A Founder Mutation in Presenilin 1 Causing Early-Onset Alzheimer Disease in Unrelated Caribbean Hispanic Families

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Context Genetic determinants of Alzheimer disease (AD) have not been comprehensively examined in Caribbean Hispanics, a population in the United States in whom the frequency of AD is higher compared with non-Hispanic whites.

Objective To identify variant alleles in genes related to familial early-onset AD among Caribbean Hispanics.

Design and Setting Family-based case series conducted in 1998-2001 at an AD research center in New York, NY, and clinics in the Dominican Republic.

Patients Among 206 Caribbean Hispanic families with 2 or more living members with AD who were identified, 19 (9.2%) had at least 1 individual with onset of AD before the age of 55 years.

Main Outcome Measure The entire coding region of the presenilin 1 gene and exons 16 and 17 of the amyloid precursor protein gene were sequenced in probands from the 19 families and their living relatives.

Results A G-to-C nucleotide change resulting in a glycine–alanine amino acid substitution at codon 206 (Gly206Ala) in exon 7 of presenilin 1 was observed in 23 individuals from 8 (42%) of the 19 families. A Caribbean Hispanic individual with the Gly206Ala mutation and early-onset familial disease was also found by sequencing the corresponding genes of 319 unrelated individuals in New York City. The Gly206Ala mutation was not found in public genetic databases but was reported in 5 individuals from 4 Hispanic families with AD referred for genetic testing. None of the members of these families were related to one another, yet all carriers of the Gly206Ala mutation tested shared a variant allele at 2 nearby microsatellite polymorphisms, indicating a common ancestor. No mutations were found in the amyloid precursor protein gene.

Conclusions The Gly206Ala mutation was found in 8 of 19 unrelated Caribbean Hispanic families with early-onset familial AD. This genetic change may be a prevalent cause of early-onset familial AD in the Caribbean Hispanic population.
patients or families of Spanish origin, but Caribbean Hispanic families typically have not been included in comprehensive studies of the genetic determinants of early-onset AD. We initiated a study of familial AD in Caribbean Hispanic families, noting several individuals who developed the disease before the age of 55 years. We hypothesized that a genetic variation in presenilin 1 or the amyloid precursor protein would be associated with AD in these families.

**METHODS**

**Subjects and Setting**

Between 1998 and 2001, 225 Caribbean Hispanic families with a history of AD in at least 2 living first-degree relatives were recruited. Participants identified themselves as Caribbean Hispanic if the proband or his/her family members were born in, or originated from, one of the Caribbean islands. Patients and their families were recruited from multiple sources: the Alzheimer’s Disease Research Center-Memory Center at Columbia University in New York; a population-based epidemiological study of aging and dementia in northern Manhattan (New York); and physicians in the Dominican Republic (H.R., M.M., M.T.).

In families meeting inclusion criteria, the history was expanded until generations no longer included affected individuals. Children were included if they were older than 30 years or if they were suspected of having AD. All the probands contacted participated. Nineteen families (8.4%) were subsequently ineligible because the diagnosis of AD could not be confirmed, leaving 206 families. Twenty-three relatives refused to participate, although other members of their families were included. Only 3 refusals were in families with early-onset AD.

To estimate the frequency of presenilin 1 variants in a group more representative of the general population, 319 individuals randomly chosen from a community study of aging and dementia in 2126 Medicare recipients in the Washington Heights section of Manhattan were also genotyped. This included 147 Caribbean Hispanics (115 healthy, 32 with dementia), 118 non-Hispanic blacks (95 healthy, 23 with dementia), and 54 non-Hispanic whites (46 healthy, 8 with dementia). All patients with dementia met criteria for probable AD.

The Institutional Review Board of Columbia Presbyterian Medical Center and Columbia University Health Sciences and the Bioethics National Committee for Research in the Dominican Republic approved the study. Informed consent was obtained from either the participant or a family member when the individual had dementia.

