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Original Article

The presenilin 1 p.Gly206Ala mutation is a frequent cause of early-onset Alzheimer's disease in Hispanics in Florida

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Abstract: Mutations in the gene encoding the presenilin-1 protein (PSEN1) were first discovered to cause Alzheimer's disease (AD) 20 years ago. Since then more than 200 different pathogenic mutations have been reported, including a p.Gly206Ala founder mutation in the Hispanic population. Here we report mutation analysis of known AD genes in a cohort of 27 early-onset (age of onset ≤65, age of death ≤70) Hispanic patients ascertained in Florida. The *PSEN1* p.Gly206Ala mutation was identified in 13 out of 27 patients (48.1%), emphasizing the importance of this specific mutation in the etiology of early-onset AD in this population. One other patient carried the known *PSEN1* p.Gly378Val mutation. Genotyping of the *PSEN1* p.Gly206Ala and p.Gly378Val mutations in 63 late-onset Hispanic AD patients did not identify additional mutation carriers. All p.Gly206Ala mutation carriers shared rare alleles at two microsatellite markers flanking *PSEN1* supporting a common founder. This study confirms the p.Gly206Ala variant as a frequent cause of early onset AD in the Hispanic population and for the first time reports the high frequency of this mutation in Hispanics in Florida.

Keywords: Alzheimer's disease, early-onset, presenilin 1, founder mutation, diagnosis, Hispanic

Introduction

Alzheimer's disease (AD) is a polygenic neurodegenerative disorder that is the most common cause of dementia. AD is clinically characterized by memory impairment and cognitive defects, and pathologically by β-amyloid aggregates in extracellular senile plaques, and the presence of hyper-phosphorylated microtubule associated protein tau in intracellular neurofibrillary tangles [1]. Mutations in three genes are responsible for the majority of familial AD: amyloid precursor protein (*APP*), presenillin 1 (*PSEN1*) and presenillin 2 (*PSEN2*) [2-6]. However, the complex genetic nature of AD stretches far beyond these three genes with mutations in the genes encoding microtubule-associated protein tau (*MAPT*), progranulin (*GRN*) and sortilin-related receptor (*SORL1*), as well as repeat expansions in the chromosome 9 open reading frame 72 (*C9ORF72)* gene and copy number variants in *APP* being shown to cause clinical AD at a lower frequency [7-11]. Several additional genes, mostly identified via genome-wide association studies (GWAS) [12], were found to increase the risk to develop AD with the E4 allele of the apolipoprotein E (*APOE*) gene conferring the greatest risk [13, 14].

Genetic studies of AD have predominantly centered on the Caucasian populations of Europe and North-America. However, Hispanic populations have been shown to have a higher frequency of AD than their non-Hispanic white counterparts [15-19]. Interestingly, these studies also showed that the age of AD symptom presentation in the Hispanic population is earlier (6.8 years earlier in Hispanics compared to

Table 1. Hispanic EOAD cohort

Sample	Gender	Family history of AD*	Age of Onset	Age of Death	*PSEN1* Variant	*APOE* Genotype
MC36	F	YES	NA	51	c.1131 G>T p.G378V	E3E3
MC50	F	YES	42	50	c.617 G>C p.G206A	E3E3
MC59	M	YES	50	67	c.617 G>C p.G206A	E3E3
MC61	M	YES	52	62	c.617 G>C p.G206A	E3E4
MC1	M	YES	51	64	c.617 G>C p.G206A	E4E4
MC16	M	YES	53	NA	c.617 G>C p.G206A	E2E3
MC19	F	YES	53	NA	c.617 G>C p.G206A	E3E4
MC24	M	YES	54	64	c.617 G>C p.G206A	E3E4
MC65	M	YES	57	70	c.617 G>C p.G206A	E3E3
FPADS1	M	YES	57	NA	c.617 G>C p.G206A	E3E4
FPADS2	M	YES	58	NA	c.617 G>C p.G206A	E3E3
MC84	M	NO	61	69	c.617 G>C p.G206A	E3E3
MC22	M	YES	61	NA	c.617 G>C p.G206A	E3E3
MC8	F	NO	63	NA	c.617 G>C p.G206A	E3E3
MC21	M	YES	39	NA		E3E3
FPADS5	M	YES	47	NA		E4E4
FPADS6	F	YES	47	NA		E4E4
MC20	M	YES	48	NA		E3E4
MC3	M	NO	52	NA		E3E3
FPADS3	M	YES	54	NA		E3E3
FPADS4	M	NO	59	NA		E3E3
FPADS7	F	NO	61	NA		E3E4
MC2	F	YES	64	NA		E4E4
MC23	M	YES	NA	59		E4E4
MC28	M	UNK	NA	63		E3E4
MC37	F	YES	NA	65		E3E4
MC64	M	YES	NA	66		E3E3

*Family history was considered positive if a first or second degree relative presented with Alzheimer's disease.

