ISSN- 0301-1216
Indian J. Prev. Soc. Med. Vol. 56, No.4, 2025

Apolipoprotein E Polymorphism Status with Neurological Diseases in North Indian Population

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ABSTRACT

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), non-AD dementia, Parkinson's disease (PD), stroke, Multiple sclerosis (MS) and Wilson's diseases (WD).

A cross-sectional study was performed on 113 non diseased and 350 diseased subjects (AD, PD & stroke) from Institute of Human Behavior & Allied Sciences (IHBAS), New Delhi (India). ApoE genotyping was done in all subjects by ARMS-PCR method.

In AD, non-AD dementia, PD, stroke, MS and WD groups, 103 subjects, 43, 36, 112, 24 and 32 subjects were included respectively in the study, whereas non-diseased 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, ε3/3 was found to be the most predominant genotype in both groups. In association study ε4 allele was identified as a risk factor in all neurological diseases. Specifically, ε4 was a significant risk factor in AD with 2.5 times increased risk (COR: 2.73, CI: 1.32 – 5.65). However, ApoEε2 emerged as a protective factor in AD, but a risk factor in non-AD dementia, PD and stroke (COR: 2.10, CI: 0.93 – 4.76).

ε4 allele has been found strongly associated with Alzheimer's disease and Parkinson's disease, whereas ε2 emerged out as a risk factor for stroke, non-AD dementia and PD.

Keywords: ApoE, ε4, Alzheimer's disease, Parkinson's disease, Stroke, Multiple sclerosis, Wilson's disease

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Citation: Agarwal R., Tripathi CB., Kushwaha S. Apolipoprotein E Polymorphism Status with Neurological Diseases in North Indian Population. Indian J Prev Soc Med, 2025; 56 (4): **542-550.**
DOI: https://doi.org/10.5281/zenodo.18068140

Sequence of Article: **Submission:** 15.10.2025 **Accepted:** 21.11.2025 **Published:** 31.12.2025

Prior Publication: Nil; Source of Funding: Nil; Conflicts of Interest: None, Article # 917/1466

Introduction

Apolipoprotein E (ApoE), located in 19q13.2, with three common isoforms termed ε2, ε3, and ε4, deriving six genotypes, produces apolipoprotein E consisting of 299 amino acids. It is primarily expressed in liver followed by the brain, where it plays critical role in lipid transport, neuronal repair and various neuropathological processes[1]. In humans, three polymorphic forms of ApoE: ApoE ε2 (Cys-112, Cys-158), ApoE ε3 (Cys-112, Arg-158), and ApoE ε4 (Arg-112, Arg-158) differ in two key amino acid positions at 112 and 158 that significantly alter the charge and structural properties of the protein, ultimately influencing it's functional properties like cholesterol transport, antioxidant function, neurite extension, protection from cognitive decline, maintenance of cytoskeletal integrity, mitochondrial function and prevention of neurodegeneration [1, 2].

Several lines of evidence have consistently shown associations between ApoE genotypes and the prevalence and clinical outcomes of various neurodegenerative diseases[3]. The different mechanisms by which ApoE could influence these neurological disorders include modulation of neuro-inflammation, lipid metabolism, synaptic plasticity, and neuronal toxicity[4,5]. The major neurodegenerative disorders where ApoE polymorphism has been involved in the disease pathogenesis and disease outcome are

Alzheimer's disease (AD), Parkinson's disease (PD), Multiple Sclerosis (MS), Wilson's Disease (WD), other dementia & cognitive impairment, cerebral amyloid angiopathy, amylotrophic lateral sclerosis etc.

In Alzheimer's disease (AD), the ε4 allele is a known risk factor that increases susceptibility in a dose-dependent manner, while the ε2 allele offers a protective effect[6]. Carriers of the ApoE ε4 allele face a 29% lifetime risk of developing AD, with mechanisms suggested to include different binding capacities for amyloid-beta (Aβ) peptide and tau protein, leading to increased amyloid deposition in the brain[7]. Meanwhile, some studies observed association of ε4 with increased peripheral lipid levels, decreased cerebral glucose metabolism, and increased Aβ deposition and neurofibrillary tangles formation in the brain[8], along with contemporary environmental conditions such as high carbohydrates, fat and low fibers intake, and reduced physical activity, increases the susceptibility of ε4 carriers to developing AD[9]. Not only AD, studies have underlined that ε4 carriers have an increased risk to develop non-AD dementia such as LBD and frontotemporal Dementia also [10].

