

APOE Polymorphism And Susceptibility To Dementia Of The Alzheimer's Type In The Indian Population

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Abstract

Objective: The study aimed at the estimation of the prevalence of APOE polymorphism among Indians suffering from dementia of the Alzheimer's type and its association with disease risk as compared to the non-demented individuals. Another aim was to generate baseline data for future studies, as no study is available for those from Indian race/ethnicity.

Methods: The APOE genotypes of 100 patients with Alzheimer's Disease (AD) and 36 age-matched controls were determined by isotyping the polymorphic region from genomic DNA. PCR amplified APOE DNA fragment of 244 bp was digested with HhaI and fragments were analyzed on 15% PAGE.

Results: The allele and genotype frequencies were found to be significantly higher in AD patients as compared to the controls and the E4 allele was found to affect the risk of the disease in a dose-dependent manner. The frequency of the E4 allele was significantly increased in the patient group (0.47) as compared to age-matched controls (0.13). More pronounced effects were observed in genotype comparison.

Conclusions: The effect of APOE E4 was dosage dependent and the risk of AD in individuals with the E4E4 genotype was 20-fold greater than in individuals with the E3E4 genotype (10-fold), as compared to the E3E3 genotype. The risk was higher even after adjusting for age and sex. The E4 allele is an allele for developing AD in Indians.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the major cause of dementia in the elderly and is characterized by the presence of senile plaques and neurofibrillary tangles in selected regions of the brain. Age at onset has long been used to classify the disease as either early-onset Alzheimer's disease or late-onset Alzheimer's disease; usually with 60 years of age as an arbitrary cut-off. However, age alone is not a determinant of the clinical features. Early-onset AD (EOAD) may be explained by mutations in presenilin-1 (ps-1), presenilin-2 (PS-2), and Amyloid precursor protein (APP) [1]. The majority of cases (90-95%) are late-onset AD (LOAD), in which several factors have been implicated. Of these, the E4 allele of the lipid transport protein apoE (APOE gene, apoE protein) is the major factor [2]

Several lines of evidence suggest that apoE plays an important role in lipid transport, degeneration and regulation

in nervous tissue. Although the pathogenesis of amyloid deposition in AD is not completely elucidated, studies suggest the involvement of apoE in this process [3]. The presence of apoE in the extracellular senile plaques and the neurofibrillary tangles of AD suggest that apoE may be involved in the formation of these lesions. In cerebrospinal fluid, apoE avidly binds to synthetic amyloid β (A β) peptide, the primary constituent of senile plaques. The mRNA for apoE is increased in the brains of AD patients [4].

Three major protein isoform of apoE (apoE2, apoE3 and apoE4) are the products of three alleles (E2, E3 and E4) at a single gene locus on the proximal long arm of chromosome 19q13.2. Other variants of APOE do exist, namely APOE1, APOE5 and APOE7, but they are extremely rare [5]. The analysis of families with LOAD resulted in the identification of a disease locus on chromosome 19 [6]. Late-onset familial and sporadic AD patients have been associated with an increased frequency of the E4 allele, suggesting an association of APOE4 with increased susceptibility to

disease [7]. In these patients, the E4 allele frequency was 0.50 ± 0.06 , compared with age-matched controls of 0.16 ± 0.03 . This result has been confirmed by other studies in various ethnic populations, involving both early- and late-onset AD [8,9,10]. A gene dosage effect was also observed [8] showing that the risk increases from 20% when no E4 alleles are present to 90% when two copies of E4 are present.

The present study is an effort to delineate these relationships in Indian AD patients and to provide baseline data for future studies involving Indian populations. Also, we explore gender differences pertaining to the proportions of various alleles in this sample.

