

Association of Apolipoprotein E with Neurodegenerative Diseases in North Indian Population

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Abstract

Background: Apolipoprotein E (ApoE) genotypes are associated with pathogenesis of various neurological diseases. This study was designed to explore the association of ApoE polymorphism with risk of various neurological diseases- Alzheimer's disease (AD), Parkinson's disease (PD), and stroke.

Methods: A cross-sectional study was performed on non-diseased and diseased subjects (AD, PD & stroke) from Institute of Human Behavior & Allied Sciences (IHBAS), New Delhi (India). 251 patients diseased and 113 non-diseased individuals were included in the study. ApoE genotyping was done in all subjects by ARMS-PCR method.

Results: In AD, PD and stroke groups, 103 subjects, 36 subjects and 112 subjects were included respectively in the study, whereas control group had 113 subjects. Average age in diseased and non-diseased groups was 61.27 years (SD= 14.07) and 57.45 years (SD= 14.25) respectively.

On genetic analysis for ApoE, ApoEε3/ε3 was found to be the most predominant genotype in both groups. Association study showed strong association of ApoEε4 allele with AD (COR: 2.73, CI: 1.32 – 5.65, $p = 0.01$), whereas weak association with PD and stroke as risk factor. However, ApoEε2 emerged as a protective factor in AD, but ε2 allele had two times more risk of stroke (COR: 2.10, CI: 0.93 – 4.76, $p=0.11$). The presence of ApoEε3 allele was a statistically significant protective factor in both AD and PD subjects, but not significant in stroke.

Conclusions: ApoEε4 allele has been found strongly associated with Alzheimer's disease and Parkinson's disease, whereas ApoEε2 emerged out as a risk factor for stroke and PD.

Keywords: Alzheimer's Disease, Parkinson's Disease, Stroke, APOE Genotyping, APOE, APOEE2, APOEE3 and APOEE4.

Introduction

Apolipoprotein E (ApoE), located in 19q13.2, encoding protein, apolipoprotein E, a lipoprotein with the highest expression in liver followed by the brain [1, 2]. It is a cholesterol carrier involved in lipid transportation and injury repair in the brain.

In humans, there are three polymorphic forms of ApoE: ApoEε2 (Cys-112, Cys-158), ApoEε3 (Cys-112, Arg-158), and ApoEε4

(Arg-112, Arg-158), influencing differently the functional properties of ApoE like cholesterol transport, antioxidant function, neurite extension, protection from cognitive decline, maintenance of cytoskeletal integrity, function of mitochondria, and prevention of neurodegeneration [1-3].

Several lines of evidence have proved beyond doubt that the ApoE genotypes are associated with the occurrence and clinical

outcome of neurodegenerative diseases [4]. ApoE is associated with pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), stroke, other dementia etc by modulating neuroinflammation, lipid metabolism, synaptic plasticity and neuronal toxicity [5-9].

ApoE ϵ 4 increases the risk of AD in a dose-dependent manner, in contrast to ApoE ϵ 2, which plays a protective role [10, 11]. It is estimated that the lifetime risk of developing AD increases to 29% for carriers for ApoE ϵ 4 allele by increasing peripheral lipid levels, decreased cerebral glucose metabolism, and increased A β deposition and neurofibrillary tangles formation in the brain [6]. Along with contemporary environmental conditions such as high carbohydrates, fat and low fibres intake, and reduced physical activity, increases the susceptibility of ApoE ϵ 4 carriers in developing AD [12-14].

Several groups investigated the association of ApoE and PD onset or PD dementia (PDD) as clinical and pathological features of PD and AD frequently overlap. A meta-analysis of 22 studies reported a positive association between the ApoE ϵ 2 allele frequency and PD risk, while no such association was found in ApoE ϵ 3 or ApoE ϵ 4 allele carriers [15].

Given that ApoE ϵ 2 appears to increase PD risk in some studies, it is likely that the role of ApoE in PD may be mechanistically distinct from that in other neurological disorders associated with ApoE ϵ 4.

Stroke, another neurological disorder shares several risk factors with heart disease [16, 17]. Recently stroke prevalence has been demonstrated to be significantly greater in ApoE ϵ 4 carriers due to its association with increased levels of LDL and cholesterol [18].