In Parkinson's disease (PD), associations between ApoE genotype and disease susceptibility or age of onset or PD dementia (PDD) are less clear as studies show that the clinical and pathological features of PD and AD frequently overlap[11].While, a meta-analysis of 22 studies reported a positive association between the ApoE ε2 allele frequency and PD risk, while no such association was found in ε3 or ε4 allele carriers[12], another report suggests that ApoE plays no role in modulating the clinical features of PDD, suggesting that the underlying causes of dementia in PD differs from that of AD[11]. Given that ε2 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 ε4.

Stroke, another neurological disorder shares several risk factors with heart disease[13]. Recently stroke prevalence has been demonstrated to be significantly greater in ApoEε4 carriers due to its association with increased levels of LDL and cholesterol[14].

As ApoE plays significant role in myelin repair, neuronal plasticity, and cerebral inflammatory processes, Multiple sclerosis (MS), being a chronic inflammatory demyelinating disorder of the central nervous system, ApoE may be relevant in MS[15]. In MS, the association of ApoE genotype with MS is supported by many pathological changes occurring in MS. Firstly, astrogliosisis a major feature of active lesions in MS, whereas astrocytes are the major site of production of ApoE in CNS. Secondly, a potential mechanism of demyelination in MS involves free radical damage via nitric oxide[16] and Apo E may protect CNS against oxidative insult. Though an initial study reported a higher frequency of MS in ε4 homozygous individuals from a Danish population playing a role as a progression modifier of MS, with negative effect on brain pathology, cognitive dysfunction, and severity[17]. However, some studies found no association between ε4 allele frequency and MS susceptibility[18].

Progressive neuro-degeneration is an important signature among neural varieties of Wilson's disease (WD) patients. It has been reported that ε4 has least copper chelating activity[19]. As WD patients show abnormal copper deposition, ε4 could influence neuronal vulnerability, thereby leading to aggravated clinical manifestations, and diminished age of onset. Initial studies suggested association of allele with early onset of WD[20, 21], whereas some studies indicated that ApoE ε3/ε3 might confer neuro-protection by delaying the onset of the disease[22].

As the potential effects of ApoE isoforms on lipid metabolism, cell signalling, and neurotoxicity in CNS have potential to influence both AD and other neurological disorders, further investigation is warranted to explore ApoE as risk factor. Hence the present study was designed to observe the various genotypes of ApoE in neurological diseases like dementia including Alzheimer's disease (AD), non-AD dementia, Parkinson's disease (PD), stroke, Multiple Sclerosis and Wilson's disease and to explore the association of various ApoE alleles with the risk of them.

Material & Methods

A cross-sectional study was performed on non-diseased and diseased subjects with various neurological diseases, recruited from outpatient services of Psychiatry & Neurology departments of Institute of Human Behaviour & Allied Sciences (IHBAS), New Delhi (India). Subjects diagnosed with Alzheimer's disease (AD), non-AD dementia, Parkinson's disease (PD), stroke, Multiple Sclerosis and Wilson's disease were taken. 350 patients (137 females & 213 males) diagnosed with various neurological diseases and 113 non diseased individuals (49 females & 64 males) were included in the study.

Non diseased Group: Non diseased group was selected from subjects attending the department of Neurology in same hospital for illness other than dementias, PD, stroke, Multiple Sclerosis and Wilson's disease.

