Hum Genet (1996) 98 : 430–436 © Springer-Verlag 1996

ORIGINAL INVESTIGATION

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Polymorphisms in the human apolipoprotein-J/clusterin gene: ethnic variation and distribution in Alzheimer's disease

Received: 14 February 1996 / Revised: 15 April 1996

Abstract Apolipoprotein-J/clusterin (*APOJ/CLI*) shares many biological properties with apolipoprotein-E (*APOE*) including, but not limited to, avid binding with β-amyloid peptide. Thus, *APOJ/CLI* warrants scrutiny as a candidate Alzheimer's disease (AD) susceptibility gene. We identified seven nucleotide sequence polymorphisms in *APOJ/CLI*, two of which, in exon 7, alter the predicted amino acid sequence. The JVIIB variant is an asparagine-to-histidine substitution, which deletes a glycosylation signal at amino acid 317; the JVIIC variant is an aspartate-to-asparagine substitution, which forms a new glycosylation signal at position 328. Both of these coding variants, as well as two neutral polymorphisms in exon 2, were more frequent in African-Americans than Hispanics and were rare in Caucasians. However, no individual coding or non-coding variant was consistently associated with AD. At the population level, *APOJ/CLI* polymorphisms are frequent among persons of African descent, but probably do not alter susceptibility to AD.

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Introduction

Alzheimer's disease (AD) is a complex genetic disease that affects 10% or more of people over the age of 65 and shows a definite but limited familial component. Several genes have been associated with AD: the amyloid precursor protein (*APP*) gene on chromosome 21 (reviewed in Ashall and Goate 1994), the *APOE* gene on chromosome 19 (Strittmatter et al. 1992) and the presenilin genes on chromosomes 14 and 1, which encode predicted transmembrane proteins of uncertain function (Sherrington et al. 1995; Levy-Lahad et al. 1995a,b). Chromosome 21 and 1 mutations are rare and associated with familial AD with onset of symptoms between ages 50 and 60, whereas mutations in chromosome 14 have earlier age-at-onset. In contrast, the *APOE* gene, in particular the e4 allelic variant, is associated with sporadic and familial AD of both late and early onset (reviewed in Weisgraber et al. 1994).

The ApoJ protein (also referred to as clusterin, SGP-2, TRPM-2, AR, SP-40,40 and GpIII) can regulate complement function (Jenne and Tschopp 1989; Kirszbaum et al. 1989), and shares certain interesting properties with ApoE, including cholesterol binding in apolipoprotein complexes (de Silva et al. 1990b; Jenne et al. 1991), production by astrocytes in response to inflammatory cytokines (Zwain et al. 1994), increased expression after experimental brain injury in animals (May et al. 1990; Pasinetti and Finch 1991; Laping et al. 1991), localization to the pathological amyloid-core plaques of AD (Choi-Miura et al. 1992), binding to soluble amyloid beta peptide (Ghiso et al. 1993; Zlokovic et al. 1994) and increased expression in AD brains (Duguid et al. 1989; May et al. 1990). In addition, the ApoJ protein is internalized by cells via the gp330 membrane glycoprotein (Kounnas et al. 1995), a receptor that can also internalize ApoE-con-

taining lipoproteins (Willnow et al. 1992; Kounnas et al. 1995) and that is homologous to the LRP protein, which can also act as a receptor for ApoE and for a secreted form of APP (Beisiegel et al. 1989; Kounnas et al. 1995). By virtue of these biological similarities of its gene product with ApoE, the *APOJ/CLI* gene, on chromosome 8p21 (Minoshima et al. 1991; Fink et al. 1993), could be considered a candidate gene for susceptibility to AD.

Because isoelectric focusing (IEF) polymorphisms in serum ApoJ protein have been reported in an African population and Americans of African descent (Kamboh et al. 1991), we searched for DNA sequence polymorphisms in the human *APOJ/CLI* gene with the objective of analyzing the frequencies of these polymorphisms and their possible association with AD in a large sample of elderly African-American, Hispanic and Caucasian/non-Hispanic subjects. Here we describe a series of coding and noncoding nucleotide sequence polymorphisms in *APOJ* and characterize their distribution among AD cases and controls in these three ethnic groups.