**Clinical Assessments**

A standardized medical and neurological history and examination, the Blessed Dementia Rating Scale, which assessed daily function, and a comprehensive, neuropsychological test battery were given in Spanish. Results of these examinations were reviewed by a group of physicians and neuropsychologists, shielded from genotype and family information, for consensus regarding the presence or absence of dementia using published criteria for AD and vascular dementia. Age at onset was based on the report of the patient and family. Severity of AD was rated using the Clinical Dementia Rating Scale.

**Presenilin 1 and Amyloid Precursor Protein Mutation Analysis**

All exons in presenilin 1 and exons 16 and 17 in the amyloid precursor protein gene were amplified from genomic DNA by polymerase chain reaction (PCR). Standard cycling parameters of denaturation at 94°C for 30 seconds, annealing at a specific temperature for 45 seconds, and extension at 72°C for 1 minute were used. (The primer sets selected for each exon and the annealing conditions used are available from the author on request.) Presenilin 1 exon annotation followed that by the Alzheimer’s Disease Collaborative Group and public genetic databases.

All PCR products were analyzed both by denaturing high-performance liquid chromatography (WAVE, Transgenic, Omaha, Neb) and by direct DNA sequencing in the proband, and then other family members, of each family. The PCR products were purified and subjected to direct sequencing. For denaturing high-performance liquid chromatography, sequences were amplified as above, except the final extension in the PCR was followed by denaturation and reannealing to allow heteroduplex formation. Denaturing high-performance liquid chromatography parameters used for analysis of each exon were calculated using a predictive algorithm supplied by the manufacturer.

**Analysis of Polymorphisms Flanking Presenilin 1**

Founder effects were investigated using 2 previously undescribed, highly polymorphic microsatellite markers that flank presenilin 1. The first is a GT-repeat at position 33117 (Genbank accession No. AF109907). The second is a CA-repeat at position 23000 of this same sequence. To amplify the GT-repeat from genomic DNA of each proband and unaffected control, we used forward primer 5’-GAGGAGATAGACACTGTATGGCG-3’ and reverse primer 5’-CTAGGCTTAACACCTGGGTGATG-3’. To amplify the CA-repeat, we used a forward primer 5’-TCTGTCCATTTCCGTATACGAAGACAC-3’ and reverse primer 5’-GTCAGATGGCG-3’, both in a reaction mixture containing Platinum Taq DNA polymerase (Life Technologies, Rockville, Md) and dNTPs with alpha-32P labeled dCTP (Amersham Biosciences, Piscataway, NJ). The 32P-labeled PCR products were electrophoresed on denaturing 6% acrylamide sizing gels.

To determine whether certain haplotypes segregated with AD in the families, we constructed haplotypes using the Viterbi algorithm in GENEHUNTER. We also conducted a parametric linkage analysis assuming an autosomal dominant model with a disease allele frequency of 0.0001 and age-dependent liability classes (<40, 40-50, 51-55, 56-65, and >65). Multipoint linkage analysis that included the GT-repeat, CA-
repeat, and presenilin 1 alleles was completed using MULTI-LINK.28

To determine whether or not the distribution of putative disease-related haplotypes in probands differed from that in unrelated Caribbean Hispanic controls, we estimated and compared haplotype frequencies in all probands and in Caribbean Hispanic controls from the Washington Heights community study using the EHPlus program.27,28 In addition, we used a Monte-Carlo method to compute empirical P values based on 2000 replicates, to reduce the potential problems associated with the conventional χ² test applied to a contingency table with sparse data.

Functional Analysis of Presenilin 1 Mutation

HEK293 cells were stably transfected with BAPP_Swedish and either empty vector in the pcDNA3 vector (mock transfected), wild type PS1 (negative control), Leu392Val mutant PS1 (positive control), or Gly206Ala mutant presenilin 1 plasmids as previously described.30 For each double transfection, at least 3 independent stable clones were selected and replated. Amyloid β peptide levels (Aβ40 and Aβ42) were then measured by ELISA in 16-hour conditioned media as previously described.31 Paired 1-sided Student t tests were used to compare Aβ secretion by wild type cells (independent clones, n = 5) and PS1 mutant cells (independent clones for each mutant, n = 6).