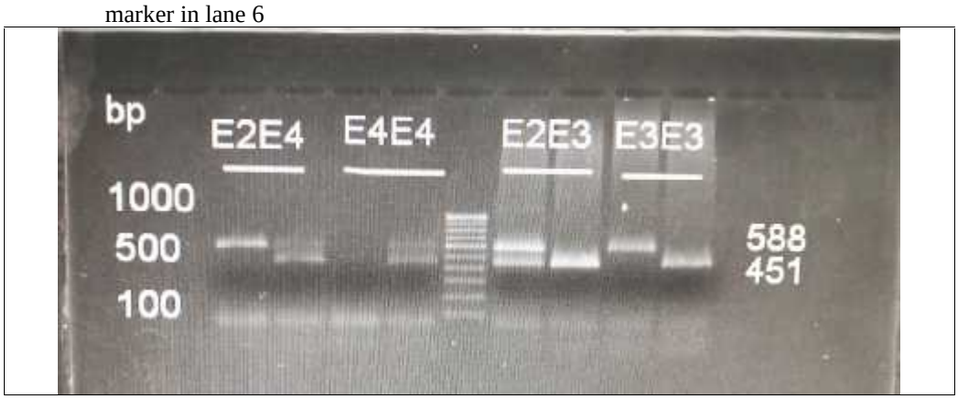
All subjects included in the study were informed about the study and blood samples were taken after written consent was obtained from individuals who agreed to be included in the present study. They underwent neurological examination along with routine biochemical, hormonal and radiological examinations.

Blood Collection: 5-8 ml non fasting venous blood sample was taken in evacuated tubes containing EDTA for DNA study and in plain tubes for biochemical studies. Samples collected in plain vial were centrifuged within 60 minutes of collection to separate the serum and were examined for routine biochemistry, whereas samples collected in EDTA containing vial were stored at -20^{0}C until DNA was extracted for ApoE genotyping.

Apo E Genotyping: Blood sample was drawn from the cubital vein of the subjects and was collected in EDTA evacuated tubes. DNA was extracted from the whole blood using salting out method (23). The quantity and quality of DNA samples were assessed using gel electrophoresis (1%) and UV-VIS spectroscopy. DNA samples ran as single band on the gel and optical density (OD) was measured at wavelengths of 230, 260 & 280 nm. Average 260/280 ratio was 1.7± 0.2 while 260/230 ratio was 1.5 ± 0.8. Extracted DNA samples had a final concentration ranging from 20–80 ng/µL. DNA samples were stored at -20^{0}C until processed.

Apo E genotyping was performed using amplification refractory mutation system polymerase chain reaction (ARMS-PCR) as described elsewhere (24) as shown in Figure 1.

Fig.-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



PCR reaction was performed in two tubes, containing the specific cys primers (Cys112 and Cys158) in one while the specific Arg primers (Arg112 and Arg158) in another tube with common reverse primer (Table 1). Reaction volume (20-µl) included 50 ng genomic DNA, 0.4µM Cys primers and Arg primers, 0.4 µM reverse primer (common primer), 1.6 µl dimethylsulfoxide (DMSO), 10 µl of 2X master mix [Taq DNA polymerase (0.05 U/µL), 4 mM $MgCl_2$, 0.4 mM of each dNTP and reaction buffer, Thermo Fisher Scientific, Waltham, Massachusetts, United States], and 4.8 µl nuclease free water.

Table-1: Primers for ARMS-PCR

Primer name	Primer sequence	Product length (bp)
Arg112 (forward)	5'-CGCGGACATGGAGGACGTTC-3'	588
Arg158 (forward)	5'- ATGCCGATGACCTGCAGACGC-3'	451
Cys112 (forward)	5'- CGCGGACATGGAGGACGTTT-3'	588
Cys158 (forward)	5'- ATGCCGATGACCTGCAGACGT-3'	451
Common primer (reverse)	5'- GTTCAGTGATTGTCGCTGGGCA-3'	

Amplification was initiated by denaturation for 5 min at 95˚C, followed by 35 cycles of 95˚C for 30 sec, 63˚C for 30 sec and 72˚C for 30 sec, and final extension at 72˚C for 10 min. Amplified nucleotides were resolved by 2% agarose gel electrophoresis with a 1 Kbp ladder (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

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.