Materials and methods

Subjects, diagnosis and ethnicity

The design and selection of cases and controls have been previously described (Maestre et al. 1995; Tang et al. 1996). Gender did not differ in cases and controls, but patients were older than controls and had less education (Table 2). None of the controls were spouses or relatives of cases. A second group of African-American patients and controls was ascertained from a previously described series as a replicate sample (Hendrie et al. 1995). The 32 patients with AD and the 49 controls were identified in a community-based study using criteria for AD that were identical.

Data analysis

Allele frequencies for patients with AD and controls were determined by counting alleles and calculating sample proportions. Frequencies of *APOJ* and *APOE* alleles in patients and controls were compared using the chi-square test. We estimated both simple and stratified (by ethnic group) odds ratios for AD associated with the presence of each variant allele (homozygous and heterozygous), using subjects with the most common (JIIA, JVIIA) genotypes as the reference group. This analysis was also performed for each possible combination of exon II and exon VII genotypes. The frequencies for the demographic categories, including ethnic groups, were compared among cases and controls using chi-square analysis where required. Both univariate and multivariate odds ratios for AD with particular genotypes were also calculated from logistic regression adjusting for age and ethnic group. The association between polymorphisms in exons 2 and 7 was examined using chi-square statistic and the likelihood ratio test of linkage equilibrium using the EH program with and without the case-control option (Terwilliger and Ott 1994). Power to detect linkage disequilibrium was estimated using methods suggested by Olson and Wijsman (1994).

PCR and single-strand conformation polymorphism (SSCP) analysis

Genomic DNA, 100 ng, was subjected to PCR with upstream and downstream intronic primers flanking individual *APOJ*-coding exons in standard PCR buffer (Perkin-Elmer, Branchburg, NJ) with 1.25 mM of each dNTP and 1 U of *Taq* polymerase in a volume of 50 μl. Thermal cycling consisted of initial denaturation for 4 min at 94°C followed by 30 cycles of annealing at 54°C for 30 s, extension at 72°C for 45 s and denaturation at 94°C for 1 min, with a final extension at 72°C for 5 min. Aliquots of the PCR products were visualized on ethidium-stained 1.4% agarose gels to confirm successful amplification and lack of extraneous products. SSCP analysis was a modification of the procedure of Orita et al. (1989). An aliquot of PCR product was diluted 1:20 into water and then 1:10 into fresh PCR reagents containing an 80-fold reduced concentration of dNTPs and including α32P-dCTP (1 μCi/10 μl). Radiolabeling was carried out for six PCR cycles, and the radiolabeled product was diluted 1:20 into 0.1% SDS/10 mM EDTA, heated to 65°C for 5 min, diluted 1:1 into standard sequencing stop solution containing 50% formamide, heated to 75°C for 3 min and loaded on a nondenaturing 6% acrylamide gel maintained at 4°C. Electrophoresis was at 400 V for 16–20 h.

DNA sequencing

For direct sequencing, PCR products were gel-isolated using GlassPacTM (National Scientific, San Rafael, Calif.) and subjected to cycle-sequencing using reagents and *Taq* polymerase from the fmolTM system (Promega, Madison, Wis.) with appropriate ^{32}P-end-labeled primers. Cycling conditions were as above except that the buffer contained 5% dimethylsulfoxide, annealing was at 52°C and extension was at 70°C. After 30 PCR cycles the sequence reactions were analyzed on 6% acrylamide/7 M urea gels. In some cases the gel-isolated PCR products were cloned in a plasmid vector (TA cloning vector, Invitrogen, San Diego, Calif.) and individual clones were subjected to standard dideoxy sequencing using ^{35}S-dATP and T7 DNA polymerase (Sequenase, U.S.B, Cleveland, Ohio).