Apolipoprotein E Genotyping

The apolipoprotein E (ApoE) genotype was determined using the method of Hixon and Vernier22 and Maestre et al.33

Age at Onset

The mean age at onset was compared using Student t test and proportional hazards model with Kaplan-Meier plots34 among individuals with and without presenilin 1 mutations.

RESULTS

Clinical examinations and blood samples were obtained from 763 individuals in 206 families. In 19 (9.2 %) families, at least 1 person had disease onset before the age of 55 years (Table 1 and Figure 1). A proband from 8 (42%) of these 19 families had a G-to-C nucleotide change at position 865 bp in exon 7 of the longest splice form of presenilin 1 (Genbank accession L42110, NM_000021, sequence in Figure 2) resulting in a glycine–alanine amino acid substitution at codon 206 (Gly206Ala).35,36 No presenilin 1 mutations were observed in the probands of the other 11 families (Figure 1).

The Gly206Ala mutation was found in 23 (52%) of the 44 individuals tested in the 8 families (Table 2); 21 of the 23 carriers had AD at the time they were examined (mean [SD] age at onset, 54.5 [7.1] years). The other 2 individuals, 57 and 52 years of age (Table 2), had cognitive impairment but no functional impairment. These 2 individuals would meet criteria for mild cognitive impairment.37 Five others had AD without the Gly206Ala mutation. Their mean (SD) age at onset was significantly later (68.9 [9.5] years; t = 3.7, P = 0.001), and 3 were homozygous for apolipoprotein E ε4 (ApoE ε4) and 2 were heterozygous. The ApoE ε4 allele was present in 11 of the 21 patients with the Gly206Ala mutation, but had no effect on the age at onset (with ε4: 54.7 years; without ε4: 54.2 years, P = .9).

Additional Families and a Community Study

No presenilin 1 mutations were observed in probands from another 28 (13.5%) of the 206 Caribbean Hispanic families with onset between the ages of 56 and 65 years (mean, 61.9 years) (Figure 1). However, among the 319 unrelated subjects from the Washington Heights community study, a Caribbean Hispanic with AD beginning at 58 years of age had the Gly206Ala mutation and a family history of AD. This person was not a known relative of any of the Caribbean Hispanic families described above. No African Americans or whites had the presenilin mutation.

The Gly206Ala mutation was not found in the 2 public AD mutation databases.22,23 However, while our initial work was under review, the same mutation was reported in 4 unrelated probands and 1 sibling with early-onset AD from a series of 414 patients referred for genetic testing for AD.38 All of these additional cases were also of the Hispanic ethnic group. Whether they are of Caribbean origin is unknown. Another 5 Hispanic probands have since been identified with
Figure 1. Sources of Patients and Families Identified in This Study

![Sources of Patients and Families](image)

AD indicates Alzheimer disease.

**Figure 2. Nucleotide and Amino Acid Sequence of the Region of Presenilin Exon 7 That Includes the Caribbean Hispanic Mutation**

```
L  ATC  TGG  AAT  TTT  GGT  GTG  GGA  ATG  ATT
W  N    F    A    V    G    V    G    M    I
```

The altered nucleotide and amino acid are in bold.

This mutation, but DNA was not available for additional analysis (W.K.S., written communication, August 2001).

**Functional Assessment of the Mutation**

The effect of the Gly206Ala mutation on the secretion of amyloid β peptide isoforms (Aβ40 and Aβ42) was tested in HEK293 cell lines stably overexpressing this mutation. Cells expressing wild type presenilin 1 secreted 11 ± 0.08 ng/mL of Aβ40 and 3.63 ± 1.29 ng/mL of Aβ42. Cells expressing the Gly206Ala mutant or another known mutation, Leu392Val, secreted similar quantities of Aβ40 (11.32 ± 0.11 ng/mL and 12.22 ± 0.78 ng/mL, respectively). The Gly206Ala mutation caused a 2.2-fold increase in the amount of amyloid Aβ42 secretion (8.03 ± 2.48 ng/mL, P = .003), while the Leu392Val resulted in a 5-fold increase (18.47 ± 9.31 ng/mL, P < .001). This provides evidence that the conservative glycine to alanine substitution is a pathogenic mutation.