Results

Baseline Characteristics: In the present study, a total of 463 study subjects were recruited from North India consisting of 350 diseased (mean age: 56.63 ± 20.71 years; 137 females & 213 males) and 113 non-diseased individuals (mean age: 57.45 ± 14.25 years; 49 females & 64 males). A detail of subjects with different neurological diseases within the diseased group is presented in Table 2. Diseased and non-diseased groups were homogenous with respect to gender, habitat, dietary habit, smoking habit, and alcohol intake habits (data not shown).

Distribution of ApoE variants in various neurological diseases: Genetic analysis was conducted to assess the frequency of distribution of ApoE alleles and six possible genotypes in diseased and non-diseased subjects (Table 2). The most prevalent genotype identified was ε3ε3 in diseased (70.28%) and non-diseased (79.60%) group, followed by ε3ε4 genotype, with a frequency of 15.14% among diseased individuals and 10.60% among those in the non-diseased group. Notably, the least common genotype across both groups was ε2ε4, while the ε2ε2 genotype was absent in all study subjects.

Table -2: Characteristic features of Study group

Baseline Characteristics		**Disease Status**			
		Diseased (N = 350)		**Non-diseased (N =113)**	
		No.	**%**	**No.**	**%**
Gender	Male	213	60.85	64	56.60
	Female	137	39.14	49	43.40
Diagnosis	Non-AD Dementia (VaD, FTD, PDD, LBD)	43	12.28	--	--
	Alzheimer's Disease (AD)	103	29.42	--	--
	Parkinson's Disease (PD)	36	10.28	--	--
	Stroke	112	9.00	--	--
	Multiple Sclerosis (MS)	24	6.85	--	--
	Wilson's Disease (WD)	32	9.14	--	--
APOE Genotyping	e2e3	33	9.42	10	8.80
	e2e4	06	1.71	0	0.0
	e3e3	246	70.28	90	79.60
	e3e4	53	15.14	12	10.60
	e4e4	12	3.42	01	0.90

The distribution of ApoE genotype across various neurological diseases including non-AD dementia, AD, PD, stroke, Multiple Sclerosis and Wilson's disease is detailed in Table 3. Multiple sclerosis exhibited the highest frequency of ε3ε3 at 83.33%, closely followed by Wilson's disease (81.25%). Conversely, non-AD dementia had least frequency (60.46%).

Table -3: Apolipoprotein E Genotypes in various Neurological Diseases (Diseased group)

Apo E variants	**Diagnosis (N=350)**					
	Non-AD Dementia (N=43)	**AD (N=103)**	**PD (N=36)**	**Stroke (N=112)**	**MS (N=24)**	**WD (N=32)**
ε 2 ε3	07	03	05	17	00	01
ε 2 ε4	00	04	00	02	00	00
ε 3 ε3	26	74	22	78	20	26
ε 3 ε4	09	14	06	15	04	05
ε 4 ε4	01	08	03	00	00	00

Among the 350 subjects, 08 subjects with AD, 01 with non-AD dementia, and 03 with PD possessed ε4ε4 genotype; however, this genotype was not present in subjects with stroke, MS and Wilson's disease. The ε3ε4 genotype frequency was highest in subjects with non-AD dementia and PD. The ε2ε4 genotype was rare, observed only in patients with AD and stroke, while it was absent in all other diseased group.

These findings highlight the genetic variability within the studied populations and suggest that specific ApoE genotypes may be associated with different neurological conditions, potentially indicating a genetic predisposition to these diseases. Further investigation may be necessary to understand the underlying mechanisms and implications of these genotype distributions in relation to disease risk and progression.

Association of Apolipoprotein E alleles with risk of Neurological diseases: 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 various neurological diseases.

In AD group, ε2 appeared as a protective factor (COR: 0.75, CI: 0.28 -2.05); however, it was statistically insignificant (p = 0.76), whereas in non-AD dementia ε2 was a risk factor (COR: 2.0, CI: 0.71, 5.65). In contrast, within the stroke group, the presence of the ε2 allele was associated with an increased risk (Table-4), as individuals with the ε2 allele faced approximately twice the risk of stroke (COR: 2.10, CI: 0.93 – 4.76) compared to subjects with other ApoE allele, though this finding was also not statistically significant (p= 0.11). Similar observations were noted in the PD group (COR; 1.66, CI: 0.53 – 5.23, p = 0.58).