Slot-blotting with allele-specific oligonucleotides (ASOs)

PCR products, 6 μl, were denatured by addition of 1 μl of 4 N NaOH/10 mM EDTA and incubation for 10 min at room temperature, neutralized by addition of 150 μl of ice-cold 1 M ammonium acetate and subjected to duplicate transfer (50 μl/slot) to nylon membrane using a vacuum manifold apparatus. The membrane was rinsed briefly in 2 × SSC, UV crosslinked at 0.3 J/cm3, baked at 80°C for 1 h in a vacuum oven and then cut into strips corresponding to the duplicate transfers for hybridization with two allelic oligonucleotide probes. Oligonucleotides, 150 ng, were end-labeled in reaction volume of 10 μl using 10 U of T4 polynucleotide kinase and 5 μCi of γ32P-ATP. Prehybridization and hybridization were carried out for 6–16 h each in 6 × SSC containing 0.1% SDS, 3% formamide, 5 mM sodium pyrophosphate and 1% blocking reagent from the GeniusTM Kit (Boehringer-Mannheim, Indianapolis, Ind.) at 42°C. Blots were washed for 15 min at room temperature in two changes of 6 × SSC and then at 50°C for 5 min in 6 × SSC.

Primers and ASOs

APOJ PCR primers were:

exon 1:	(upstream)	TCCGCGGCATTCTTTGGGC
	(downstream)	TGCCCGCCCATCCGTCCTG
exon 2:		CGTGCAAAGACTCCAGAA;
		TGGCCAGAGGAACATCAT
exon 3:		CTCTTGCACTTCTCTTGC;
		TCCAGTGGGATGGTCAAG
exon 4:		AGCCTTGTGTCTTCCTGT;
		GCATATTTCACTAGGCTC
exon 5:		GAGCTTCTCCTAACTGTG;
		AAAGGCCATGAGCTTCCA
exon 6:		CTGGATGACTGACTCTTC;
		TCCATAAAGGCAGCACCA

exon 7: CTTCCCTTCACACTTCTC;
downstream (a) TCCATAAAGGCAGCACCA
downstream (b) GACTTTAGCAGCTCGTTG
exon 8: CCACAGTGTTTCAGCTCT;
TTTTGTGGCTCCCAGAGA
exon 9: GGATGTTTTACTTTGGAGG
AGAGGCTGGGCGGAGTTGG

The exon 7 3′(a) primer was used in the initial screening for polymorphisms by SSCP and direct sequencing, and the exon 7 3′(b) primer, which brackets the coding polymorphisms more closely, was used for subsequent PCR, SSCP and slot-blotting. ASOs were:

J7.1 (JVIIA-specific): TGTTCCACCAACAACCC
J7.2 (JVIIB-specific): TGTTCCACCAACCACCC

Genotyping for *APOE* alleles was as previously described (Maestre et al. 1995).

Results

DNA polymorphisms

To screen for polymorphisms by SSCP, we initially amplified each of the nine *APOJ* exons from DNAs of 20 African-Americans by PCR using flanking intronic primers. SSCP variants were observed for exons 2, 5, 6 and 7. Sequencing of the PCR products showed that the exon 6 variant was due to a single nucleotide insertion in the intron sequence upstream of the 3′ primer and that the exon 2 and exon 5 variants were due to nucleotide substitutions in exon sequences that were neutral with respect to amino acid coding potential (Table 1). In contrast, two of the exon 7 SSCP variants corresponded to nucleotide substitutions that altered the predicted amino acid sequence (Table 1, Fig. 1). Assigning the most common allele, corresponding to the published sequence (Wong et al. 1994), as JVIIA, we designate the next most common exon 7 allele as JVIIB and the more rare allele as JVIIC. The JVIIB allele is an A-to-C replacement at nucleotide position 1025 and corresponds to a substitution of histidine for asparagine at amino acid position 317; the JVIIC allele is a G-to-A replacement at nucleotide position 1058 and corresponds to a substitution of asparagine for aspartate at amino acid position 328. Both of these exon 7 sequence variants, as well as the noncoding polymorphisms in exon 2, could be distinguished by their characteristic SSCP band patterns, as indicated by the reproducibility of these patterns and the precise correlation between the SSCP results and direct sequencing in 20 subjects with the variant genotypes. Allele-specific oligonucleotides (ASOs) that differed at a single position corresponding to the JVIIB allelic nucleotide substitution also allowed rapid scoring of this coding polymorphism when applied as hybridization probes to slot-blots of the exon 7 PCR products. In each case the slot-blot result was concordant with the SSCP pattern (Fig. 1C). Since the exon 2 and 7 PCR products from several hundreds of individuals were subjected to SSCP analysis (Table 3) and since the exon 7 and exon 2 PCR products from 20 subjects with the variant alleles were directly sequenced, it is likely that all of the common polymorphisms in these two exons were identified. However, since our SSCP analysis of the other exons involved fewer individuals and since fewer of the PCR products from these exons were sequenced, we cannot rule out the presence of additional rare *APOJ* sequence variants.