**Analysis of a Genetic Founder Effect**

If the Gly206Ala mutation occurred in a recent common ancestor of the individuals with this variant, alleles at nearby markers should be shared on chromosomes with this mutation. Every individual tested with the Gly206Ala mutation, the (GT)21 and the (CA)19 always segregated together (pedigrees available from the authors on request).

**Haplotype-Phenotype Relationship**

To determine how the haplotype (Gly206Ala mutation, the (GT)21, and the (CA)19) segregated with AD, we conducted multipoint parametric linkage analysis in the 8 families. The haplotype segregated strongly with AD in these families in all but 2 of the haplotype carriers, who did not yet have AD but did have mild cognitive impairment. Multipoint parametric linkage analysis that included the Gly206Ala mutation, the (GT)21 and the (CA)19 yielded a maximum limit of detection (LOD) score of 2.16 (P = .00081). The estimated haplotype frequencies differed significantly between probands from all 13 families (9 in this report and 4 from the referral series) and unrelated Caribbean Hispanic controls (P = .000032).
Amyloid Precursor Protein Gene

No variants were observed in exons 16 and 17 of the amyloid precursor protein gene among the 19 Caribbean Hispanic probands with AD onset before the age of 55 years.

COMMENT

The Gly206Ala mutation in presenilin 1 was observed in 8 Caribbean Hispanic families with onset of familial AD before the age of 55 years. The identical mutation was also found in a Caribbean Hispanic patient with familial early-onset AD by mutation screening of individuals in the Washington Heights community study in northern Manhattan. The mutation was not identified while screening chromosomes from 256 normal in-

Table 2. Characteristics of Families With Early-Onset Familial Alzheimer Disease and the Presenilin 1 Gly206Ala Mutation

<table>
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<tr>
<th>Family No.*</th>
<th>Origin</th>
<th>Person</th>
<th>Sex</th>
<th>Alzheimer Disease</th>
<th>Gly206Ala Mutation</th>
<th>ApoE Genotype†</th>
<th>Current Age or Age at Onset, y‡</th>
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<td>+</td>
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<tr>
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*All families labeled with “NY” prefix were part of an investigation of Caribbean Hispanic families. The individual labeled with the “RM” prefix was identified from a random sample of Medicare recipients participating in a study of aging and dementia in northern Manhattan. The individual has a family history of Alzheimer disease, but no relatives have been examined.

†ApoE indicates apolipoprotein E.

‡Age at onset if demented.

§Individual meets criteria for mild cognitive impairment.
Examples of Genotyping for the GT-repeat and CA-repeat Microsatellite Markers Upstream of the Presenilin-1 Gene in Probands and Unrelated Caribbean Hispanic Controls

The shared alleles for each marker are indicated with arrowheads. All probands had the identical number of repeats in both microsatellite markers. The (GT)\textsubscript{21} allele at the GT-repeat marker and the (CA)\textsubscript{19} allele at the CA-repeat marker were present in all probands, while less than 15% of the controls carry either allele. P indicates proband; +, unrelated control carrying the shared allele. ("Shadow bands," which are often seen as a technical artifact with dinucleotide markers, are seen with the GT-repeat, but not with the CA-repeat.)

Individuals, including 115 healthy elderly Caribbean Hispanics.

The same mutation has been reported in 5 Hispanic patients, 4 probands and 1 sibling, with AD referred for genetic testing. Moreover, a laboratory (Athena Diagnostics Inc, Worcester, Mass) identified another 5 probands with the same mutation. The individuals in these 18 families (9 from this report and 9 from the referral series\cite{38}) were not related to one another. Based on the triplet genetic code, a given amino acid can change in principle, be caused by various nucleotide changes. All mutation carriers in these families shared the identical nucleotide change. We further observed shared alleles at 2 polymorphic repeat sequences flanking presenilin 1 in all individuals with the Gly206Ala mutation, strengthening the likelihood that it was inherited from a common ancestor. Evidence supporting the segregation of the haplotype containing the Gly206Ala mutation, the (GT)\textsubscript{21} allele at the GT-repeat microsatellite, and the (CA)\textsubscript{19} allele at the CA-repeat microsatellite with AD was based on the constructed haplotypes and multipoint linkage analysis in our original 8 families and in the comparison of estimated haplotypes from the probands and unrelated Caribbean Hispanic controls.