Table-4: Association of Apolipoprotein E 4 Allele with Risk of Alzheimer's diseases, Parkinson's disease& Stroke, Crude Odds Ratio (COR) and their 95 % Confidence Interval (CI)

Variables	Disease Status		COR	95% CI	Disease Status		COR	95% CI	Disease Status		COR	95% CI
	Non-diseased (N=113)	Diseased (AD: N=103)			Non-diseased (N=113)	Diseased (AD: N=112)			Non-diseased (N=113)	Diseased (AD: N=36)		
ApoE ε2 Allele												
Absent	103	96	1.00	0.28	103	93	1.00	0.93	103	31	1.00	0.53
Present	10	07	0.75	2.05	10	19	2.10	4.76	10	05	1.66	5.23
ApoE ε3 Allele												
Absent	01	12	1.00	0.01	01	02	1.00	9.04	01	03	1.00	0.01
Present	112	91	0.07	0.53	112	110	0.49	5.49	112	33	0.10	0.98
ApoE ε4 Allele												
Absent	100	76	1.00	1.32	100	95	1.00	0.63	100	27	1.00	0.99
Present	13	27	2.73	5.65	13	17	1.38	2.99	13	09	2.56	6.63

Conversely, ε3 allele showed significant protective effect. In both AD and PD groups, the presence of ε3 allele was a statistically significant in reducing the disease risk (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).

Table-5: Association of Apolipoprotein E 4 Allele with Risk of Non-AD dementia, Multiple Sclerosis & Wilson's disease, Crude Odds Ratio (COR) and their 95% Confidence Interval (CI)

Variables	Disease Status		COR	95% CI	Disease Status		COR	95% CI	Disease Status		COR	95% CI
	Non-diseased (N=113)	Non-AD Dementia (N=43)			Non-diseased (N=113)	Multiple Sclerosis (N=24)			Non-diseased (N=113)	Wilson's Disease N=32)		
ApoE ε2 Allele												
Absent	07	10	2.00	0.71,	00	10	0.20	0.01,	01	10	0.33	0.04,
Present	36	103		5.65	24	103		3.55	31	103		2.70
ApoE ε3 Allele												
Absent	42	112	0.38	0.02,	24	112	0.65	0.03,	32	112	0.87	0.03,
Present	01	01		6.13	00	01		16.52	00	01		21.79
ApoE ε4 Allele												
Absent	10	13	2.33	0.94	04	13	1.54	0.45	05	13	1.54	0.45
Present	33	100		5.81	20	100		5.21	20	100		5.21

In stroke group, although ε3 was identified as a protective factor (COR: 0.49, CI: 0.04 – 5.49), this association was not statistically significant (p=0.62). No significant association was observed between Apo E alleles and other neurological diseases including non-AD dementia, Multiple sclerosis and Wilson's disease.

When impact of ε4 was examined across all diseased groups compared to non-diseased group, the presence of ε4 allele was identified as a risk factor in all neurological diseases studied. Specifically, ε4 was a significant risk factor in AD, showing more than 2.5 times increased risk (COR: 2.73, CI: 1.32 – 5.65, p = 0. 01). A similar trend was observed in non-AD dementia (COR: 2.33, CI: 0.94, 5.81) and PD (COR: 2.56, CI: 0.99 – 6.63, p = 0.09), although these results were not statistically significant. In the stroke group, the contribution of the ε4 allele as a risk factor was minimal and not statistically significant in stroke subjects (COR: 1.38, CI: 0.63–2.99, p =0.54), MS (COR: 1.54, CI: 0.45, 5.21) and Wilson's disease (COR: 1.54, CI: 0.45, 5.21) compared to non-diseased group.

These findings underscore the complex role of ApoE alleles in neurological diseases, with distinct protective and risk associations that vary across conditions.