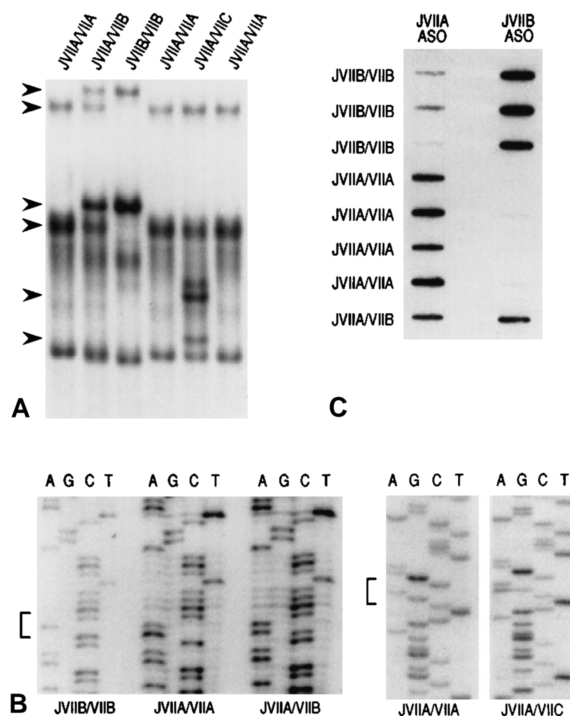

Fig. 1 A–C Detection and sequencing of *APOJ* coding polymorphisms. **A** Single-strand conformation polymorphism (SSCP) analysis of exon 7 PCR products. The diagnostic bands for the allelic variants are indicated by the *arrowheads*. Genotypes, confirmed by sequencing, are indicated above the *lanes*. The exon 7 3'(b) primer was used as the downstream primer. **B** Direct sequencing of exon 7 PCR products. The allelic nucleotide substitutions are indicated by the *brackets*. Genotypes are indicated below each sequence set. **C** Slot-blot analysis of exon 7 PCR products with allele-specific oligonucleotides (ASOs). Genotypes, confirmed by SSCP and direct PCR sequencing, are indicated on the *left*

Table 1 *APOJ* nucleotide sequence variants. Numbering of nucleotides is according to Wong et al. 1994.

Allele	Position	Coding change
IIC	Exon II; nt160/codon28 gac→gat	None
IIB	Intron II; +8 from exon II splice donor site; g→a	None
VB	Exon V; nt865/codon263 cac→cat	None
VIB	Intron V; -2 from exon VI splice acceptor site; insertion t	None
VIIB	Exon VII; nt1025/codon317 aac→cac	Asn→His
VIIC	Exon VII; nt1058/codon328 gac→aac	Asp→Asn
VIID	Exon VII; nt1030/codon318 ccc→ccg	None

Table 2 Demographics and *APOJ* allele frequency among patients with Alzheimer's disease and controls by ethnic group in Washington Heights

Subjects	Age	IIC[a]	IIB	IIA	VIIC	VIIB	VIIA
Washington Heights African-American[a]							
Alzheimer's disease	78 (7.4)	0.25	0.15	0.60	0.03	0.21	0.76
Control	73 (5.8)*	0.20	0.10	0.70	0.02	0.21	0.77
Caucasian							
Alzheimer's disease	70 (10.5)	0	0	1.00	0	0.009	0.99
Control	74 (8.6)	0	0	1.00	0.01	0.02	0.97
Hispanic							
Alzheimer's disease	77 (8.2)	0.06	0.03	0.91	0.01	0.07	0.92
Control	73 (6.1)*	0.07	0.03	0.90	0.01	0.05	0.94
Indiana African-Americans[a]							
Alzheimer's disease	79 (5.9)	0.19	0.083	0.727	0.03	0.25	0.72
Control	83 (9.0)*	0.18	0.074	0.745	0	0.26	0.74