Caucasians), and the occurrence of the E4 allele in *APOE* is lower (38% in Hispanics AD cases, 59% in Caucasian AD cases) [15-19]. These factors make this an interesting population to further identify novel AD causing variants, genetic risk factors or potentially protective variants associated with the disease.

The most comprehensive studies into genetic variants in Hispanic AD cases so far have identified one highly penetrant mutation in *PSEN1* as a founder mutation in the Hispanic population (p.Gly206Ala, c.617G>C , dbSNP ID: rs63750082) [18]. This mutation, although predicted to be conservative, was shown to increase amyloid $A\beta_{42}$ secretion over 2-fold. This functional effect combined with familial segregation in separate genetic studies provided strong evidence in support of the pathogenicity of this mutation [20-22]. Importantly, the high occurrence of this variant in Hispanics has already been used to determine the role of genetic modifiers on the age of onset in this population [23].

Here we performed mutation analysis of 27 early-onset Hispanic AD patients ascertained in Florida through the Neurology Department at Mayo Clinic Florida, Jacksonville, the Mayo Clinic Florida brain bank and the Florida Presenile Alzheimer's Disease Subjects (FPADS) registry and identified the p.Gly206Ala mutation as a major cause of AD in this population. The identification of these p.Gly206Ala mutation carriers with varying ages of onset will be a powerful resource in future studies aimed at the identification of risk factors influencing disease onset and progression.

Methods

Subjects

We performed mutation analysis on a cohort of 27 early-onset (EOAD) (age of onset ≤65, age of death ≤70) Hispanic AD patients of presumed Puerto Rican descent (**Table 1**), of which 9 patients were seen at the Department of Neurology at Mayo Clinic Florida between 1997 and 2015, 12 cases were collected through the Mayo Clinic Brain Bank (one clinic patient is now deceased and in the Brain Bank), and 7 patients recruited through the FPADS registry, a recently established network of five clinical centers in Florida enrolling early-onset AD patients into research studies (patients included in this study were recruited at the University of Miami and the Wien Center at Mount Sinai Medical Center). The average age at onset in this cohort was 53.7 (range 39-64; $n_{available}$=22)

Table 2. Genotypes of potential genetic modifiers in *PSEN1* p.Gly206Ala carriers

Patient information			Studied variant with previously reported effect on onset age			
Sample	Age of Onset	Age of Death	*SNX25*-rs11730401	*SORBS2*-rs13130022	*SH3RF3*-rs6542814	*NPHP1*-rs906815
			G allele later-onset	C-allele later onset	A-allele later onset	A-allele earlier onset
MC50	42	50	AA	CT	GG	GG
MC59	50	67	AA	CT	GA	GG
MC1	51	64	AA	TT	GA	GA
MC61	52	62	AA	TT	GG	GG
MC16	53	NA	AA	TT	GA	GG
MC19	53	NA	AA	TT	GA	GG
MC24	54	64	AA	TT	GG	GG
FPADS1	57	NA	AG	CT	GG	GA
MC65	57	70	AA	CT	GG	GA
FPADS2	58	NA	AA	CT	GG	GG
MC22	61	NA	AG	TT	GA	GG
MC84	61	69	AA	CT	AA	GG
MC8	63	NA	AA	TT	GG	GG

and the average age at death 62.5 (range 50-70; $n_{available}$=12). An additional 63 Hispanic late-onset AD (LOAD) patients ascertained at Mayo Clinic Florida (n=13) and the Mayo Clinic brain bank (n=50) were studied for the presence of the p.Gly206Ala and p.Gly378Val mutation. For clinical patients the diagnosis of AD was given according to the NINCDS-ADRDA criteria, NIA-Reagan criteria were used to confirm AD diagnosis in all pathology cases. The average age at onset in this late-onset cohort was 77.2 (range 66-87; $n_{available}$=13) and the average age at death 81.1 (range 71-91; $n_{available}$=50). Institutional review board-approved protocols, including informed consent, were followed to obtain all DNA samples.