Hence ApoE ϵ 4 may be the best candidate for studying the interrelationship between genetic and acquired risk factors along with other alleles of ApoE. This study was designed to observe the various genotypes of ApoE in neurological diseases like Alzheimer's disease (AD), Parkinson's disease (PD) and stroke and to explore the association of ApoE alleles with the risk of Alzheimer's disease, Parkinson's disease and stroke.

Materials and Methods

Subjects

A cross-sectional study was performed on non-diseased and diseased subjects with various neurological diseases, recruited from Psychiatry & Neurology departments of Institute of Human Behaviour & Allied Sciences (IHBAS), New Delhi (India). Subjects diagnosed with Alzheimer's disease, Parkinson's disease and strokes were taken. Patients presenting with other dementias were not taken.

251 patients (mean age: 61.3 ± 14.1 years; 100 females & 151 males) diagnosed with various neurological diseases and 113

non-diseased individuals (mean age: 57.45 ± 14.3 years; 49 females & 64 males) were included in the study. The study was approved by the Institutional Ethics Committee.

AD Group

In AD group, 103 subjects (mean age 67.1 ± 11.2 years; 48 females & 55 males) were recruited who were fulfilling the criteria of the National Institute of Neurological and Communicative Disorders Association (NINCDS-ADRDA) and Mini Mental State Examination (MMSE) [19]. AD was confirmed by cerebral MRI studies which showed generalised atrophy with selective temporoparietal atrophy in all patients.

PD Group

In PD group, 36 subjects (mean age 55.9 ± 11.8 years; 11 females & 25 males) were included in the study. PD cases were diagnosed using Unified Parkinson Disease Rating Scale (UPDRS) and Mini Mental State Examination (MMSE) [20].

Stroke Group

In stroke group, there were 112 subjects (Mean age 57.6 ± 15.2 years; 41 females & 71 males). All the subjects with acute onset of persistent neurological deficit were diagnosed as stroke and was confirmed by neuroimaging (CT/MRI). The patients of head injury presenting with stroke were excluded.

Non-Diseased Group

Non diseased group had 113 patients (mean age: 57.5 ± 14.3 years; 49 females & 64 males) without any memory complaints who were attending the department of Neurology in same hospital for illness other than dementias, PD or stroke.

All subjects included in the study were informed about the study and blood samples were taken after written consent was taken from individuals who agreed to be included in the present study. They underwent neurological examination along with routine biochemical, hormonal and radiological examinations.

Blood Collection

Non fasting venous blood samples were taken in the evacuated tubes containing EDTA for DNA study and in plain tubes for biochemical studies. Samples collected in EDTA containing vial was stored at -70°C until DNA was extracted for ApoE genotyping.

DNA Extraction

DNA was extracted from the whole blood using salting out method [21]. Extracted DNA samples were stored at -200°C until processed.

Apo E Genotyping

Apo E genotyping was performed using amplification refractory mutation system polymerase chain reaction (ARMS-PCR) as described by You et al. 2015 (Figure 1) [22].



Figure 1: Gel picture showing ApoE genotyping. ApoEε2/4 in lane 1 and 2, ApoEε4/4 in lane 3 and 4, ApoEε2/3 in lane 6 and 7, ApoEε3/3 in lane 8 and 9 & molecular weight marker in lane 6.

Statistical Analysis

The descriptive statistics (Mean, Standard deviation, Frequency and Percentage) were calculated to describe the data. The Student t test for independent sample was used to find out the difference in averages for continuous variable age and the Chi-square test was applied to explore the association between categorical variables.

The Crude Odds Ratio (COR) along with 95% Confidence Interval was also calculated to find out the risk of association. The multivariable binary logistic regression analysis was done to

find out the absolute contribution of each independent variables and result was explained on the basis of Adjusted Odds Ratio (AOR) and 95% Confidence Interval.

Results

In the present study, a total of 364 study subjects were recruited from North India comprising of 251 diseased and 113 non-diseased subjects as shown in. Among 251 diseased subjects, the 103 (41.0%) had AD whereas PD had 36 (14.3%) & stroke in 112 (44.6%) subjects.