Discussion

The synthesis and distribution of plasma Apolipoprotein E (ApoE) reveal its crucial role in cholesterol metabolism and thereby, its involvement in various neurological diseases. Primarily ApoE is synthesized by the astrocytes, followed by oligodendrocytes, microglia and injured and stressed neurons in the brain; it plays a major role in the distribution of cholesterol from cells during membrane synthesis, neurotic extension, growth and repair[25]. There are notable differences in the impact of ApoE alleles on plasma cholesterol levels, with ε2 and ε3 reducing its levels, while ε4 increases them. Beyond its role in cholesterol metabolism, ApoE also has antioxidant properties, with efficacy ranked as ε 2> ε3> ε4.[26]. Studies show that ε4 allele poses greater risk compared to ε2 and ε3, highlighting its potential use as a prognostic marker in neurodegenerative diseases like AD, PD, stroke and MS as well as an emerging therapeutic target [1, 27, 28]. As ApoE allele shows distinguished difference in distribution among the population throughout the world, understanding of ethnic variation of ApoE alleles worldwide is crucial to elucidate their role in these diseases. Studies have established that ε3 is most prevalent allele globally, with ε4 serving as an ancestral allele, while ε2 allele frequency is inconsistent, notably absent in Native American populations and rare among Southern Europeans[29, 30]. Additionally, ε3 is prevalent in European and Asian population, whereasε4 is markedly high in Oceanians and Africans [29].

A number of studies have explored the distribution of ApoE genotyping and their association with various neurological diseases across different populations in the world. However, the current study stands out as it offers insight into the role of ApoE genotypes as risk factors for multiple neurological diseases in a large North Indian cohort. In the present study the predominant genotype observed was ε3ε3 in both diseased (70.28%) and non-diseased (79.60%) group. Theε3ε4 genotype followed, with frequencies of 15.14% in diseased and 10.60% in the non-diseased subjects, while ε2ε2 was not present in any study groups, aligning with the findings from other Indian studies[31]. Moreover, the highest frequency of ε3ε3 was noted in patients with Multiple Sclerosis (83.33%), while non-AD dementia and PD presented with the lowest frequencies (60.46% and 61.11%, respectively). In the AD group, of the 103 subjects, eight carried the ε4ε4 genotype, which was absent in stroke patients, a finding noted by Genaie et al. 2020 also [13].

The presence of the ε4 allele is identified as a significant risk factor in the AD group, increasing the risk more than 2.5-fold compared to non-diseased individuals. Similar findings were observed in PD, with ε4 demonstrating a smaller, non-significant contribution to stroke risk. Singh et al 2012 earlier has reported that the ε4 allele had 5.7 times greater susceptibility to AD in North Indian population[32]. A meta-analysis conducted in 2014 confirmed these associations, identifying the ε4 allele as a significant risk factor for AD due to its higher prevalence[33]. The ε4 allele is believed to promote AD pathogenesis through mechanisms involving amyloid-β (Aβ) accumulation, aggregation, and impaired clearance, thus contributing to cerebral amyloid angiopathy and is predominantly associated with late-onset AD [25, 34]. The reduced antioxidant activity of ε4 with impaired neuronal metabolism may increase the risk of AD in individuals carrying ε4 allele[25], which further increases when two copies are present[34]. Unlike ε4, the ε2 allele appears protective against AD, as supported by various studies [26, 35] in their studies, which suggested a substantial reduction in AD risk linked to this allele. Farrer et al[26] also noted decreased risk of AD in carriers of ε2/ε2 or ε2/ε3 as

compared to carriers of ε3/ε3. Another study reported[36] delayed onset of AD in carriers of ε2 allele suggesting it's protective role in AD. Conversely, ε2 and ε4 were found to be risk factors in non-AD dementia group which is supported by Mishra et al. They reported an association between ApoE and FTD with a protective effect for ε2 and an increased risk conferred by ε4,[37] by accelerating neuro-degeneration and tau-pathology independent of amyloid β. However, Jairani et al 2016[38] observed no such association. In PD, while ε4 has emerged as a risk factor, a recent meta-analysis performed with 22 association studies of PD suggest that ε2 may confer increased risk, contradicting previous reports about ε4 as a risk factor for PD as it showed no such effect[12]. Ghebremedhin et al (2006)[39] reported that increased ε4 frequency correlated with advanced PD pathology stages, emphasizing ε4's potential role in severity or progression of PD pathology, thereby influencing the PD risk, rather than initiation. In contrast to ε4, ε2 was observed as a risk factor in PD in our study, which is similar to a meta-analysis performed by Huang et al, [12]. They reported that ε2 allele increases significant risk for PD. Ghebremedhin et al (2006)[39] also found trend toward increased frequency of ε2 allele with increasing PD stages.