Five individuals with older-onset AD did not have the Gly206Ala mutation but had at least 1 \textit{ApoE} ε4 allele. Several family members also had the Gly206Ala mutation. Among those individuals with the Gly206Ala mutation, the \textit{ApoE} ε4 allele did not influence the age at onset.

Aldudo et al\cite{39} reported novel mutations in presenilin 1 mutations (Met232Leu, Ala409Thr, Leu282Arg) with both familial and sporadic AD from Spain. Presenilin 1 mutations were observed in exon 5 (Asn135Asp)\cite{40} and in exon 6 (Leu171Pro) in Mexican families,\cite{41} and in exon 8 (Glu280Ala) in a large family from South America.\cite{42,43} None of these mutations were found in the Caribbean Hispanics we investigated.

Presenilin 1 mutations in exons 4, 5, 6, 8, and 11 have been found in 3 or more families, but only 2, both in exon 4, have been attributed to a common founder.\cite{44,45,46} The frequency of some presenilin mutations in individuals from unrelated families and from diverse ethnic backgrounds suggests either that these mutations are very old and have persisted for several generations, or that they may have arisen as independent mutations at different times. For example, the Ghu280Ala mutation in exon 8 has been observed in more than 12 unrelated families of Japanese, Spanish, and European ethnic groups.\cite{47,48,49} The Gly206Ala mutation in presenilin 1 represents a conservative amino acid change, but a similarly conservative mutation has been reported in 2 unrelated families in exon 11, Gly384Ala.\cite{50,51} Both the Gly206Ala and Gly384Ala mutations selectively increase the production of the neurotoxic \textit{Aβ}\textsubscript{42} isoform of amyloid β-peptide. Consistent with functional importance, the glycine at codon 206 is evolutionarily conserved in all animal presenilins (including vertebrate presenilin 1 and presenilin 2 and their orthologues in invertebrates) although it is not conserved in the Aradopsis homologue.\cite{52}

It is intriguing to speculate on the origin of the Gly206Ala mutation in exon 7 in presenilin 1. It has not been reported in patients from Spain, Mexico, or Colombia, therefore it may have originated in earlier generations of Caribbean Hispanics. Caribbean Hispanics from the Dominican Republic and Puerto Rico share a rich and complex heritage. First invaded by the Spanish explorers, many of the indigenous peoples of these islands were killed and those who survived experienced political domination by Spanish, French, and British colonists during the next 500 years. The Gulf Stream sailing route used by the Europeans to settle the Caribbean islands tracks the western coast of Africa before crossing the Atlantic almost directly to the Caribbean islands. The Gly206Ala mutation therefore also could have been introduced when Africans were brought as slaves to the Caribbean islands. These same currents also have provided a means by which the progeny of a single founder could have dispersed among the Caribbean islands in the remote past.\cite{53}

Mutation screening in this study was limited to a population sample of Caribbean Hispanics in northern Manhattan. Screening for this or other presenilin 1 mutations in a healthy population would not be practical because early-onset AD is a rare disorder. However, the finding of the Gly206Ala mutation in presenilin 1 in 18 unrelated Caribbean Hispanic families identified by completely different methods, including a series of patients referred to a commercial laboratory,\cite{54} implies that it may account for a substantial percentage of early-onset familial AD in the Caribbean Hispanic population. Genetic testing is currently of limited use for late-onset and sporadic AD.\cite{55} However, in research settings and in diagnosis or ge-
nomic counseling of a Caribbean Hispanic patient with early-onset familial dementia, identifying a Gly206Ala mutation may be useful.

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