Table 1: Characteristics of Study Population

Variables	Disease Status	
	Diseased (n = 251)	Non-diseased (n =113)
Age (Mean, SD), years	61.27, 14.07	57.45, 14.25
Gender, n (%)		
Male	151 (60.15%)	64 (56.60%)
Female	100 (39.84%)	49 (43.40%)
Diagnosis, n (%)		
Alzheimer's Disease (AD)	103 (41.03%)	-
Parkinson's Disease (PD)	36 (14.34%)	-
Stroke	112 (44.62%)	-
ApoE Genotype, n (%)		
ε2 ε3	25 (9.96%)	10 (8.80%)
ε2 ε4	06 (2.39%)	00 (0.00%)
ε3 ε3	174 (69.32%)	90 (79.60%)
ε3 ε4	35 (13.94%)	12 (10.60%)
ε4 ε4	11 (4.38%)	01 (0.90%)

Average age in diseased and non diseased groups was 61.27 years (± 14.07) and 57.45 years (± 14.25) respectively. Cases and controls were homogenous in terms of gender, habitat, dietary habit, smoking habit, and alcohol intake habits (data not shown).

ApoE Genotyping

Genetic analysis was performed for identifying the frequency of distribution of ApoE alleles and six possible genotypes

in diseased and non-diseased subjects as shown in. The most common genotype was ApoEε3/3 in both diseased (69.3%) and non-diseased (79.6%) group, whereas the least common genotype observed was ε2/4 in both groups.

Table 2: Frequency of ApoE Genotypes in Various Neurological Diseases

ApoE Genotype	Diagnosis (n =251)			
	AD (n=103)	Stroke (n= 112)	PD (n= 36)	Total
ε2ε3	03	17	05	25
ε2ε4	04	02	00	06
ε3ε3	74	78	22	174
ε 3ε4	14	15	06	35
ε4ε4	08	00	03	11

Distribution of ApoE genotype in various neurological diseases (AD, PD & Stroke) studied is given in Table 2. Highest frequency of ApoEε3/3 was observed in stroke (78 subjects). Out of 103 subjects, 08 subjects with AD had ApoEε4/4 genotype whereas it was absent in stroke. However, the frequency of ApoEε3/4 genotype was almost similar in AD (14 subjects) and stroke (15 subjects) and 06 PD subjects had ApoEε3/4 genotype. The fre-

quency of ApoEε2/4 genotype was very small in all three diseased subjects.

In the present study all the subjects were gender matched in PD & stroke groups, whereas in AD group, age was significantly higher as compared to non diseased group. However distribution of males and females were found to be almost similar across all the groups (Table 3).

Table 3: Association of Apolipoprotein E 4 Allele with Risk Of AD, PD & Stroke, p-value, Crude Odds Ratio (COR) and their 95 % Confidence Interval (CI)

Variables	Disease status		p-value	COR (95% CI)	Disease status		p-value	COR (95% CI)	Disease status		p-value	COR (95% CI)
	Non-diseased (n= 113)	Diseased (AD: n=103)			Non-diseased (n= 113)	Diseased (Stroke: n= 112)			Non-diseased (n =113)	Diseased (PD: n=36)		
Age (Mean, SD)	57.45, 14.25	67.12, 11.24	0.00	—	57.45, 14.25	57.61, 15.21	0.94	—	57.45, 14.25	55.94, 11.83	0.57†	—
Gender												
Male	64	55	0.73	1.00	64	71	0.37	1.00	64	26	0.14†	1.00
Female	49	48		1.14 (0.67 – 1.95)	49	41		0.75 (0.44 – 1.29)	49	10		0.50 (0.22 – 1.14)
ApoE ε2 Allele												
Absent	103	96	0.76	1.00	103	93	0.11	1.00	103	31	0.58†	1.00
Present	10	07		0.75 (0.28 - 2.05)	10	19		2.10 (0.93- 4.76)	10	05		1.66 (0.53 – 5.23)
ApoE ε3 Allele												
Absent	01	12	0.00	1.00	01	02	0.62	1.00	01	03	0.04*	1.00
Present	112	91		0.07 (0.01 - 0.53)	112	110		0.49 (0.04 – 5.49)	112	33		0.10 (0.01 – 0.98)
ApoE ε4 Allele												
Absent	100	76	0.01	1.00	100	95	0.54	1.00	100	27	0.09†	1.00
Present	13	27		2.73 (1.32 - 5.65)	13	17		1.38 (0.63 – 2.99)	13	09		2.56 (0.99 - 6.63)