Sequencing analysis

All 27 Hispanic EOAD patients were sequenced for the coding regions of *PSEN1* (exons 3-12), *PSEN2* (exons 3-12) and exons 16 and 17 of *APP*. Each exon was PCR amplified using Apex products, purified using the Agencourt Ampure system (Agencourt Bioscience Corporation), and sequenced using Big Dye Terminator V3.1 products (Applied Biosystems). Sequencing purification was performed using the Agencourt CleanSEQ method (Agencourt Bioscience Corporation), and ran on an ABI3730 DNA-analyzer (Applied Biosystems). Sequencing analysis was performed using Sequencher (Genecodes). Sequencing of *PSEN1* exon 11 was also performed in all LOAD patients to determine the presence of additional *PSEN1* p.Gly378Val mutation carriers. Finally, sequencing was performed in the subgroup of 13 PSEN1 p.Gly206Ala mutation carriers for 5 PCR fragments encompassing previously reported modifier variants in SNX25 (rs-11730401), PDILM3 (rs-28522047), SORBS2 (rs-13130022), SH3RF3 (rs-6542814) and NPHP1 (rs-906815). All primer sequences are available upon request.

Genotyping

APOE genotyping in the EOAD cohort was determined using predesigned TaqMan SNP genotyping assays for rs7412 (C_904973_10) and rs429358 (C_3084793_20) (Applied Biosystems) and analyzed on an ABI 7900HT Fast Real Time PCR system using Sequence Detection System (SDS) v2.2.2 software (Applied Biosystems). Custom TaqMan SNP genotyping assays were used to determine the presence of the *MAPT* p.Arg406Trp mutation (in the EOAD cohort) and *PSEN1* p.Gly206Ala mutations (in the LOAD cohort).

APP copy-number analysis

To detect genomic *APP* copy-number mutations in the EOAD cohort, real-time PCR analysis was performed with a made to order TaqMan assay (Hs01547105_cn, Applied Biosystems) and analyzed on ABI7900HT Fast Real Time PCR system using SDS 2.2.2 software (ΔΔct method). Genomic DNA (20ng) from each EOAD patient was run in duplicate and normalized to the Copy Number Reference Assay RNase P (cat: 4403326, Applied Biosystems). Two patients previously identified to be *APP* duplication carriers were included as positive controls.

Screening for GGGGCC repeat expansions in C9ORF72

EOAD patients were screened for the presence of the GGGGCC hexanucleotide repeat expansion in *C9ORF72* using a two-step PCR based protocol, as previously described [24]. Briefly, the hexanucleotide repeat was amplified in all samples using one fluorescently labeled PCR primer. Next, fragment length analysis was performed on an automated ABI3730 DNA analyzer using GeneMapper software (Applied Biosystems). All patients that appeared homozygous in this assay were next analyzed using a repeat primed PCR method where characteristic stutter amplification pattern on electropherogram was considered evidence of a pathogenic *C9ORF72* expansion.

Microsatellite analysis

Two polymorphic microsatellite markers surrounding *PSEN1* were analyzed in all 90 Hispanic AD patients to determine the presence of a founder haplotype in p.Gly206Ala mutation carriers. Marker 1 is a $(TC)_n$ repeat located at D14S77, chr14: 73570594-73570656 (GRCh37/hg19) (Forward primer: 5'GCGTGAGTCACTGTGCC 3', Reverse primer: 5'CAGACAGAAATTAACCAGAGTTGAA 3'). Marker 2 is a $(TG)_n$ repeat located at chr14: 73581862-73581897 (GRCh37/hg19) (Forward primer: 5'GAGGAGATAGAACATCTGATGGC 3', Reverse primer: 5'CTAGGCTTAACACCTGGGTGATG 3'). Each marker was PCR amplified using one FAM fluorescently labelled primer using APEX products. PCR products were subsequently diluted 1:150 in water and quantified using a GENESCAN 400HD [ROX] size standard on an ABI3730 DNA-analyzer (Applied Biosystems). Data interpretation was performed using GeneMapper (Applied Biosystems).