*$P < 0.01$

[a] Note the significant difference ($P < 0.00001$) between African-Americans and the other ethnic groups. Allelic frequencies did not differ significantly between cases and controls within and across ethnic groups. The total number of patients and controls genotyped is indicated in Table 3

The ApoJ protein is subject to glycosylation, including the addition of negatively charged sialic acid, at seven asparagine residues roughly evenly distributed through the primary sequence and located within asparagine-X-threonine/serine (N-X-S/T) consensus glycosylation signal sequences (de Silva et al. 1990a). Both of the allelic variants that we have identified are potentially associated with altered glycosylation: the JVIIB allele disrupts a N-P-S sequence and the JVIIC allele creates a new potential glycosylation site with the sequence N-E-S. An interesting evolutionary comparison can be made between these human sequence variants and the mouse (Jordan-Stark et al. 1994) and rat (Collard and Griswold 1987) *ApoJ* sequences. The human JVIIB allele deletes a glycosylation site that is not present at the corresponding position in the rodent sequence but that appears to have its rodent counterpart at a slightly more C-terminal position; the human JVIIC allele produces a potential glycosylation site at precisely this position. This suggests that both positions in the protein might be accessible to glycosylation in vivo.

APOJ allele frequencies

Genotyping for the exon 7-coding polymorphisms was carried out on DNA from the northern Manhattan series of African-Americans (165 patients with AD and 273 healthy elderly unrelated controls), Hispanics (126 patients and 179 healthy elderly unrelated controls) and Caucasians (53 patients and 43 unrelated healthy controls). There were significant differences among the three ethnic groups in the distribution of *APOJ* exon 7 and exon 2 polymorphisms, with all variant alleles showing the highest frequency in African-Americans, an intermediate frequency in Hispanics and the lowest frequency in Caucasians ($\chi^2 = 43.2$, $df(4)$, $P < 0.00001$; Table 2). The JVIIB variant was observed in only 1.6% of Caucasian chromosomes; one JVIIC variant allele (0.5%) was found in this group. Differences in the frequency of JVIIB and JVIIC variants were not related to age or gender in any group. The frequencies of both the JVIIB and JVIIC variants did not differ among African-American patients with AD compared with controls (Table 2). The JVIIB and JVIIC allele frequencies observed among the African-American subjects from Indiana were similar to those found in the northern Manhattan series and also did not differ in cases and controls (Table 2). The sample size in the combined series of African-Americans ($n = 519$) gave us sufficient statistical power ($\alpha = 0.01$, $\beta = 90\%$) to be able to detect at least an 11% difference in allele frequency using the case-control design (Olson and Wijsman 1994).

While *APOJ* DNA polymorphisms have not been previously reported, in an IEF analysis of serum ApoJ protein, Kamboh et al. (1991) found a single major variant, designated "J*2", at an allele frequency of 0.237 in a series of 158 African-Americans and this isoform was not detected in a series of 240 Caucasians. The "J*2" variant carried an increased net positive charge relative to the default "J*1" isoform, both before and after desialation with neuraminidase. A single African-American individual carried a second more positively charged IEF variant, which was designated "J*3". Since our JVIIB DNA allele is found at a similar frequency to the "J*2" isoform in African-Americans, is very rare in Caucasians and is predicted to increase the net positive charge of the ApoJ protein, both by replacing asparagine with histidine and by eliminating a consensus recognition site for post-translational addition of negatively charged sialic acid residues, it is likely that it corresponds to the "J*2" IEF variant. Similarly, our JVIIC DNA allele might correspond to the "J*3" IEF variant, but since the JVIIC allele frequency (0.03) for African-Americans in our series is higher than the "J*3" IEF allele frequency (0.003) observed by Kamboh et al. (1991), it is not clear whether these rare variants represent the same allele.