*P<0.05; Significant

† Not significant

Bivariate analysis was performed in the present study to assess the association of all three alleles i.e ε2, ε3 and ε4 of ApoE with AD, PD and Stroke (Table 3). In AD group, ε2 emerged as a protective factor (COR: 0.75, CI: 0.28 - 2.05), but was statistically insignificant (p = 0.76). However in stroke group, ε2 allele was found to be a risk factor (Table 3), where presence of ε2 allele had two times more risk (COR: 2.10, CI: 0.93 – 4.76) to have stroke as compared to subjects having ApoE allele other than ε2, but it was not significant (p = 0.11).

Similar results were found in PD group (COR; 1.66, CI: 0.53 – 5.23, p = 0.58). On the other hand, ApoEε3 allele was statistically significant protective factor in both AD and PD subjects

(COR: 0.07, CI: 0.01 – 0.53, p = 0.00 in AD & COR: 0.10, CI: 0.01 – 0.98, p = 0.04 in PD). Similarly in stroke also ApoEε3 emerged as a protective (COR: 0.49, CI: 0.04 – 5.49), but was not statistically significant (p = 0.62).

When association of ApoEε4 was assessed in all diseased groups as compared to non diseased group, the presence of ApoEε4 allele was found to be a risk factor across all three neurological diseases under study. However, ApoEε4 was a significant risk factor in AD group, contributing more than two & half times risk of having AD as compared to non-diseased group (COR: 2.73, CI: 1.32 – 5.65, p = 0.01).

In PD also, ApoE ϵ 4 was found to be a risk factor (COR: 2.56, CI: 0.99 – 6.63, $p = 0.09$), whereas ApoE ϵ 4 allele had very small and non-significant contribution in stroke subjects (COR: 1.38, CI: 0.63 – 2.99, $p = 0.54$) as compared to non-diseased group.

As the dependent variable was categorical and dichotomous (Diseased & Non-diseased) in the present study, the multivariable

binary logistic regression analysis was done in all three comparative situations (AD vs. Non-diseased, Stroke vs. Non-diseased & PD vs. Non-diseased) to find out the absolute contribution of each selected independent variables (Age, Gender, ApoE ϵ 2, ApoE ϵ 3 & ApoE ϵ 4) on the dependent variable (Table 4).

Table 4: Association Of Apolipoprotein E 4 Allele with Risk Of AD, PD & Stroke, Adjusted Odds Ratio (AOR) and their 95 % Confidence Intervals (CI)

Variables	AD		Stroke		PD	
	AOR	95% CI	AOR	95% CI	AOR	95% CI
Age	1.07	1.04 -1.10	1.00	0.99 – 1.02	0.99	0.96 – 1.02
Gender						
Male	1.00		1.00		1.00	
Female	1.23	0.68 – 2.26	0.73	0.42 – 1.27	0.44	0.19 -1.05
ApoE ϵ 2 Allele						
Absent	1.00		1.00		1.00	0.72 – 7.98
Present	0.39	0.11 – 1.40	2.21	0.95 – 5.14	2.39	
ApoE ϵ 3 Allele						
Absent	1.00		1.00		1.00	
Present	0.11	0.01 – 1.20	0.86	0.06 – 12.01	0.13	0.01 – 1.64
ApoE ϵ 4 Allele						
Absent	1.00		1.00		1.00	
Present	1.95	0.80 – 4.76	1.42	0.62 – 3.23	1.87	0.62 – 5.65

Similar to earlier findings in table 3, it has been shown that the age & gender did not significantly contribute in all three neurological diseases. Although not statistically significant, the presence of allele ApoE ϵ 2 was protective for AD (AOR: 0.39, CI: 0.11 – 1.40; $p = 0.15$), whereas it emerged out as a risk factor contributing more than two times risk for stroke (AOR: 2.21, CI: 0.95 – 5.14, $p = 0.07$) and PD (AOR: 2.39, CI: 0.72 – 7.98, $p = 0.16$) but statistically not significant.. Further, it was observed that the ApoE ϵ 3 allele was a protective factor in all neurological diseased group, whereas ApoE ϵ 4 allele was found to be a risk factor for AD, PD and stroke.