Results

PSEN1 p.Gly206Ala is common in Hispanic EOAD in Florida

To determine the contribution of mutations in known neurodegenerative disease genes to a newly ascertained population of Hispanic EOAD patients from Florida, we performed mutation analysis of *APP*, *PSEN1* and *PSEN2* as well as *APP* copy-number analysis in all EOAD patients. We further screened all patients for the presence of a *C9ORF72* repeat expansion and the *MAPT* p.Arg406Trp mutation, two genetic mutations often detected in series of clinically diagnosed AD patients. Out of the 27 patients, 13 were found to carry the p.Gly206Ala mutation in *PSEN1* (48.1%) (**Table 1**). The average age of onset in mutation carriers was 54.8 years (range 42-63 years) and the average age of death 62.2 years (range 50-70 years). Sequencing analysis also identified the known p.Gly378Val pathogenic mutation in exon 11 of *PSEN1* in one other patient. No other mutations in any of the analyzed genes were observed in the remaining 13 patients. Genotyping of *PSEN1* p.Gly206Ala and *PSEN1* p.Gly378Val mutations in 63 Hispanic LOAD patients did not identify any additional carriers of these mutations.

Of the 14 *PSEN1* mutation carriers, 12 had a clear positive family history of AD. In one p.Gly206Ala carrier no family history was reported, whereas another p.Gly206Ala carriers' father died at the age of 54 of an unknown cause and mother died of lymphatic cancer at the age of 69 years. The average onset age in p.Gly206Ala mutation carriers was 54.8 years with a wide range from 42 to 63 years. Seven of the 13 p.Gly206Ala carriers came to autopsy at the Mayo Clinic Brain Bank with an average age at death of 62.2 years (range 50-70). These cases all presented with typical AD as defined by the algorithm to identify neuropathological AD subtypes [25]. All had Braak neurofibrillary tangle stage VI and Thal amyloid phase 5. Disease duration in these 7 cases was 11.5 years (range 8.3-17.0). Within the cohort of p.Gly206Ala mutation carriers, 5 of the 13 patients (38.5%) carried at least one *APOE* E4 risk allele as compared to 8/14 of the EOAD non p.Gly206Ala carriers (57.1%).

Hispanic p.Gly206Ala mutation carriers in Florida share common founder

Genotyping analysis of microsatellite markers 1 and 2 flanking *PSEN1* showed the presence of a shared allele in all p.Gly206Ala mutation carriers, which was rare in the general population. For marker 1, a 218 bp allele was present in all 13 mutation carriers (100%) as compared to 15.6% of non-mutation carriers (12/77). Marker 2 showed a 215 bp allele in all mutation carriers (100%) as compared to 5.2% (4/77) in

non-mutation carriers. None of the non-mutation carriers had both the 218 bp allele at marker 1 and the 215 bp allele at marker 2.

Study of previously reported age of onset PSEN1 modifying variants

We analyzed 5 single nucleotide variants (SNPs) recently reported to modify disease onset in p.Gly206Ala mutation carriers and LOAD patients. All p.Gly206Ala carriers were homozygous for the major allele of rs28522047 in *PDILM3*. For the other four SNPs, genotypes are summarized in **Table 2**. For *SNX25* (rs11730401) in which the minor G-allele had previously been reported to be associated with a delayed onset age, the G-allele carriers in our cohort also had a later average onset (59.0 years) as compared to non G-allele carriers (54.0 years). For the other 3 variants, onset ages were remarkably similar when patient groups were stratified by rare-allele status. Due to the small sample size, statistical analysis was not performed.

Discussion

As part of our ongoing collection of DNA samples from patients with AD in Florida, we ascertained 27 Hispanic EOAD and 63 Hispanic LOAD patients for genetic studies. Because of the higher frequency of AD in the Hispanic population and specifically those from the Caribbean Islands [15-19], these Florida-based Hispanics are of specific interest to the study of genetic factors contributing to the disease; yet, a systematic analysis of mutations in the known AD genes had not previously been performed in this population. Interestingly, we observed a strikingly high occurrence of the *PSEN1* p.Gly206Ala mutation in our EOAD cohort (13/27, 48.1%). We further observed one EOAD patient with the known *PSEN1* p.Gly378Val mutation, which had previously been reported in one non-Hispanic family from France [26]. Mutations in *APP* and *PSEN2*, repeat expansions in *C9ORF72* and the *MAPT* p.Arg406Trp mutation, previously associated with clinical AD, were excluded in the remaining 13 EOAD patients. Genotyping of the p.Gly206Ala and p.Gly378Val mutations in *PSEN1* in our LOAD population did not identify additional mutation carriers.