APOE allele frequencies

In the combined northern Manhattan and Indiana data, there were significant differences between cases and controls in the *APOE*-ε4 allele frequencies in all three

Table 3 *APOJ* VII genotypes in cases and controls by ethnic group in Washington Heights and Indiana. The number in each column represents the number of individuals with that specific genotype and the number in parentheses indicates the percentage of subjects with the specific genotype

Subjects	N	VIIA/A	VIIA/B	VIIA/C	VIIB/B	VIIB/C	VIIC/C
Washington Heights African-American							
Alzheimer's disease	165	100 (61%)	43 (26%)	9 (5.5%)	12 (7.3%)	1 (1%)	0
Control	273	158 (58%)	90 (33%)	13 (4.8%)	10 (3.7%)	2 (1%)	0
Caucasian							
Alzheimer's disease	53	52 (98%)	1 (2%)	0	0	0	0
Control	43	40 (93%)	2 (5%)	1 (2%)	0	0	0
Hispanic							
Alzheimer's disease	126	107 (85%)	14 (11%)	3 (2.4%)	2 (1.6%)	0	0
Control	179	157 (88%)	15 (8%)	6 (3.4%)	1 (0.6%)	0	0
Indiana African-American							
Alzheimer's disease	32	15 (47%)	14 (44%)	2 (6%)	1 (3%)	0	0
Control	49	26 (53%)	20 (41%)	0	3 (6%)	0	0

ethnic groups: Caucasians (0.30 vs 0.07), African-Americans (0.28 versus 0.20 in the combined groups; 0.24 vs 0.21 in the northern Manhattan group; 0.41 vs 0.15 in the Indiana group) and Hispanics (0.19 vs 0.11); $P < 0.001$ for each.

APOJ genotypes – case-control comparisons

There was no association between AD and heterozygosity for the JVIIB or JVIIC coding variants in either group of African-American subjects (northern Manhattan or Indiana; Table 3), and no JVIIC homozygotes were encountered. After we genotyped 318 African-Americans and Hispanics from northern Manhattan, we observed a significant increase in JVIIB homozygosity among cases (OR = 9.3; 1.7–52.1, $P < 0.01$). However, when a similar trend was not seen among the African-Americans from Indiana, we genotyped an additional 263 subjects from northern Manhattan. While the trend persisted it was no longer statistically significant (OR = 1.8; 95% CI 0.7–3.9, $P = 0.13$).

Since homozygosity for the e4 allele at the *APOE* locus is strongly associated with AD in all ethnic groups tested, including African-Americans and Hispanics (Maestre et al. 1995), we next wished to evaluate whether the statistical associations with AD would change if *APOE* e4 homozygotes were removed from the analysis. When these subjects were removed, there was still no evidence for a strong association of either of the two *APOJ* coding variants with AD (data not shown). We also tested for an interaction between the *APOJ* genotype and the *APOE* genotype in conferring apparent AD risk using a logistic regression model that included both *APOE* and *APOJ*, but this analysis did not show evidence for an additive or synergistic interaction (data not shown).

Noncoding polymorphisms in the *APOJ* gene would not directly influence AD susceptibility. These markers could potentially show an association with AD if they were in linkage disequilibrium with another as yet uncharacterized coding polymorphism, either in *APOJ* or in a nearby gene, or with a functional polymorphism in a *cis*-acting regulatory element such as the *APOJ* promoter or an enhancer sequence. We therefore tested the *APOJ* exon 2 polymorphisms for AD association in the northern Manhattan and Indiana series of African-Americans. Among

Table 4 *APOJ* II genotypes in cases and controls by ethnic group in Washington Heights and Indiana. The number in each column represents the number of individuals with that specific genotype and the number in parentheses indicates the percentage of subjects with the specific genotype

Subjects	N	IIA/A	IIA/B	IIA/C	IIB/B	IIB/C	IIC/C
Washington Heights African-American							
Alzheimer's disease	84	30 (36%)	19 (23%)	21 (25%)	1 (1%)	5 (6%)	8 (10%)
Control	128	63 (49%)	17 (13%)	36 (28%)	1 (1%)	6 (5%)	5 (4%)
Caucasian							
Alzheimer's disease	27	27 (100%)	0	0	0	0	0
Control	24	24 (100%)	0	0	0	0	0
Hispanic							
Alzheimer's disease	62	53 (86%)	4 (6.5%)	3 (4.8%)	0	0	2 (3.2%)
Control	68	56 (82%)	4 (6%)	7 (10.3%)	0	0	1 (1.5%)
Indiana African-Americans							
Alzheimer's disease	24	14 (59%)	2 (8%)	5 (21%)	0	2 (8%)	1 (4%)
Control	47	25 (53%)	7 (15%)	13 (28%)	0	0	2 (4%)