Discussion

Plasma ApoE is synthesised primarily by liver hepatocytes followed by brain. ApoE present in CSF and brain is primarily synthesised by the astrocytes, followed by oligodendrocytes, microglia and injured and stressed neurons and play a major role in the distribution of cholesterol from cells during membrane synthesis, neuritic extension, growth and repair [23, 24]. ApoE ϵ 2 & ϵ 3 alleles decrease the plasma levels of cholesterol, whereas ϵ 4 increases it. Also, ApoE has antioxidant activities, ranked as ϵ 2> ϵ 3> ϵ 4 [25].

Studies show that ApoE ϵ 4 is a hazardous allele as compared to ϵ 2 and ϵ 3, thereby can be used not only as prognostic marker in neurodegenerative diseases, but also as a novel therapeutic target. As ApoE allele show distinguished difference in distribution throughout the world, understanding of ethnic variation of ApoE polymorphism is crucial for further studies on role of ApoE alleles in neurodegenerative diseases. Studies show that ApoE ϵ 3 is prevalent in European and Asian population whereas ApoE ϵ 4 is markedly high in Oceanians and Africans [26, 27].

Significant number of studies have been done to assess the distribution of ApoE genotyping and association of various Apo E alleles and risk of variety of neurodegenerative diseases (AD, PD and Stroke) in different populations in the world, but no such study has been performed in such large North Indian population. In the present study the most common genotype observed was ApoE ϵ 3/3 in all the groups, followed by ApoE ϵ 3/4 genotype in diseased (13.9%) and non-diseased group (10.6%) subjects, whereas ApoE ϵ 2/2 was not observed in all the groups, which is similar to studies performed in Indian population [28, 29].

When ApoE genotyping was performed in individual neurological disease, highest frequency of ApoE ϵ 3/3 was observed in AD (71.8%), whereas PD (61.1%) had least frequency. In AD, 7.7% subjects had ApoE ϵ 4/4 genotype whereas it was absent in stroke. Similar findings have been made in stroke patients in eastern Indian population by Genaie et al, 2020 [17].

Our study shows, 39.9% of the patients with AD and 11.5 % of non-diseased carried at least 01 ApoE ϵ 4 allele, whereas 6.8 % of AD subjects and 8.8 % of non-diseased carried ApoE ϵ 2 allele. 15.2% stroke and 25% PD subjects had one ϵ 4 allele each. On the other hand ApoE ϵ 2 allele was present in 17.0 % and 13.9% of stroke and PD patients respectively. When association of all three alleles of ApoE with various AD, PD and Stroke subjects was studied, ApoE ϵ 4 allele emerged as risk factor in all three diseased groups, where as ApoE ϵ 2 allele was protective in AD group only.

ApoE ϵ 4 was noted to be a significant risk factor in AD and PD groups, contributing more than 2.5 times risk of having disease as compared to non diseased group, whereas the presence of ApoE ϵ 4 allele had insignificant contribution in stroke subjects.

In AD patients, similar findings have been reported by Singh et al 2012, where AD patient were 5.7 times more susceptible to ApoE ϵ 4 allele [30].

Similarly a meta-analysis performed showed statistically significant association of ApoE ϵ 4 allele with AD, indicating that ApoE ϵ 4 allele is a significant risk factor for AD [31]. ApoE ϵ 4 increases the risk of AD by initiating and accelerating amyloid β (A β) accumulation, aggregation and deposition in the brain and diminished clearance of A β , thereby enhancing the progression of cerebral amyloid angiopathy [16, 24]. ApoE ϵ 4 has been shown to be associated with dose dependent greater risk of hippocampal volume loss and increased atrophy before clinical presentation of AD.

The reduced antioxidant activity of ApoE ϵ 4 with impaired neuronal metabolism may increase the risk of AD in individuals carrying ApoE ϵ 4 allele [24], which further increases when two copies are present [32]. As it is mostly associated with late onset AD, in age group of 60-70 years, there is more rapid memory decline in individuals with no dementia, if ApoE ϵ 4 is present [33].