The *PSEN1* p.Gly206Ala mutation was previously reported as a founder mutation in Caribbean Hispanics ascertained in New York [22], and Puerto Rican Hispanics residing in the Philadelphia area [20] and is considered pathogenic based on familial segregation and *in-vitro* functional studies. Haplotype sharing analysis using 2 microsatellite markers flanking *PSEN1* confirmed a common ancestor for all p.Gly206Ala mutation carriers in our cohort. A comparison of alleles at the flanking markers shows that this is the same haplotype previously reported for Caribbean Hispanic carriers of the p.Gly206Ala mutation; recruited from Research Centers in New York and Philadelphia which are mostly from Puerto Rican heritage, and the same as mutation carriers recruited by physicians in the Dominican Republic [20, 21]. The mutation carriers for which specific origin was known in our study (n=10) also all had Puerto Rican heritage.

All p.Gly206Ala mutation carriers from our cohort were EOAD, defined as having an onset at or before the age of 65 or an age at death at or before the age of 70 if the onset age was unknown. The average age of onset in p.Gly206Ala mutation carriers was 54.8 ± 5.6 years (range 42-63 years). This is in line with the previous two studies which reported carriers with onset ages ranging from 40 s to 70 s [20, 21]. This wide variability in onset age is somewhat uncommon for mutations in *PSEN1* and suggests that other genetic and/or environmental factors may contribute to the disease penetrance. Despite the variable onset, most previously reported patients showed clinical and neuropsychological profiles of typical AD and neuropathological examination of one patient showed severe widespread plaque and tangle pathology without other meaningful disease lesions. Our new cohort extends these findings and reports on the pathological characterization of 7 brains of p.Gly206Ala carriers. All showed typical AD with widespread plaques and tangles. Interestingly, an average disease duration of 11.5 ± 3.2 years (range 8.3-17.0) was observed, which is somewhat longer than expected for typical AD, especially given the early onset age in these patients.

Previous studies reported that the *APOE* E4 allele did not contribute significantly to the variability in age of onset in *PSEN1* p.Gly206Ala

mutation carriers [22, 23]. In our cohort, very few mutation carriers also had an *APOE* E4 allele and therefore *APOE* is also unlikely to explain much of the variability in onset age observed in our cohort. Interestingly, Lee et. al recently focused on a cohort of 56 p.Gly206Ala mutation carriers to identify genetic modifiers of the age of onset using an unbiased genome-wide approach followed by confirmatory studies in a large LOAD (n=2888) cohort. They concluded that variants in *SNX25*, *PDLIM3*, *SORBS2*, *SH3RF3* and *NPHP1* may contribute to the variation in onset age in EOAD and LOAD. For rs11730401 (*SNX25), rs13130022* (SORBS2) and rs6542814 (*SH3RF3*), patients heterozygous for the minor allele had a delayed age of onset (8.75, 11.11, and 9.3 years respectively), whereas carrying the minor allele at rs906815 (NPHP1) and rs28522047 (PDILM3) was associated with an earlier onset (11.69 and 11.97 years respectively). We determined the genotypes of each of these potential modifiers in our cohort and observed a 5-year delay in the average age of onset of our two patients carrying the minor allele of rs11730401 (*SNX25)* as compared to non-carriers in line with the previous report. Even though we could not perform statistical analysis in our cohort due to small sample size, we have provided full details on the genotypes of all candidate variants in this manuscript so that this information can be included in future studies and meta-analyses.

The identification of another large cohort of Caribbean Hispanic *PSEN1* p.Gly206Ala mutation carriers in the US is important and provides additional patients for future studies aimed at the identification of genetic modifiers. Moreover, since the FPADS registry is a new initiative focused on the systematic recruitment of EOAD patients at five facilities in Florida, including two sites in Miami, the discovery of an even larger cohort of carriers of this mutation is inevitable due to the extensive Hispanic population in South-Florida. The additional families will aid in natural history studies of this specific mutation which could be important for future prevention and treatment studies, and could complement the work that is currently performed on the extended Colombian *PSEN1* p.Glu280Ala family [27, 28]. Finally, for individual Hispanic EOAD patients in Florida, especially those with a family history of dementia, our current findings suggest that for diagnosis or genetic counseling a targeted study of the *PSEN1* p.Gly206Ala mutation is warranted and expected to reveal a mutation in approximately 50% of patients.

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Disclosure of conflict of interest

None.

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