.51	Tolerated	Benign	208	6	2.8	256	6	2.3						
21:27394181 (rs764406483)	APP	TGTG/T	APP:NM_001136129:exon5:c.669_67 ldel:p.Thr224del	–	–	–	213	1	0.5	262	0	0						
21:27512583 (rs9305282)	APP	C/G	Start Gained	–	–	–	202	12	5.6	250	12	4.6						

Summary of the variants in EOAD genes identified in the WES discovery study and resulting in alteration of the coding sequence. All variants only observed in AD cases but not in the cognitively normal controls of the WES discovery cohort were evaluated in the replication cohort. Follow-up was done by genotyping for all but 2 variants

*(a)*which were Sanger sequenced due to inability to design genotyping probes. Chr, chromosome; Maj, major allele; Min, minor allele; N, number of observations; MAF, minor allele frequency. Amino acid change is depicted according to ANNOVAR [33] reference gene notations where available,

*(b)*University of California Santa Cruz (UCSC)

*(c)*ENSEMBL transcript notations where not available. Chromosomal positions are according to Genome Build