Similarly in PD, ApoE ϵ 4 was found to be a risk factor. But, recent meta-analysis performed with 22 association studies of PD showed ApoE ϵ 2 as a risk factor for PD with no effect of ApoE ϵ 4 [34]. However, few studies reported increased frequency of ApoE ϵ 4 (22.5%) in PD cases where advanced concomitant AD pathology were excluded. In another study performed by Ghebremedhin et al 2006 on pathologically classified as moderate to severe PD had increased frequency of ApoE ϵ 4 allele and decreased ApoE ϵ 3 allele with higher pathologic PD stages. Hence it can be concluded that ApoE ϵ 4 is associated with severity or progression of PD pathology, thereby influencing the PD risk [35-37].

Present study shows that ApoE ϵ 4 allele contributes non-significantly in stroke patients, which does not collaborate with study of Giani et al 2019 reporting 2.74 fold odds for developing ischemic stroke in patients having ApoE ϵ 4 allele. Gu et al 2013 also showed in their meta analysis 2.34-fold increase risk of ischemic stroke in ϵ 4 allele carriers. In our study, risk was not significant which could be due to non availability of data regarding cause of stroke in the patients recruited, whereas studies available in the literature have shown association of ApoE ϵ 4 in ischemic and haemorrhagic stroke separately [16, 38].

Unlike ApoE ϵ 4, presence of allele ApoE ϵ 2 was protective for AD, whereas it emerged as a risk factor contributing more than 2.0 times risk for stroke and PD in our study. Similar findings have been reported by Farrer et al 1997 [25]. They suggested that the ApoE ϵ 2 allele may be protective against AD or associated with a marked reduction in AD risk and reported decreased risk of AD in carriers of ApoE ϵ 2/2 or ϵ 2/3 compared to carriers of ApoE ϵ 3/3. Another study reported that ApoE ϵ 2 is associated with delayed onset of AD. We found ApoE ϵ 2 as a risk factor for stroke and PD [39].

Huang et al, 2004 in their meta-analysis also reported that ApoE ϵ 2 allele increases significant risk for PD [34]. Ghebremedhin et al 2006 & Pulkas et al 2011 also found trend toward increased frequency of ApoE ϵ 2 allele with increasing PD stages. However, various alleles of ApoE play variable role in stroke based on the type of stroke i.e ischemic or hemorrhagic.

Similar to our finding, Kokubo et al 2000 found that ApoE ϵ 2 had a 2-fold risk of cerebral infarction in Japanese population. Risk was greater in cortical infarction as compared to lacunar infarction [40-42].

Same study also reported increased risk of intracerebral hemorrhage (ICH) with ApoE ϵ 2/2 genotype. This association of ApoE ϵ 2 and stroke was accentuated in patients more than 70 years as compared to patients aged 40- 69 years. A meta-analysis performed on 26 studies of ischemic stroke, showed weak association of ApoE ϵ 2 with ICH [43]. This association was more strong in Asian population as compared to white population, whereas Ganie et al 2020 [16] could not find any association between ApoE ϵ 2 and ischemic stroke.

Conclusion

In our study ApoE ϵ 3 allele emerged as a protective factor in all diseased groups, as ApoE ϵ 3 allele was most frequent in both diseased and non diseased groups. Farrer et al, 1997 [25] reported low risk of AD in carriers of ApoE ϵ 3 allele as compared to those carrying ApoE ϵ 3/3. Chabra et al 2000 & Ghu et al 2013 [28,38] found ApoE ϵ 3 allele most frequently in Indian population and Corbo and Sacchi [26] reported ApoE ϵ 3 being more prevalent in all human societies. Ganie et al, 2020 [16] has also reported ApoE ϵ 3 as a protective factor, for developing stroke among the ethnic Bengali population of West Bengal.

Based on the findings of present study, it can be concluded that ApoE ϵ 4 allele was strongly associated with AD and PD, whereas ApoE ϵ 2 was protective for AD but emerged as a risk factor for stroke and PD. However, there has been number of limitations in the present study. Subjects with mild cognitive decline and other types of dementia should have been included in the study. Also, association of ApoE should have been studied separately in ischemic and hemorrhagic stroke.

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