METHODS

SUBJECTS

Participants for this study were selected from various hospitals of North Western India. These include the Institute of Human Behaviour and Allied sciences (IHBAS), in Delhi, and the Punjab Mental Hospital and Bhatia Neuropsychiatric Hospital, both in Amritsar, Punjab. Physicians using standard NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/ Alzheimer's Disease and Related Disorders Association) criteria clinically diagnosed the participants as having probable dementia of the Alzheimer's type. A total of 100 participants (41 females and 59 males) with ages ranging from 48-85 years constituted the AD sample. The control group consisted of 36 age-matched individuals (20 females and 16 males), the spouses of the patients. Information regarding education, head injury, consanguinity, family history, medical history, smoking habits, and job description, was collected along with age and sex. The spouses were not genetically related to the participants and were fit both mentally and physically. The participants and their spouses shared similar family responsibilities and dietary habits, and had exposure to similar pollutants and metal ions. Blood samples were drawn from participants with informed consent of caregivers and from control group individuals with their own consent after the procedures had been fully explained. Ethics board approval was obtained.

DNA ANALYSIS

High molecular weight genomic DNA was isolated from blood leukocytes using the following modified method [11]. A fragment of 244 bp covering the APOE polymorphic region was amplified by PCR in a DNA thermal cycler using the oligonucleotide primers F4 5' ACA GAA TTC GCC CCG GCC TGG TAC AC 3' and F6 5' TAA GCT TGG

CAC GGC TGT CCA AGG A 3' [12], and the amplified product was subjected to digestion overnight using restriction enzyme HhaI (3 units in 24 l PCR product) at 37°C. The reaction mixture was loaded onto 15% polyacrylamide with 10% glycerol, non-denaturing gel and electrophoresed under a constant voltage of 150 V. The gel after electrophoresis was stained with silver nitrate and the bands were visualized. The sizes of bands were compared with a known size marker (pUC18/Sau3AI-pUC18/TaqI digest). APOE genotypes for the patients and control groups were determined by scoring for a unique combination of fragment sizes, as described by [13].

STATISTICAL METHODS

Allele frequencies for AD cases and control groups were estimated by the gene count method. The allele and genotype frequencies were compared using two-tailed chi-squared tests. Crude and adjusted (age and sex) odds ratios (OR) using logistic regression were calculated along with their 95% confidence intervals (CI) to assess the contribution of APOE alleles for the risk of AD. SPSS release 7.5 was used for the statistical analyses [14].

RESULTS

The AD participants were divided into early AD (disease onset <60 years) and late AD (disease onset >60 years) categories. Out of the total 100 AD cases, 42 (females 17, males 25) were designated as early AD, whereas 58 cases (24 females, 34 males) were designated as late AD. The distribution of various genotypes among AD and controls is presented in Table 1.

Figure 1

Table 1: Number (Percentages) of Various Genotypes in the Disease and Control Groups in a Study of apoE Polymorphisms

	E2E2	E3E3	E4E4	E2E3	E2E4	E3E4	Total
AD	--	18 (18.00)	16 (16.00)	3 (3.00)	13 (13.00)	50 (50.00)	100
Controls	1 (2.78)	23 (63.88)	1 (2.78)	4 (11.11)	1 (2.78)	6 (16.66)	36

APOE genotyping for all the individuals revealed significant statistical differences between groups, as far as the allele frequencies were concerned. The frequencies of the E2, E3, and E4 alleles in the various groups of AD patients are presented in Table 2. The allele frequencies were compared using chi-squared analyses (degrees of freedom = 1) and statistically significant differences were observed for the E3 and E4 alleles.

Figure 2

Table 2: Distribution of Various Alleles and Comparison of Allele Frequencies Among Early-onset AD cases, Late-onset AD cases, and Controls

Alleles	Distribution of alleles			Controls	Comparison of various groups		
	Early AD	Late AD	Total AD		Early AD/Controls (χ^2)	Late AD/Controls (χ^2)	Total AD/Controls (χ^2)
E2	0.06	0.09	0.08	0.09	0.29	0.05	0.19
E3	0.44	0.45	0.45	0.78	7.94*	9.04*	10.54*
E4	0.50	0.46	0.47	0.13	16.67*	14.83*	16.68*

*Significant at $p < 0.05$

The gender distribution of the various alleles along with the statistical comparisons is summarized in Table 3.