heterozygotes, there was no evidence of a consistent association of either of these polymorphisms with AD (Table 4). Alleles on exon 2 and 7 were strongly associated with each other, particularly JIIC-JVIIB (χ^2 = 256, 8 *df*, $P <$ 0.0000), but this was independent of case-control status.

Homozygosity for the JIIC-JVIIB haplotype was somewhat more prevalent among cases than controls (6.8% vs 2.6%). In the combined northern Manhattan and Indiana groups the odds ratio for AD associated with JIIC-JVIIB homozygosity was increased compared to all other genotypes combined (*OR* = 3.8; 95% *CI* 1.1–13.1, $P < 0.01$). However, the relationship was no longer statistically significant in the absence of *APOE*-e4 (*OR* = 4.3; 95% *CI* 0.8–23.5, $P = 0.09$). There was also no evidence that the combination of an *APOE*-e4 allele and the JIIC-JVIIB haplotype had an additive effect on the risk of AD, that is the odds ratio for AD associated with one or more *APOE*-e4 alleles in conjunction with JIIC-JVIIB homozygosity (*OR* = 6.3; 95% *CI* 1.2–33.6, $P < 0.03$) had an overlapping 95% confidence interval with that for JIIC-JVIIB homozygosity without considering *APOE* status.

Discussion

There is considerable circumstantial evidence implicating the ApoJ protein in the neuropathological changes of AD (May 1993). To test *APOJ* as a candidate AD susceptibility gene, we have characterized coding and non-coding DNA sequence polymorphisms in this gene. As predicted from the previous study of IEF variants of the ApoJ protein (Kamboh et al. 1991), we find that the frequency of *APOJ* DNA polymorphisms is highly ethnically skewed, with the highest frequency of the variant alleles observed in individuals of African origin. Our findings of a lack of association of either of the two identified *APOJ* coding polymorphisms with AD indicates that at the population level these variants do not account for a significant component of AD susceptibility. The moderately elevated frequency of homozygosity for the JIIC-JVIIB haplotype among AD cases, while not statistically convincing in our series, probably warrants further study in larger cohorts.

While our findings do not support a major role for genetic variation in the ApoJ protein in susceptibility to AD, the DNA polymorphisms that we have described should be useful for exploring other possible disease associations. For example, ApoJ is a major prostatic gene product (Montpetit et al. 1986), and differential expression and functional data suggest a possible cytoprotective or anti-apoptotic role for this protein in prostate cancer cells (Buttyan et al. 1989; Sensibar et al. 1995). Prostate cancer has a twofold higher incidence in African-Americans relative to Caucasians (Morton 1994) and the possibility that genetic variation at the *APOJ* locus might account for a component of this susceptibility can now be investigated. From a more general point of view, the *APOJ* DNA polymorphisms described here should be a useful addition to the growing panel of available genetic markers that show markedly different allele frequencies in genetically distinct racial/ethnic groups (Dean et al. 1994). Such markers have been used for anthropo-genetic studies and more recently have shown theoretical promise for application in mapping by the admixture linkage disequilibrium method, which seeks to capitalize on linkage disequilibrium of markers with genetic traits or diseases in recently racially admixed populations such as Hispanics and African-Americans (Stephens et al. 1994).

Acknowledgements The authors wish to thank Long Zhao, Luwa Yuan, Marcel Kempowski, Adria Gonzales, John Shinin, Maria Gonzales and Harold Brown for their technical assistance in this project. We also thank Kathleen Hall, who is the project coordinator of the Indiana Alzheimer's Disease Project, and Michael Shelanski, M.D., Ph.D., who provided important scientific critique and encouragement. The work was supported by NIH grants AG07232, AG10963, AG08702 and RR00645 and by the Charles S. Robertson Memorial Gift for Alzheimer's Disease Research from the Banbury Fund.

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