In stroke patients, our study found no significant contribution from ε4 allele, unlike Giani et al, who reported2.74-fold odds for developing ischemic stroke among ε4 carriers. Studies from Gu *et al.* 2013 in their meta analysis[40] suggested a 2.34-fold increase risk of ischemic stroke among ε4 allele carriers. The lack of significant findings in our study may be attributed to insufficient data regarding stroke etiology in the enrolled patients, while studies available in the literature have shown association of ApoE ε4 in ischemic and haemorrhagic stroke separately[13]. Like PD, ε2 was observed as a risk factor in stroke also. In Japanese population, Kokubo et al (2000)[41] found that ε2 had a 2-fold risk of cerebral infarction, risk was greater in cortical infarction as compared to lacunar infarction. Also, same study reported that ε2/ε2 genotype had an increased risk of intracerebral hemorrhage (ICH). This association of ε2 and stroke was accentuated in patients more than 70 years as compared to patients aged 40- 69 years. In meta-analysis performed on 26 studies on ischemic stroke, ε2 was found to be associated with ICH, but strength of association was poor[42]. This association was stronger in Asian population as compared to white population. However, another meta-analysis performed on 11 case control studies with ICH found no significant association between the risk of ICH and the ε2 allele[43] which was similar to findings of Ganiae et al[13]. The relationship between ApoE alleles and stroke risk has been explored variably across different studies, indicating a more nuanced understanding of these associations is needed.

In patients of Multiple Sclerosis, 16.66% carried ε4 allele, while the ε2 was absent in our study. Study conducted by Rafiei et al (2012)[44] indicated a association of ε4 with MS susceptibility and protective role of ε2 in MS development, whereas first meta-analysis performed by Xuan et al in (2011)[15] on 5472 cases and 4727 controls found no association between the ε4, ε2 alleles and MS susceptibility. In Wilson's diseases (WD) group, ApoE had no impact on disease presentation. Although Litwin et al (2012)[20] observed that women with ε4 presented with WD symptoms earlier than women with ε3/ε3 genotype, the effect of ε4-positive genotype was more pronounced in ATP7B p.H1069Q homozygous women. Roy et al 2017[21] observed significantly overrepresentation among WD patients than in controls. Thus, individuals bearing ε4 allele could present with compromised copper binding and increased copper-induced toxicity.

In the present studyε3 allele emerged as a protective factor in AD, PD, Stroke and WD. As mentioned earlier, ε3 allele was most frequent in both diseased and non diseased groups. The prevalence of this allele aligns with its distribution in different populations, as highlighted by previous studies indicating low risk associated with ε3 carriers. Ongoing research in diverse populations will enhance our understanding of ApoE's role in modulating disease risk and progression, potentially guiding novel therapeutic approaches.

Conclusion

In conclusion, the current study establishes a significant association between ApoE gene polymorphism and various neurological diseases, particularly in patients with Alzheimer's disease (AD), Parkinson's disease (PD), and stroke within the North Indian population. The ε4 allele is strongly linked to increased risk for both AD and PD, highlighting its role as a major genetic risk factor. Conversely, the ε3 allele appears protective across all studied neurological conditions, reinforcing its beneficial role in mitigating disease risk. Notably, while ε2 has shown protective effects against AD, it paradoxically emerged as a risk factor for both stroke and PD.

These findings underscore the complexity of ApoE's influence on neurological health and suggest potential avenues for genetic screening and targeted interventions. However, further research involving larger sample sizes is essential to validate these associations and enhance our understanding of the underlying mechanisms. Such studies could provide more robust insights into how ApoE polymorphisms contribute to neurodegenerative diseases, inform public health strategies, and guide therapeutic development for affected populations.

Ethical Committee Approval No.: 2022/5/24/V-1

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