Figure 3

Table 3: Gender Distribution and Comparison of APOE Alleles in Early- and Late-onset AD

Alleles	Females			Males		
	Early AD	Late AD	Early/Late AD (χ^2)	Early AD	Late AD	Early/Late AD (χ^2)
E2	0.06	0.17	1.90	0.06	0.04	0.14
E3	0.38	0.33	0.14	0.48	0.53	0.14
E4	0.56	0.50	0.13	0.46	0.43	0.07

Odds ratios were calculated for the total AD group as compared to matched controls (Table 4). With the E3/E3 genotype as reference (as this is the most common allele in all populations of the world and also considered the parent allele), the crude odds ratios for the AD cases as compared to matched controls were statistically significant for the E2/E4, E3/E4, and E4/E4 genotypes. After adjusting for age and sex, these significant results were maintained.

Figure 4

Table 4: Comparison of Genotypes Among AD Cases and Controls With Crude and Adjusted Odds Ratios

Variables	Crude Analysis OR (95% CI)	Adjusted Analysis OR (95% CI)
Age	----	0.29 (0.09, 0.91)
Sex (Female)	----	2.04 (0.77, 5.39)
E3/E3	1.0 (ref)	1.0 (ref)
E2/E3 or E2/E2	0.77 (0.16, 3.64)	1.00 (0.19, 5.23)
E2/E4	16.61 (1.98, 139.09)	25.61 (2.82, 232.25)
E3/E4	10.65 (3.74, 30.36)	10.99 (3.68, 32.85)
E4/E4	20.43 (2.47, 168.84)	23.27 (2.67, 202.76)

DISCUSSION

The proportion of E3 and E4 alleles differs significantly between diseased and control groups. These results are consistent with observations made by others [15, 16, 17]. To estimate the contribution of various alleles towards the risk

of disease development, odds ratios (OR) were calculated. The risk associated with different APOE4 genotypes was significantly higher in the AD as compared to the control group. Similar results have also been reported [18, 19, 20].

Similar findings based on a meta-analysis were reported [21], revealing population differences in the odds for developing AD. The results of the present study present a similar worldwide trend in the occurrence of various genotypes. Therefore, this comparison demonstrates a significant association between the APOE4 allele and AD in the present study. Although a small sample size, a suggestion for a protective effect of the E2 allele was observed, which was limited to the E2E3 and E2E2 genotypes. The E2E3 genotype appears to be equally protective (OR=0.6) in all the populations compared by [21] and the present study. Another finding of this study is that the genotypes E2E4 and E3E3 are not equivalent in terms of AD risk, consistent with evidence provided by [21]. The E2E4 genotype has emerged as a risk associated genotype, as far as development of AD is concerned in the Indian population.

The allele E4 of apolipoprotein was found to be associated with AD in a dose-dependent fashion in a randomly selected elderly population of Kuopio, in eastern Finland [22]. The researchers reported a 2.7-fold increase in the risk for AD with the presence of one allele and a 9.3-fold increase with the presence of two alleles. Also, the genotype frequencies were compared by [19] and the risk of developing AD in E4 homozygotes was 10-fold greater than in E4 heterozygotes (2-fold), when compared to those without the E4 allele. In another study [23], individuals with the E4E4 or E3E4 genotype had 2.27 (95% CI, 1.06-4.89) times the odds of developing AD as compared to those with the E3E3 genotype. These studies are consistent with our data, showing 10 to 20-fold increases, from one to two copies of E4 in the analyses.

The increase in the E4 allele in AD patients could be explained by elucidating the role of APOE in the pathogenesis of AD. Studies [4, 5] have convincingly shown that apoE binds avidly to synthetic A peptide and also that the apoE4 binds more rapidly to -amyloid peptide (at physiological pH), than the E3 form. It was also observed that patients with one or two E4 alleles have increased amyloid deposition in the brain as compared to those who lack this allele. Whatever the role apolipoprotein E plays in the pathogenesis of AD, it seems that there is an increased prevalence of the E4 allele in patients with AD. The results

of the present study also confirmed the involvement of APOE4 allele in the development of AD. The emergence of the E2E4 genotype as a risk factor raises many questions and probably this may be because of racial and ethnic differences. Although E4 is a proved risk factor for the disease, the detailed information regarding the genetic makeup of the individuals should be considered while diagnosing AD. Thus, the major predisposing factor can be the genetic makeup of the individuals. One major study limitation is the small number of subjects in each category. Detailed ethnicity-based studies involving large population groups and targeting other genes involved [24], can provide further insight.

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